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TOPIC HIGHLIGHT

2016 Colorectal Cancer: Global view

Clinical efficacy and drug resistance of anti-epidermal growth factor receptor therapy in colorectal cancer

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Abstract

Colorectal cancer (CRC) ranked third in cancer related death and its incidence has been increasing worldwide. In recent decades important therapeutic advances have been developed in treatment of metastatic CRC (mCRC), such as monoclonal antibodies against epidermal growth factor receptor (anti-EGFR), which provided additional clinical benefits in mCRC. However, anti-EGFR therapies have limited usage due to approximately 95% of patients with *KRAS* mutated mCRC do not response to anti-EGFR treatment. Thus, *KRAS* mutation is predictive of nonresponse to anti-EGFR therapies but it alone is not a sufficient basis to decide who should not be received such therapies because; approximately fifty percent (40%-60%) of CRC patients with wild-type *KRAS* mutation also have poor response to anti-EGFR based treatment. This fact leads us to suspect that there must be other molecular determinants of response to anti-EGFR therapies which have not been identified yet. Current article summarizes the clinical efficacy of anti-EGFR therapies and also evaluates its resistance mechanisms.

Key words: Colorectal cancer; Epidermal growth factor receptor; *KRAS* mutation; Anti-epidermal growth factor receptor antibody; Drug resistance

Core tip: Molecular targeting agents, such as monoclonal antibodies against epidermal growth factor receptor (anti-EGFR), provide additional clinical benefits in metastatic colorectal cancer (CRC). However, anti-EGFR therapies have limited usage due to approximately 95% of patients with *KRAS* mutated metastatic CRC do not response to anti-EGFR treatment. Thus, *KRAS* mutation is predictive of nonresponse to anti-EGFR therapies but it alone is not a sufficient basis to decide who should not be received such therapies because approximately fifty percent (40%-60%) of CRC patients with wild-type *KRAS* mutation also have poor response to anti-EGFR based treatment. This fact leads us to suspect that there must be other molecular determinants of response to anti-EGFR therapies which have not been identified yet. Current article summarizes the clinical efficacy of

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INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers in both genders (second in females and third in males)[1]; and it is also ranked third in cancer related death in both genders with approximately 15.1 deaths per 100000[2,3]. While the mortality rate of CRC has been decreasing in Western countries, its incidence has been increasing worldwide, except United States[4]. Despite of decreasing death rates, approximately fifty percent of patients with CRC are diagnosed with metastatic disease in their initial assessments[5].

Several chemotherapeutic agents [*e.g.*, pyrimidine analogs (*e.g.*, 5-fluorouracil), platinum-based antineoplastic agents, and topoisomerase inhibitors] have become available in the past and thus survival rate of CRC patients significantly increased. Also, recently developed molecular targeting agents, such as monoclonal antibodies against epidermal growth factor receptor (EGFR) (*e.g.*, cetuximab and panitumumab)[6,7], provided additional clinical benefits in metastatic CRC (mCRC)[8-10].

In several types of cancer, including CRC, EGFR is overexpressed or amplified. Monoclonal antibodies keep EGFR in an inactive state by binding to and occluding the ligand-binding site of EGFR when the ligand is unbound (acting as competitive antagonists). This leads an inhibition of intracellular signaling pathways of EGFR (RAS/RAF/MAPK and PI3K/PTEN/AKT) that involved in several cellular activities including cell proliferation, motility, invasion, and survival[11].

KRAS, a signal transduction molecule, transduces the signal from ligand-bound EGFR to the nucleus. Prospective randomized trials elucidated that presence of mutation in *KRAS* gene leads to non-response to anti-EGFR based treatment[6-10,12-14]. Therefore, it is highly recommended that *KRAS* mutation status should be known before initiating anti-EGFR based treatment in mCRC patients. Thus, *KRAS* mutation is predictive of nonresponse to anti-EGFR therapies but it alone is not a sufficient basis to decide who should not be received such therapies because almost 60% of CRC patients with wild-type (WT) *KRAS* mutation also have poor response to anti-EGFR based treatment[15]. This fact leads us to suspect that there must be other molecular determinants of response to anti-EGFR therapies which have not been identified yet. Current article summarizes the clinical efficacy of anti-EGFR therapies and also evaluates its resistance mechanisms.

CLINICAL EFFICACY OF ANTI-EGFR ANTIBODY IN MCRC

Both Cetuximab, an IgG1 type chimeric monoclonal antibody, and panitumumab, an IgG2 type fully human monoclonal antibody, induce apoptosis by inhibiting downstream signaling pathways of EGFR (RAS/RAF/MAPK and PI3K/PTEN/AKT). Also, these molecules, especially cetuximab, activate antibody-dependent cellular cytotoxicity which consequently improves their cytotoxic actions and therapeutic effectiveness[16].

The recent published randomized non-inferiority phase Ⅲ study showed median overall survival (OS) was similar in patients with mCRC who treated with panitumumab alone and with cetuximab alone[17]. The incidences of any grade and grade 3-4 adverse events were similar in both treatment groups, however, the incidence of grade 3-4 infusion reaction was lower and grade 3-4 hypomagnesaemia was higher in panitumumab group than in cetuximab group[18]. In some studies, cetuximab and panitumumab have been investigated in combination with FOLFIRI (folinic acid, fluorouracil, and irinotecan) and FOLFOX (folinic acid, fluorouracil, and oxaliplatin) as initial therapy option for treatment of mCRC. And a meta-analysis of these 14 randomized studies concluded that there is a clear benefit to the use EGFR inhibitors in patients with WT *KRAS* mCRC[18]. An updated analysis (CRYSTAL trial) demonstrated that adding cetuximab to FOLFIRI as first-line therapy improves survival in patients with WT *KRAS* mCRC[19]. Also another randomized phase Ⅲ study showed that the combination of panitumumab and FOLFIRI significantly improves progression-free survival (PFS), but not OS, in mCRC patients with WT *KRAS*[9]. Three other trials have evaluated the addition of cetuximab to FOLFOX in first line treatment of patients WT *KRAS* mCRC. In randomized phase Ⅱ OPUS study, combination of FOLFOX and cetuximab was associated with increased response rate and PFS. However, this treatment had no benefit in median OS[12]. In the Medical Research Council (MRC) COIN study, adding cetuximab to oxaliplatin-based chemotherapy in patients with WT *KRAS* mCRC increased response rate with no benefit in PFS or OS[20]. Similarly, another phase Ⅲ study (NORDIC-Ⅶ) showed that cetuximab did not add significant benefit when combined with FOLFOX in treatment of patients with WT *KRAS* mCRC[21]. In contrast to earlier studies, the recent published randomized phase Ⅲ CALGB/SWOG 80405 trial demonstrated that addition of cetuximab to FOLFOX or FOLFIRI chemotherapy was significantly increased PFS and OS in treatment of patients with all RAS-WT mCRC[22]. In the study by Douillard *et al*[23] (the PRIME study), which compared panitumumab plus FOLFOX and FOLFOX alone in mCRC patients with WT *KRAS*/NRAS,

panitumumab plus FOLFOX group showed a statistically significant improvement in PFS and OS.

Based on this knowledge, all patients with newly diagnosed mCRC should be tested for *KRAS* mutation. Also screening of *KRAS* mutations seems essential in mCRC patients to initiate anti-EGFR based treatment. But *KRAS* mutation alone is not a sufficient basis to decide who should not be received such therapies because almost 60% of CRC patients with WT *KRAS* mutation also have poor response to anti-EGFR based treatment[15]. Also 5%-9% of CRC patients have a specific mutation in *BRAF* gene (V600E)[24,25]. But the use of BRAF as a predictive marker for response to anti-EGFR based treatment is unclear. This fact leads us to suspect that there must be other molecular determinants of response to anti-EGFR therapies which have not been identified yet.

MECHANISMS OF RESISTANCE TO ANTI-EGFR TREATMENT

KRAS/NRAS/BRAF mutations

Approximately 40% of CRC patients have mutation in exon 2 of the coding of the *KRAS* gene[26,27]. Prospective randomized studies showed that *KRAS* mutations are predictive of non-response to anti-EGFR based treatment[6-10,12-14]. These studies showed that tumors with a mutation in codon 12 or 13 of exon 2 of the *KRAS* gene are essentially unresponsive to anti-EGFR based treatment. Recent studies demonstrated that mutation in *KRAS* outside of exon 2 and mutation in *NRAS* are also predictive for unresponsiveness to ant-EGFR treatment[23,28]. Recently, a study assessed the superiority of FOLFOX plus panitumumab to FOLFOX alone according to *RAS* (*KRAS* or *NRAS*) or *BRAF* (B-type Raf kinase) mutation status. In that study, 17% of patients with non-mutated *KRAS* exon 2 had other RAS mutation which has been shown to be associated with inferior survival with panitumumab plus FOLFOX treatment[23]. Cetuximab or panitumumab treatments seem to be eligible for selected patients with WT *KRAS* tumors who also have BRAF-WT mutations[29].

BRAF oncogene encodes BRAF protein which is a member of RAS/RAF/MAPK (mitogen-activated protein kinase) pathway[27]. Mutations in *BRAF* and *KRAS* genes are mutually exclusive[30]. Approximately 9% (5%-9%) of patients with CRC have a mutation in *BRAF* gene (V600E)[24,25]. CRYSTAL and PETACC-3 studies demonstrated that patients with BRAF mutation have a worse prognosis than those with the WT tumors[19,31]. However, the use of BRAF as a predictive marker is unclear. CRYSTAL study elucidated that BRAF mutation does not seem to be strong predictive biomarker for the addition cetuximab to FOLFIRI in the first line treatment of WT mCRC[19]. Also, subset analysis of the PRIME study found that BRAF mutation indicates poor prognosis but it may not be predictive of the benefit of adding panitumumab to FOLFOX in the first line treatment of mCRC[8]. Tol *et al*[25] demonstrated that *BRAF* mutation is a negative indicator for prognosis in mCRC patients and in contrast to *KRAS* mutation, this feature is not restricted to the outcome of the cetuximab. In subsequent lines of therapy elucidated that *BRAF* mutation is a marker of resistance to anti-EGFR treatment in the non-first line setting of mCRC[29,32,33].

Vemurafenib is orally administered selective inhibitor of BRAF V600 kinase but using it alone in BRAF–mutated CRC patients results insufficient activity[34]. Studies suggested that feedback activation of EGFR signaling might be responsible of the resistance to Vemurafenib in CRC[35,36]. In a cohort study by Hyman *et al*[37], median PFS and OS did not change with vemurafenib monotherapy or vemurafenib and cetuximab combination therapy in patients with CRC (Table 1).

HYPERACTIVATION OF PI3K-PTEN AXIS

Interestingly, 41% of patients do not have *KRAS* or BRAF mutation, and they do not respond to anti-EGFR treatment[29]. Some studies suggested that anti-EGFR downstream pathways other than RAS/RAF/MAPK [*e.g.*, phosphoinositide 3-kinase/phosphatase and tensin homolog pathway (PI3K/PTEN)], might be responsible for the resistance to anti-EGFR based therapy. It was shown that mutation in PI3KCA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) or loss of PTEN is associated with resistance to anti-EGFR based treatment[38-40]. Tural *et al*[41] investigated the effect of oncogenic activation of the members of EGFR downstream pathways (*e.g.*, PI3K, PTEN and BRAF) on response to anti-EGFR therapy. They have showed that PI3K expression and PTEN loss might be used as predictive to the response to anti-EGFR treatment in mCRC patients with WT *KRAS*. According to this study, BRAF negative, PTEN expressing and PI3K non-expressing CRCs have higher response rate and longer PFS and OS than all others. Most studies evaluated PI3K mutation in response to cetuximab based treatments in CRC patients[38,42-45]. In these studies, *PI3K* mutation has been suggested as predictive of resistance to anti-EGFR-based therapies. On the other hand, the role of PI3K mutation in response is conflict. Perrone *et al*[38] has investigated *PI3KCA* gene mutations in CRC patients and they suggested that mutation in PI3KCA causes resistance to anti-EGFR therapies. Also Prenen *et al*[45] analyzed PI3CA and *KRAS* mutations status in chemo-refractory mCRC patients who treated with anti-EGFR based treatment and they did not determine any correlation between PI3KCA mutation and response to anti-EGFR treatment. Nevertheless, most of studies have suggested that PTEN inactivation is a negative predictor of response to anti-EGFR therapy[38-40]. Bardellie *et al*[46] stated that PI3K expression and PTEN loss are correlated with decreased survival and are predictors of poor response to anti-EGFR therapy. Based on these studies, it is well known that activating mutation in PI3KCA or inactivation of PTEN phosphates

Table 1 Clinical trials of targeted agents in combination with chemotherapy as first-line treatments for metastatic colorectal cancer

Ref.	Year	Population	Patient number	Regimen	Median PFS (mo)	P^1	Median OS (mo)	P^1	Response rate (%)	P^1
CRYSTAL[19]	2009	All	599	FOLFIRI	8.0	0.048	18.6	0.31	38.7	0.0038
			599	FOLFIRI + Cetuximab	8.9		19.9		46.9	
		KRAS WT	350	FOLFIRI	8.4	0.0012	20	0.0093	39.7	< 0.001
		subgroup	316	FOLFIRI + Cetuximab	9.9		23.5		57.3	
		KRAS MT	183	FOLFIRI	7.7	0.26	16.7	0.75	36.1	0.35
		subgroup	214	FOLFIRI + Cetuximab	7.4		16.2		31.3	
OPUS[12]	2009	All	168	FOLFOX4	7.2	0.62	18	0.91	36	0.064
			169	FOLFOX4 + Cetuximab	7.2		18.3		46	
		KRAS WT	97	FOLFOX4	7.2	0.0064	18.5	0.39	34	0.0027
		subgroup	82	FOLFOX4 + Cetuximab	8.3		22.8		57	
		KRAS MT	59	FOLFOX4	8.6	0.0153	17.5	0.2	53	0.029
		subgroup	77	FOLFOX4 + Cetuximab	5.5		13.4		34	
COIN[20]	2011	KRAS WT	367	FOLFOX/XELOX	8.6	0.60	17.9	0.68	57	0.049
		group	362	FOLFOX/XELOX + Cetuximab	8.6		17		64	
		KRAS WT	127	FOLFOX	9.2	0.056	-	-	-	-
		group	117	FOLFOX + Cetuximab	9.0		-		-	
		KRAS WT	240	XELOX	8.0	0.56	-	-	-	-
		group	245	XELOX + Cetuximab	8.4		-		-	
		KRAS MT	268	FOLFOX/XELOX	-	-	14.8	0.8	-	-
		group	297	FOLFOX/XELOX + Cetuximab	-		13.6		-	
NORDIC-VII[21]	2012	All	185	Nordic FLOX (control group)	7.9	-	20.4	-	41	-
			194	FLOX + Cetuximab	8.3	0.31	19.7	0.67	49	0.15
			187	intermittent FLOX + Cetuximab	7.3	NA	20.3	0.79	47	NA
		KRAS WT	97	Nordic FLOX (control group)	8.7	-	22	-	47	-
		subgroup	97	FLOX + Cetuximab	7.9	0.66	20.1	0.48	46	0.89
			109	intermittent FLOX + Cetuximab	7.5	NA	21.4	0.66	51	NA
		KRAS MT	58	Nordic FLOX (control group)	7.8	-	20.4	-	40	-
		subgroup	72	FLOX + Cetuximab	9.2	0.07	21.1	0.89	49	0.31
			65	intermittent FLOX + Cetuximab	7.2	NA	20.5	0.84	42	NA
CALGB/ SWOG[22]	2014	KRAS WT group	578	FOLFIRI or mFOLFOX6 + Cetuximab	10.45	NA	29.93	0.34	-	-
80405 (study is ongoing)			559	FOLFIRI or mFOLFOX6 + Bevacizumab	10.84		29.04		-	
PRIME[8]	2010	KRAS WT	331	FOLFOX4	8.0	0.02	19.7	0.072	48	0.068
		group	325	FOLFOX4 + Panitumumab	9.6		23.9		55	
		KRAS MT	219	FOLFOX4	8.8	0.02	19.3	0.068	40	-
		group	221	FOLFOX4 + Panitumumab	7.3		15.5		40	
Hyman *et al*[37]	2015	BRAF V600	10	Vemurafenib	4.5	-	9.3	-	0	-
		group	27	Vemurafenib + Cetuximab	3.7		7.1		4	
Reidy *et al*[51]	2010	All	23	IMC-A12 (anti-IGF-1R antibody)	5.9	-	5.2	-	0	-
			21	IMC-A12 (anti-IGF-1R antibody) + Cetuximab	6.1		4.5		5	
		KRAS WT	20	IMC-A12 (anti-IGF-1R antibody) + Cetuximab	9.4		10.9		0	
		group								

[1]95%CI. PFS: Progression-free survival; OS: Overall survival; All: All patients group; WT: Wild type; MT: Mutant type; NA: Not available; KRAS: KRAS exon 2, codons 12 and 13; FOLFIRI: Irinotecan, fluorouracil, and leucovorin; FOLFOX: Fluorouracil, leucovorin, and oxaliplatin; XELOX: Capecitabine and oxaliplatin; FLOX: Fluorouracil, leucovorin, and oxaliplatin.

can deregulate PI3K signaling pathway[46]. Two studies demonstrated that PI3KCA mutation and PTEN loss which cause PI3K pathway activation are significant predictors of response to anti-EGFR treatment[38,42]. Also, Tural *et al*[41] indicated that PI3K expression and PTEN loss together are correlated with significantly worse outcome.

HYPEREXPRESSION OR HYPERACTIVATION OF TYPE 1 INSULIN LIKE GROWTH FACTOR RECEPTOR

The type 1 insulin like growth factor receptor (IGF-1R) belongs to the class of tyrosine kinase receptors. IGF-1R functions by activating downstream signaling pathways which include MAPK and PI3K/AKT. Previous studies showed that IGF-1R overexpression results neoplastic transformation of cultured cells[47]. Also IGF-1R overexpression was seen in several types of human tumors[48] and its downregulation has been shown to be able to inhibit the growth of these cells[49]. These findings make IGF-1R an attractive candidate as therapeutic target in anti-tumor therapies. A previous study showed that combination therapy of antibodies against to IGF-1R and anti-EGFR results in further inhibition of CRC cell line growth[50]. A phase Ⅱ study evaluated the safety and the efficacy of human anti-IGF-1R monoclonal antibody

(either alone or in combination with cetuximab) in mCRC patients, and both treatment modalities was reported as insufficient in chemorefractory mCRC patients[51] (Table 1).

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385 DOI: 10.3322/caac.20006]

3 **Alberts SR**, Wagman LD. Chemotherapy for colorectal cancer liver metastases. *Oncologist* 2008; **13**: 1063-1073 [PMID: 18838438 DOI: 10.1634/theoncologist.2008-0142]

4 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]

5 **Centers for Disease Control and Prevention**. United States Cancer Statistics: US Cancer Statistics Working Group, 2013. Available from: URL: http://www.cdc.gov/uscs

6 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]

7 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313 DOI: 10.1056/NEJMoa033025]

8 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010; **28**: 4697-4705 [PMID: 20921465 DOI: 10.1200/JCO.2009.27.4860]

9 **Peeters M**, Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, André T, Chan E, Lordick F, Punt CJ, Strickland AH, Wilson G, Ciuleanu TE, Roman L, Van Cutsem E, Tzekova V, Collins S, Oliner KS, Rong A, Gansert J. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol* 2010; **28**: 4706-4713 [PMID: 20921462 DOI: 10.1200/JCO.2009.27.6055]

10 **Van Cutsem E**, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, Canon JL, Van Laethem JL, Maurel J, Richardson G, Wolf M, Amado RG. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol* 2007; **25**: 1658-1664 [PMID: 17470858 DOI: 10.1200/JCO.2006.08.1620]

11 **Ciardiello F**, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med* 2008; **358**: 1160-1174 [PMID: 18337605 DOI: 10.1056/NEJMra0707704]

12 **Bokemeyer C**, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donea S, Ludwig H, Schuch G, Stroh C, Loos AH, Zubel A, Koralewski P. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 663-671 [PMID: 19114683 DOI: 10.1200/JCO.2008.20.8397]

13 **Karapetis CS**, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; **359**: 1757-1765 [PMID: 18946061 DOI: 10.1056/NEJMoa0804385]

14 **Heinemann V**, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, Heintges T, Lerchenmüller C, Kahl C, Seipelt G, Kullmann F, Stauch M, Scheithauer W, Hielscher J, Scholz M, Müller S, Link H, Niederle N, Rost A, Höffkes HG, Moehler M, Lindig RU, Modest DP, Rossius L, Kirchner T, Jung A, Stintzing S. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet Oncol* 2014; **15**: 1065-1075 [PMID: 25088940 DOI: 10.1016/S1470-2045(14)70330-4]

15 **Linardou H**, Dahabreh IJ, Kanaloupiti D, Siannis F, Bafaloukos D, Kosmidis P, Papadimitriou CA, Murray S. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. *Lancet Oncol* 2008; **9**: 962-972 [PMID: 18804418 DOI: 10.1016/S1470-2045(08)70206-7]

16 **Scott AM**, Wolchok JD, Old LJ. Antibody therapy of cancer. *Nat Rev Cancer* 2012; **12**: 278-287 [PMID: 22437872 DOI: 10.1038/nrc3236]

17 **Price TJ**, Peeters M, Kim TW, Li J, Cascinu S, Ruff P, Suresh AS, Thomas A, Tjulandin S, Zhang K, Murugappan S, Sidhu R. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. *Lancet Oncol* 2014; **15**: 569-579 [PMID: 24739896 DOI: 10.1016/S1470-2045(14)70118-4]

18 **Vale CL**, Tierney JF, Fisher D, Adams RA, Kaplan R, Maughan TS, Parmar MK, Meade AM. Does anti-EGFR therapy improve outcome in advanced colorectal cancer? A systematic review and meta-analysis. *Cancer Treat Rev* 2012; **38**: 618-625 [PMID: 22118887 DOI: 10.1016/j.ctrv.2011.11.002]

19 **Van Cutsem E**, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; **29**: 2011-2019 [PMID: 21502544 DOI: 10.1200/JCO.2010.33.5091]

20 **Maughan TS**, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, Idziaszczyk S, Harris R, Fisher D, Kenny SL, Kay E, Mitchell JK, Madi A, Jasani B, James MD, Bridgewater J, Kennedy MJ, Claes B, Lambrechts D, Kaplan R, Cheadle JP. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011; **377**: 2103-2114 [PMID: 21641636 DOI: 10.1016/S0140-6736(11)60613-2]

21 **Tveit KM**, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, Sigurdsson F, Kure E, Ikdahl T, Skovlund E, Fokstuen T, Hansen F, Hofsli E, Birkemeyer E, Johnsson A, Starkhammar H, Yilmaz MK, Keldsen N, Erdal AB, Dajani O, Dahl O, Christoffersen T. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. *J Clin Oncol* 2012; **30**: 1755-1762 [PMID: 22473155 DOI: 10.1200/JCO.2011.38.0915]

22 **Venook AP**, Niedzwiecki D, Lenz H-J, Innocenti F, Mahoney MR, O'Neil BH, Shaw JE, Polite BN, Hochster HS, Atkins JN, Goldberg RM, Mayer RJ, Schilsky RL, Bertagnolli MM, Blanke CD, (Alliance) CaLGB. CALGB/SWOG 80405: Phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with KRAS wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (MCRC). *J Clin Oncol* 2014; **32**: 5s, 2014 (suppl; abstr LBA3)

23 **Douillard JY**, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F,

Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; **369**: 1023-1034 [PMID: 24024839 DOI: 10.1056/NEJMoa1305275]

24 **Cutsem EV**, Folprecht IL, Nowacki M, Barone C, Shchepotin I, Maurel J, Cunningham D, Celik I, Kohne C. Cetuximab plus FOLFIRI: Final data from the CRYSTAL study on the association of KRAS and BRAF biomarker status with treatment outcome. *J Clin Oncol* 2010; **28** (May 20 Supply): 3570

25 **Tol J**, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. *N Engl J Med* 2009; **361**: 98-99 [PMID: 19571295 DOI: 10.1056/NEJMc0904160]

26 **Amado RG**, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 1626-1634 [PMID: 18316791 DOI: 10.1200/JCO.2007.14.7116]

27 **Wan PT**, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004; **116**: 855-867 [PMID: 15035987]

28 **Sorich MJ**, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. *Ann Oncol* 2015; **26**: 13-21 [PMID: 25115304 DOI: 10.1093/annonc/mdu378]

29 **Di Nicolantonio F**, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 5705-5712 [PMID: 19001320 DOI: 10.1200/JCO.2008.18.0786]

30 **Benvenuti S**, Sartore-Bianchi A, Di Nicolantonio F, Zanon C, Moroni M, Veronese S, Siena S, Bardelli A. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res* 2007; **67**: 2643-2648 [PMID: 17363584 DOI: 10.1158/0008-5472.CAN-06-4158]

31 **Roth AD**, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010; **28**: 466-474 [PMID: 20008640 DOI: 10.1200/JCO.2009.23.3452]

32 **Laurent-Puig P**, Cayre A, Manceau G, Buc E, Bachet JB, Lecomte T, Rougier P, Lievre A, Landi B, Boige V, Ducreux M, Ychou M, Bibeau F, Bouché O, Reid J, Stone S, Penault-Llorca F. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol* 2009; **27**: 5924-5930 [PMID: 19884556 DOI: 10.1200/JCO.2008.21.6796]

33 **Loupakis F**, Ruzzo A, Cremolini C, Vincenzi B, Salvatore L, Santini D, Masi G, Stasi I, Canestrari E, Rulli E, Floriani I, Bencardino K, Galluccio N, Catalano V, Tonini G, Magnani M, Fontanini G, Basolo F, Falcone A, Graziano F. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer* 2009; **101**: 715-721 [PMID: 19603018 DOI: 10.1038/sj.bjc.6605177]

34 **Kopetz S**, Desai J, Chan E, Hecht JR, O'Dwyer PJ, Lee RJ, Nolop KB, Saltz L. PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors. *J Clin Oncol* 2010; **28** Suppl: 15s. abstract

35 **Prahallad A**, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012; **483**: 100-103 [PMID: 22281684 DOI: 10.1038/nature10868]

36 **Corcoran RB**, Ebi H, Turke AB, Coffee EM, Nishino M, Cogdill AP, Brown RD, Della Pelle P, Dias-Santagata D, Hung KE, Flaherty KT, Piris A, Wargo JA, Settleman J, Mino-Kenudson M, Engelman JA. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov* 2012; **2**: 227-235 [PMID: 22448344 DOI: 10.1158/2159-8290.CD-11-0341]

37 **Hyman DM**, Puzanov I, Subbiah V, Faris JE, Chau I, Blay JY, Wolf J, Raje NS, Diamond EL, Hollebecque A, Gervais R, Elez-Fernandez ME, Italiano A, Hofheinz RD, Hidalgo M, Chan E, Schuler M, Lasserre SF, Makrutzki M, Sirzen F, Veronese ML, Tabernero J, Baselga J. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. *N Engl J Med* 2015; **373**: 726-736 [PMID: 26287849 DOI: 10.1056/NEJMoa1502309]

38 **Perrone F**, Lampis A, Orsenigo M, Di Bartolomeo M, Gevorgyan A, Losa M, Frattini M, Riva C, Andreola S, Bajetta E, Bertario L, Leo E, Pierotti MA, Pilotti S. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. *Ann Oncol* 2009; **20**: 84-90 [PMID: 18669866 DOI: 10.1093/annonc/mdn541]

39 **Frattini M**, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M, Camponovo A, Etienne LL, Cavalli F, Mazzucchelli L. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer* 2007; **97**: 1139-1145 [PMID: 17940504 DOI: 10.1038/sj.bjc.6604009]

40 **Loupakis F**, Pollina L, Stasi I, Ruzzo A, Scartozzi M, Santini D, Masi G, Graziano F, Cremolini C, Rulli E, Canestrari E, Funel N, Schiavon G, Petrini I, Magnani M, Tonini G, Campani D, Floriani I, Cascinu S, Falcone A. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 2622-2629 [PMID: 19398573 DOI: 10.1200/JCO.2008.20.2796]

41 **Tural D**, Batur S, Erdamar S, Akar E, Kepil N, Mandel NM, Serdengeçti S. Analysis of PTEN, BRAF and PI3K status for determination of benefit from cetuximab therapy in metastatic colorectal cancer patients refractory to chemotherapy with wild-type KRAS. *Tumour Biol* 2014; **35**: 1041-1049 [PMID: 23996432 DOI: 10.1007/s13277-013-1138-8]

42 **Saridaki Z**, Tzardi M, Papadaki C, Sfakianaki M, Pega F, Kalikaki A, Tsakalaki E, Trypaki M, Messaritakis I, Stathopoulos E, Mavroudis D, Georgoulias V, Souglakos J. Impact of KRAS, BRAF, PIK3CA mutations, PTEN, AREG, EREG expression and skin rash in ≥ 2 line cetuximab-based therapy of colorectal cancer patients. *PLoS One* 2011; **6**: e15980 [PMID: 21283802 DOI: 10.1371/journal.pone.0015980]

43 **Mao C**, Yang ZY, Hu XF, Chen Q, Tang JL. PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in KRAS wild-type metastatic colorectal cancer: a systematic review and meta-analysis. *Ann Oncol* 2012; **23**: 1518-1525 [PMID: 22039088 DOI: 10.1093/annonc/mdr464]

44 **De Roock W**, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; **11**: 753-762 [PMID: 20619739 DOI: 10.1016/S1470-2045(10)70130-3]

45 **Prenen H**, De Schutter J, Jacobs B, De Roock W, Biesmans B, Claes B, Lambrechts D, Van Cutsem E, Tejpar S. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. *Clin Cancer Res* 2009; **15**: 3184-3188 [PMID: 19366826 DOI: 10.1158/1078-0432.CCR-08-2961]

46 **Bardelli A**, Siena S. Molecular mechanisms of resistance to

cetuximab and panitumumab in colorectal cancer. *J Clin Oncol* 2010; **28**: 1254-1261 [PMID: 20100961 DOI: 10.1200/JCO.2009.24.6116]

47 **Kaleko M**, Rutter WJ, Miller AD. Overexpression of the human insulinlike growth factor I receptor promotes ligand-dependent neoplastic transformation. *Mol Cell Biol* 1990; **10**: 464-473 [PMID: 2153917]

48 **Ouban A**, Muraca P, Yeatman T, Coppola D. Expression and distribution of insulin-like growth factor-1 receptor in human carcinomas. *Hum Pathol* 2003; **34**: 803-808 [PMID: 14506643]

49 **Hailey J**, Maxwell E, Koukouras K, Bishop WR, Pachter JA, Wang Y. Neutralizing anti-insulin-like growth factor receptor 1 antibodies inhibit receptor function and induce receptor degradation in tumor cells. *Mol Cancer Ther* 2002; **1**: 1349-1353 [PMID: 12516969]

50 **Reinmuth N**, Liu W, Fan F, Jung YD, Ahmad SA, Stoeltzing O, Bucana CD, Radinsky R, Ellis LM. Blockade of insulin-like growth factor I receptor function inhibits growth and angiogenesis of colon cancer. *Clin Cancer Res* 2002; **8**: 3259-3269 [PMID: 12374697]

51 **Reidy DL**, Vakiani E, Fakih MG, Saif MW, Hecht JR, Goodman-Davis N, Hollywood E, Shia J, Schwartz J, Chandrawansa K, Dontabhaktuni A, Youssoufian H, Solit DB, Saltz LB. Randomized, phase II study of the insulin-like growth factor-1 receptor inhibitor IMC-A12, with or without cetuximab, in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer. *J Clin Oncol* 2010; **28**: 4240-4246 [PMID: 20713879 DOI: 10.1200/JCO.2010.30.4154]

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TOPIC HIGHLIGHT

2016 Gastric Cancer: Global view

Robot-assisted surgery for gastric cancer

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Abstract

Minimally invasive surgery for gastric cancer is a relatively new research field, with convincing results mostly stemming from Asian countries. The use of the robotic surgery platform, thus far assessed as a safe procedure, which is also easier to learn, sets the background for a wider spread of minimally invasive technique in the treatment of gastric cancer. This review will cover the literature published so far, analyzing the pros and cons of robotic surgery and highlighting the remaining study questions.

Key words: Gastric cancer survival; Robotic surgery; Gastric cancer surgery; Lymphadenectomy; Minimally invasive surgery

Core tip: An important problem remains regarding the selection of the appropriate technique for a given gastric cancer case. Encouraging results are being published using the robotic technique, but the lack of homogenous study groups in terms of staging, comorbidities and adjuvant and neoadjuvant therapies makes it hard to establish a clear indication for the robotic gastrectomy in gastric cancer. Carefully weighing the treatment options is especially important since there are more and more groups publishing acceptable results with the robotic technique.

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INTRODUCTION

Surgery is unanimously considered the mainstay curative treatment in gastric cancer. Technically, the possibilities range from open surgery to minimally invasive methods like laparoscopy or robotic surgery. However, the newer laparoscopic techniques have only proven their effectiveness in early gastric cancer[1]. The current challenge for robotic surgery in gastric cancer is to prove its benefit as a treatment option, ideally in the form of a survival advantage. Up until now studies only proved its non-inferiority compared with existent techniques.

Technologic progress has clearly had an impact in medicine and surgery, in particular. However the newest developments in the field of technology are not always the best ones and examples can easily be found in the last decades. Rejecting a new technique altogether is, however, not an option in the field of surgery. It would possibly mean closing the roads to a new development that could allow for patients to benefit from procedures which are not easily or not at all undertaken at the moment.

MINIMALLY INVASIVE SURGERY FOR GASTRIC CANCER

The existence of so many treatment options for gastric cancer suggests that currently there is no consensus regarding the adequate therapeutic conduct. Thus far, the following objectives for gastric cancer surgery have been made clear and should be pursued in any case: (1) If surgery can be performed, it must proceed, usually as a part of multimodal cancer treatment[1]. The surgical approach is based on the Virchow-Halsted theory of centrifugal dissemination of carcinomas. This mechanistic theory dating from the end of the 19th century is based on the fact that cancer was believed to begin in the target organ and then spread in an orderly fashion through lymphatic drainage routes invading lymph nodes along the way[2,3]; (2) The tumor must be resected according to oncological safety limits[1]; and (3) An adequate lymphadenectomy must be performed. Its extent varies depending on the location and stage of the tumor[1]. Reaching these objectives correlates with a higher survival rate and a lower rate of recurrence[4,5].

Modern day gastric cancer treatment was definitely impacted by technological progress. The laparoscopy revolution was quickly introduced in this field, with the first laparoscopic gastrectomy performed by Kitano *et al*[6]. Experience accumulated with bariatric surgery must not be neglected either, as it led to an improvement in the technique required to perform intracorporeal anastomoses. The consequence was a rapid development of laparoscopic surgery for gastric cancer beginning, of course, with the early stages. On the other hand, the treatment options for gastric cancer were also enriched by the development of endoscopy, which limited the indications for video-assisted surgery.

Nonetheless, minimally invasive surgery failed to disseminate with great speed worldwide owing mostly to the fact that it is a technically demanding procedure. It is currently particularly favored in Asian countries[7,8] where it is gaining terrain as a treatment for early gastric cancer, but it is interesting to note that laparoscopic surgery for gastric cancer is still an investigational procedure even in countries like Japan[1]. To advance this type of surgery into the category of standard procedures, results of large randomized controlled studies like KLASS-01[9], KLASS-02[10] and JCOG 0912[11] comparing the results of open and laparoscopic surgery are still awaited.

At the moment, the benefits of laparoscopy are still being debated, despite all the published studies which seemingly accrue "pro" arguments at a constant rate. In our opinion, the main objection is that these studies presenting good postoperative as well as oncological outcomes, mostly come from highly experienced large-volume surgical centers, which offer a standard of care that is not easily reproducible everywhere in the world.

ROBOTIC SURGERY

The robotic technologies were brought about to circumvent some of the difficulties of laparoscopic surgery. The laparoscopic procedures for gastric cancer have indeed been associated with improved postoperative outcomes and oncological results[12-15], but the platform itself imposes a series of technical shortcomings. The two-dimensional views coupled with the fulcrum effect and the inherent tremor reduce the surgical range of motion and prolong the learning curve especially for large scale procedures such as gastrectomy. The robotic system comes with a three-dimensional view enabling depth perception, the EndoWrist® technology which allows for seven degrees of freedom and tremor filtration. Additionally, images can be enlarged enabling the performance of delicate steps such as lymph node dissection along great vessels which are essential in achieving a D2 dissection, suturing or knotting. These features could enable the performance of relatively complicated procedures such as function-preserving gastrectomy or extended resections for advanced gastric cancer using a minimally invasive method. Nonetheless this technique also has its disadvantages: Costs, duration of the procedures, the necessary trainings.

The use of the robotic platforms in general surgery did not enjoy the same success as it did in urologic surgery, and the field of gastric cancer is no exception. There are a series of shortcomings of the robotic platform explaining this situation. First of all the lack of robotic staplers and robotic seal and cut devices like

LigaSure™ is a considerable inconvenience. Second, due to the costs, the robotic platform cannot be used to cover the whole spectrum of procedures normally performed by a general surgeon[12].

Current status of robotic surgery in early gastric cancer

Studies evaluating robotic surgery for early gastric cancer alone are scarce and stem mostly from Asian countries. The higher incidence of gastric cancer in these countries, together with the wide extent and increased efficacy of the national gastric cancer screening programs fueled the search for minimally invasive treatment modalities for the early stages of the disease. This led not only to the development of endoscopic resection, but also to a large pool of surgeons well versed in minimally invasive gastrectomies. The encouraging results published in small non-randomized comparative studies of laparoscopic *vs* open surgery for early gastric cancer[13-15] were followed by the increased use of laparoscopy in clinical practice. Japan reports that at least 20% of the gastrectomies for early gastric cancer in its hospitals are now being performed laparoscopically[1]. The need for better statistical evidence supporting the minimally invasive treatment of early gastric cancer was answered by starting two major randomized controlled trials which are now underway in Japan and South Korea comparing laparoscopy and open surgery[9,11].

Following the foot-steps of laparoscopic surgery, robotics was first introduced in the treatment of early stage patients by the same surgeons who had acquired experience in the field of laparoscopic gastrectomies. After the first robotic gastrectomy reported in 2003 by Hashizume *et al*[16], a series of encouraging reports on robotic surgery for gastric carcinomas began to appear in literature (Table 1).

In keeping with the trends of gastric cancer incidence in the eastern and western continents, Asian studies focus on mixed cohorts of gastric cancer patients with a high prevalence of the early stages or on early gastric cancer patients alone (Table 1). The largest cohort of early-stage gastric cancer to date was published by Woo *et al*[17]. A total of 827 patients were included in this nonrandomized comparative study of robotic (236 patients) and laparoscopic surgery (591 patients) for stage Ia and Ib gastric carcinomas. The total operative time was significantly increased for the robotic procedures compared with laparoscopy (219.5 min *vs* 170.7 min, $P < 0.001$), but the robotic group also showed a lower estimated blood loss (91.6 mL *vs* 147.8 mL, $P = 0.02$). The length of hospital stay was slightly in favor of the laparoscopic group (7 d *vs* 7.7 d, $P = 0.004$) and there were no differences regarding morbidity and mortality. In terms of oncological principles, the number of retrieved lymph nodes was not different and all the patients in the robotic group had negative resection margins[17].

Other studies comparing robotic surgery to laparoscopy in the treatment of gastric cancer show the same operative outcomes. The operative times are always significantly longer for the robotic group (Table 2). This has been attributed to longer docking times necessary for the robot. However, a learning curve effect can be derived from the two studies separating the laparoscopic surgery group into an initial and a recent subgroup[18,19]. The operating times reported for the initial laparoscopic technique subgroup are even longer than those of the robotic subgroup. That is no longer the case for the recent laparoscopy subgroup which yields the shortest operating time between the three subgroups (Table 2). In the study of Song *et al*[19] the difference between these mean operative times were 289.5, 230 and 134 min, respectively with a statistically significant difference. The decrease of the mean operative times between the initial and the latter robotic cases (231 min *vs* 208 min) in the large cohort published by Woo *et al*[17] indicates that shortening the operating times is also a matter of exercise, as was the case when the laparoscopic gastrectomies were introduced.

Regarding the estimated blood loss and the number of retrieved lymph nodes, there are conflicting results stemming from most of the cohorts comparing laparoscopy to robotic surgery (Table 2). A meta-analysis performed by Shen *et al*[20] including the studies which also appear in our retrospective tables (Tables 1-3) comparing robotics and laparoscopy also found no statistically significant difference on the number of retrieved lymph nodes. However, a significantly lower blood loss was found in favor of the robotic group.

Current status of robotic surgery in advanced gastric cancer

Papers stemming from Europe, on the other hand, have a large prevalence of advanced gastric cancer cases in their study groups. In the largest study up to date (5839 patients) comparing robotic (436 patients), laparoscopic (861 patients) and open surgery (4542 patients) performed for stage Ⅰ, Ⅱ and Ⅲ gastric cancer by Kim KM *et al*[47], overall safety of these three types of surgery was the main focus. The overall complication rate was the same between the three groups (OG 10.7% LG 9.4% and RG 10.1%, $P = 0.494$) and so was their severity ($P = 0.424$). However, robotic surgery was prone to complications related to leaks ($P = 0.017$), whereas ileus and abscesses were more prevalent in open surgery ($P = 0.001$, $P = 0.013$ respectively). The authors explain that stapling lines were not reinforced with sutures in minimally invasive surgery, as opposed to open surgery and that the patients included in the open surgery group were mainly patients with more advanced disease for whom the complexity of the resections was higher. The robotic group showed a faster recovery with a shorter time to starting the soft diet and a shorter postoperative stay ($P < 0.001$ for both parameters) (Table 3). This study also showed an increased duration of the procedure compared to laparoscopic and open surgery (224 min *vs* 176 min *vs* 158 min, $P < 0.001$) combined with a lower estimated blood loss for the robotic group ($P < 0.001$). The

Table 1 Summary of studies reporting use of robotic surgery for gastric cancer

Ref.	Year	Type of study	Type of surgery	Stage[1]	Type of resection	No. of patients			
						Total	R	L	O
Patriti *et al*[21]	2008	CS	R	6 patients Ⅰ, 6 patients Ⅱ, 1 patient Ⅲ	8 DG, 4 TG, 1 PG	13	13		
Lee *et al*[22]	2011	CS	R	Ⅰ	DG	12	12		
D'Annibale *et al*[23]	2011	CS	R	17 patients Ⅰ, 6 patients Ⅱ, 1 patient Ⅲ	11 TG, 13 DG	24	24		
Isogaki *et al*[24]	2011	CS	R	N/A	46 DG, 14 TG, 1 PG	61	61		
Kim *et al*[25]	2013	CS	R	11 patients Ⅰ, 1 patient Ⅲ	N/A	12	12		
Liu *et al*[26]	2013	CS	R	26 patients Ⅰ, 32 patients Ⅱ, 46 patients Ⅲ	38 DG, 54 TG, 12 PG	104	104		
Park *et al*[27]	2013	CS	R	178 patients Ⅰ, 22 patients Ⅱ or more advanced	154 STG, 46 TG	200	200		
Tokunaga *et al*[28]	2014	CS	R	Ⅰ A	18 DG	18	18		
Anderson *et al*[29]	2007	CS	R	Early GC	7 STG	7	7		
Song *et al*[30]	2009	CS	R	Early GC	67 STG, 33 TG	100	100		
Hur *et al*[31]	2010	CS	R	N/A	5 STG, 2 TG	7	7		
Uyama *et al*[32]	2012	CS	R	18 patients Ⅰ A, 7 patients Ⅱ A to ⅢC	25 DG	25	25		
Yu *et al*[33]	2012	CS	R	N/A	29 DG, 12 TG	41	41		
Jiang *et al*[34]	2012	CS	R	24 patients Ⅰ, 28 patients Ⅱ, 68 patients Ⅲ[2]	62 DG, 35 TG, 23 PG	120	120		
Hyung *et al*[18]	2007	NC	R *vs* L	N/A	N/A	30	10	20	
Song *et al*[19]	2009	NC	R *vs* L	R: 20 patients Ⅰ, L: 37 patients Ⅰ, 3 patients Ⅱ	R: 20 DG, L: 40 DG	60	20	40	
Pugliese *et al*[35]	2010	NC	R *vs* L	37 patients early GC, 33 patients advanced GC	64 STG	64	16	48	
Woo *et al*[17]	2011	NC	R *vs* L	827 patients Ⅰ a or Ⅰ b	R: 172 DG, 62, 2 CT; L: 481 DG, 108 TG, 2 CT	827	236	591	
Eom *et al*[36]	2012	NC	R *vs* L	R: 25 patients Ⅰ, 3 patients Ⅱ, 2 patients Ⅲ, L: 56 patients Ⅰ, 6 patients Ⅱ	DG both groups	92	30	62	
Park *et al*[37]	2012	NC	R *vs* L	R: 27 patients Ⅰ, 3 patients Ⅱ; L: 108 patients Ⅰ, 11 patients Ⅱ, 1 patient Ⅲ	DG both groups	150	30	120	
Yoon *et al*[38]	2012	NC	R *vs* L	R: 29 patients Ⅰ, 7 patients Ⅱ, L: 55 patients Ⅰ, 7 patients Ⅱ, 3 patients Ⅲ	TG both groups	101	36	65	
Kang *et al*[39]	2012	NC	R *vs* L	R: 82 patients Ⅰ, 11 patients Ⅱ, 7 patients Ⅲ	R: 84 STG, 16 TG	382	100	282	
Hyun *et al*[40]	2013	NC	R *vs* L	R: 30 patients Ⅰ, 5 patients Ⅱ, 3 patients Ⅲ; L: 67 patients Ⅰ, 9 patients Ⅱ, 7 patients Ⅲ	R: 29 DG, 9 TG; L: 65 DG, 18 TG	121	38	83	
Noshiro *et al*[41]	2014	NC	R *vs* L	R: 18 patients Ⅰ, 3 patients Ⅱ-Ⅳ, L: 113 patients Ⅰ, 47 patients Ⅱ-Ⅳ	DG both groups	181	21	160	
Han *et al*[42]	2014	NC	R *vs* L	R: 59 patients Ⅰ, 8 patients Ⅱ, 1 patient Ⅲ, L: 66 patients Ⅰ, 2 patients Ⅱ	PPG both groups	136	68	68	
Junfeng *et al*[43]	2014	NC	R *vs* L	R: 29 patients Ⅰ, 36 patients Ⅱ, 55 patients Ⅲ, L: 115 patients Ⅰ, 98 patients Ⅱ, 181 patients Ⅲ	R: 92 DG, 26 TG, 2 PG; L: 261 DG, 118 TG, 15 PG	510	120	394	
Kim *et al*[44]	2014	NC	R *vs* L	R: 145 patients Ⅰ, 27 patients Ⅱ and Ⅲ; L: 422 patients Ⅰ, 59 patients Ⅱ and Ⅲ	N/A	653	172	481	
Kim *et al*[45]	2010	NC	R *vs* L *vs* O	Lower than cT2N1M0	STG all groups	39	16	11	12
Huang *et al*[46]	2012	NC	R *vs* L *vs* O	R: 29 patients Ⅰ, 7 patients Ⅱ, 3 patients Ⅲ; L: 55 patients Ⅰ, 9 patients Ⅱ, O: 198 patients Ⅰ, 106 patients Ⅱ, 282 patients Ⅲ	R: 32 STG, 7 TG; L: 57 STG, 7 TG; O: 407 STG, 179 TG	689	39	64	586
Kim *et al*[47]	2012	NC	R *vs* L *vs* O	R: 3 patients 0, 350 patients Ⅰ, 51 patients Ⅱ, 32 patients Ⅲ; L: 8 patients 0, 714 patients Ⅰ, 96 patients Ⅱ, 43 patients Ⅲ, O: 28 patients 0, 2376 patients Ⅰ, 823 patients Ⅱ, 1313 patients Ⅲ	R: 327 DG, 109 TG, L: 703 DG, 158 TG; O: 3309 DG, 1232 TG	5839	436	861	4542
Pernazza *et al*[48]	2006	NC	R *vs* O	R: 2 patients 0, 20 patients Ⅰ, 12 patients Ⅱ, 5 patients Ⅲ, 6 patients Ⅳ	R: 21 DG, 24 TG	90	45	0	45
Caruso *et al*[49]	2011	NC	R *vs* O	R: 13 patients Ⅰ, 9 patients Ⅱ, 4 patients Ⅲ, 3 patients Ⅳ, O: 57 patients Ⅰ, 18 patients Ⅱ, 33 patients Ⅲ, 12 patients Ⅳ	R: 16 DG, 12 TG, 1 PG; O: 83 DG, 37 TG	149	29	0	120
Procopiuc *et al*[50]	2015	NC	R *vs* O	R: 9 patients Ⅱ, 9 patients Ⅲ, O: 15 patients Ⅱ, 14 patients Ⅲ	R: 7 DG, 10 TG, 1 PG; O: 6 DG, 23 TG	47	18		29

[1]Data as reported by the authors from preoperative evaluation; [2]Postoperatively obtained staging. CS: Clinical series; NC: Nonrandomized comparative study; R: Robotic surgery; L: Laparoscopic surgery; O: Open surgery; TG: Total gastrectomy; STG: Subtotal gastrectomy; DG: Distal gastrectomy; PG: Proximal gastrectomy; CT: Completion total gastrectomy; PPG: Pylorus-preserving gastrectomy; GC: Gastric cancer.

Table 2 Main operative outcomes in studies reporting use of robotic surgery for gastric cancer

Ref.	OP time (min)	Estimated blood loss (mL)	No. of harvested lymph nodes	Conversions
Patriti *et al*[21]	286	103	28.1	0
Lee *et al*[14]	253	135	46	0
D'Annibale *et al*[23]	267.5	30	28	0
Isogaki *et al*[24]	TG > DG 520 > 388	TG > DG 150 > 61.8	TG approximately equal DG 43 approximately equal 42	0
Kim *et al*[25]	234.7	46.4	42.4	
Liu *et al*[26]	272.52	80.78	23.1	1.8
Park *et al*[27]	248.8	146.1	37.9	3.5
Tokunaga *et al*[28]	331.5	32.5	40	0
Anderson *et al*[29]	420	300	24	0
Song *et al*[30]	231.3	128.2	36.7	0
Hur *et al*[31]	205			
Uyama *et al*[32]	361	51.8	44.3	0
Yu *et al*[33]	TG > DG 285 > 225	TG > DG 180 > 150	34.2	4.8
Jiang *et al*[34]	245	70	22.5	
Hyung *et al*[18]	Initial L > R > Recent L 337 > 253 > 164	-	Recent L > R > Initial L 37.8 > 34 > 29.2	0
Song *et al*[19]	Initial L > R > Recent L 289.5 > 230 > 134 ss	R > Recent L 94.8 > 39.5	Recent L > R > Initial L 42.7 > 35.3 > 31.5	0
Pugliese *et al*[35]	R > L 344 > 235 ss	L > R 148 > 90 ss	L > R 31 > 25	L > R 3 > 2
Woo *et al*[17]	R > L 219.5 > 170.7 ss	L > R 147.9 > 91.6 ss	R > L 39 > 37.4	0 = 0
Eom *et al*[36]	R > L 229.1 > 189.4 ss	R > L 152.8 > 88.3	L > R 33.4 > 30.2	
Park *et al*[37]	R > L 218 > 140 ss	R > L 75 > 60	R approximately equal L 34 approximately equal 35	0
Yoon *et al*[38]	R > L 305.8 > 210.2 ss		R > L 42.8 > 39.4	
Kang *et al*[39]	R > L 202 > 173 ss	L > R 173.4 > 93.2 ss		
Hyun *et al*[40]	R > L 234.4 > 220	R approximately equal L 131.3 approximately equal 130.4	R approximately equal L 32.8 approximately equal 32.6	0 = 0
Noshiro *et al*[41]	R > L 439 > 315 ss	L > R 115 > 96	R > L 44 > 40	R = L 0 = 0
Han *et al*[42]	R > L 258 > 193 ss		L > R 36.5 > 33.4	0
Junfeng *et al*[43]	R > L 234.8 > 221.3 ss	L > R 137.6 > 118.3 ss	R > L 34.6 > 32.7 ss	
Kim *et al*[44]	R > L 206.4 > 167.1 ss	L > R 134.9 > 59.8 ss	R approximately equal L 37.3 approximately equal 36.8	R = L 0 = 0
Kim *et al*[45]	R > L > O 259.2 > 203.9 > 126.7 ss	O > L > R 78.8 > 44.7 > 30.3 ss	O > R > L 43.3 > 41.1 > 37.4	0 = 0
Huang *et al*[46]	R > L > O 430 > 350 > 320	O > L > R 400 > 100 > 50 ss	O > R > L 34 > 32 > 26	
Kim *et al*[47]	R > L > O 226 > 176 > 158 ss	O > L > R 182 > 112 > 85	O > R > L 40.5 > 40.2 > 37.6 ss	
Pernazza *et al*[48]	R > O 293.8 > 224.6		R 34.2	
Caruso *et al*[49]	R > O 290 > 222	O > R 386.1 > 197.6	O > R 31.7 > 28	
Procopiuc *et al*[50]	R > O 320.83 > 243.36 ss	O > R 564.62 > 208.26 ss	O > R 25 > 22	0

R: Robotic surgery; L: Laparoscopic surgery; O: Open surgery; TG: Total gastrectomy; DG: Distal gastrectomy; ss: Statistically significant.

number of harvested lymph nodes was no different between open and robotic surgery.

In the experience of our group, the robotic platform is a versatile tool in the surgical approach of advanced gastric cancer. Our study[50] enrolled 47 patients who were exclusively advanced gastric cancer patients and went on to receive either open (n = 29) or robotic (n = 18) surgery. Significantly longer mean operating times (320.83 min *vs* 243.36 min), but significantly lower blood loss (208.26 mL *vs* 546.62 mL) and shorter hospital stay (11.04 d *vs* 8.1 d) were obtained for the robotic group (Table 3). We found no difference in the number of retrieved lymph nodes or the rate of complications. After a mean follow up time of 31.66 mo for the open surgery group and a 24.72 for the robotic surgery group, the Kaplan-Meier analysis of the survival data revealed no statistically significant difference between the two cohorts (P = 0.177).

The authors consider that special emphasis needs to be placed on the long-term results of robotic surgery in advanced gastric cancer. The MAGIC trial[51] published in 2006 showed a survival benefit for gastric cancer patients receiving epirubicin, cisplatin and fluorouracil perioperatively when compared with patients treated with surgery alone. But the study also reported that 34% of the patients enrolled in the perioperative chemotherapy group, were unable to receive the regimen after surgery owing, among others, to postoperative complications. This creates a need for less invasive surgery like robotic surgery even in the treatment of the advanced gastric cancer patients. Patients would be thus enabled to receive the complete chemotherapy regimen, which would positively impact their survival prognosis[51].

Another reason to investigate robotic surgery in the treatment of advanced gastric cancer would be the imperfect staging systems currently available. Studies report a considerable amount of patients staged as EGC perioperatively who turn out intraoperatively to suffer from advanced gastric cancer[52,53]. Given these numbers Pugliese *et al*[35] even proposed that all gastrectomies be performed including a D2 lymphadenectomy regardless

Table 3 Main postoperative outcomes in studies reporting use of robotic surgery for gastric cancer

Ref.	Time to first flatus (d)	Time to oral feeding (d)	Postoperative hospital stay (d)	Morbidity (%)	Mortality (%)	Follow up time (mo)
Patriti *et al*[21]			11.2	41.4	0	12.2
Lee *et al*[22]	2.4	4.6	6.6	8.3	0	
D'Annibale *et al*[23]		5	6	2	0	48
Isogaki *et al*[24]				4	1	
Kim *et al*[25]			6	0	0	
Liu *et al*[26]	2.5	4.1	6.2	11.8	0	
Park *et al*[27]			8	19	1	
Tokunaga *et al*[28]			8	22.22		
Anderson *et al*[29]		4 (2-8)	4 (3-9)	14.3		
Song *et al*[30]	2.9 ± 0.5	4.2	7.8	13	0	
Hur *et al*[31]						
Uyama *et al*[32]		3.56	12.1	8	0	11
Yu *et al*[33]	3.1	3.7		4.8	0	11
Jiang *et al*[34]			6.3	5	0	
Hyung *et al*[18]	Recent L > Initial L > R 3.3 > 3.1 > 2.9	Initial L > Recent L > R 4.8 > 4.3 > 4	Initial L > R = Recent L 6.9 > 6 = 6			
Song *et al*[19]	Recent L > Initial L = R 3.25 > 3 = 3	Initial L > Recent L > R 4.95 > 4.1 > 4	Initial L > Recent L > R 7.7 > 6.2 > 5.7	Recent L > Initial L = R 10 > 5 = 5		
Pugliese *et al*[35]			R = L 10 = 10	L > R 12.5 > 6.2	R > L 6.2 > 2	53
Woo *et al*[17]			R > L 7.7 > 7 ss	L > R 13.7 > 11	R approximately equal L 0.3 approximately equal 0.4	
Eom *et al*[36]	R = L 3.4 = 3.4		R approximately equal L 7.9 approximatelyequal 7.8	R > L 13 > 6		
Park *et al*[37]				R > L 17 > 7.5	R = L 0 = 0	
Yoon *et al*[38]	L > R 4.9 > 4.2		L > R 10.3 > 8.8	R > L 16.7 > 15.4		
Kang *et al*[39]			R > L 9.8 > 8.1 ss	R > L 14 > 10.3	R = L 0 = 0	
Hyun *et al*[40]			L > R 11.9 > 10.5	R > L 47.3 > 38.5	R = L 0 = 0	
Noshiro *et al*[41]			L > R 13 > 8 ss	L > R 10 > 9.5	0	
Han *et al*[42]		L > R 5 > 4.4	L > R 9.1 > 8.6	L > R 22.1 > 19.1	R = L 0 = 0	R > L 22.7 > 19.3
Junfeng *et al*[43]	L > R 3.3 > 3.1	L > R 4.1 > 3.9	L approximately equal R 7.9 approximately equal 7.8	R > L 5.8 > 4.3	R > L 32.2 > 30.1	L > R 19 > 15
Kim *et al*[44]			R > L 7.1 > 6.7	R > L 5.2 > 4.2	L > R 0.6 > 0	
Kim *et al*[45]	L > O > R 3.6 > 3.4 > 3.2		O > L > R 6.7 > 6.5 > 5.1 ss		R = L 0 = 0	
Huang *et al*[46]				L > R > O 15.6 > 15.4 > 14.7		
Kim *et al*[47]		O > L > R 5.7 > 4.7 > 4.4 ss	O > L > R 10.2 > 7.8 > 7.5 ss	O > R > L 10.7 > 10.1 > 934	0.4 ND	
Pernazza *et al*[48]				R > O 24.5 > 13.3	O > R 8.9 > 4.4	R = O 26 = 26
Caruso *et al*[49]			O > R 13.4 > 9.6	O > R 42.5 > 41.4	O > R 3.3 > 0	O > R 44 > 25
Procopiuc *et al*[50]			O > R 11.04 > 8.1 ss	O > R 27.58 > 22.22	O = R 0 = 0	O > R 31.6 > 24.7

R: Robotic surgery; L: Laparoscopic surgery; O: Open surgery; TG: Total gastrectomy; DG: Distal gastrectomy; ss: Statistically significant; ND: No statistical difference.

of the initial tumoral staging.

TECHNICAL ASPECTS

Combined resections

There has been a lack of studies specifically focused on the possible benefits of robotic multivisceral resections for advanced gastric cancer. Previous research by surgeons experienced in minimally invasive surgery suggests that the precision offered by the robotic platform might be of more use in large, technically-challenging procedures like multivisceral resections, rather than in cases requiring less complex surgery[54,55].

Lymphadenectomy

To put forth robotic surgery as a viable surgical technique in gastric cancer treatment, its contribution to performing an extended lymphadenectomy needs to be made clear.

In laparoscopy, one of the major sources of intraoperative bleeding was shown to be lymph node dissection, especially when occurring around the large vessels[56,57]. In our experience with the robotic platform owing to the elimination of physiologic tremor, the 3D steady view, and the 7 degrees of freedom of the EndoWrist® instruments lymph node dissection along the celiac trunk, the left gastric artery and the hepatic pedicle which are usually associated with increased bleeding, are now performed in a more precise and safe environment[50].

The cohorts of Hyung[18] and Song *et al*[30] both included an initial and a recent laparoscopy group thus

allowing the assessment of the evolution of surgery parameters along the learning curve for this type of surgery and their comparison to the initial experience in robotic surgery. Although not statistically significant, recent laparoscopy showed the highest number of retrieved lymph nodes, with initial robotic cases coming second, in front of the initial laparoscopic cases. This comes to support the view that laparoscopy has a steeper learning curve than robotic surgery and that even inexperienced surgeons may obtain easily reproducible, high quality results faster with the robotic platform. This difference between the two techniques may not be important in the east, where experienced laparoscopic surgeons show no difficulties in quickly adjusting to the robotic platform, but it could bring a significant advantage to the western surgeons who simply cannot benefit from the same training in laparoscopy for gastric cancer due to the particular epidemiology of this disease.

The majority of the studies listed in Tables 1-3 show a higher number of retrieved lymph nodes for robotic procedures, which is an encouraging result given the extent of the preoperative under staging reported until now and the probable need to perform D2 lymphadenectomies for all patients until a reliable method for precise preoperative staging is introduced.

Digestive tract reconstruction

Key moments for the anastomosis are as follows: (1) closure of the duodenal stump; (2) closure of the stomach stump in subtotal gastrectomy or that of the esophageal stump in total gastrectomy; and (3) preparing the jejunum for the gastro-jejuno anastomosis or the eso-jejunoanastomosis. We generally opt for a Roux-en-Y anastomosis[58].

The reconstruction solutions after total or subtotal gastrectomy can be grouped into two large categories. First, the extracorporeal anastomoses by the robot-assisted surgery require the performance of a minila-parotomy (smaller than 6 cm) through which the ends that need to be anastomosed are brought out and continuity of the digestive tract is reestablished, usually using circular stapler. This technique is not suitable for obese patients for whom the incision may need to be larger than 6 cm to perform the proximal resection and the purse-string suture on the esophageal stump.

To fully take advantage of the minimal invasiveness provided by the robotic platform, several techniques for intracorporeal anastomoses have been developed. They avoid the laparotomy and imply sectioning the esophagus under video control and then performing the anastomosis with a specific technique not requiring an abdominal incision. One option is using the OrVil™ device (Covidien, Mansfield, MA, United States). This consists of a foldable stapler anvil forming a 170° angle with the adjoining PVC tube. The OrVil™ device is introduced through the mouth and into the esophageal stump at which point the anvil is unfolded and connected to the circular stapler introduced abdominally. For this technique our team uses a 21 mm anvil followed by a Roux-en-Y reconstruction with good postoperative results[58]. Similar to this is the technique described by Hiki *et al*[59] in which the anvil of a circular stapler is attached to a nasogastric tube using sutures and then introduced trans-orally. Another technique was described by Inaba *et al*[60] and involves the creation of a side-to-side anastomosis using a linear stapler. Yet another option would be the manual sewing of the anastomosis, which we do not recommend, since it would prolong operating times unnecessarily, given the fact that the available mechanical devices are reliable alternatives.

The role of the assistant surgeon

In a study published by our team[61], we assessed the role of the patient-side surgeon in robotic surgery. We found obvious benefits for the team when highly-trained assistants were involved in the procedure. Remarkable improvements were seen in handling the robot (docking and undocking times), the speed and precision in manipulating laparoscopic devices like the LigaSure or clip applier devices. Our data show that maintaining the same members of the team throughout more procedures and including assistants who undertook a structured, formal training program are more likely to warrant for fast and safe interventions.

OPEN QUESTIONS OF RESEARCH

An important problem remains regarding the selection of the appropriate technique for a given gastric cancer case. Thus far indications for robotic gastrectomy were: (1) a diagnosis of early gastric cancer without evidence of lymph node involvement; (2) T1 cancer with perigastric lymph node involvement; and (3) serosa-negative gastric cancer without lymph node metastasis. However, many of the patients were understaged preoperatively. This raises the need to study the outcomes of robotic surgery on large patient cohorts in randomized prospective studies not only for early gastric cancer, but also for tumors possibly requiring the D2 lymphadenectomy.

A recently published study surveying gastric cancer surgery techniques in United States academic medical centers[62] shows that the number of robotic gastrectomies for gastric cancer has remained constant in 2011, 2012 and 2013. The study also mentioned that the robotic technique was utilized in the patients with the highest risk of mortality and severity of illness, in keeping with the fact that minimally invasive surgery has a lower impact on patient performance status and immune response mechanisms postoperatively[62-64]. Therefore, extending the indications of robotic surgery to advanced gastric cancer is also a valid study point, especially in the West.

The option between endoscopic, laparoscopic, robotic or open surgery must be made based on well-established diagnostic criteria. This is not easy and one must take into account the caveats of evidence based

medicine and randomized controlled trials. The case of the results published by Bonenkamp *et al*[65] and Cuschieri *et al*[66,67] regarding the survival benefit of the D2 lymphadenectomy and the controversies thereafter have marked a decade of debate regarding the strategies for gastric cancer treatment. Carefully weighing the treatment options is especially important since there are more and more groups publishing acceptable results with the robotic technique.

CONCLUSION

Encouraging results are being published using the robotic technique, but the lack of homogenous study groups in terms of staging, comorbidities and adjuvant and neo-adjuvant therapies makes it hard to establish a clear indication for the robotic gastrectomy in gastric cancer.

Robotic surgery has proven to be safe and feasible thus far, but more convincing large volume prospective studies are needed to put it on the treatment list of early and advanced gastric cancer.

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REFERENCES

1 **Japanese Gastric Cancer Association.** Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer* 2011; **14**: 113-123 [PMID: 21573742 DOI: 10.1007/s10120-011-0042-4]
2 **Vasilescu C**. [Current problems in surgical oncology: 1. Theoretical models in oncology and their implications in surgical therapy]. *Chirurgia* (Bucur) 2011; **106**: 17-22 [PMID: 21523955]
3 **Baum M**. Modern concepts of the natural history of breast cancer: a guide to the design and publication of trials of the treatment of breast cancer. *Eur J Cancer* 2013; **49**: 60-64 [PMID: 22884336 DOI: 10.1016/j.ejca.2012.07.005]
4 **Wu CW**, Hsiung CA, Lo SS, Hsieh MC, Chen JH, Li AF, Lui WY, Whang-Peng J. Nodal dissection for patients with gastric cancer: a randomised controlled trial. *Lancet Oncol* 2006; **7**: 309-315 [PMID: 16574546 DOI: 10.1016/S1470-2045(06)70623-4]
5 **Sasako M**, Sano T, Yamamoto S, Kurokawa Y, Nashimoto A, Kurita A, Hiratsuka M, Tsujinaka T, Kinoshita T, Arai K, Yamamura Y, Okajima K. D2 lymphadenectomy alone or with para-aortic nodal dissection for gastric cancer. *N Engl J Med* 2008; **359**: 453-462 [PMID: 18669424 DOI: 10.1056/NEJMoa0707035]
6 **Kitano S**, Iso Y, Moriyama M, Sugimachi K. Laparoscopy-assisted Billroth I gastrectomy. *Surg Laparosc Endosc* 1994; **4**: 146-148 [PMID: 8180768]
7 **Etoh T**, Inomata M, Shiraishi N, Kitano S. Minimally invasive approaches for gastric cancer-Japanese experiences. *J Surg Oncol* 2013; **107**: 282-288 [PMID: 22504947 DOI: 10.1002/jso.23128]
8 **Yang HK**, Suh YS, Lee HJ. Minimally invasive approaches for gastric cancer-Korean experience. *J Surg Oncol* 2013; **107**: 277-281 [PMID: 22806494 DOI: 10.1002/jso.23179]
9 **Kim HH**, Han SU, Kim MC, Hyung WJ, Kim W, Lee HJ, Ryu SW, Cho GS, Kim CY, Yang HK, Park do J, Song KY, Lee SI, Ryu SY, Lee JH. Prospective randomized controlled trial (phase III) to comparing laparoscopic distal gastrectomy with open distal gastrectomy for gastric adenocarcinoma (KLASS 01). *J Korean Surg Soc* 2013; **84**: 123-130 [PMID: 23396494 DOI: 10.4174/jkss.2013.84.2.123]
10 **Lee JH**, Son SY, Lee CM, Ahn SH, Park do J, Kim HH. Morbidity and mortality after laparoscopic gastrectomy for advanced gastric cancer: results of a phase II clinical trial. *Surg Endosc* 2013; **27**: 2877-2885 [PMID: 23404155 DOI: 10.1007/s00464-013-2848-0]
11 **Nakamura K**, Katai H, Mizusawa J, Yoshikawa T, Ando M, Terashima M, Ito S, Takagi M, Takagane A, Ninomiya M, Fukushima N, Sasako M. A phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastric Cancer (JCOG0912). *Jpn J Clin Oncol* 2013; **43**: 324-327 [PMID: 23275644 DOI: 10.1093/jjco/hys220]
12 **Inabnet WB**. Robotic thyroidectomy: must we drive a luxury sedan to arrive at our destination safely? *Thyroid* 2012; **22**: 988-990 [PMID: 23025541 DOI: 10.1089/thy.2012.2210.com2]
13 **Hayashi H**, Ochiai T, Shimada H, Gunji Y. Prospective randomized study of open versus laparoscopy-assisted distal gastrectomy with extraperigastric lymph node dissection for early gastric cancer. *Surg Endosc* 2005; **19**: 1172-1176 [PMID: 16132323 DOI: 10.1007/s00464-004-8207-4]
14 **Lee JH**, Han HS, Lee JH. A prospective randomized study comparing open vs laparoscopy-assisted distal gastrectomy in early gastric cancer: early results. *Surg Endosc* 2005; **19**: 168-173 [PMID: 15580441 DOI: 10.1007/s00464-004-8808-y]
15 **Huscher CG**, Mingoli A, Sgarzini G, Sansonetti A, Di Paola M, Recher A, Ponzano C. Laparoscopic versus open subtotal gastrectomy for distal gastric cancer: five-year results of a randomized prospective trial. *Ann Surg* 2005; **241**: 232-237 [PMID: 15650632 DOI: 10.1097/01.sla.0000151892.35922.f2]
16 **Hashizume M**, Sugimachi K. Robot-assisted gastric surgery. *Surg Clin North Am* 2003; **83**: 1429-1444 [PMID: 14712877 DOI: 10.1016/S0039-6109(03)00158-0]
17 **Woo Y**, Hyung WJ, Pak KH, Inaba K, Obama K, Choi SH, Noh SH. Robotic gastrectomy as an oncologically sound alternative to laparoscopic resections for the treatment of early-stage gastric cancers. *Arch Surg* 2011; **146**: 1086-1092 [PMID: 21576595 DOI: 10.1001/archsurg.2011.114]
18 **Hyung WJ**. [Robotic surgery in gastrointestinal surgery]. *Korean J Gastroenterol* 2007; **50**: 256-259 [PMID: 18159190]
19 **Song J**, Kang WH, Oh SJ, Hyung WJ, Choi SH, Noh SH. Role of robotic gastrectomy using da Vinci system compared with laparoscopic gastrectomy: initial experience of 20 consecutive cases. *Surg Endosc* 2009; **23**: 1204-1211 [PMID: 19263147 DOI: 10.1007/s00464-009-0351-4]
20 **Shen WS**, Xi HQ, Chen L, Wei B. A meta-analysis of robotic versus laparoscopic gastrectomy for gastric cancer. *Surg Endosc* 2014; **28**: 2795-2802 [PMID: 24789136 DOI: 10.1007/s00464-014-3547-1]
21 **Patriti A**, Ceccarelli G, Bellochi R, Bartoli A, Spaziani A, Di Zitti L, Casciola L. Robot-assisted laparoscopic total and partial gastric resection with D2 lymph node dissection for adenocarcinoma. *Surg Endosc* 2008; **22**: 2753-2760 [PMID: 18813994 DOI: 10.1007/s00464-008-0129-0]
22 **Lee HH**, Hur H, Jung H, Jeon HM, Park CH, Song KY. Robot-assisted distal gastrectomy for gastric cancer: initial experience. *Am J Surg* 2011; **201**: 841-845 [PMID: 21741513 DOI: 10.1016/j.amjsurg.2010.05.013]
23 **D'Annibale A**, Pende V, Pernazza G, Monsellato I, Mazzocchi P, Lucandri G, Morpurgo E, Contardo T, Sovernigo G. Full robotic gastrectomy with extended (D2) lymphadenectomy for gastric cancer: surgical technique and preliminary results. *J Surg Res* 2011; **166**: e113-e120 [PMID: 21227455 DOI: 10.1016/j.jss.2010.11.881]
24 **Isogaki J**, Haruta S, Man-I M, Suda K, Kawamura Y, Yoshimura F, Kawabata T, Inaba K, Ishikawa K, Ishida Y, Taniguchi K, Sato S, Kanaya S, Uyama I. Robot-assisted surgery for gastric cancer: experience at our institute. *Pathobiology* 2011; **78**: 328-333 [PMID: 22104204 DOI: 10.1159/000330172]
25 **Kim YM**, Baek SE, Lim JS, Hyung WJ. Clinical application of image-enhanced minimally invasive robotic surgery for gastric cancer: a prospective observational study. *J Gastrointest Surg* 2013; **17**: 304-312 [PMID: 23207683 DOI: 10.1007/s11605-012-2094-0]
26 **Liu XX**, Jiang ZW, Chen P, Zhao Y, Pan HF, Li JS. Full robot-

assisted gastrectomy with intracorporeal robot-sewn anastomosis produces satisfying outcomes. *World J Gastroenterol* 2013; **19**: 6427-6437 [PMID: 24151361 DOI: 10.3748/wjg.v19.i38.6427]

27 **Park JY**, Kim YW, Ryu KW, Eom BW, Yoon HM, Reim D. Emerging Role of Robot-assisted Gastrectomy: Analysis of Consecutive 200 Cases. *J Gastric Cancer* 2013; **13**: 255-262 [PMID: 24511422 DOI: 10.5230/jgc.2013.13.4.255]

28 **Tokunaga M**, Sugisawa N, Kondo J, Tanizawa Y, Bando E, Kawamura T, Terashima M. Early phase II study of robot-assisted distal gastrectomy with nodal dissection for clinical stage IA gastric cancer. *Gastric Cancer* 2014; **17**: 542-547 [PMID: 24005955 DOI: 10.1007/s10120-013-0293-3]

29 **Anderson C**, Ellenhorn J, Hellan M, Pigazzi A. Pilot series of robot-assisted laparoscopic subtotal gastrectomy with extended lymphadenectomy for gastric cancer. *Surg Endosc* 2007; **21**: 1662-1666 [PMID: 17345142 DOI: 10.1007/s00464-007-9266-0]

30 **Song J**, Oh SJ, Kang WH, Hyung WJ, Choi SH, Noh SH. Robot-assisted gastrectomy with lymph node dissection for gastric cancer: lessons learned from an initial 100 consecutive procedures. *Ann Surg* 2009; **249**: 927-932 [PMID: 19474671 DOI: 10.1097/01.sla.0000351688.64999.73]

31 **Hur H**, Kim JY, Cho YK, Han SU. Technical feasibility of robot-sewn anastomosis in robotic surgery for gastric cancer. *J Laparoendosc Adv Surg Tech A* 2010; **20**: 693-697 [PMID: 20809816 DOI: 10.1089/lap.2010.0246]

32 **Uyama I**, Kanaya S, Ishida Y, Inaba K, Suda K, Satoh S. Novel integrated robotic approach for suprapancreatic D2 nodal dissection for treating gastric cancer: technique and initial experience. *World J Surg* 2012; **36**: 331-337 [PMID: 22131088 DOI: 10.1007/s00268-011-1352-8]

33 **Yu PW**, Tang B, Zeng DZ, Zhao YL, Shi Y, Hao YX, Qian F. [Robotic-assisted radical gastrectomy using da Vinci robotic system: a report of 41 cases]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2012; **15**: 121-124 [PMID: 22368015]

34 **Jiang ZW**, Zhao K, Wang G, Bao Y, Xie LF, Liu FT, Pan HF, Zhang XL, Ruan H, Li N, Li JS. [Application of surgical robotic system in patients with gastric cancer: a report of 120 cases]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2012; **15**: 801-803 [PMID: 22941680]

35 **Pugliese R**, Maggioni D, Sansonna F, Costanzi A, Ferrari GC, Di Lernia S, Magistro C, De Martini P, Pugliese F. Subtotal gastrectomy with D2 dissection by minimally invasive surgery for distal adenocarcinoma of the stomach: results and 5-year survival. *Surg Endosc* 2010; **24**: 2594-2602 [PMID: 20414682 DOI: 10.1007/s00464-010-1014-1]

36 **Eom BW**, Yoon HM, Ryu KW, Lee JH, Cho SJ, Lee JY, Kim CG, Choi IJ, Lee JS, Kook MC, Rhee JY, Park SR, Kim YW. Comparison of surgical performance and short-term clinical outcomes between laparoscopic and robotic surgery in distal gastric cancer. *Eur J Surg Oncol* 2012; **38**: 57-63 [PMID: 21945625 DOI: 10.1016/j.ejso.2011.09.006]

37 **Park JY**, Jo MJ, Nam BH, Kim Y, Eom BW, Yoon HM, Ryu KW, Kim YW, Lee JH. Surgical stress after robot-assisted distal gastrectomy and its economic implications. *Br J Surg* 2012; **99**: 1554-1561 [PMID: 23027072 DOI: 10.1002/bjs.8887]

38 **Yoon HM**, Kim YW, Lee JH, Ryu KW, Eom BW, Park JY, Choi IJ, Kim CG, Lee JY, Cho SJ, Rho JY. Robot-assisted total gastrectomy is comparable with laparoscopically assisted total gastrectomy for early gastric cancer. *Surg Endosc* 2012; **26**: 1377-1381 [PMID: 22083338 DOI: 10.1007/s00464-011-2043-0]

39 **Kang BH**, Xuan Y, Hur H, Ahn CW, Cho YK, Han SU. Comparison of Surgical Outcomes between Robotic and Laparoscopic Gastrectomy for Gastric Cancer: The Learning Curve of Robotic Surgery. *J Gastric Cancer* 2012; **12**: 156-163 [PMID: 23094227 DOI: 10.5230/jgc.2012.12.3.156]

40 **Hyun MH**, Lee CH, Kwon YJ, Cho SI, Jang YJ, Kim DH, Kim JH, Park SH, Mok YJ, Park SS. Robot versus laparoscopic gastrectomy for cancer by an experienced surgeon: comparisons of surgery, complications, and surgical stress. *Ann Surg Oncol* 2013; **20**: 1258-1265 [PMID: 23080320 DOI: 10.1245/s10434-012-2679-6]

41 **Noshiro H**, Ikeda O, Urata M. Robotically-enhanced surgical anatomy enables surgeons to perform distal gastrectomy for gastric cancer using electric cautery devices alone. *Surg Endosc* 2014; **28**: 1180-1187 [PMID: 24202713 DOI: 10.1007/s00464-013-3304-x]

42 **Han DS**, Suh YS, Ahn HS, Kong SH, Lee HJ, Kim WH, Yang HK. Comparison of Surgical Outcomes of Robot-Assisted and Laparoscopy-Assisted Pylorus-Preserving Gastrectomy for Gastric Cancer: A Propensity Score Matching Analysis. *Ann Surg Oncol* 2015; **22**: 2323-2328 [PMID: 25361887 DOI: 10.1245/s10434-014-4204-6]

43 **Junfeng Z**, Yan S, Bo T, Yingxue H, Dongzhu Z, Yongliang Z, Feng Q, Peiwu Y. Robotic gastrectomy versus laparoscopic gastrectomy for gastric cancer: comparison of surgical performance and short-term outcomes. *Surg Endosc* 2014; **28**: 1779-1787 [PMID: 24385251 DOI: 10.1007/s00464-013-3385-6]

44 **Kim HI**, Park MS, Song KJ, Woo Y, Hyung WJ. Rapid and safe learning of robotic gastrectomy for gastric cancer: multidimensional analysis in a comparison with laparoscopic gastrectomy. *Eur J Surg Oncol* 2014; **40**: 1346-1354 [PMID: 24080199]

45 **Kim MC**, Heo GU, Jung GJ. Robotic gastrectomy for gastric cancer: surgical techniques and clinical merits. *Surg Endosc* 2010; **24**: 610-615 [PMID: 19688399 DOI: 10.1007/s00464-009-0618-9]

46 **Huang KH**, Lan YT, Fang WL, Chen JH, Lo SS, Hsieh MC, Li AF, Chiou SH, Wu CW. Initial experience of robotic gastrectomy and comparison with open and laparoscopic gastrectomy for gastric cancer. *J Gastrointest Surg* 2012; **16**: 1303-1310 [PMID: 22450954 DOI: 10.1007/s11605-012-1874-x]

47 **Kim KM**, An JY, Kim HI, Cheong JH, Hyung WJ, Noh SH. Major early complications following open, laparoscopic and robotic gastrectomy. *Br J Surg* 2012; **99**: 1681-1687 [PMID: 23034831 DOI: 10.1002/bjs.8924]

48 **Pernazza G**, Gentile E, Felicioni L, Tumbiolo S, Giulianotti PC. Improved Early Survival After Robotic Gastrectomy in Advanced Gastric Cancer. *Surg Laparosc Endosc Percutan Tech* 2006; **286** [DOI: 10.1097/00129689-200608000-00024]

49 **Caruso S**, Patriti A, Marrelli D, Ceccarelli G, Ceribelli C, Roviello F, Casciola L. Open vs robot-assisted laparoscopic gastric resection with D2 lymph node dissection for adenocarcinoma: a case-control study. *Int J Med Robot* 2011; **7**: 452-458 [PMID: 21984205 DOI: 10.1002/rcs.416]

50 **Procopiuc L,** Tudor S, Manuc M, Diculescu M, Vasilescu C. Open vs robotic radical gastrectomy for locally advanced gastric cancer(†). *Int J Med Robot* 2015; [Epub ahead of print] [PMID: 26096708 DOI: 10.1002/rcs.1674]

51 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20 [PMID: 16822992 DOI: 10.1056/NEJMoa055531]

52 **Shimizu S**, Uchiyama A, Mizumoto K, Morisaki T, Nakamura K, Shimura H, Tanaka M. Laparoscopically assisted distal gastrectomy for early gastric cancer: is it superior to open surgery? *Surg Endosc* 2000; **14**: 27-31 [PMID: 10653231 DOI: 10.1007/s0046649900005]

53 **Ganpathi IS**, So JB, Ho KY. Endoscopic ultrasonography for gastric cancer: does it influence treatment? *Surg Endosc* 2006; **20**: 559-562 [PMID: 16446988 DOI: 10.1007/s00464-005-0309-0]

54 **Uyama I**, Suda K, Satoh S. Laparoscopic surgery for advanced gastric cancer: current status and future perspectives. *J Gastric Cancer* 2013; **13**: 19-25 [PMID: 23610715 DOI: 10.5230/jgc.2013.13.1.19]

55 **Marano A**, Choi YY, Hyung WJ, Kim YM, Kim J, Noh SH. Robotic versus Laparoscopic versus Open Gastrectomy: A Meta-Analysis. *J Gastric Cancer* 2013; **13**: 136-148 [PMID: 24156033 DOI: 10.5230/jgc.2013.13.3.136]

56 **Jin SH**, Kim DY, Kim H, Jeong IH, Kim MW, Cho YK, Han SU. Multidimensional learning curve in laparoscopy-assisted gastrectomy for early gastric cancer. *Surg Endosc* 2007; **21**: 28-33 [PMID: 16960676 DOI: 10.1007/s00464-005-0634-3]

57 **Kim MC**, Choi HJ, Jung GJ, Kim HH. Techniques and

complications of laparoscopy-assisted distal gastrectomy (LADG) for gastric cancer. *Eur J Surg Oncol* 2007; **33**: 700-705 [PMID: 17399938 DOI: 10.1016/j.ejso.2007.02.018]

58 **Vasilescu C**, Procopiuc L. Robotic surgery of locally advanced gastric cancer: a single-surgeon experience of 41 cases. *Chirurgia* (Bucur) 2012; **107**: 510-517 [PMID: 23025119]

59 **Hiki N**, Fukunaga T, Yamaguchi T, Nunobe S, Tokunaga M, Ohyama S, Seto Y, Muto T. Laparoscopic esophagogastric circular stapled anastomosis: a modified technique to protect the esophagus. *Gastric Cancer* 2007; **10**: 181-186 [PMID: 17922097 DOI: 10.1007/s10120-007-0433-8]

60 **Inaba K**, Satoh S, Ishida Y, Taniguchi K, Isogaki J, Kanaya S, Uyama I. Overlap method: novel intracorporeal esophagojejunostomy after laparoscopic total gastrectomy. *J Am Coll Surg* 2010; **211**: e25-e29 [PMID: 21036074 DOI: 10.1016/j.jamcollsurg.2010.09.005]

61 **Sgarbura O**, Vasilescu C. The decisive role of the patient-side surgeon in robotic surgery. *Surg Endosc* 2010; **24**: 3149-3155 [PMID: 20495980 DOI: 10.1007/s00464-010-1108-9]

62 **Glenn JA**, Turaga KK, Gamblin TC, Hohmann SF, Johnston FM. Minimally invasive gastrectomy for cancer: current utilization in US academic medical centers. *Surg Endosc* 2015; **29**: 3768-3775 [PMID: 25791064 DOI: 10.1007/s00464-015-4152-7]

63 **Bobocea AC**, Trandafir B, Bolca C, Cordoş I. Minimally invasive surgery in cancer. Immunological response. *Chirurgia* (Bucur) 2012; **107**: 154-157 [PMID: 22712341]

64 **Tudor S**, Giza DE, Lin HY, Fabris L, Yoshiaki K, D'Abundo L, Toale KM, Shimizu M, Ferracin M, Challagundla KB, Cortez MA, Fuentes-Mattei E, Tulbure D, Gonzalez C, Henderson J, Row M, Rice TW, Ivan C, Negrini M, Fabbri M, Morris JS, Yeung SC, Vasilescu C, Calin GA. Cellular and Kaposi's sarcoma-associated herpes virus microRNAs in sepsis and surgical trauma. *Cell Death Dis* 2014; **5**: e1559 [PMID: 25476907 DOI: 10.1038/cddis.2014.515]

65 **Bonenkamp JJ**, Hermans J, Sasako M, van de Velde CJ, Welvaart K, Songun I, Meyer S, Plukker JT, Van Elk P, Obertop H, Gouma DJ, van Lanschot JJ, Taat CW, de Graaf PW, von Meyenfeldt MF, Tilanus H. Extended lymph-node dissection for gastric cancer. *N Engl J Med* 1999; **340**: 908-914 [PMID: 10089184 DOI: 10.1056/NEJM199903253401202]

66 **Cuschieri A**, Fayers P, Fielding J, Craven J, Bancewicz J, Joypaul V, Cook P. Postoperative morbidity and mortality after D1 and D2 resections for gastric cancer: preliminary results of the MRC randomised controlled surgical trial. The Surgical Cooperative Group. *Lancet* 1996; **347**: 995-999 [PMID: 8606613]

67 **Cuschieri A**, Weeden S, Fielding J, Bancewicz J, Craven J, Joypaul V, Sydes M, Fayers P. Patient survival after D1 and D2 resections for gastric cancer: long-term results of the MRC randomized surgical trial. Surgical Co-operative Group. *Br J Cancer* 1999; **79**: 1522-1530 [PMID: 10188901 DOI: 10.1038/sj.bjc.6690243]

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TOPIC HIGHLIGHT

2016 Pancreatic Cancer: Global view

MicroRNA in pancreatic ductal adenocarcinoma and its precursor lesions

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the 4th deadliest cancer in the United States, due to its aggressive nature, late detection, and resistance to chemotherapy. The majority of PDAC develops from 3 precursor lesions, pancreatic intraepithelial lesions (PanIN), intraductual papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm. Early detection and surgical resection can increase PDAC 5-year survival rate from 6% for Stage Ⅳ to 50% for Stage Ⅰ. To date, there are no reliable biomarkers that can detect PDAC. MicroRNAs (miRNA) are small noncoding RNAs (18-25 nucleotides) that regulate gene expression by affecting translation of messenger RNA (mRNA). A large body of evidence suggests that miRNAs are dysregulated in various types of cancers. MiRNA has been profiled as a potential biomarker in pancreatic tumor tissue, blood, cyst fluid, stool, and saliva. Four miRNA biomarkers (miR-21, miR-155, miR-196, and miR-210) have been consistently dysregulated in PDAC. MiR-21, miR-155, and miR-196 have also been dysregulated in IPMN and PanIN lesions suggesting their use as early biomarkers of this disease. In this review, we explore current knowledge of miRNA sampling, miRNA dysregulation in PDAC and its precursor lesions, and advances that have been made in using miRNA as a biomarker for PDAC and its precursor lesions.

Key words: Pancreatic cancer; MicroRNA; Biomarkers; Pancreatic intraepithelial lesions; Intraductal papillary mucinous neoplasm

Core tip: Reliable biomarkers are needed to detect pancreatic ductal adenocarcinoma (PDAC) early in order to decrease mortality. In this review, we discuss what the current knowledge is on microRNA (miRNA) in PDAC and its precursor lesions. MiR-21, miR-155, miR-196, miR-210 are dysregulated in tissue, serum, cyst fluid, and stool of PDAC patients. MiR-21, miR-155, and miR-196 are dysregulated in intraductual papillary

mucinous neoplasm and pancreatic intraepithelial lesions demonstrating that these miRNAs may serve as potential biomarkers for early stage lesions and cancer.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the 4th deadliest cancer in the United States, due to its aggressive nature, late detection, and resistance to chemotherapy[1,2]. The majority of PDAC develops from 3 precursor lesions, pancreatic intraepithelial lesions (PanIN), intraductual papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN)[3]. The cystic precursor lesions of the pancreas are detectable by certain imaging modalities such as Endoscopic ultrasound (EUS)[4-6], Magnetic Resonance Imaging of the Abdomen with Cholangiopancreatography[7], and computerized tomography scan[8,9]. To date, there is no modality that clearly detects PanIN lesions, although studies have suggested a correlation between multifocal PanIN and lobular atrophy of the pancreas on EUS[10].

Early detection and surgical resection can increase PDAC 5-year survival rate from 6% for Stage Ⅳ to 50% for Stage Ⅰ [11,12]. Detection and surgical removal of precursor lesions has the potential to be curative. Because of this, there has been much research focused on identification of individuals at high-risk of PDAC, detection of early stage lesions, and on the discovery of reliable biomarkers of this deadly disease. Carbohydrate antigen (CA) 19-9 is a poor biomarker of PDAC, as it is elevated in 30%-40% of benign diseases of the pancreas[13,14] with a sensitivity of 79% (70%-90%) and specificity of 82% (68%-91%)[15] for PDAC.

MicroRNA (miRNA) expression has been studied in tumor detection, cancer development and progression, and prognosis[16]. MiRNAs are small noncoding RNAs (18-25 nucleotides) that regulate gene expression by affecting translation of messenger RNA (mRNA)[16-18]. MiRNA function to stabilize mRNA transcripts *via* post-transcriptional gene silencing *via* inhibition of the translation process or cleavage of their target mRNAs[16,19]. Over the last decade, the role of miRNA in cancer development and detection has evolved. MiRNA is very stable in tissue, plasma, stool, and other fluids and can be quantified in very small sample sizes, making it an excellent potential biomarker for the detection of PDAC. Current priorities include: (1) identification of miRNAs that are reliably dysregulated in PDAC; (2) determining which sample source(s) are easily accessible and have the highest yield for detecting these biomarkers; and (3) development of novel ways in which to use this information to detect early onset PDAC and precursor lesions. Array-based analysis is used to evaluate the expression levels of thousands of miRNAs in various tissue types. Subsequent trials have validated these findings by performing quantitative real time PCR (QRT-PCR) and performed receiver operating characteristics (ROC) analysis to determine the sensitivity and specificity of these miRNAs as potential biomarkers for early or advanced disease. Some miRNAs, such as miR-21, miR-155, miR-196a, and miR-210 have stood out as potential biomarkers of this highly fatal disease[20-24]. Our review is aimed at exploring current knowledge of miRNA sampling, miRNA dysregulation in PDAC and its precursor lesions, and advances that have been made in using miRNA as a biomarker for PDAC and its precursor lesions.

ROLE OF MIRNA IN PDAC DETECTION: SAMPLES FROM TISSUE, SERUM, PANCREATIC JUICE, STOOL AND SALIVA

Attention has been paid to circulating serological signatures, autoantibodies, epigenetic markers, circulating tumor cells (TCs), and miRNAs in order to detect PDAC at an earlier stage of disease[25,26]. The use of miRNA for diagnosis and screening is still an evolving field; in the right patient population, an ideal miRNA test would be highly sensitive and specific, minimally-invasive and cost-effective. MiRNA expression in PDAC was first examined in PDAC tissue cells[27]. Now miRNA has been found in serum, blood, whole plasma, stool, saliva, and cyst fluid (Table 1). Current knowledge is described below.

MiRNA in whole pancreas tissue or PDAC biopsies

Szafranska *et al*[27] performed the first analysis comparing miRNA expression in normal pancreas tissue, chronic pancreatitis (CP) tissue and PDAC tissue. On imaging, it can be challenging to distinguish CP from PDAC given the thick stroma and inflammation that may be found in both of these conditions. Furthermore, it is unclear if the aberrant expression of particular miRNAs is secondary to the desmoplastic reaction in CP and PDAC, and not related to tumorigenesis itself. They and others have found that miRNA-216 and miRNA-217 are significantly down-regulated in PDAC and miRNA-143, miR-145, miR-146a, miR-148a, miR-150, miR-155, miR-196a, miR-196b, miR-210, miR-222, miR-223, miR-31 are up-regulated in PDAC[24,27-29]. However, this study also demonstrated that dysregulation of miRNA-196a, miR-196b, miR-203, miR-210, miR-222, miR-217, and miR-375 were found only in PDAC, whereas miRNA-29c, miR-96, miR-143, miR-145, miR-148b, and miR-150 were abnormally expressed in both CP and PDAC. This may suggest that the latter are responsible for causing the desmoplastic reaction as opposed to tumorigenesis.

Table 1 MiRNA in pancreatic ductal adenocarcinoma

MiRNA	Whole pancreas	Serum and plasma	Saliva	Stool	Pancreatic juice
miR-10b		↑[54]			
miR-16		↑[52]			
miR-18a		↑[29,56,57]			
miR-20a		↑[55,134]			↓[62]
miR-21	↑[22,24,27,32,34,38,55,71]	↑[55,71]		↑[63]↔[71]	↑[61,62,71]
miR-24		↑[55]			
miR-27a-3p		↑[134]			↑[62]
miR-29c	↓[27]				
miR-30a-3p	↓[27]				
miR-30c		↓[37]			
miR-31	↑[27]				
miR-34a		↑[37]			
miR-96	↓[27,135]				↓[62]
miR-99a		↑[52]			
miR-101	↑[32]				
miR-106b		↑[54]			
miR-130b	↓[27]				
miR-135b	↑[31]				
miR-139-3p		↓[37,58]			
miR-141	↓[27]				
miR-143	↑[27,71]			↓[71]	
miR-145	↑[27]				
miR-146a	↑[27]				↑[62]
miR-148a	↓[27,29]				
miR-148b	↓[27-29]				
miR-150	↑[27]				
miR-155	↑[22-24,27,32,66,71]	↑[22,54]		↑[63]↓[71]	↑[61,71]
miR-181a,b,d	↑[24]			↑[72]	
miR-185		↑[52,55,134]			
miR-191		↑[55]			
miR-192		↑[37,58]			
miR-194		↑[37]			
miR-196a	↑[22,23,27,49,52,71]	↑[21,22,52,71]		↑[72]↓[71]	↑[62,71]
miR-196b	↑[27]				↑[60]
miR-200a		↑[136]			↑[62]
miR-200b		↑[136]			
miR-205					
miR-210	↑[27,71,137,138]	↑[137]		↑[72], ↔ [71]	↑[60,71]
miR-212	↑[38]	↑[37]			
miR-216	↓[27,38]			↓[63]↓[71]	
miR-217	↓[27]				↓[62]
miR-222	↑[24,27,38]				
miR-223	↑[27]				
miR-375	↓[27,71]			↔[71]	
miR-492		↓[59]			↑[60]
miR-494	↓[27,74]				
miR-508-5p		↓[37]			
miR-513a-5p		↓[37]			
miR-602		↑[37]			
miR-630		↓[37]			
miR-663a		↓[59]			
miR-801		↑[37]			
miR-887		↓[37]			
miR-923		↓[37]			
miR-940			↑[74]	↑[74]	
miR-1290	↑[30]				
miR-1427					↑[60]
miR-3679-5p			↓[74]	↓[72,74]	

miR: MicroRNA; ↑: Up-regulated; ↓: Down-regulated; ↔: Unchanged.

MiR-1290 is elevated in early stage PDAC compared to normal controls[30]. Additionally, miR-135b has been shown to be an effective biomarker for distinguishing PDAC from CP with high sensitivity and specificity[31].

MiR-21, MiR-155, and miR-196 have been demonstrated by multiple groups to differentiate PDAC from non-cancerous lesions of the pancreas[20-24,27,32]. Special attention has been placed on the role of miR-21 in

PDAC, as it has been implicated in tumorigenesis, TC invasion, the desmoplastic reaction, and metastasis of TC[33-36]. Further studies did not demonstrate that miR-21 expression in stromal cells correlated with tumor stage.

MiR-192 has also been found to be present in pancreatic TC, but is seldom seen in stromal cells and not found in adjacent normal pancreas tissue[37]. In this same study, miR-194 expression was detected in PDAC tissue, but not found in the surrounding normal pancreatic tissue. Unfortunately, despite these findings, no significant difference was found between the serum levels of miR-194 in patients with PDAC and healthy controls.

One proposed mechanism for PDAC development includes signaling between the molecular markers of the desmoplastic reaction and TCs[38-41]. Liffers *et al*[29] demonstrated that miR-148a is down-regulated in microdissected PDAC tissue and when over-expressed prevents tumor growth. This suggests that miR-148a may have a crucial role in the molecular signaling by which tumorigenesis occurs. While it is important to find biomarkers that are deregulated in PDAC, it is also important to understand which miRNAs are involved in these aberrant signaling pathways.

MiRNA in serum and plasma of PDAC patients

MiRNAs are known to have organ-specific expression in many human cancers[42,43]. Less than a decade ago, studies found that miRNA could reliably be detected in the serum in both animal models and humans[44,45], and since that time, there has been much research dedicated to identifying which miRNAs have differential expression and the implications of these findings in the detection, staging, treatment, and prognosis of cancers[46-50].

Attempts to use miRNA biomarkers in conjunction with CA19-9 have yielded mixed results. A study examining 847 different miRNAs in patients with PDAC found increased expression of miR-375 in PDAC as opposed to controls. MiR-375 did not improve detection nor predict prognosis in patients with PDAC when compared to CA19-9 alone[51]. Liu *et al*[52] found that using serum miR-16 and miR-196a in combination with CA19-9 increased detection of PDAC and Stage Ⅰ lesions when compared to either modality alone, which suggests that miR-16 and miR-196a may be deregulated early in PDAC. These biomarkers were also up-regulated in studies performed on pancreas tissue, demonstrating that miR-16 and miR-196a can be used as peripheral biomarkers of PDAC. Gao, *et al*[53] also demonstrated that miR-16, when combined with CA19-9, served as a potential biomarker for detection of PDAC when compared to patients with CP.

One limitation of CA19-9 as a biomarker is that it is elevated in a large portion of patients with benign pancreatic diseases. Because of this limitation, studies have evaluated the miRNA expression of patients with PDAC compared to those with benign diseases such as CP or choledocolithiasis. They found that miR-10b, miR-155, and miR-106b were consistently elevated in the serum of patients with PDAC but not in those with benign pancreatic disease[54]. Liu *et al*[55] have demonstrated that up-regulation of miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-191 can be used to distinguish PDAC from healthy controls and CP. Additionally, miR-135b has been shown to be an effective biomarker for distinguishing PDAC from CP with high sensitivity and specificity[31]. MiR-18a levels have also been shown to have increased expression in patients with PDAC and interestingly decrease after surgical resection suggesting that miR-18a levels may be a good marker to not only detect disease but also to monitor disease recurrence[29,56,57]. Zhang *et al*[37] also demonstrated that miR-194, miR-192, miR-602, miR-801, miR-212, miR-34a are up-regulated in PDAC, while miR-923, miR-139-3p, miR-513a-5p, miR-630, miR-30c-1, miR-887, miR-508-5p, and miR-139a-5p were down-regulated in PDAC specimens[37,58]. From these data, they demonstrated that miR-192 is neither present in the stromal cells of the pancreas nor the serum, but it is up-regulated in PDAC TCs and is involved in cell proliferation of PANC-1 TC lines *in vitro*[58]. Lin *et al*[59] performed microarray on 1711 serum miRNAs and found that 23 were down-regulated and 22 were up-regulated in the serum of PDAC patients when compared to normal controls. Of these, miR-492 and miR-663a were found to have decreased expression that was statistically significant in PDAC; however, only miR-663a was found to have a positive correlation with stage of disease[59]. Further studies are needed to determine which miRNAs will be clinically relevant.

Pancreatic juice miRNA

Pancreatic juice sampling requires an invasive endoscopic procedure, but studying the miRNA concentrations of patients with PDAC, benign pancreatic lesions, and healthy controls can shed light on potential biomarkers for detecting disease as they are found in high concentration in cyst fluid. As EUS and endoscopic retrograde cholangiopancreatogram (ERCP) are two methods by which pancreatic masses are frequently detected and sampled, these specimens could be sent for miRNA analysis in order to determine the malignant potential of these lesions. Wang *et al*[60] performed microarray of 49 miRNAs on secretin-stimulated pancreatic juice of a group of patients with PDAC, CP, and normal controls. They demonstrated that miR-205, miR-210, miR-492, and miR-1427 are all significantly elevated in PDAC when compared to controls; however, this statistical significance does not exist when compared to patients with CP[60]. Additionally, by using ROC curves, they determined that combining these 4 miRNAs with serum CA19-9 the sensitivity and specificity of PDAC detection is 91% and 100% respectively, though this analysis was limited by a sample size of 6. Other groups have evaluated the pancreatic juice of patients with PDAC pre-operatively *via* ERCP and from post-operative specimens[61,62]. Sadakari *et al*[61] analyzed the expression

of miR-155 and miR-21 in pancreatic juice sampled *via* ERCP and found that these miRNAs were significantly elevated when compared to patients with CP and healthy controls, though the levels did not correlate with pancreatic juice cytology[61]. Again these findings are consistent with those from pancreatic tissue and serum. Hong *et al*[62] evaluated 158 miRNAs in post-operative fine needle aspiration specimens and found by qRT-PCR that miR-21, miR-27a, miR-146a, and miR-186a were significantly over-expressed in PDAC tissue and miR-217, miR-20a, and miR-96 were significantly down-regulated in PDAC tissue when compared to normal controls[62]. These two studies have demonstrated the feasibility of detecting miRNA from pancreatic juice, thus indicating the potential for using pancreatic juice biomarkers to detect early lesions given the higher concentration of miRNA in this fluid sample.

MiRNA in stool specimens

Frozen stool specimens may serve as potential non-invasive biomarker samples for PDAC. Over-expressed miRNAs from gastrointestinal cancers are shed from the exfoliative cells of the gastrointestinal tract. Intraluminal release of pancreatic juice also allows for detection of miRNAs in the stool[63-69]. Previous studies have largely focused on genetic markers of tumorigenesis and not miRNA[70]. Yang *et al*[63] performed a feasibility study of using stool miRNAs as a potential screening tool for detection of PDAC. They evaluated expression of 5 miRNAs that had been previously shown to be over-expressed in PDAC and found that miRNA-21 and miR-155 were over-expressed and miR-216 was under-expressed in all PDAC stool specimens when compared to normal controls and CP patients. These findings are consistent with what has been found in whole pancreas, pancreatic cyst fluid, and serum specimens. Additionally, with ROC analysis they demonstrated that combining miR-21 and miR-155 in stool samples there was a sensitivity of 93.33% and specificity of 66.67%. When they combined all 3 miRNAs (miR-21, miR-155, and miR-216), the sensitivity and specificity were 83.33% each. Link *et al*[71] selected 7 miRNAs (miR-21, miR-210, miR-143, miR-155, miR-196a, miR-216a, miR-375) and determined that like Yang's group miR-216a was found in lower concentrations in the stool of patients with PDAC. However, unlike Yang's group, they found that miR-155 was down-regulated in this population and miR-21 was unchanged in the stool of controls compared to CP or PDAC[71].

Ren *et al*[72] also evaluated the expression of miR-21, miR-155, miR-181a, miR-196a, and miR-210 and found that miR-181b, miR-196a, and miR-210 were significantly over-expressed in PDAC patients when compared to controls, but only miR-181b and miR-210 were elevated in CP patients, though these elevations were not significant. Ren *et al*[72] established a positive correlation between miR-196a levels and tumor size, which had not been previously described in studies of the serum or stool. Overall, while studies of fecal miRNA have demonstrated feasibility, conflicting data have emerged on which miRNAs are differentially expressed in the stool of PDAC, benign pancreatic disease, and normal controls.

Salivary miRNA

The field of salivanomics has been developing since blood molecules have been found in saliva[73]. As with stool miRNA, salivary miRNA may serve as a non-invasive biomarker for PDAC. Xie *et al*[74] is the only group to have evaluated salivary miRNA in PDAC. They conducted a microarray of 2006 miRNAs and noted that 10, including miR-4433-5p, miR-4665-3p, miR-940, miR-1273g-3p, miR-3676-5p, miR-3679-5p, miR-3940-5p, miR-4327, miR-4442, and miR-5100, were up-regulated or down-regulated in salivary samples. Of these, only miR-940 was significantly up-regulated and miR-3679-5p was significantly down-regulated in the PDAC specimens during the validation phase of the study. Until now, neither has been implicated in PDAC in the serum, stool, whole pancreas, or pancreatic juice. More studies are needed in this area.

ROLE OF MIRNA IN DETECTION OF PRECURSOR LESIONS

The absence of symptoms in early disease makes PDAC a cancer that is detected at very late stages when mortality approaches 100%. Much research has been dedicated to detecting miRNA in patients with PDAC as a novel biomarker for the presence of disease. Given the aggressive nature of PDAC, detection of precursor lesions with malignant potential would be critical to increasing the survival of these patients. PanIN lesions are microscopic PDAC precursor lesions that are graded 1-3 and are categorized based on the level of architectural and cytological atypia that is present[75,76]. Grade 1a is early intraepithelial proliferative lesions that have flat architecture, while grade 1b lesions have papillary architecture. PanIN-2 lesions have moderate abnormalities and PanIN-3 lesions have severe abnormalities, though none of these lesions invade the basement membrane[75,76]. IPMN lesions are mucin-producing cystic tumors, which arise from the epithelium of the pancreatic ducts and have the potential for malignant transformation[77,78]. They are categorized by main duct type (MD) or branch duct type (BD) and histologically are classified as having low-, intermediate-, and high-grade dysplasia[3]. Their malignant potential differs based on their location within the pancreatic ducts, and MD-IPMN carry a 44%-48% risk compared to BD-IPMNs, which only carry a 11%-17% risk of malignant transformation[79-82]. MCN are also mucin-producing epithelial neoplasms with ovarian-type stroma occur primarily in middle-aged females and are located in the body and tail of the pancreas and carry a 12% chance of tumor progression[83-86]. Cystic fluid is analyzed

Table 2 MiRNA in precursor lesions

MiRNA	IPMN	PanIN-1	PanIN-2	PanIN-3
miR-10b			↑[101]	↑[101]
miR-21	↑[23,32,87,88]	↑[100,101]	↑[98,99]	↑[98,99]
miR-92a	↑[93]			
miR-99a	↓[91,93]			
miR-99b	↓[91]			
miR-100	↓[91,93]			
miR-101	↓[32]			
miR-125b	↑[93]			
miR-126	↓ [91]			
miR-130a	↓ [91]			
miR-145	↑[93]			
miR-148			↓[101]	↓[101]
miR-155	↑[23,32]	↑[101]	↑[98]	↑[98]
miR-182		↑[101]		
miR-196a	↑[90]		↑[97]	↑[97]
miR-196b			↑[97]	↑[97]
miR-200a		↑[101]		
miR-200b		↑[101]		
miR-212	↓[93]			
miR-217			↓[101]	↓[101]
miR-221	↑[87]			
miR-296-5p		↑[101]		
miR-342-3p	↓[91]			
miR-483-3p	↑[88,93]			

miR: MicroRNA; ↑: Up-regulated; ↓: Down-regulated; PanIN: Pancreatic intraepithelial lesions; IPMN: Intraductal papillary mucinous neoplasm.

for CEA and amylase as other tumor markers have not demonstrated reliability in detecting malignant lesions.

As cystic neoplasms of the pancreas carry the risk of malignant transformation, determining a way to accurately predict which will progress to invasive carcinomas may guide surgical management and treatment decisions. MiRNA has been examined in PanIN lesions and IPMN as a potential candidate for early detection and the likelihood of progression to cancer. Understanding which miRNAs become deregulated early in the disease process may lead to advances for treatment decisions (Table 2).

MiRNA in IPMN lesions

Given the increased use of abdominal imaging, more pancreatic cystic lesions are being detected. There are guidelines in place to help guide management based on cystic characteristics that are consistent with malignancy[77]. The first study looking at miRNA expression levels in precursor lesions of the pancreas was performed by Habbe *et al*[23] who determined that miR-155 and miR-21 were over-expressed in the IPMN neoplastic epithelium, specifically those with carcinoma-in-situ[23,87]. MiR-155 was also significantly elevated in the pancreatic juice of these patients. While the levels of up-regulation of miRNA-21 and miR-155 correlated with the degree of cellular atypia found in the IPMN lesions, the study lacked long-term outcome data, highlighting the need for large and more longitudinal studies. Caponi *et al*[32] established a relationship between expression of miR-21 in invasive IPMNs and clinical outcome and observed that higher levels of miR-21 were correlated with worse overall and disease-free survival. Furthermore, they demonstrated that miR-155 and miR-21 had higher expression levels in invasive IPMN lesions when compared to non-invasive lesions, suggesting that these miRNAs may serve as early markers of malignant transformation[32,88]. MiR-101 has been shown to be down-regulated in invasive IPMNs when compared to non-invasive IPMNs and normal tissue. This deregulation of MiR-101 has not been described in PDAC samples, which may indicate that miR-101 plays a role in tumor invasion[32,89]. As with studies of miRNA in the serum and tissue of PDAC patients, miR-196a was found to be up-regulated in the pancreatic juice of intestinal-type IPMN[90]. In a recent study miR-100, miR-99b, miR-99a, miR-342-3p, miR-126, miR-130a were found to be down-regulated were up-regulated in high-risk *vs* low-risk IPMN lesions[91]. Furthermore, low miR-99b in IPMN fluid was associated with MD involvement, which is associated with a greater risk for transformation into a malignant neoplasm. Abue *et al*[88] found that miR-483-3p was up-regulated in PDAC cells and plasma when compared to IPMN lesions and may also serve as a useful biomarker in differentiating IPMN lesions with malignant potential from normal tissue and PDAC. The down-regulated miRNAs correlated with high-risk IPMNs and may be involved in cyst invasion and progression. Lee *et al*[92] found that miRNA expression varied amongst pancreatic cystic neoplasm. Specifically, miR-31-5p, miR-4830-5p, miR-99a-5p, and miR-375 were characteristic of serous cyst adenomas (SCA), whereas miR-10-5p, miR-202-3p, miR-210, and miR-375 differentiated MCN from SCA, IPMN, and PDAC[92]. It is unclear why this overlap in miR-375 occurs[92]. Henry *et al*[93] found that miR-92a, miR-99a, miR-100, miR-125b, miR-145, miR-212 and miR-483 were differentially expressed between benign and pre-malignant and malignant lesions of the pancreas and they suggested that a high amount of RNA present in the cystic fluid may suggest the presence of malignant transformation[93]. As previously described miR-21, miR-155, miR-196a have been implicated in both PDAC and IPMN and given these widely replicated results, studies aimed at detecting these biomarkers in serum, saliva, and stool could help to determine, in a non-invasive way, if they are increased in pre-malignant lesions.

MiRNA in panin lesions

Currently, PanIN lesions are found in the neighboring pancreatic tissue of patients with PDAC; however, there is no consistent way to detect the presence of these lesions[10,94-96]. Identifying a biomarker to detect PanIN lesions may be critical in early detection of PDAC. Slater *et al*[97] demonstrated that miRNA-196a and miR-196b were elevated in PDAC and PanIN-2/3 lesions in both animal models of PDAC and humans with PDAC. Ryu *et al*[98] demonstrated that miR-155

is up-regulated in PanIN-2 and PanIN-3 lesions when compared to neighboring healthy pancreatic tissue, but not in PanIN-1 lesions. Furthermore, miR-21 has been shown to be over-expressed in PanIN-2[98,99] and PanIN-3[98,99], but not PanIN-1 lesions suggesting that this is a marker for later disease. These are significant findings as miR-155 and miR-21 have been shown to be up-regulated in IPMN lesions and PDAC suggesting that they are early markers for cells with malignant potential. Yu *et al*[100] found that miR-196b was up-regulated in PanIN-3 lesions, which correlates with previous studies that have found that miR-196b is up-regulated in PDAC lesions[100,101]. Importantly, miR-21, miR-155, miR200a, miR-200b, miR-182, and miR-296-5p were deregulated as early as PanIN-1 lesions and remained deregulated until progression to PDAC, with the exception of miR-200c that normalized in PanIN-3 lesions. A recent publication describing miRNA expression in PanIN lesions found that miRNA-148a and miR-217 were down-regulated while miR-10b was up-regulated in PanIN-2 and PanIN-3 lesions[101]. While miR-21 has been shown repeatedly to be over-expressed in PDAC, there are conflicting studies on its deregulation in early PanIN lesions suggesting that it may represent a later and more aggressive dysregulation in the progression to PDAC. A non-invasive method to detect advanced PanIN lesions would represent a significant advance in the field.

DISCUSSION

While PDAC is the fourth most common cause of cancer-related deaths, there is still no reliable way to detect early disease and patients present with late-stage disease with a nearly 100% mortality. Research in the field of biomarkers shows a great deal of promise as current research aims to understand the molecular mechanisms and stromal microenvironment of this deadly tumor. MiRNA are small nucleotides that control the genetic expression in all cells and importantly in an organ-specific manner. Abberant miRNA expression has been identified in various cancers[102-106], and factors such as transcriptional deregulation, epigenetic alterations, mutations, DNA copy number abnormalities, and defects in the miRNA biogenesis pathway may account for these differences in expression[107,108]. C-myc and p53 are two transcriptional factors that have been associated transcriptional deregulation of miRNA[109-111], Epigenetic regulation of miRNAs by DNA methylation and histone tail modification play a role in miRNA expression through chromatin remodeling[105,112-114]. Both germ-line and somatic mutations are responsible for miRNA expression levels in various types of cancers[115-117]. It has been described by Calin *et al*[118], that miRNAs are located a fragile sites on the chromosome, minimal regions of loss of heterozygosity, minimal regions of amplifications, and common breakpoints, thus increasing the risk for DNA copy abnormalities. DNA copy abnormalities have been found in melanoma, breast cancer, ovarian cancer, leukemia, colorectal cancer[119-122]. Lastly, defects in miRNA biogenesis pathway may contribute to varying expression levels and cancer phenotype as miRNA undergoes complex processing intracellulary prior to reaching its mature form[123-127]. In addition to the aforementioned mechanisms, dietary components, such as folate, retinoids, curcumin, and Vitamin D have been implicated in the modulation of miRNA expression[128-130]. Some miRNAs have been shown to increase muscle loss in cancer cachexia and specifically, increased miR-21 levels have been shown to increase muscle breakdown in pancreatic cancer[131,132]. Deeper understanding of the regulatory mechanisms of miRNA expression will hopefully give new insight to the factors responsible for miRNA deregulation and lead to miRNA-based diagnostic testing and miRNA-directed therapy for PDAC.

Some limitations that exist with the current miRNA research at this time include standardization of extraction, reproducibility of testing, diagnostic yield in the various sample methods, and small sample sizes. Additionally, despite finding biomarkers for this disease, there is limited evidence that miRNA will impact PDAC-related mortality. Dysregulation of miRNA affects the cell cycle, proliferation, apoptosis, epigenetics, oncogenesis, tumor differentiation, tumor invasion, tumor metastasis and migration, prognosis, and chemoresistance in numerous cancers[133]. Increased efforts to understand the biological function of miRNA expression and its effects on cancer development are needed.

Despite these limitations, great advances have been made in this field and now miRNA expression is being analyzed not just in pancreatic tissue and cystic fluid, but also in stool, saliva, and serum; which would lead to non-invasive ways by which to analyze the expression levels of miRNA in patients at high risk. There have been great efforts to identify which of the greater than 2000 miRNAs are deregulated in PDAC and its precursor lesions and miRNA-21, miR-155, and miR-196b seem to be dysregulated in both early lesions and advanced cancer and show promise as potential screening tools in the future.

REFERENCES

1 **Li D**, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**: 1049-1057 [PMID: 15051286 DOI: 10.1016/S0140-6736(04)15841-8]

2 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]

3 **Klöppel G**, Basturk O, Schlitter AM, Konukiewitz B, Esposito I. Intraductal neoplasms of the pancreas. *Semin Diagn Pathol* 2014; **31**: 452-466 [PMID: 25282472 DOI: 10.1053/j.semdp.2014.08.005]

4 **Rösch T**, Lorenz R, Braig C, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Endoscopic ultrasound in pancreatic tumor diagnosis. *Gastrointest Endosc* 1991; **37**: 347-352 [PMID: 2070987 DOI: 10.1016/S0016-5107(91)70729-3]

5 **Müller MF**, Meyenberger C, Bertschinger P, Schaer R, Marincek B. Pancreatic tumors: evaluation with endoscopic US, CT, and MR imaging. *Radiology* 1994; **190**: 745-751 [PMID: 8115622 DOI: 10.1148/radiology.190.3.8115622]

6 **Chang KJ**. Endoscopic ultrasound-guided fine needle aspiration in

the diagnosis and staging of pancreatic tumors. *Gastrointest Endosc Clin N Am* 1995; **5**: 723-734 [PMID: 8535620]
7 **Park MJ**, Kim YK, Choi SY, Rhim H, Lee WJ, Choi D. Preoperative detection of small pancreatic carcinoma: value of adding diffusion-weighted imaging to conventional MR imaging for improving confidence level. *Radiology* 2014; **273**: 433-443 [PMID: 24991989 DOI: 10.1148/radiol.14132563]
8 **Canto MI**, Goggins M, Hruban RH, Petersen GM, Giardiello FM, Yeo C, Fishman EK, Brune K, Axilbund J, Griffin C, Ali S, Richman J, Jagannath S, Kantsevoy SV, Kalloo AN. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. *Clin Gastroenterol Hepatol* 2006; **4**: 766-781; quiz 665 [PMID: 16682259 DOI: 10.1016/j.cgh.2006.04.003]
9 **Canto MI**, Goggins M, Yeo CJ, Griffin C, Axilbund JE, Brune K, Ali SZ, Jagannath S, Petersen GM, Fishman EK, Piantadosi S, Giardiello FM, Hruban RH. Screening for pancreatic neoplasia in high-risk individuals: an EUS-based approach. *Clin Gastroenterol Hepatol* 2004; **2**: 606-621 [PMID: 15224285 DOI: 10.1016/S1542-3565(04)00244-7]
10 **Brune K**, Abe T, Canto M, O'Malley L, Klein AP, Maitra A, Volkan Adsay N, Fishman EK, Cameron JL, Yeo CJ, Kern SE, Goggins M, Hruban RH. Multifocal neoplastic precursor lesions associated with lobular atrophy of the pancreas in patients having a strong family history of pancreatic cancer. *Am J Surg Pathol* 2006; **30**: 1067-1076 [PMID: 16931950]
11 **Egawa S**, Takeda K, Fukuyama S, Motoi F, Sunamura M, Matsuno S. Clinicopathological aspects of small pancreatic cancer. *Pancreas* 2004; **28**: 235-240 [PMID: 15084963 DOI: 10.1097/00006676-200404000-00004]
12 **Ishikawa O**, Ohigashi H, Imaoka S, Nakaizumi A, Uehara H, Kitamura T, Kuroda C. Minute carcinoma of the pancreas measuring 1 cm or less in diameter--collective review of Japanese case reports. *Hepatogastroenterology* 1999; **46**: 8-15 [PMID: 10228758]
13 **Satake K**, Kanazawa G, Kho I, Chung Y, Umeyama K. Evaluation of serum pancreatic enzymes, carbohydrate antigen 19-9, and carcinoembryonic antigen in various pancreatic diseases. *Am J Gastroenterol* 1985; **80**: 630-636 [PMID: 2411125]
14 **Rosty C**, Goggins M. Early detection of pancreatic carcinoma. *Hematol Oncol Clin North Am* 2002; **16**: 37-52 [PMID: 12063828 DOI: 10.1016/S0889-8588(01)00007-7]
15 **Goonetilleke KS**, Siriwardena AK. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. *Eur J Surg Oncol* 2007; **33**: 266-270 [PMID: 17097848 DOI: 10.1016/j.ejso.2006.10.004]
16 **Sethi S**, Kong D, Land S, Dyson G, Sakr WA, Sarkar FH. Comprehensive molecular oncogenomic profiling and miRNA analysis of prostate cancer. *Am J Transl Res* 2013; **5**: 200-211 [PMID: 23573364]
17 **Sethi A**, Sholl LM. Emerging Evidence for MicroRNAs as Regulators of Cancer Stem Cells. *Cancers* (Basel) 2011; **3**: 3957-3971 [PMID: 24213119 DOI: 10.3390/cancers3043957]
18 **Keller A**, Leidinger P, Borries A, Wendschlag A, Wucherpfennig F, Scheffler M, Huwer H, Lenhof HP, Meese E. miRNAs in lung cancer - studying complex fingerprints in patient's blood cells by microarray experiments. *BMC Cancer* 2009; **9**: 353 [PMID: 19807914 DOI: 10.1186/1471-2407-9-353]
19 **Hassan O**, Ahmad A, Sethi S, Sarkar FH. Recent updates on the role of microRNAs in prostate cancer. *J Hematol Oncol* 2012; **5**: 9 [PMID: 22417299 DOI: 10.1186/1756-8722-5-9]
20 **Tang S**, Bonaroti J, Unlu S, Liang X, Tang D, Zeh HJ, Lotze MT. Sweating the small stuff: microRNAs and genetic changes define pancreatic cancer. *Pancreas* 2013; **42**: 740-759 [PMID: 23774697 DOI: 10.1097/MPA.0b013e3182854ab0]
21 **Liu M**, Du Y, Gao J, Liu J, Kong X, Gong Y, Li Z, Wu H, Chen H. Aberrant expression miR-196a is associated with abnormal apoptosis, invasion, and proliferation of pancreatic cancer cells. *Pancreas* 2013; **42**: 1169-1181 [PMID: 24048456 DOI: 10.1097/MPA.0b013e3182962acb]
22 **Wang J**, Chen J, Chang P, LeBlanc A, Li D, Abbruzzesse JL, Frazier ML, Killary AM, Sen S. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. *Cancer Prev Res* (Phila) 2009; **2**: 807-813 [PMID: 19723895 DOI: 10.1158/1940-6207.CAPR-09-0094]
23 **Habbe N**, Koorstra JB, Mendell JT, Offerhaus GJ, Ryu JK, Feldmann G, Mullendore ME, Goggins MG, Hong SM, Maitra A. MicroRNA miR-155 is a biomarker of early pancreatic neoplasia. *Cancer Biol Ther* 2009; **8**: 340-346 [PMID: 19106647 DOI: 10.4161/cbt.8.4.7338]
24 **Bloomston M**, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, Liu CG, Bhatt D, Taccioli C, Croce CM. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 2007; **297**: 1901-1908 [PMID: 17473300 DOI: 10.1001/jama.297.17.1901]
25 **Yi JM**, Guzzetta AA, Bailey VJ, Downing SR, Van Neste L, Chiappinelli KB, Keeley BP, Stark A, Herrera A, Wolfgang C, Pappou EP, Iacobuzio-Donahue CA, Goggins MG, Herman JG, Wang TH, Baylin SB, Ahuja N. Novel methylation biomarker panel for the early detection of pancreatic cancer. *Clin Cancer Res* 2013; **19**: 6544-6555 [PMID: 24088737 DOI: 10.1158/1078-0432.CCR-12-3224]
26 **He XY**, Yuan YZ. Advances in pancreatic cancer research: moving towards early detection. *World J Gastroenterol* 2014; **20**: 11241-11248 [PMID: 25170208 DOI: 10.3748/wjg.v20.i32.11241]
27 **Szafranska AE**, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, Labourier E, Hahn SA. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. *Oncogene* 2007; **26**: 4442-4452 [PMID: 17237814 DOI: 10.1038/sj.onc.1210228]
28 **Hanoun N**, Delpu Y, Suriawinata AA, Bournet B, Bureau C, Selves J, Tsongalis GJ, Dufresne M, Buscail L, Cordelier P, Torrisani J. The silencing of microRNA 148a production by DNA hypermethylation is an early event in pancreatic carcinogenesis. *Clin Chem* 2010; **56**: 1107-1118 [PMID: 20431052 DOI: 10.1373/clinchem.2010.144709]
29 **Liffers ST**, Munding JB, Vogt M, Kuhlmann JD, Verdoodt B, Nambiar S, Maghnouj A, Mirmohammadsadegh A, Hahn SA, Tannapfel A. MicroRNA-148a is down-regulated in human pancreatic ductal adenocarcinomas and regulates cell survival by targeting CDC25B. *Lab Invest* 2011; **91**: 1472-1479 [PMID: 21709669 DOI: 10.1038/labinvest.2011.99]
30 **Li A**, Yu J, Kim H, Wolfgang CL, Canto MI, Hruban RH, Goggins M. MicroRNA array analysis finds elevated serum miR-1290 accurately distinguishes patients with low-stage pancreatic cancer from healthy and disease controls. *Clin Cancer Res* 2013; **19**: 3600-3610 [PMID: 23697990 DOI: 10.1158/1078-0432.CCR-12-3092]
31 **Munding JB**, Adai AT, Maghnouj A, Urbanik A, Zöllner H, Liffers ST, Chromik AM, Uhl W, Szafranska-Schwarzbach AE, Tannapfel A, Hahn SA. Global microRNA expression profiling of microdissected tissues identifies miR-135b as a novel biomarker for pancreatic ductal adenocarcinoma. *Int J Cancer* 2012; **131**: E86-E95 [PMID: 21953293 DOI: 10.1002/ijc.26466]
32 **Caponi S**, Funel N, Frampton AE, Mosca F, Santarpia L, Van der Velde AG, Jiao LR, De Lio N, Falcone A, Kazemier G, Meijer GA, Verheul HM, Vasile E, Peters GJ, Boggi U, Giovannetti E. The good, the bad and the ugly: a tale of miR-101, miR-21 and miR-155 in pancreatic intraductal papillary mucinous neoplasms. *Ann Oncol* 2013; **24**: 734-741 [PMID: 23139258 DOI: 10.1093/annonc/mds513]
33 **Kadera BE**, Li L, Toste PA, Wu N, Adams C, Dawson DW, Donahue TR. MicroRNA-21 in pancreatic ductal adenocarcinoma tumor-associated fibroblasts promotes metastasis. *PLoS One* 2013; **8**: e71978 [PMID: 23991015 DOI: 10.1371/journal.pone.0071978]
34 **Dillhoff M**, Liu J, Frankel W, Croce C, Bloomston M. MicroRNA-21 is overexpressed in pancreatic cancer and a potential predictor of survival. *J Gastrointest Surg* 2008; **12**: 2171-2176 [PMID: 18642050 DOI: 10.1007/s11605-008-0584-x]
35 **Giovannetti E**, Funel N, Peters GJ, Del Chiaro M, Erozenci LA, Vasile E, Leon LG, Pollina LE, Groen A, Falcone A, Danesi R, Campani D, Verheul HM, Boggi U. MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic

aspects underlying its role in the modulation of gemcitabine activity. *Cancer Res* 2010; **70**: 4528-4538 [PMID: 20460539 DOI: 10.1158/0008-5472.CAN-09-4467]

36 **Hwang JH**, Voortman J, Giovannetti E, Steinberg SM, Leon LG, Kim YT, Funel N, Park JK, Kim MA, Kang GH, Kim SW, Del Chiaro M, Peters GJ, Giaccone G. Identification of microRNA-21 as a biomarker for chemoresistance and clinical outcome following adjuvant therapy in resectable pancreatic cancer. *PLoS One* 2010; **5**: e10630 [PMID: 20498843 DOI: 10.1371/journal.pone.0010630]

37 **Zhang J**, Zhao CY, Zhang SH, Yu DH, Chen Y, Liu QH, Shi M, Ni CR, Zhu MH. Upregulation of miR-194 contributes to tumor growth and progression in pancreatic ductal adenocarcinoma. *Oncol Rep* 2014; **31**: 1157-1164 [PMID: 24398877]

38 **Lee EJ**, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ, Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. *Int J Cancer* 2007; **120**: 1046-1054 [PMID: 17149698 DOI: 10.1002/ijc.22394]

39 **Lou E**, Subramanian S, Steer CJ. Pancreatic cancer: modulation of KRAS, MicroRNAs, and intercellular communication in the setting of tumor heterogeneity. *Pancreas* 2013; **42**: 1218-1226 [PMID: 24152947 DOI: 10.1097/MPA.0000000000000007]

40 **Rowley DR**. Reprogramming the tumor stroma: a new paradigm. *Cancer Cell* 2014; **26**: 451-452 [PMID: 25314074 DOI: 10.1016/j.ccell.2014.09.016]

41 **Apte MV**, Wilson JS, Lugea A, Pandol SJ. A starring role for stellate cells in the pancreatic cancer microenvironment. *Gastroenterology* 2013; **144**: 1210-1219 [PMID: 23622130 DOI: 10.1053/j.gastro.2012.11.037]

42 **Liu CG**, Calin GA, Meloon B, Gamliel N, Sevignani C, Ferracin M, Dumitru CD, Shimizu M, Zupo S, Dono M, Alder H, Bullrich F, Negrini M, Croce CM. An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. *Proc Natl Acad Sci USA* 2004; **101**: 9740-9744 [PMID: 15210942 DOI: 10.1073/pnas.0403293101]

43 **He L**, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. A microRNA polycistron as a potential human oncogene. *Nature* 2005; **435**: 828-833 [PMID: 15944707 DOI: 10.1038/nature03552]

44 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]

45 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]

46 **Ali S**, Saleh H, Sethi S, Sarkar FH, Philip PA. MicroRNA profiling of diagnostic needle aspirates from patients with pancreatic cancer. *Br J Cancer* 2012; **107**: 1354-1360 [PMID: 22929886 DOI: 10.1038/bjc.2012.383]

47 **Qazi AM**, Gruzdyn O, Semaan A, Seward S, Chamala S, Dhulipala V, Sethi S, Ali-Fehmi R, Philip PA, Bouwman DL, Weaver DW, Gruber SA, Batchu RB. Restoration of E-cadherin expression in pancreatic ductal adenocarcinoma treated with microRNA-101. *Surgery* 2012; **152**: 704-711; discussion 711-713 [PMID: 22943841 DOI: 10.1016/j.surg.2012.07.020]

48 **Brunet Vega A**, Pericay C, Moya I, Ferrer A, Dotor E, Pisa A, Casalots À, Serra-Aracil X, Oliva JC, Ruiz A, Saigí E. microRNA expression profile in stage III colorectal cancer: circulating miR-18a and miR-29a as promising biomarkers. *Oncol Rep* 2013; **30**: 320-326 [PMID: 23673725 DOI: 10.3892/or.2013.2475]

49 **Kong X**, Du Y, Wang G, Gao J, Gong Y, Li L, Zhang Z, Zhu J, Jing Q, Qin Y, Li Z. Detection of differentially expressed microRNAs in serum of pancreatic ductal adenocarcinoma patients: miR-196a could be a potential marker for poor prognosis. *Dig Dis Sci* 2011; **56**: 602-609 [PMID: 20614181 DOI: 10.1007/s10620-010-1285-3]

50 **Ulivi P**, Foschi G, Mengozzi M, Scarpi E, Silvestrini R, Amadori D, Zoli W. Peripheral blood miR-328 expression as a potential biomarker for the early diagnosis of NSCLC. *Int J Mol Sci* 2013; **14**: 10332-10342 [PMID: 23681013 DOI: 10.3390/ijms140510332]

51 **Carlsen AL**, Joergensen MT, Knudsen S, de Muckadell OB, Heegaard NH. Cell-free plasma microRNA in pancreatic ductal adenocarcinoma and disease controls. *Pancreas* 2013; **42**: 1107-1113 [PMID: 24048453 DOI: 10.1097/MPA.0b013e318296bb34]

52 **Liu J**, Gao J, Du Y, Li Z, Ren Y, Gu J, Wang X, Gong Y, Wang W, Kong X. Combination of plasma microRNAs with serum CA19-9 for early detection of pancreatic cancer. *Int J Cancer* 2012; **131**: 683-691 [PMID: 21913185 DOI: 10.1002/ijc.26422]

53 **Gao L**, He SB, Li DC. Effects of miR-16 plus CA19-9 detections on pancreatic cancer diagnostic performance. *Clin Lab* 2014; **60**: 73-77 [PMID: 24600978]

54 **Cote GA**, Gore AJ, McElyea SD, Heathers LE, Xu H, Sherman S, Korc M. A pilot study to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select miRNA in plasma and bile. *Am J Gastroenterol* 2014; **109**: 1942-1952 [PMID: 25350767 DOI: 10.1038/ajg.2014.331]

55 **Liu R**, Chen X, Du Y, Yao W, Shen L, Wang C, Hu Z, Zhuang R, Ning G, Zhang C, Yuan Y, Li Z, Zen K, Ba Y, Zhang CY. Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer. *Clin Chem* 2012; **58**: 610-618 [PMID: 22194634 DOI: 10.1373/clinchem.2011.172767]

56 **Komatsu S**, Ichikawa D, Takeshita H, Morimura R, Hirajima S, Tsujiura M, Kawaguchi T, Miyamae M, Nagata H, Konishi H, Shiozaki A, Otsuji E. Circulating miR-18a: a sensitive cancer screening biomarker in human cancer. *In Vivo* 2014; **28**: 293-297 [PMID: 24815829]

57 **Morimura R**, Komatsu S, Ichikawa D, Takeshita H, Tsujiura M, Nagata H, Konishi H, Shiozaki A, Ikoma H, Okamoto K, Ochiai T, Taniguchi H, Otsuji E. Novel diagnostic value of circulating miR-18a in plasma of patients with pancreatic cancer. *Br J Cancer* 2011; **105**: 1733-1740 [PMID: 22045190 DOI: 10.1038/bjc.2011.453]

58 **Zhao C**, Zhang J, Zhang S, Yu D, Chen Y, Liu Q, Shi M, Ni C, Zhu M. Diagnostic and biological significance of microRNA-192 in pancreatic ductal adenocarcinoma. *Oncol Rep* 2013; **30**: 276-284 [PMID: 23612862 DOI: 10.3892/or.2013.2420]

59 **Lin MS**, Chen WC, Huang JX, Gao HJ, Sheng HH. Aberrant expression of microRNAs in serum may identify individuals with pancreatic cancer. *Int J Clin Exp Med* 2014; **7**: 5226-5234 [PMID: 25664025]

60 **Wang J**, Raimondo M, Guha S, Chen J, Diao L, Dong X, Wallace MB, Killary AM, Frazier ML, Woodward TA, Wang J, Sen S. Circulating microRNAs in Pancreatic Juice as Candidate Biomarkers of Pancreatic Cancer. *J Cancer* 2014; **5**: 696-705 [PMID: 25258651 DOI: 10.7150/jca.10094]

61 **Sadakari Y**, Ohtsuka T, Ohuchida K, Tsutsumi K, Takahata S, Nakamura M, Mizumoto K, Tanaka M. MicroRNA expression analyses in preoperative pancreatic juice samples of pancreatic ductal adenocarcinoma. *JOP* 2010; **11**: 587-592 [PMID: 21068491]

62 **Hong TH**, Park IY. MicroRNA expression profiling of diagnostic needle aspirates from surgical pancreatic cancer specimens. *Ann Surg Treat Res* 2014; **87**: 290-297 [PMID: 25485236 DOI: 10.4174/astr.2014.87.6.290]

63 **Yang JY**, Sun YW, Liu DJ, Zhang JF, Li J, Hua R. MicroRNAs in stool samples as potential screening biomarkers for pancreatic ductal adenocarcinoma cancer. *Am J Cancer Res* 2014; **4**: 663-673 [PMID: 25520858]

64 **Leung WK**, To KF, Man EP, Chan MW, Hui AJ, Ng SS, Lau JY, Sung JJ. Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in fecal samples of patients with colorectal cancer or polyps. *Am J Gastroenterol* 2007; **102**: 1070-1076 [PMID: 17378912 DOI: 10.1111/j.1572-0241.2007.01108.x]

65 **Nagasaka T**, Tanaka N, Cullings HM, Sun DS, Sasamoto H,

Uchida T, Koi M, Nishida N, Naomoto Y, Boland CR, Matsubara N, Goel A. Analysis of fecal DNA methylation to detect gastrointestinal neoplasia. *J Natl Cancer Inst* 2009; **101**: 1244-1258 [PMID: 19700653 DOI: 10.1093/jnci/djp265]

66 **Link A**, Balaguer F, Shen Y, Nagasaka T, Lozano JJ, Boland CR, Goel A. Fecal MicroRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1766-1774 [PMID: 20551304 DOI: 10.1158/1055-9965.EPI-10-0027]

67 **Deschner EE**. Early proliferative changes in gastrointestinal neoplasia. *Am J Gastroenterol* 1982; **77**: 207-211 [PMID: 7072690]

68 **Kalimutho M**, Del Vecchio Blanco G, Di Cecilia S, Sileri P, Cretella M, Pallone F, Federici G, Bernardini S. Differential expression of miR-144* as a novel fecal-based diagnostic marker for colorectal cancer. *J Gastroenterol* 2011; **46**: 1391-1402 [PMID: 21863218 DOI: 10.1007/s00535-011-0456-0]

69 **Kanaoka S**, Yoshida K, Miura N, Sugimura H, Kajimura M. Potential usefulness of detecting cyclooxygenase 2 messenger RNA in feces for colorectal cancer screening. *Gastroenterology* 2004; **127**: 422-427 [PMID: 15300574 DOI: 10.1053/j.gastro.2004.05.022]

70 **Haug U**, Wente MN, Seiler CM, Jesenofsky R, Brenner H. Stool testing for the early detection of pancreatic cancer: rationale and current evidence. *Expert Rev Mol Diagn* 2008; **8**: 753-759 [PMID: 18999925 DOI: 10.1586/14737159.8.6.753]

71 **Link A**, Becker V, Goel A, Wex T, Malfertheiner P. Feasibility of fecal microRNAs as novel biomarkers for pancreatic cancer. *PLoS One* 2012; **7**: e42933 [PMID: 22905187 DOI: 10.1371/journal.pone.0042933]

72 **Ren Y**, Gao J, Liu JQ, Wang XW, Gu JJ, Huang HJ, Gong YF, Li ZS. Differential signature of fecal microRNAs in patients with pancreatic cancer. *Mol Med Rep* 2012; **6**: 201-209 [PMID: 22504911]

73 **Patel RS**, Jakymiw A, Yao B, Pauley BA, Carcamo WC, Katz J, Cheng JQ, Chan EK. High resolution of microRNA signatures in human whole saliva. *Arch Oral Biol* 2011; **56**: 1506-1513 [PMID: 21704302 DOI: 10.1016/j.archoralbio.2011.05.015]

74 **Xie Z**, Yin X, Gong B, Nie W, Wu B, Zhang X, Huang J, Zhang P, Zhou Z, Li Z. Salivary microRNAs show potential as a noninvasive biomarker for detecting resectable pancreatic cancer. *Cancer Prev Res* (Phila) 2015; **8**: 165-173 [PMID: 25538087 DOI: 10.1158/1940-6207.CAPR-14-0192]

75 **Hruban RH**, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, Kern SE, Klimstra DS, Klöppel G, Longnecker DS, Lüttges J, Offerhaus GJ. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. *Am J Surg Pathol* 2001; **25**: 579-586 [PMID: 11342768 DOI: 10.1097/00000478-200105000-00003]

76 **Hruban RH**, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Biankin AV, Biankin SA, Compton C, Fukushima N, Furukawa T, Goggins M, Kato Y, Klöppel G, Longnecker DS, Lüttges J, Maitra A, Offerhaus GJ, Shimizu M, Yonezawa S. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol* 2004; **28**: 977-987 [PMID: 15252303 DOI: 10.1097/01.pas.0000126675.59108.80]

77 **Tanaka M**, Fernández-del Castillo C, Adsay V, Chari S, Falconi M, Jang JY, Kimura W, Levy P, Pitman MB, Schmidt CM, Shimizu M, Wolfgang CL, Yamaguchi K, Yamao K. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. *Pancreatology* 2012; **12**: 183-197 [PMID: 22687371 DOI: 10.1016/j.pan.2012.04.004]

78 **Adsay NV**, Bagci P, Tajiri T, Oliva I, Ohike N, Balci S, Gonzalez RS, Basturk O, Jang KT, Roa JC. Pathologic staging of pancreatic, ampullary, biliary, and gallbladder cancers: pitfalls and practical limitations of the current AJCC/UICC TNM staging system and opportunities for improvement. *Semin Diagn Pathol* 2012; **29**: 127-141 [PMID: 23062420 DOI: 10.1053/j.semdp.2012.08.010]

79 **Loftus EV**, Olivares-Pakzad BA, Batts KP, Adkins MC, Stephens DH, Sarr MG, DiMagno EP. Intraductal papillary-mucinous tumors of the pancreas: clinicopathologic features, outcome, and nomenclature. Members of the Pancreas Clinic, and Pancreatic Surgeons of Mayo Clinic. *Gastroenterology* 1996; **110**: 1909-1918 [PMID: 8964418 DOI: 10.1053/gast.1996.v110.pm8964418]

80 **Furukawa T**, Hatori T, Fujita I, Yamamoto M, Kobayashi M, Ohike N, Morohoshi T, Egawa S, Unno M, Takao S, Osako M, Yonezawa S, Mino-Kenudson M, Lauwers GY, Yamaguchi H, Ban S, Shimizu M. Prognostic relevance of morphological types of intraductal papillary mucinous neoplasms of the pancreas. *Gut* 2011; **60**: 509-516 [PMID: 21193453 DOI: 10.1136/gut.2010.210567]

81 **Valsangkar NP**, Morales-Oyarvide V, Thayer SP, Ferrone CR, Wargo JA, Warshaw AL, Fernández-del Castillo C. 851 resected cystic tumors of the pancreas: a 33-year experience at the Massachusetts General Hospital. *Surgery* 2012; **152**: S4-12 [PMID: 22770958 DOI: 10.1016/j.surg.2012.05.033]

82 **Rodriguez JR**, Salvia R, Crippa S, Warshaw AL, Bassi C, Falconi M, Thayer SP, Lauwers GY, Capelli P, Mino-Kenudson M, Razo O, McGrath D, Pederzoli P, Fernández-Del Castillo C. Branch-duct intraductal papillary mucinous neoplasms: observations in 145 patients who underwent resection. *Gastroenterology* 2007; **133**: 72-79; quiz 309-310 [PMID: 17631133 DOI: 10.1053/j.gastro.2007.05.010]

83 **Goh BK**, Tan YM, Chung YF, Chow PK, Cheow PC, Wong WK, Ooi LL. A review of mucinous cystic neoplasms of the pancreas defined by ovarian-type stroma: clinicopathological features of 344 patients. *World J Surg* 2006; **30**: 2236-2245 [PMID: 17103100 DOI: 10.1007/s00268-006-0126-1]

84 **Crippa S**, Fernández-Del Castillo C, Salvia R, Finkelstein D, Bassi C, Domínguez I, Muzikansky A, Thayer SP, Falconi M, Mino-Kenudson M, Capelli P, Lauwers GY, Partelli S, Pederzoli P, Warshaw AL. Mucin-producing neoplasms of the pancreas: an analysis of distinguishing clinical and epidemiologic characteristics. *Clin Gastroenterol Hepatol* 2010; **8**: 213-219 [PMID: 19835989 DOI: 10.1016/j.cgh.2009.10.001]

85 **Zamboni G**, Scarpa A, Bogina G, Iacono C, Bassi C, Talamini G, Sessa F, Capella C, Solcia E, Rickaert F, Mariuzzi GM, Klöppel G. Mucinous cystic tumors of the pancreas: clinicopathological features, prognosis, and relationship to other mucinous cystic tumors. *Am J Surg Pathol* 1999; **23**: 410-422 [PMID: 10199470 DOI: 10.1097/00000478-199904000-00005]

86 **Crippa S**, Fernández-del Castillo C. Management of intraductal papillary mucinous neoplasms. *Curr Gastroenterol Rep* 2008; **10**: 136-143 [PMID: 18462599 DOI: 10.1007/s11894-008-0034-7]

87 **Ryu JK**, Matthaei H, Dal Molin M, Hong SM, Canto MI, Schulick RD, Wolfgang C, Goggins MG, Hruban RH, Cope L, Maitra A. Elevated microRNA miR-21 levels in pancreatic cyst fluid are predictive of mucinous precursor lesions of ductal adenocarcinoma. *Pancreatology* 2011; **11**: 343-350 [PMID: 21757972 DOI: 10.1159/000329183]

88 **Abue M**, Yokoyama M, Shibuya R, Tamai K, Yamaguchi K, Sato I, Tanaka N, Hamada S, Shimosegawa T, Sugamura K, Satoh K. Circulating miR-483-3p and miR-21 is highly expressed in plasma of pancreatic cancer. *Int J Oncol* 2015; **46**: 539-547 [PMID: 25384963]

89 **Nakahara O**, Takamori H, Iwatsuki M, Baba Y, Sakamoto Y, Tanaka H, Chikamoto A, Horino K, Beppu T, Kanemitsu K, Honda Y, Iyama K, Baba H. Carcinogenesis of intraductal papillary mucinous neoplasm of the pancreas: loss of microRNA-101 promotes overexpression of histone methyltransferase EZH2. *Ann Surg Oncol* 2012; **19** Suppl 3: S565-S571 [PMID: 21932133 DOI: 10.1245/s10434-011-2068-6]

90 **Aso T**, Ohtsuka T, Matsunaga T, Kimura H, Watanabe Y, Tamura K, Ideno N, Osoegawa T, Takahata S, Shindo K, Ushijima Y, Aishima S, Oda Y, Ito T, Mizumoto K, Tanaka M. "High-risk stigmata" of the 2012 international consensus guidelines correlate with the malignant grade of branch duct intraductal papillary mucinous neoplasms of the pancreas. *Pancreas* 2014; **43**: 1239-1243 [PMID: 25036910 DOI: 10.1097/MPA.0000000000000199]

91 **Permuth-Wey J**, Chen YA, Fisher K, McCarthy S, Qu X, Lloyd MC, Kasprzak A, Fournier M, Williams VL, Ghia KM, Yoder SJ, Hall L, Georgeades C, Olaoye F, Husain K, Springett GM, Chen

DT, Yeatman T, Centeno BA, Klapman J, Coppola D, Malafa M. A genome-wide investigation of microRNA expression identifies biologically-meaningful microRNAs that distinguish between high-risk and low-risk intraductal papillary mucinous neoplasms of the pancreas. *PLoS One* 2015; **10**: e0116869 [PMID: 25607660 DOI: 10.1371/journal.pone.0116869]

92 **Lee LS**, Szafranska-Schwarzbach AE, Wylie D, Doyle LA, Bellizzi AM, Kadiyala V, Suleiman S, Banks PA, Andruss BF, Conwell DL. Investigating MicroRNA Expression Profiles in Pancreatic Cystic Neoplasms. *Clin Transl Gastroenterol* 2014; **5**: e47 [PMID: 24476997 DOI: 10.1038/ctg.2013.18]

93 **Henry JC**, Bassi C, Giovinazzo F, Bloomston M. MicroRNA from pancreatic duct aspirate differentiates cystic lesions of the pancreas. *Ann Surg Oncol* 2013; **20** Suppl 3: S661-S666 [PMID: 23884752 DOI: 10.1245/s10434-013-3138-8]

94 **Brosens LA**, Hackeng WM, Offerhaus GJ, Hruban RH, Wood LD. Pancreatic adenocarcinoma pathology: changing "landscape". *J Gastrointest Oncol* 2015; **6**: 358-374 [PMID: 26261723 DOI: 10.3978/j.issn.2078-6891.2015.032]

95 **Andea A**, Sarkar F, Adsay VN. Clinicopathological correlates of pancreatic intraepithelial neoplasia: a comparative analysis of 82 cases with and 152 cases without pancreatic ductal adenocarcinoma. *Mod Pathol* 2003; **16**: 996-1006 [PMID: 14559982 DOI: 10.1097/01.MP.0000087422.24733.62]

96 **Shi C**, Klein AP, Goggins M, Maitra A, Canto M, Ali S, Schulick R, Palmisano E, Hruban RH. Increased Prevalence of Precursor Lesions in Familial Pancreatic Cancer Patients. *Clin Cancer Res* 2009; **15**: 7737-7743 [PMID: 19996207 DOI: 10.1158/1078-0432.CCR-09-0004]

97 **Slater EP**, Strauch K, Rospleszcz S, Ramaswamy A, Esposito I, Klöppel G, Matthäi E, Heeger K, Fendrich V, Langer P, Bartsch DK. MicroRNA-196a and -196b as Potential Biomarkers for the Early Detection of Familial Pancreatic Cancer. *Transl Oncol* 2014; **7**: 464-471 [PMID: 24956938 DOI: 10.1016/j.tranon.2014.05.007]

98 **Ryu JK**, Hong SM, Karikari CA, Hruban RH, Goggins MG, Maitra A. Aberrant MicroRNA-155 expression is an early event in the multistep progression of pancreatic adenocarcinoma. *Pancreatology* 2010; **10**: 66-73 [PMID: 20332664 DOI: 10.1159/000231984]

99 **du Rieu MC**, Torrisani J, Selves J, Al Saati T, Souque A, Dufresne M, Tsongalis GJ, Suriawinata AA, Carrère N, Buscail L, Cordelier P. MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions. *Clin Chem* 2010; **56**: 603-612 [PMID: 20093556 DOI: 10.1373/clinchem.2009.137364]

100 **Yu J**, Li A, Hong SM, Hruban RH, Goggins M. MicroRNA alterations of pancreatic intraepithelial neoplasias. *Clin Cancer Res* 2012; **18**: 981-992 [PMID: 22114139 DOI: 10.1158/1078-0432.CCR-11-2347]

101 **Xue Y**, Abou Tayoun AN, Abo KM, Pipas JM, Gordon SR, Gardner TB, Barth RJ, Suriawinata AA, Tsongalis GJ. MicroRNAs as diagnostic markers for pancreatic ductal adenocarcinoma and its precursor, pancreatic intraepithelial neoplasm. *Cancer Genet* 2013; **206**: 217-221 [PMID: 23933230 DOI: 10.1016/j.cancergen.2013.05.020]

102 **Volinia S**, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006; **103**: 2257-2261 [PMID: 16461460 DOI: 10.1073/pnas.0510565103]

103 **Cummins JM**, Velculescu VE. Implications of micro-RNA profiling for cancer diagnosis. *Oncogene* 2006; **25**: 6220-6227 [PMID: 17028602 DOI: 10.1038/sj.onc.1209914]

104 **Yanaihara N**, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006; **9**: 189-198 [PMID: 16530703 DOI: 10.1016/j.ccr.2006.01.025]

105 **Zhang L**, Volinia S, Bonome T, Calin GA, Greshock J, Yang N, Liu CG, Giannakakis A, Alexiou P, Hasegawa K, Johnstone CN, Megraw MS, Adams S, Lassus H, Huang J, Kaur S, Liang S, Sethupathy P, Leminen A, Simossis VA, Sandaltzopoulos R, Naomoto Y, Katsaros D, Gimotty PA, DeMichele A, Huang Q, Bützow R, Rustgi AK, Weber BL, Birrer MJ, Hatzigeorgiou AG, Croce CM, Coukos G. Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. *Proc Natl Acad Sci USA* 2008; **105**: 7004-7009 [PMID: 18458333 DOI: 10.1073/pnas.0801615105]

106 **Esquela-Kerscher A**, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer* 2006; **6**: 259-269 [PMID: 16557279 DOI: 10.1038/nrc1840]

107 **Deng S**, Calin GA, Croce CM, Coukos G, Zhang L. Mechanisms of microRNA deregulation in human cancer. *Cell Cycle* 2008; **7**: 2643-2646 [PMID: 18719391 DOI: 10.4161/cc.7.17.6597]

108 **Breving K**, Esquela-Kerscher A. The complexities of microRNA regulation: mirandering around the rules. *Int J Biochem Cell Biol* 2010; **42**: 1316-1329 [PMID: 19800023 DOI: 10.1016/j.biocel.2009.09.016]

109 **Deng W**, Cao X, Chen J, Zhang Z, Yu Q, Wang Y, Shao G, Zhou J, Gao X, Yu J, Xu X. MicroRNA Replacing Oncogenic Klf4 and c-Myc for Generating iPS Cells via Cationized Pleurotus eryngii Polysaccharide-based Nanotransfection. *ACS Appl Mater Interfaces* 2015; **7**: 18957-18966 [PMID: 26269400 DOI: 10.1021/acsami.5b06768]

110 **He L**, He X, Lowe SW, Hannon GJ. microRNAs join the p53 network--another piece in the tumour-suppression puzzle. *Nat Rev Cancer* 2007; **7**: 819-822 [PMID: 17914404 DOI: 10.1038/nrc2232]

111 **Chang TC**, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, Dang CV, Thomas-Tikhonenko A, Mendell JT. Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat Genet* 2008; **40**: 43-50 [PMID: 18066065 DOI: 10.1038/ng.2007.30]

112 **Lomberk GA**, Urrutia R. The Triple-Code Model for Pancreatic Cancer: Cross Talk Among Genetics, Epigenetics, and Nuclear Structure. *Surg Clin North Am* 2015; **95**: 935-952 [PMID: 26315515 DOI: 10.1016/j.suc.2015.05.011]

113 **Santosh B**, Varshney A, Yadava PK. Non-coding RNAs: biological functions and applications. *Cell Biochem Funct* 2015; **33**: 14-22 [PMID: 25475931 DOI: 10.1002/cbf.3079]

114 **Rodríguez-Vicente AE**, Díaz MG, Hernández-Rivas JM. Chronic lymphocytic leukemia: a clinical and molecular heterogenous disease. *Cancer Genet* 2013; **206**: 49-62 [PMID: 23531595 DOI: 10.1016/j.cancergen.2013.01.003]

115 **Shi L**, Jackstadt R, Siemens H, Li H, Kirchner T, Hermeking H. p53-induced miR-15a/16-1 and AP4 form a double-negative feedback loop to regulate epithelial-mesenchymal transition and metastasis in colorectal cancer. *Cancer Res* 2014; **74**: 532-542 [PMID: 24285725 DOI: 10.1158/0008-5472.CAN-13-2203]

116 **Vos S**, Vesuna F, Raman V, van Diest PJ, van der Groep P. miRNA expression patterns in normal breast tissue and invasive breast cancers of BRCA1 and BRCA2 germ-line mutation carriers. *Oncotarget* 2015; **6**: 32115-32137 [PMID: 26378051]

117 **Wu M**, Jolicoeur N, Li Z, Zhang L, Fortin Y, L'Abbe D, Yu Z, Shen SH. Genetic variations of microRNAs in human cancer and their effects on the expression of miRNAs. *Carcinogenesis* 2008; **29**: 1710-1716 [PMID: 18356149 DOI: 10.1093/carcin/bgn073]

118 **Calin GA**, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004; **101**: 2999-3004 [PMID: 14973191 DOI: 10.1073/pnas.0307323101]

119 **Zhang L**, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, Liang S, Naylor TL, Barchetti A, Ward MR, Yao G, Medina A, O'brien-Jenkins A, Katsaros D, Hatzigeorgiou A, Gimotty PA, Weber BL, Coukos G. microRNAs exhibit high frequency genomic alterations in human cancer. *Proc Natl Acad Sci USA* 2006; **103**: 9136-9141 [PMID: 16754881 DOI: 10.1073/pnas.0508889103]

120 **Mehrotra M**, Luthra R, Ravandi F, Sargent RL, Barkoh BA, Abraham R, Mishra BM, Medeiros LJ, Patel KP. Identification of clinically important chromosomal aberrations in acute myeloid

leukemia by array-based comparative genomic hybridization. *Leuk Lymphoma* 2014; **55**: 2538-2548 [PMID: 24446873 DOI: 10.3109/10428194.2014.883073]

121 **Eldai H**, Periyasamy S, Al Qarni S, Al Rodayyan M, Muhammed Mustafa S, Deeb A, Al Sheikh E, Afzal M, Johani M, Yousef Z, Aziz MA. Novel genes associated with colorectal cancer are revealed by high resolution cytogenetic analysis in a patient specific manner. *PLoS One* 2013; **8**: e76251 [PMID: 24204606 DOI: 10.1371/journal.pone.0076251]

122 **Pallasch CP**, Patz M, Park YJ, Hagist S, Eggle D, Claus R, Debey-Pascher S, Schulz A, Frenzel LP, Claasen J, Kutsch N, Krause G, Mayr C, Rosenwald A, Plass C, Schultze JL, Hallek M, Wendtner CM. miRNA deregulation by epigenetic silencing disrupts suppression of the oncogene PLAG1 in chronic lymphocytic leukemia. *Blood* 2009; **114**: 3255-3264 [PMID: 19692702 DOI: 10.1182/blood-2009-06-229898]

123 **Hammond SM**. An overview of microRNAs. *Adv Drug Deliv Rev* 2015; **87**: 3-14 [PMID: 25979468 DOI: 10.1016/j.addr.2015.05.001]

124 **Lee Y**, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003; **425**: 415-419 [PMID: 14508493 DOI: 10.1038/nature01957]

125 **Yi R**, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 2003; **17**: 3011-3016 [PMID: 14681208 DOI: 10.1101/gad.1158803]

126 **Lund E**, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science* 2004; **303**: 95-98 [PMID: 14631048 DOI: 10.1126/science.1090599]

127 **Bohnsack MT**, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 2004; **10**: 185-191 [PMID: 14730017 DOI: 10.1261/rna.5167604]

128 **Davis CD**, Ross SA. Evidence for dietary regulation of microRNA expression in cancer cells. *Nutr Rev* 2008; **66**: 477-482 [PMID: 18667010 DOI: 10.1111/j.1753-4887.2008.00080.x]

129 **Wang WL**, Chatterjee N, Chittur SV, Welsh J, Tenniswood MP. Effects of 1α,25 dihydroxyvitamin D3 and testosterone on miRNA and mRNA expression in LNCaP cells. *Mol Cancer* 2011; **10**: 58 [PMID: 21592394 DOI: 10.1186/1476-4598-10-58]

130 **Kutmon M**, Coort SL, de Nooijer K, Lemmens C, Evelo CT. Integrative network-based analysis of mRNA and microRNA expression in 1,25-dihydroxyvitamin D3-treated cancer cells. *Genes Nutr* 2015; **10**: 484 [PMID: 26276506 DOI: 10.1007/s12263-015-0484-0]

131 **He WA**, Calore F, Londhe P, Canella A, Guttridge DC, Croce CM. Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7. *Proc Natl Acad Sci USA* 2014; **111**: 4525-4529 [PMID: 24616506 DOI: 10.1073/pnas.1402714111]

132 **Soares RJ**, Cagnin S, Chemello F, Silvestrin M, Musaro A, De Pitta C, Lanfranchi G, Sandri M. Involvement of microRNAs in the regulation of muscle wasting during catabolic conditions. *J Biol Chem* 2014; **289**: 21909-21925 [PMID: 24891504 DOI: 10.1074/jbc.M114.561845]

133 **Wang N**, Xia S, Chen K, Xiang X, Zhu A. Genetic alteration regulated by microRNAs in biliary tract cancers. *Crit Rev Oncol Hematol* 2015; **96**: 262-273 [PMID: 26095617 DOI: 10.1016/j.critrevonc.2015.05.015]

134 **Wang WS**, Liu LX, Li GP, Chen Y, Li CY, Jin DY, Wang XL. Combined serum CA19-9 and miR-27a-3p in peripheral blood mononuclear cells to diagnose pancreatic cancer. *Cancer Prev Res* (Phila) 2013; **6**: 331-338 [PMID: 23430754 DOI: 10.1158/1940-6207.CAPR-12-0307]

135 **Yu S**, Lu Z, Liu C, Meng Y, Ma Y, Zhao W, Liu J, Yu J, Chen J. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. *Cancer Res* 2010; **70**: 6015-6025 [PMID: 20610624 DOI: 10.1158/0008-5472.CAN-09-4531]

136 **Li A**, Omura N, Hong SM, Vincent A, Walter K, Griffith M, Borges M, Goggins M. Pancreatic cancers epigenetically silence SIP1 and hypomethylate and overexpress miR-200a/200b in association with elevated circulating miR-200a and miR-200b levels. *Cancer Res* 2010; **70**: 5226-5237 [PMID: 20551052 DOI: 10.1158/0008-5472.CAN-09-4227]

137 **Ho AS**, Huang X, Cao H, Christman-Skieller C, Bennewith K, Le QT, Koong AC. Circulating miR-210 as a Novel Hypoxia Marker in Pancreatic Cancer. *Transl Oncol* 2010; **3**: 109-113 [PMID: 20360935 DOI: 10.1593/tlo.09256]

138 **Schultz NA**, Werner J, Willenbrock H, Roslind A, Giese N, Horn T, Wøjdemann M, Johansen JS. MicroRNA expression profiles associated with pancreatic adenocarcinoma and ampullary adenocarcinoma. *Mod Pathol* 2012; **25**: 1609-1622 [PMID: 22878649 DOI: 10.1038/modpathol.2012.122]

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REVIEW

Antitumor effects of the benzophenanthridine alkaloid sanguinarine: Evidence and perspectives

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Abstract

Historically, natural products have represented a significant source of anticancer agents, with plant-derived drugs becoming increasingly explored. In particular, sanguinarine is a benzophenanthridine alkaloid obtained from the root of *Sanguinaria canadensis*, and from other poppy *Fumaria* species, with recognized anti-microbial, anti-oxidant and anti-inflammatory properties. Recently, increasing evidence that sanguinarine exibits anticancer potential through its capability of inducing apoptosis and/or antiproliferative effects on tumor cells, has been proved. Moreover, its antitumor seems to be due not only to its pro-apoptotic and inhibitory effects on tumor growth, but also to its antiangiogenic and anti-invasive properties. Although the precise mechanisms underlying the antitumor activity of this compound remain not fully understood, in this review we will focus on the most recent findings about the cellular and molecular pathways affected by sanguinarine, together with the rationale of its potential application in clinic. The complex of data currently available suggest the potential application of sanguinarine as an adjuvant in the therapy of cancer, but further pre-clinical studies are needed before such an antitumor strategy can be effectively translated in the clinical practice.

Key words: Sanguinarine; Cancer; Apoptosis; Cell-cycle; Chemotherapy

Core tip: Sanguinarine is a benzophenanthridine alkaloid isolated from the root of *Sanguinaria canadensis*, and other poppy *Fumaria* species, which exibits a clear-cut anticancer potential by inducing apoptosis and/or antiproliferative effects on tumor cells. Sanguinarine also shows antiangiogenic and anti-invasive properties, as demonstrated *in vitro* and *in vivo*. In consideration of the multiple biological effects of sanguinarine, which suggest its possible use in cancer therapy, further detailed pharmacokinetic and toxicologic studies are required to assess both the efficacy and safety of the compound before proposing a possible translation into the clinic.

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INTRODUCTION

Tumor initiation is the result of multiple genetic and epigenetic events. Transformed cells are characterized by indefinite proliferation, apoptosis-resistance and the capability to metastasize and support angiogenesis[1].

Chemotherapy, irradiation and/or immunotherapy represent the gold standard approach for the treatment of cancer worldwide. The increased frequency of tumor relapse and the toxicity of the anticancer drugs, however, often reduce the therapeutical effectiveness of several antitumor therapy protocols. Therefore, the identification of more effective therapeutic protocols is needed and, in this direction, phytochemicals may represent an attractive alternative because of their low toxicity and low cost[2]. In this scenario, sanguinarine (Figure 1) and chelerythrine are the principal members of quaternary benzo[c]phenanthridine alkaloids (QBAs)[3] obtained from *Sanguinaria canadensis, Chelidonium majus,* and *Macleaya cordata.* Alkaloids include a large group of secondary metabolites (SMs) that differ in relation to structure, function and biodistribution[4]. In the past, QBAs have attracted the attention of many pharmacologists because of their own low toxicity[5,6] and their multiple biological activities, such as the antitumor[7], antimicrobial[8,9], anti-inflammatory[10], anti-HIV[11], anti-platelet[12], anti-angiogenesis[13], and antiparasitic activities[14-16]. The influence of QBAs on the activity of various important biological enzymatic pathways has been also demonstrated[7]. For long times, sanguinarine-containing herbs were believed to possess anticancer activity but only recently evidence that sanguinarine possesses a strong anti-neoplastic activity, which is mediated mainly by the induction of tumor cell apoptosis has been proved.

This review summarize the most recent findings on the molecular mechanisms underlying the antitumor activity of sanguinarine both *in vitro,* in a variety of human tumor cells, and *in vivo* in selected experimental tumor models, together with the rationale of its potential application in clinical practice.

SANGUINARINE INDUCES APOPTOSIS IN TUMOR CELLS

Physiologically, the human body controls homeostasis by eliminating damaged and aged cells by means of a genetically programmed process named apoptosis[17,18]. Tumor cells evade apoptosis and grow indefinitely. Several proteins, among which are caspases, pro-apoptotic Bax and anti-apoptotic B cell lymphoma (Bcl)-2, cytochrome c, and apoptotic protease activating factor -1, carry out the apoptotic programme either by intrinsic or extrinsic pathways. The first one is dependent on mitochondria, whereas the second one is initiated by the so-called death receptors (DRs). Selected anti-apoptotic proteins, among which Bcl-2, have been found over-expressed in different types of cancers. The down-regulation of anti-apoptotic proteins in cancer cells represents a promising therapeutic strategy of intervention in cancer therapy.

A number of plant-derived agents, have been shown to be capable of hampering disease progression by inducing cell apoptosis in multiple types of human and experimental cancers. Recently QBAs, and particularly sanguinarine, have been indicated as potential anti-cancer compounds. In detail, it has been reported that micromolar concentrations of sanguinarine are capable of inhibiting tumor cell growth, and this inhibitory effect is associated with cell cycle arrest and induction of apoptosis[19-22]. The anti-proliferative and/or pro-apoptotic activities of sanguinarine have been demonstrated in *in vitro* studies on several cancer cell types including epidermal[23], keratinocyte[24,25], prostate[26-28], cervical[29], breast[20,30-33], leukaemia[34,35], lymphoma[36], melanoma[37-39], colon[40,41], colorectal[21], gastric[42], pancreatic[19], lung[22], neuroendocrine[43], osteosarcoma[44], and human neuroblastoma cells[45]. By contrast, there are few studies on the *in vivo* effectiveness of sanguinarine administration *per os*[46,47] in animal tumor models[33,48].

It has been reported that sanguinarine exerts an antiproliferative activity on murine melanoma cells both *in vitro* and *in vivo* (B16 melanoma 4AS in the syngeneic host C57BL/mice), as well as in A375 human melanoma xenografts in athymic nude mice[48]. We also have conducted a study aimed at evaluating the anti-tumor effect of sanguinarine both *in vitro* and *in vivo* in a rat colorectal cancer model (DHD/K12/TRb cell line)[49]. We found that the *in vitro* addition of sanguinarine has a dose-dependent inhibitory effect on the proliferation of DHD/K12/TRb cells and induces tumor cell apoptosis. Sanguinarine also showed a clear-cut *in vivo* anti-tumor activity, leading to an inhibition of tumor growth higher than 70%[49]. The sanguinarine-induced inhibition of tumor growth was associated with its pro-apoptotic effect on tumor cells, as confirmed by the *ex-vivo* histopathological examinations performed on experimental tumor sections and by TUNEL assay[49].

It is known that sanguinarine-induced apoptosis occurs through multiple pathways, including the activation of nuclear factor-κB (NF-κB)[50], the mitochondrial damage resulting in activation of the caspase machinery[24] and the cell cycle arrest[27]. In detail, the sanguinarine-induced apoptosis occur either *via* a mithocondrial pathway dependent on caspase-9 or by the DR pathways, with the activation of caspase 8. The activation of caspase 3, which represents a key factor for apoptosis execution in both pathways, and the following

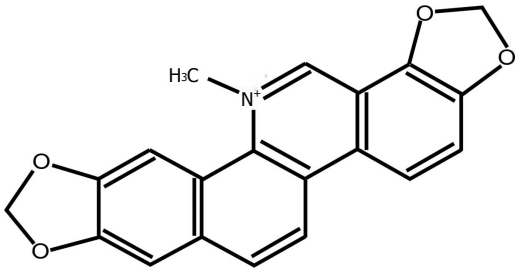

Figure 1 Chemical structure of sanguinarine.

cleavage of PARP together with the down-regulation of Bcl-2 and c-FLIP, may play a very important role in the apoptosis induced by sanguinarine[26,51,52]. Studies performed in human neuroblastoma cells SH-SY5Y have shown that sanguinarine reduces the expression of anti-apoptotic genes, particularly of NOL3, BCL2, and HRK genes[45]. A down-regulation of pro-caspase 3, Bcl-2, cIAP2, XIAP, and c-FLIPs[20,52] has been also observed in basal cell-like MDA-MB-231 human breast carcinoma cells treated with sanguinarine. The effect of sanguinarine treatment has been evaluated also on the expression levels of Bax and Bcl-2 proteins in immortalized human keratinocytes (HaCaT)[24,25], human leukaemia JM1 and K562 cells[35] and in Hela and SiHa human cervical tumor cells[53]. These findings indicate that sanguinarine, depending on the dose employed, down-regulates the expression levels of Bcl-2 protein while increasing those of Bax protein, which is a key regulator of mitochondrial damage. Notably, Bax expression has been associated with an increased sensitivity of cancer cells to chemotherapy[54], whereas an increase of Bcl-2 has been associated with the occurrence of drug-resistance phenomena[55].

It has been proved that sanguinarine is capable of inducing DNA damage, acting as an intercalating agent[56,57], and also a very rapid cell apoptosis which does not seem to be mediated by a p53-dependent DNA damage signalling in human colon cancer[41] and in malignant melanoma cells[38].

The concentration of sanguinarine plays a key role in the induction of cell death. Consistently, both apoptotic and non-apoptotic cell death pathways have been observed in response to sanguinarine. Thus, a sanguinarine-related and bimodal cell death effect, which consists of two different types of cell death, *i.e.,* by apoptosis (induced by low SA concentration; characterized by caspase 3 and PARP positivity) and oncosis (induced by high SA concentration; characterized by caspase 3 and PARP negativity), has been demonstrated in various cancer cells types[52].

SANGUINARINE INDUCES ALTERATIONS IN CELL CYCLE

Tumor cells are characterized by deregulated proliferation. Conversely, normal cells proliferation is the results of the action of selected growth signals [cyclins and cyclin-dependent kinases (CDKs)] and anti-growth signals (p21 and p27 proteins). Cyclins and CDKs cooperate in G1 for the initiation of the S phase and in G2 for inducing mitosis, whereas p21 and p27 selectively block the catalytic activity of CDK. Following addition of anti-mitogenic compounds or DNA injury, p21 and p27 bind to cyclin-CDK complex blocking their catalytic activity and consequently the cell cycle progression.

Actually a number of inhibitors and/or regulators of the cell cycle, among which sanguinarine, are suggested as potential antitumor agents. Sanguinarine treatment (0.2-2 mol/L for 24 h) blocks cell cycle by enhancing the expression of CDK inhibitors and by reducing not only cyclin D1, D2 and E, but also CDK2, 4 and 6 in human prostate cancer cells[27]. This alkaloid also up-regulates p27 and down-regulates cyclin D1, while inhibiting the activation of STAT3, as demonstrated *in vitro* in basal cell-like MDA-MB-231 human breast cancer cells and *in vivo* in a murine breast cancer model[33]. Holy *et al*[31] studied the effects of sanguinarine (5-10 μmol/L) on the cell cycle regulatory molecules, by immune-cytochemistry, that visualized the cyclin D1 and topoisomerase Ⅱ in MCF-7 breast cancer cells. They reported that sanguinarine-mediated cellular events induce cell cycle arrest in G0/G1 and inhibit cell proliferation, which is associated with a striking re-localization of cyclin D1 and topoisomerase Ⅱ from the nucleus to the cytoplasm.

SANGUINARINE-INDUCED APOPTOSIS THROUGH THE GENERATION OF REACTIVE OXYGEN SPECIES

Apoptosis induced by sanguinarine has been associated also with the production of reactive oxygen species (ROS)[20,36,52,58]. ROS are a group of highly reactive molecules, among which are superoxide anion radical, hydrogen peroxide, singlet oxygen, and hydroxyl radical. ROS are the products of the oxygen metabolism within the cell. ROS are known as key regulators of normal cell proliferation and differentiation, however, high levels of ROS have also been associated with damage of DNA and proteins and thus with the occurrence of apoptosis[59,60]. Moreover, an overdone oxidative stress has been shown capable of inducing a reduction of the normal mitochondrial membrane potential, which in turn leads to apoptosis[21,61-63]. It has been shown that ROS generation, is crucial for the apoptosis induced by sanguinarine in human breast cancer[52], SK-Mel-2 human melanoma[37], human prostate cancer[25] and in both HCT-116[21] and HT-29 human colon cancer cells[40]. Consistently, pre-treatment of tumor cells with antioxidants such as *N-acetylcysteine* or glutathione counteracts the apoptosis induced by sanguinarine[21,32,37,52]. Moreover, the over-expression of cyclooxygenase-2 (COX-2) also rescues prostate cancer cells from sanguinarine-induced apoptosis by

inhibiting the activity of NO synthase, thus suggesting the possibility to use a combination of COX-2 inhibitors and sanguinarine in the treatment of human prostate cancer[28].

SANGUINARINE-MEDIATED INHIBITION OF NF-κB

The molecular pathways associated with carcinogenesis are linked also with chronic inflammation, which emerges as an important co-factor in tumor development. The NF-κB controls the inflammatory gene expression and recently it has been suspected to be involved also in the control of tumor development[64]. Resting NF-κB localizes within the cell cytoplasm in the form of a heterodimer composed by p50, p65, and the inhibitory subunit IkBα[65]. Following activation, the IkBα protein is phosphorylated, ubiquitinated and finally degradated. Then, the p50 and p65 reach the nucleus of cell, where they interact with selected DNA sequences localized in the promoter region of various genes, leading to their transcription. Consistently, the NF-κB signalling pathway has been indicated as a key-target for the development of new chemotherapeutic approaches in cancer.

Sanguinarine has been suggested as a potential actor in the control of NF-κB-dependent pathological responses by blocking phosphorylation and degradation of IkBα. Studies by Chaturvedi *et al*[50] showed that in human myeloid ML-1a cells, the treatment with sanguinarine is capable of abrogating, dose- and time-dependently, the activation of NF-κB induced by tumor necrosis factor.

INHIBITION OF TUMOR ANGIOGENESIS BY SANGUINARINE

Many reports indicate that sanguinarine exerts antitumor activity not only by inhibiting tumor cells migration and/or invasion, but also by repressing angiogenesis[22,66]. Since solid tumors require active angiogenesis, the inhibition of endothelial cell proliferation result in the inhibition of tumor growth and progression. The best known angiogenic growth factor is represented by VEGF. Several studies have explored the relationship existing among sanguinarine, angiogenesis and metastatization. In particular, Eun and Koh[13] showed that sanguinarine inhibits the VEGF-induced endothelial cell migration, sprouting and survival *in vitro*, and blocks blood vessel formation *in vivo* in different experimental models. Furthermore, Basini *et al*[67] showed that sanguinarine is capable of blocking the VEGF-induced blood vessel growth. Depending on the concentration used, sanguinarine also inhibits VEGF secretion in human microvascular endothelial cells HMVEC as well as in A549 lung cancer cells[68]. This inhibitory effect has been associated with the suppression of the phosphorilation of Akt, p38 and VE-cadherin, which are well known modulators of the VEGF signal transduction pathway[67,69]. Moreover, sanguinarine enhances apoptosis in human mammary adenocarcinoma MCF-7 through the inhibition of VEGF release, induced by generation of ROS[32]. Sanguinarine also inhibits angiogenesis in preclinical experimental tumor models, such as mouse melanoma[48] and rat colorectal cancer, as we reported previously[49]. In both the experimental studies, the therapeutic efficacy of sanguinarine could not be attributed only to a direct anti-proliferative activity but also to the inhibition of tumor angiogenesis induced by this alkaloid.

The rationale of using VEGF-targeted therapies in the treatment of cancer lies in the possibility they offer to counteract the over-expression of VEGF provoked by chemotherapeutic drugs and radiation[70]. Consistently, dacarbazine, which is used in the therapy of human melanoma, induces increased VEGF-A production[71], and dacarbazine-resistant melanoma cells show an increased *in vivo* growth together with an increased microvessel density[72]. These studies suggest the potential application of sanguinarine, alone or in association with other VEGF inhibitors, in the control of both angiogenesis and metastatization of solid tumors.

INHIBITION OF TUMOR CELL INVASION BY SANGUINARINE

In solid tumors, neoplastic cells can penetrate the basement membrane by proteolysis and initiate metastatization, which accounts for the majority of cancer deaths. Metastatization is the result of the cooperation between cancer cells and a sort of "inflammed" microenvironment[73]. Consistently, inflammatory cells are an important source of proteases capable of causing a degradation of extracellular matrix, which represents a crucial event in the initiation of cancer cell invasion. Matrix metalloproteinases (MMPs) are an example of agents capable to degrade the extracellular matrix[74,75] and an over-production of these enzymes has been detected in various metastatic cancers[76-78]. Indeed, there is a strong evidence that increased expression and activation of MMP-2 and MMP-9 is present in tumor tissues but not in normal tissues in patients with breast cancer[79] and that MMP-2 induces cancer cell migration by means of its interaction with collagen[80].

Recent findings show that sanguinarine inhibits the tetradecanoylphorbolmyristate acetate (TPA)-induced breast cancer cell migration and invasion while inhibiting the expression of MMP-9, NF-κB and AP-1 signaling pathways[81]. Moreover, previous studies by Sun *et al*[66] have showed that sanguinarine reduces prostate cancer cell growth and invasion by the inhibition of STAT3 activation. STAT3 is constitutively active in human prostate cancer metastases and has a key role in the phenomena of tumor cell migration and invasion in different types of cancer[82-84]. Since the invasivity and/or metastatic potential of a tumor parallel its maligncy, the above findings indicate that sanguinarine may play a crucial role as a therapeutic agent in anticancer therapy

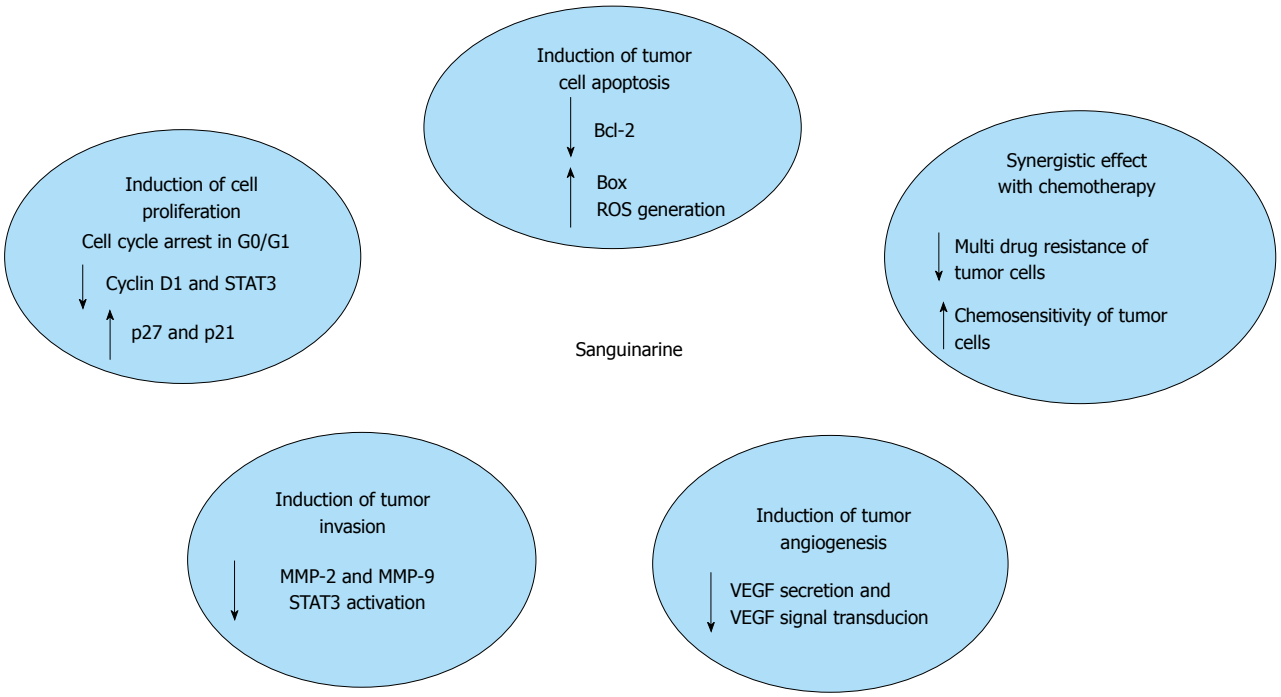

Figure 2 Cellular and molecular mechanisms underlying the antitumor activity of sanguinarine, as assessed by means of *in vitro* and *in vivo* experimental studies. ROS: Reactive oxygen species; Bcl: B cell lymphoma; VEGF: Vascular endothelial growth factor; MMP: Matrix metalloproteinase; STAT: Signal transducer and activator of transcription.

not only for its ability to induce apoptosis but also for its own "anti-invasive" properties.

SYNERGISTIC INTERACTION OF SANGUINARINE WITH CHEMOTHERAPEUTIC AGENTS

Several plant SMs are capable of influencing effectively the multidrug resistance phenomenon in tumor cells and are able also to "chemo-sensitize" them[85-89]. Some clinical studies have explored the possible advantage of combining natural products with classical chemotherapeutic regimens[90-92]. Phytotherapy, which employs plants extracts, is still used worldwide for the treatment of various human diseases. However, evidence has been proved that combinations of individual SM in an extract may exert synergistic effects. As an example, a recent study demonstrates that the combined use of non-toxic concentrations of sanguinarine and digitonin with doxorubicin, synergistically sensitizes Caco-2 (human colorectal adenocarcinoma) and CEM/ADR5000 adryamicin-resistant leukemia cells and increases the cytotoxicity of the chemotherapeutic agent doxorubicin[93]. In this regard, it is worth mentioning that the main advantage of combination therapies is represented by the possibility of reducing the doses and thus the toxicity of chemotherapy, while retaining its own efficacy. Thus, because of its potential synergistic interaction with chemotherapeutic agents, the therapeutic use of sanguinarine as an adjuvant, in association with chemotherapy, might be considered as a theoretical option in cancer therapy.

CONCLUSION

A successful resolution to the design of antitumor drugs relies, at least in part, on the possibility to overcome the intrinsic resistance to undergo apoptosis detected in many transformed cells. Findings from the studies above mentioned show that sanguinarine is capable of inhibiting tumor growth through different molecular pathways (Figure 2). A summary of the results is shown in Table 1. In conclusion, despite sanguinarine has been extensively studied, the precise mechanisms responsible for its antitumor effects still have not been completely elucidated and are strictly dependent on the cell type studied. According to the results obtained so far, it can be said that the anti-tumor action of this alkaloid is the result of a combined effect both on proliferation and invasiveness of tumor cells, that on regulation of the complex phenomena of tumor angiogenesis. In particular, owing to its pro-apoptotic potential, sanguinarine is a good candidate for the development of new anticancer therapies either when used alone or in combination with other chemotherapeutic regimens. More extensive investigation and greater caution are needed, however, to clarify the following important issues. First of all, most of the studies above mentioned have been performed *in vitro* using cancer cell lines, whereas there are only a few *in vivo* studies validating the efficacy and safety of sanguinarine administration in animal tumor models. The results of our *in vivo* studies confirm the effectiveness and safety of using oral sanguinarine administration to control tumor growth in rats[49]. Similar results had been previously reported in a murine melanoma model[48]. In that study, and

Table 1 The antitumor activity of sanguinarine

Sanguinarine induces apoptosis in tumor cells through multiple pathways, including the activation of NF-κB, the mitochondrial damage and cell cycle arrest
Sanguinarine-induced apoptosis is associated with the decrease of Bcl-2 and the increase of Bax proteins and the generation of reactive oxygen species
Sanguinarine causes cell cycle arrest by increasing the expression of p27 and decreasing cyclin D1, D2 and E, and CDK2, 4 and 6
Sanguinarine inhibits tumor progression associated with chronic inflammation *via* the inhibition of NF-κB
Sanguinarine inhibits tumor angiogenesis through the inhibition of VEGF secretion and VEGF signal transduction (Akt, p38 and VE-cadherin)
Sanguinarine has an inhibitory effect on tumor cell migration by the inhibition of MMP-9 and STAT3 activation
Sanguinarine exerts a synergistic effect with chemotherapeutic agents and enhances the chemosensitivity of Caco 2 and CEM/ADR5000 adryamicin-resistant leukemia cells

NF-κB: Nuclear factor-κB; CDK: Cyclin-dependent kinase; Bcl: B cell lymphoma; VEGF: Vascular endothelial growth factor; STAT: Signal transducer and activator of transcription.

in agreement with our findings, the anti-proliferative and anti-angiogenic effects of the oral sanguinarine administration were observed at a dosage, *i.e.*, 5 mg/kg, devoid of apparent toxicity. On the other hand, an increase of serum levels of transaminases and LDH, hepatic vacuolization, lipid accumulation and peroxidation in the liver and a reduction of triglycerides, were observed in mice treated with high-dose sanguinarine (10 mg/kg), suggesting liver injury[94]. Previous studies showed that sanguinarine can cause physiological dysfunction in skeletal, smooth and cardiac muscles[95-97]. More recent studies clearly indicate that sanguinarine acts as a pro-apoptotic factor and alters mouse normal embryonic development at a physiological dosage, *i.e.*, 0.5-2 μmol/L, which are obtained *via* dietary intake[98]. These experimental results need further confirmation in view of the possible administration of the compound in pregnancy, although at present no teratogenic effects have been reported in humans.

Most of the studies actually known have reported that sanguinarine exerts cytotoxic activity selectively on cancer cells. Consistently, sanguinarine is a negative regulator of human epidermoid carcinoma cells (A431) but not of normal epidermal keratinocytes[23]. Evidence of this differential activity have been reported recently, showing that mouse lymphocytic leukemic cells are more sensitive to sanguinarine than normal splenocytes[99].

It is a matter of fact, however, that sanguinarine has been listed as responsible for the toxicity of *Argemone mexicana* seed oil[100-102]. Das *et al*[103] reported that topical use of argemone oil (0.15-0.3 mL) or sanguinarine (4.5-18 μmol/L) followed by application of TPA induces tumor development in a murine experimental model. Ansari *et al*[104] also reported that intraperitoneal administration of sanguinarine induces DNA damage in Swiss albino mice. Sanguinarine in argemone oil, is suspected to cause glaucoma[101,102]. Argemone oil increases incidence of bladder cancer in animal models[103] and of gall bladder cancer in humans[104]. Furthermore, sanguinarine extract from bloodroot (*Sanguinaria canadensis*), previously used in oral hygiene products, was discontinued until a link between product administration and occurrence of leukoplakia was established[105,106]. Hepatic microsomes transform sanguinarine in a mutagenic epoxide and the same sanguinarine is capable of activating polycyclic aromatic hydrocarbon signaling[107]. However, related to this topic, the results available in literature are not univocal[3]. So that is still not clear if sanguinarine may act as a carcinogenic without the cooperation of other risk factors or it is capable of acting in concert with various co-carcinogens. In light of the above facts, the possibility of obtaining beneficial effects in humans by using sanguinarine remains largely unpredictable.

Finally, since at present there is increasing interest in nanotechnology application in cancer therapy and in order to prevent the potential toxic and/or side effects induced by sanguinarine administration, *in vivo* studies might be performed in experimental tumor models by encapsulating the alkaloid in tumor-targeted nanoparticles[108], which accumulate preferentially in tumors recognizing single cancer cells for diagnosis and treatment. Actually, the administration of sanguinarine (10 mg/kg) *per os* and encapsulated by lipid nanoparticles (SG-SLNs), has been shown to induce an anti-inflammatory effect in an LPS-induced endotoxin shock murine model, and the pharmacokinetic studies have proved that the $AUC_{0\text{-}24}$ and C_{max} of SG-SLNs were significantly increased when compared to those of sanguinarine alone[109].

In conclusion, several studies indicate the potential application of sanguinarine as an adjuvant in the therapy of cancer, but further detailed pharmacokinetic and toxicology studies, which have to be conducted in appropriate experimental tumor models, are absolutely required to assess the efficacy and safety of this compound before such an antitumor strategy can be translated in clinical trials.

REFERENCES

1 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70 [PMID: 10647931 DOI: 10.1016/S0092-8674(00)81683-9]

2 **Reddy L**, Odhav B, Bhoola KD. Natural products for cancer prevention: a global perspective. *Pharmacol Ther* 2003; **99**: 1-13 [PMID: 12804695 DOI: 10.1016/S0163-7258(03)00042-1]

3 **Dvorak Z**, Kuban V, Kledjus B, Hlavac J, Vicar J, Ulrichova J, Simanek V. Quaternary benzo[c]phenanthridines sanguinarine and chelerythrine: a review of investigations from chemical and biological studies. *Heterocycles* 2006; **68**: 2403-2422 [DOI: 10.1002/chin.200709233]

4 **Dostàl J**, Slavik J. Some aspects of the chemistry of quaternary benzo[c]phenanthridines alkaloids. *Stud Nat Prod Chem* 2002; **27**:

155-184 [DOI: 10.1016/S1572-5995(02)80036-9]
5 **Psotova J**, Vecera R, Zdarilova A, Anzebacherova E, Kosina P, Svobodova A, Hrbac J, Jirovsky D, Stiborova M, Lichnovsky V. Safety assessment of sanguitrin, alkaloid fraction of Macleaya cordata, in rats. *Vet Med* 2006; **51**: 145-155
6 **Kosina P**, Walterová D, Ulrichová J, Lichnovský V, Stiborová M, Rýdlová H, Vicar J, Krecman V, Brabec MJ, Simánek V. Sanguinarine and chelerythrine: assessment of safety on pigs in ninety days feeding experiment. *Food Chem Toxicol* 2004; **42**: 85-91 [PMID: 14630132 DOI: 10.1007/s11101-013-9290-8]
7 **Slaninova I**, Pencikova K, Urbanova J, Slanina J, Taborska E. Antitumor activities of sanguinarine and related alkaloids. *Phytochem Rev* 2014; **13**: 51-68 [DOI: 10.1007/s1101-013-9290-8]
8 **Miao F**, Yang XJ, Zhou L, Hu HJ, Zheng F, Ding XD, Sun DM, Zhou CD, Sun W. Structural modification of sanguinarine and chelerythrine and their antibacterial activity. *Nat Prod Res* 2011; **25**: 863-875 [PMID: 21491327 DOI: 10.1080/14786419.2010.482055]
9 **Yang XJ**, Miao F, Yao Y, Cao FJ, Yang R, Ma YN, Qin BF, Zhou L. In vitro antifungal activity of sanguinarine and chelerythrine derivatives against phytopathogenic fungi. *Molecules* 2012; **17**: 13026-13035 [PMID: 23124471 DOI: 10.3390/molecules171113026]
10 **Lenfeld J**, Kroutil M, Marsálek E, Slavík J, Preininger V, Simánek V. Antiinflammatory activity of quaternary benzophenanthridine alkaloids from Chelidonium majus. *Planta Med* 1981; **43**: 161-165 [PMID: 7312984]
11 **Tan GT**, Pezzuto JM, Kinghorn AD, Hughes SH. Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. *J Nat Prod* 1991; **54**: 143-154 [PMID: 1710653]
12 **Tsai IL**, Wun MF, Teng CM, Ishikawa T, Chen IS. Anti-platelet aggregation constituents from Formosan Toddalia asiatica. *Phytochemistry* 1998; **48**: 1377-1382 [PMID: 9720317 DOI: 10.1016/S0031-9422(97)00678-X]
13 **Eun JP**, Koh GY. Suppression of angiogenesis by the plant alkaloid, sanguinarine. *Biochem Biophys Res Commun* 2004; **317**: 618-624 [PMID: 15063803 DOI: 10.1016/j.bbrc.2004.03.077]
14 **Miao F**, Yang XJ, Ma YN, Zheng F, Song XP, Zhou L. Structural modification of sanguinarine and chelerythrine and their in vitro acaricidal activity against Psoroptes cuniculi. *Chem Pharm Bull* (Tokyo) 2012; **60**: 1508-1513 [PMID: 23000953 DOI: 10.1248/cpb.c12-00618]
15 **Yao JY**, Li XL, Shen JY, Pan XY, Hao GJ, Xu Y, Ying WL, Ru HS, Liu XL. Isolation of bioactive components from Chelidonium majus L. with activity against Trichodina sp. *Aquaculture* 2011; **318**: 235-238 [DOI: 10.1016/j.aquaculture.2011.04.035]
16 **Wang GX**, Zhou Z, Jiang DX, Han J, Wang JF, Zhao LW, Li J. In vivo anthelmintic activity of five alkaloids from Macleaya microcarpa (Maxim) Fedde against Dactylogyrus intermedius in Carassius auratus. *Vet Parasitol* 2010; **171**: 305-313 [PMID: 20413222 DOI: 10.1016/j.vetpar.2010.03.032]
17 **Steller H**. Mechanisms and genes of cellular suicide. *Science* 1995; **267**: 1445-1449 [PMID: 7878463 DOI: 10.1126/science.7878463]
18 **Meier P**, Finch A, Evan G. Apoptosis in development. *Nature* 2000; **407**: 796-801 [PMID: 11048731 DOI: 10.1038/35037734]
19 **Ahsan H**, Reagan-Shaw S, Breur J, Ahmad N. Sanguinarine induces apoptosis of human pancreatic carcinoma AsPC-1 and BxPC-3 cells via modulations in Bcl-2 family proteins. *Cancer Lett* 2007; **249**: 198-208 [PMID: 17005319 DOI: 10.1016/j.canlet.2006.08.018]
20 **Choi WY**, Kim GY, Lee WH, Choi YH. Sanguinarine, a benzophenanthridine alkaloid, induces apoptosis in MDA-MB-231 human breast carcinoma cells through a reactive oxygen species-mediated mitochondrial pathway. *Chemotherapy* 2008; **54**: 279-287 [PMID: 18667818 DOI: 10.1159/000149719]
21 **Han MH**, Kim GY, Yoo YH, Choi YH. Sanguinarine induces apoptosis in human colorectal cancer HCT-116 cells through ROS-mediated Egr-1 activation and mitochondrial dysfunction. *Toxicol Lett* 2013; **220**: 157-166 [PMID: 23660334 DOI: 10.1016/j.toxlet.2013.04.020]
22 **Jang BC**, Park JG, Song DK, Baek WK, Yoo SK, Jung KH, Park GY, Lee TY, Suh SI. Sanguinarine induces apoptosis in A549 human lung cancer cells primarily via cellular glutathione depletion. *Toxicol In Vitro* 2009; **23**: 281-287 [PMID: 19135517 DOI: 10.1016/j.tiv.2008.12.013]
23 **Ahmad N**, Gupta S, Husain MM, Heiskanen KM, Mukhtar H. Differential antiproliferative and apoptotic response of sanguinarine for cancer cells versus normal cells. *Clin Cancer Res* 2000; **6**: 1524-1528 [PMID: 10778985]
24 **Adhami VM**, Aziz MH, Mukhtar H, Ahmad N. Activation of prodeath Bcl-2 family proteins and mitochondrial apoptosis pathway by sanguinarine in immortalized human HaCaT keratinocytes. *Clin Cancer Res* 2003; **9**: 3176-3182 [PMID: 12912970]
25 **Reagan-Shaw S**, Breur J, Ahmad N. Enhancement of UVB radiation-mediated apoptosis by sanguinarine in HaCaT human immortalized keratinocytes. *Mol Cancer Ther* 2006; **5**: 418-429 [PMID: 16505117 DOI: 10.1158/1535-7163.MCT-05-0250]
26 **Malíková J**, Zdarilová A, Hlobilková A, Ulrichová J. The effect of chelerythrine on cell growth, apoptosis, and cell cycle in human normal and cancer cells in comparison with sanguinarine. *Cell Biol Toxicol* 2006; **22**: 439-453 [PMID: 16964588 DOI: 10.1007/s10565-006-0109-x]
27 **Adhami VM**, Aziz MH, Reagan-Shaw SR, Nihal M, Mukhtar H, Ahmad N. Sanguinarine causes cell cycle blockade and apoptosis of human prostate carcinoma cells via modulation of cyclin kinase inhibitor-cyclin-cyclin-dependent kinase machinery. *Mol Cancer Ther* 2004; **3**: 933-940 [PMID: 15299076]
28 **Huh J**, Liepins A, Zielonka J, Andrekopoulos C, Kalyanaraman B, Sorokin A. Cyclooxygenase 2 rescues LNCaP prostate cancer cells from sanguinarine-induced apoptosis by a mechanism involving inhibition of nitric oxide synthase activity. *Cancer Res* 2006; **66**: 3726-3736 [PMID: 16585199 DOI: 10.1158/0008-5472.CAN-05-4033]
29 **Ding Z**, Tang SC, Weerasinghe P, Yang X, Pater A, Liepins A. The alkaloid sanguinarine is effective against multidrug resistance in human cervical cells via bimodal cell death. *Biochem Pharmacol* 2002; **63**: 1415-1421 [PMID: 11996882 DOI: 10.1016/S0006-2952(02)00902-4]
30 **Debiton E**, Madelmont JC, Legault J, Barthomeuf C. Sanguinarine-induced apoptosis is associated with an early and severe cellular glutathione depletion. *Cancer Chemother Pharmacol* 2003; **51**: 474-482 [PMID: 12700925]
31 **Holy J**, Lamont G, Perkins E. Disruption of nucleocytoplasmic trafficking of cyclin D1 and topoisomerase II by sanguinarine. *BMC Cell Biol* 2006; **7**: 13 [PMID: 16512916 DOI: 10.1186/147-2121-7-13]
32 **Dong XZ**, Zhang M, Wang K, Liu P, Guo DH, Zheng XL, Ge XY. Sanguinarine inhibits vascular endothelial growth factor release by generation of reactive oxygen species in MCF-7 human mammary adenocarcinoma cells. *Biomed Res Int* 2013; **2013**: 517698 [PMID: 23762849 DOI: 10.1155/2013/517698]
33 **Kalogris C**, Garulli C, Pietrella L, Gambini V, Pucciarelli S, Lucci C, Tilio M, Zabaleta ME, Bartolacci C, Andreani C, Giangrossi M, Iezzi M, Belletti B, Marchini C, Amici A. Sanguinarine suppresses basal-like breast cancer growth through dihydrofolate reductase inhibition. *Biochem Pharmacol* 2014; **90**: 226-234 [PMID: 24875448 DOI: 10.1016/j.bcp.2014.05.014]
34 **Han MH**, Yoo YH, Choi YH. Sanguinarine-induced apoptosis in human leukemia U937 cells via Bcl-2 downregulation and caspase-3 activation. *Chemotherapy* 2008; **54**: 157-165 [PMID: 18560221 DOI: 10.1159/000140359]
35 **Weerasinghe P**, Hallock S, Tang SC, Liepins A. Role of Bcl-2 family proteins and caspase-3 in sanguinarine-induced bimodal cell death. *Cell Biol Toxicol* 2001; **17**: 371-381 [PMID: 11787859]
36 **Hussain AR**, Al-Jomah NA, Siraj AK, Manogaran P, Al-Hussein K, Abubaker J, Platanias LC, Al-Kuraya KS, Uddin S. Sanguinarine-dependent induction of apoptosis in primary effusion lymphoma cells. *Cancer Res* 2007; **67**: 3888-3897 [PMID: 17440103 DOI: 10.1158/0008-5472.CAN-06-3764]
37 **Burgeiro A**, Bento AC, Gajate C, Oliveira PJ, Mollinedo F. Rapid human melanoma cell death induced by sanguinarine through

oxidative stress. *Eur J Pharmacol* 2013; **705**: 109-118 [PMID: 23499690 DOI: 10.1016/j.ejphar.2013.02.035]

38 **Hammerová J**, Uldrijan S, Táborská E, Slaninová I. Benzo[c] phenanthridine alkaloids exhibit strong anti-proliferative activity in malignant melanoma cells regardless of their p53 status. *J Dermatol Sci* 2011; **62**: 22-35 [PMID: 21324654 DOI: 10.1016/j.jdermsci.2011.01.006]

39 **Serafim TL**, Matos JA, Sardão VA, Pereira GC, Branco AF, Pereira SL, Parke D, Perkins EL, Moreno AJ, Holy J, Oliveira PJ. Sanguinarine cytotoxicity on mouse melanoma K1735-M2 cells--nuclear vs. mitochondrial effects. *Biochem Pharmacol* 2008; **76**: 1459-1475 [PMID: 18692024 DOI: 10.1016/j.bcp.2008.07.013]

40 **Lee JS**, Jung WK, Jeong MH, Yoon TR, Kim HK. Sanguinarine induces apoptosis of HT-29 human colon cancer cells via the regulation of Bax/Bcl-2 ratio and caspase-9-dependent pathway. *Int J Toxicol* 2012; **31**: 70-77 [PMID: 22215411 DOI: 10.1177/1091581811423845]

41 **Matkar SS**, Wrischnik LA, Hellmann-Blumberg U. Sanguinarine causes DNA damage and p53-independent cell death in human colon cancer cell lines. *Chem Biol Interact* 2008; **172**: 63-71 [PMID: 18243168 DOI: 10.1016/j.cbi.2007.12.006]

42 **Choi WY**, Jin CY, Han MH, Kim GY, Kim ND, Lee WH, Kim SK, Choi YH. Sanguinarine sensitizes human gastric adenocarcinoma AGS cells to TRAIL-mediated apoptosis via down-regulation of AKT and activation of caspase-3. *Anticancer Res* 2009; **29**: 4457-4465 [PMID: 20032392]

43 **Larsson DE**, Wickström M, Hassan S, Oberg K, Granberg D. The cytotoxic agents NSC-95397, brefeldin A, bortezomib and sanguinarine induce apoptosis in neuroendocrine tumors in vitro. *Anticancer Res* 2010; **30**: 149-156 [PMID: 20150630]

44 **Park H**, Bergeron E, Senta H, Guillemette K, Beauvais S, Blouin R, Sirois J, Faucheux N. Sanguinarine induces apoptosis of human osteosarcoma cells through the extrinsic and intrinsic pathways. *Biochem Biophys Res Commun* 2010; **399**: 446-451 [PMID: 20678472 DOI: 10.1016/j.bbrc.2010.07.114]

45 **Cecen E**, Altun Z, Ercetin P, Aktas S, Olgun N. Promoting effects of sanguinarine on apoptotic gene expression in human neuroblastoma cells. *Asian Pac J Cancer Prev* 2014; **15**: 9445-9451 [PMID: 25422239 DOI: 10.7314/APJCP.2014.15.21.9445]

46 **Deroussent A**, Ré M, Hoellinger H, Cresteil T. Metabolism of sanguinarine in human and in rat: characterization of oxidative metabolites produced by human CYP1A1 and CYP1A2 and rat liver microsomes using liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal* 2010; **52**: 391-397 [PMID: 19804952 DOI: 10.1016/j.jpba.2009.09.014]

47 **Lopez Lozano MJ**, Rios Santos V, Bullon Fernandez P. [Effectiveness of chemical products as antiplaque agents]. *Rev Eur Odontoestomatol* 1991; **3**: 115-122 [PMID: 1867730]

48 **De Stefano I**, Raspaglio G, Zannoni GF, Travaglia D, Prisco MG, Mosca M, Ferlini C, Scambia G, Gallo D. Antiproliferative and antiangiogenic effects of the benzophenanthridine alkaloid sanguinarine in melanoma. *Biochem Pharmacol* 2009; **78**: 1374-1381 [PMID: 19643088 DOI: 10.1016/j.bcp.2009.07.011]

49 **Pica F**, Balestrieri E, Serafino A, Sorrentino R, Gaziano R, Moroni G, Moroni N, Palmieri G, Mattei M, Garaci E, Sinibaldi-Vallebona P. Antitumor effects of the benzophenanthridine alkaloid sanguinarine in a rat syngeneic model of colorectal cancer. *Anticancer Drugs* 2012; **23**: 32-42 [PMID: 21849887 DOI: 10.1097/CAD.0b013e32834a0c8e]

50 **Chaturvedi MM**, Kumar A, Darnay BG, Chainy GB, Agarwal S, Aggarwal BB. Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-kappaB activation, IkappaBalpha phosphorylation, and degradation. *J Biol Chem* 1997; **272**: 30129-30134 [PMID: 9374492 DOI: 10.1074/jbc.272.48.30129]

51 **Malikova J**, Zdarilova A, Hlobilkova A. Effects of sanguinarine and chelerythrine on the cell cycle and apoptosis. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2006; **150**: 5-12 [PMID: 16936897]

52 **Kim S**, Lee TJ, Leem J, Choi KS, Park JW, Kwon TK. Sanguinarine-induced apoptosis: generation of ROS, down-regulation of Bcl-2, c-FLIP, and synergy with TRAIL. *J Cell Biochem* 2008; **104**: 895-907 [PMID: 18189268 DOI: 10.1002/jcb.21672]

53 **Xu JY**, Meng QH, Chong Y, Jiao Y, Zhao L, Rosen EM, Fan S. Sanguinarine inhibits growth of human cervical cancer cells through the induction of apoptosis. *Oncol Rep* 2012; **28**: 2264-2270 [PMID: 22965493 DOI: 10.3892/or.2012.2024]

54 **Xu ZW**, Friess H, Büchler MW, Solioz M. Overexpression of Bax sensitizes human pancreatic cancer cells to apoptosis induced by chemotherapeutic agents. *Cancer Chemother Pharmacol* 2002; **49**: 504-510 [PMID: 12107556 DOI: 10.1007/s00280-002-0435-5]

55 **Reed JC**. Bcl-2: prevention of apoptosis as a mechanism of drug resistance. *Hematol Oncol Clin North Am* 1995; **9**: 451-473 [PMID: 7642473]

56 **Slaninová I**, Slanina J, Táborská E. Quaternary benzo[c]phenanthridine alkaloids--novel cell permeant and red fluorescing DNA probes. *Cytometry A* 2007; **71**: 700-708 [PMID: 17549765 DOI: 10.1002/cyto.a.20423]

57 **Saran A**, Srivastava S, Coutinho E, Maiti M. 1H NMR investigation of the interaction of berberine and sanguinarine with DNA. *Indian J Biochem Biophys* 1995; **32**: 74-77 [PMID: 7642203]

58 **Chang MC**, Chan CP, Wang YJ, Lee PH, Chen LI, Tsai YL, Lin BR, Wang YL, Jeng JH. Induction of necrosis and apoptosis to KB cancer cells by sanguinarine is associated with reactive oxygen species production and mitochondrial membrane depolarization. *Toxicol Appl Pharmacol* 2007; **218**: 143-151 [PMID: 17196629 DOI: 10.1016/j.taap.2006.10.025]

59 **Carmody RJ**, Cotter TG. Signalling apoptosis: a radical approach. *Redox Rep* 2001; **6**: 77-90 [PMID: 11450987 DOI: 10.1179/135100001101536085]

60 **Pelicano H**, Carney D, Huang P. ROS stress in cancer cells and therapeutic implications. *Drug Resist Updat* 2004; **7**: 97-110 [PMID: 15158766 DOI: 10.1016/j.drup.2004.01.004]

61 **Simon L**, Szilágyi G, Bori Z, Telek G, Magyar K, Nagy Z. Low dose (-)deprenyl is cytoprotective: it maintains mitochondrial membrane potential and eliminates oxygen radicals. *Life Sci* 2005; **78**: 225-231 [PMID: 16242156 DOI: 10.1016/j.lfs.2005.04.078]

62 **Skulachev VP**. Bioenergetic aspects of apoptosis, necrosis and mitoptosis. *Apoptosis* 2006; **11**: 473-485 [PMID: 16532373 DOI: 10.1007/s10495-006-5881-9]

63 **Kim IH**, Kim SW, Kim SH, Lee SO, Lee ST, Kim DG, Lee MJ, Park WH. Parthenolide-induced apoptosis of hepatic stellate cells and anti-fibrotic effects in an in vivo rat model. *Exp Mol Med* 2012; **44**: 448-456 [PMID: 22581380 DOI: 10.3858/emm.2012.44.7.051]

64 **Karin M**. NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol* 2009; **1**: a000141 [PMID: 20066113 DOI: 10.1101/cshperspect.a000141]

65 **Ghosh S**, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002; **109** Suppl: S81-S96 [PMID: 11983155 DOI: 10.1016/S0092-8674(02)00703-1]

66 **Sun M**, Liu C, Nadiminty N, Lou W, Zhu Y, Yang J, Evans CP, Zhou Q, Gao AC. Inhibition of Stat3 activation by sanguinarine suppresses prostate cancer cell growth and invasion. *Prostate* 2012; **72**: 82-89 [PMID: 21538419 DOI: 10.1002/pros.21409]

67 **Basini G**, Bussolati S, Santini SE, Grasselli F. Sanguinarine inhibits VEGF-induced angiogenesis in a fibrin gel matrix. *Biofactors* 2007; **29**: 11-18 [PMID: 17611290]

68 **Xu JY**, Meng QH, Chong Y, Jiao Y, Zhao L, Rosen EM, Fan S. Sanguinarine is a novel VEGF inhibitor involved in the suppression of angiogenesis and cell migration. *Mol Clin Oncol* 2013; **1**: 331-336 [PMID: 24649171 DOI: 10.3892/mco.2012.41]

69 **Basini G**, Santini SE, Bussolati S, Grasselli F. The plant alkaloid sanguinarine is a potential inhibitor of follicular angiogenesis. *J Reprod Dev* 2007; **53**: 573-579 [PMID: 17310078]

70 **Ellis LM**, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 2008; **8**: 579-591 [PMID: 18596824 DOI: 10.1038/nrc2403]

71 **Lev DC**, Ruiz M, Mills L, McGary EC, Price JE, Bar-Eli M. Dacarbazine causes transcriptional up-regulation of interleukin 8 and vascular endothelial growth factor in melanoma cells: a possible escape mechanism from chemotherapy. *Mol Cancer Ther* 2003; **2**:

753-763 [PMID: 12939465]
72 **Lev DC**, Onn A, Melinkova VO, Miller C, Stone V, Ruiz M, McGary EC, Ananthaswamy HN, Price JE, Bar-Eli M. Exposure of melanoma cells to dacarbazine results in enhanced tumor growth and metastasis in vivo. *J Clin Oncol* 2004; **22**: 2092-2100 [PMID: 15123733 DOI: 10.1200/JCO.2004.11.070]
73 **Grivennikov SI**, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883-899 [PMID: 20303878 DOI: 10.1016/j.cell.2010.01.025]
74 **Sternlicht MD**, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; **17**: 463-516 [PMID: 11687497 DOI: 10.1146/annurev.cellbio.17.1.463]
75 **Jiang MC**, Liao CF, Lee PH. Aspirin inhibits matrix metalloproteinase-2 activity, increases E-cadherin production, and inhibits in vitro invasion of tumor cells. *Biochem Biophys Res Commun* 2001; **282**: 671-677 [PMID: 11401513 DOI: 10.1006/bbrc.2001.4637]
76 **Cox TR**, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. *Dis Model Mech* 2011; **4**: 165-178 [PMID: 21324931 DOI: 10.1242/dmm.004077]
77 **Deryugina EI**, Quigley JP. Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev* 2006; **25**: 9-34 [PMID: 16680569 DOI: 10.1007/s10555-006-7886-9]
78 **Jinga DC**, Blidaru A, Condrea I, Ardeleanu C, Dragomir C, Szegli G, Stefanescu M, Matache C. MMP-9 and MMP-2 gelatinases and TIMP-1 and TIMP-2 inhibitors in breast cancer: correlations with prognostic factors. *J Cell Mol Med* 2006; **10**: 499-510 [PMID: 16796815 DOI: 10.1111/j.1582-4934.2006.tb00415.x]
79 **Scorilas A**, Karameris A, Arnogiannaki N, Ardavanis A, Bassilopoulos P, Trangas T, Talieri M. Overexpression of matrix-metalloproteinase-9 in human breast cancer: a potential favourable indicator in node-negative patients. *Br J Cancer* 2001; **84**: 1488-1496 [PMID: 11384099 DOI: 10.1054/bjoc.2001.1810]
80 **Xu X**, Wang Y, Chen Z, Sternlicht MD, Hidalgo M, Steffensen B. Matrix metalloproteinase-2 contributes to cancer cell migration on collagen. *Cancer Res* 2005; **65**: 130-136 [PMID: 15665288]
81 **Park SY**, Jin ML, Kim YH, Lee SJ, Park G. Sanguinarine inhibits invasiveness and the MMP-9 and COX-2 expression in TPA-induced breast cancer cells by inducing HO-1 expression. *Oncol Rep* 2014; **31**: 497-504 [PMID: 24220687 DOI: 10.3892/or.2013.2843]
82 **Abdulghani J**, Gu L, Dagvadorj A, Lutz J, Leiby B, Bonuccelli G, Lisanti MP, Zellweger T, Alanen K, Mirtti T, Visakorpi T, Bubendorf L, Nevalainen MT. Stat3 promotes metastatic progression of prostate cancer. *Am J Pathol* 2008; **172**: 1717-1728 [PMID: 18483213 DOI: 10.2353/ajpath.2008.071054]
83 **Itoh M**, Murata T, Suzuki T, Shindoh M, Nakajima K, Imai K, Yoshida K. Requirement of STAT3 activation for maximal collagenase-1 (MMP-1) induction by epidermal growth factor and malignant characteristics in T24 bladder cancer cells. *Oncogene* 2006; **25**: 1195-1204 [PMID: 16205632 DOI: 10.1038/sj.onc.1209149]
84 **Silver DL**, Naora H, Liu J, Cheng W, Montell DJ. Activated signal transducer and activator of transcription (STAT) 3: localization in focal adhesions and function in ovarian cancer cell motility. *Cancer Res* 2004; **64**: 3550-3558 [PMID: 15150111 DOI: 10.1158/0008-5472.CAN-03-3959]
85 **Möller M**, Weiss J, Wink M. Reduction of cytotoxicity of the alkaloid emetine through P-glycoprotein (MDR1/ABCB1) in human Caco-2 cells and leukemia cell lines. *Planta Med* 2006; **72**: 1121-1126 [PMID: 16783693 DOI: 10.1055/s-2006-941546]
86 **Ma Y**, Wink M. Lobeline, a piperidine alkaloid from Lobelia can reverse P-gp dependent multidrug resistance in tumor cells. *Phytomedicine* 2008; **15**: 754-758 [PMID: 18222670 DOI: 10.1016/j.phymed.2007.11.028]
87 **El-Readi MZ**, Hamdan D, Farrag N, El-Shazly A, Wink M. Inhibition of P-glycoprotein activity by limonin and other secondary metabolites from Citrus species in human colon and leukaemia cell lines. *Eur J Pharmacol* 2010; **626**: 139-145 [PMID: 19782062 DOI: 10.1016/j.ejphar.2009.09.040]
88 **Li S**, Lei Y, Jia Y, Li N, Wink M, Ma Y. Piperine, a piperidine alkaloid from Piper nigrum re-sensitizes P-gp, MRP1 and BCRP dependent multidrug resistant cancer cells. *Phytomedicine* 2011; **19**: 83-87 [PMID: 21802927 DOI: 10.1016/j.phymed.2011.06.031]
89 **Wink M**, Ashour ML, El-Readi MZ. Secondary Metabolites from Plants Inhibiting ABC Transporters and Reversing Resistance of Cancer Cells and Microbes to Cytotoxic and Antimicrobial Agents. *Front Microbiol* 2012; **3**: 130 [PMID: 22536197 DOI: 10.3389/fmicb.2012.00130]
90 **Li JH**. [A study on treatment of lung cancer by combined therapy of traditional Chinese medicine and chemotherapy]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 1996; **16**: 136-138 [PMID: 9208532]
91 **Cai HB**, Dai FG, Min QF, Shi M, Miao JX, Luo RC. [Clinical study of the effects of radiotherapy in combination with traditional Chinese medicine on non-small cell lung cancer]. *Di Yi Jun Yi Da Xue Xue Bao* 2002; **22**: 1112-1113 [PMID: 12480588]
92 **Efferth T**, Davey M, Olbrich A, Rücker G, Gebhart E, Davey R. Activity of drugs from traditional Chinese medicine toward sensitive and MDR1- or MRP1-overexpressing multidrug-resistant human CCRF-CEM leukemia cells. *Blood Cells Mol Dis* 2002; **28**: 160-168 [PMID: 12064912 DOI: 10.1006/bcmd.2002.0492]
93 **Eid SY**, El-Readi MZ, Wink M. Synergism of three-drug combinations of sanguinarine and other plant secondary metabolites with digitonin and doxorubicin in multi-drug resistant cancer cells. *Phytomedicine* 2012; **19**: 1288-1297 [PMID: 23146422 DOI: 10.1016/j.phymed.2012.08.010]
94 **Choy CS**, Cheah KP, Chiou HY, Li JS, Liu YH, Yong SF, Chiu WT, Liao JW, Hu CM. Induction of hepatotoxicity by sanguinarine is associated with oxidation of protein thiols and disturbance of mitochondrial respiration. *J Appl Toxicol* 2008; **28**: 945-956 [PMID: 18548746 DOI: 10.1002/jat.1360]
95 **Hu CM**, Cheng HW, Cheng YW, Kang JJ. Induction of skeletal muscle contracture and calcium release from isolated sarcoplasmic reticulum vesicles by sanguinarine. *Br J Pharmacol* 2000; **130**: 299-306 [PMID: 10807666 DOI: 10.1038/sj.bjp.0703279]
96 **Hu CM**, Cheng HW, Cheng YW, Kan JJ. Mechanisms underlying the induction of vasorelaxation in rat thoracic aorta by sanguinarine. *Jpn J Pharmacol* 2001; **85**: 47-53 [PMID: 11243574]
97 **Hu CM**, Cheng YW, Liao JW, Cheng HW, Kang JJ. Induction of contracture and extracellular Ca2+ influx in cardiac muscle by sanguinarine: a study on cardiotoxicity of sanguinarine. *J Biomed Sci* 2005; **12**: 399-407 [PMID: 15920678 DOI: 10.1007/s11373-005-3007-y]
98 **Chan WH**. Embryonic toxicity of sanguinarine through apoptotic processes in mouse blastocysts. *Toxicol Lett* 2011; **205**: 285-292 [PMID: 21722720 DOI: 10.1016/j.toxlet.2011.06.018]
99 **Kaminskyy V**, Lin KW, Filyak Y, Stoika R. Differential effect of sanguinarine, chelerythrine and chelidonine on DNA damage and cell viability in primary mouse spleen cells and mouse leukemic cells. *Cell Biol Int* 2008; **32**: 271-277 [PMID: 18029203 DOI: 10.1016/j.cellbi.2007.09.004]
100 **Das M**, Khanna SK. Clinicoepidemiological, toxicological, and safety evaluation studies on argemone oil. *Crit Rev Toxicol* 1997; **27**: 273-297 [PMID: 9189656 DOI: 10.3109/10408449709089896]
101 **Hakim SA**. Argemone oil, sanguinarine, and epidemic-dropsy glaucoma. *Br J Ophthalmol* 1954; **38**: 193-216 [PMID: 13149763 DOI: 10.1136/bjo.38.4.193]
102 **Hakim SA**. Argemone oil, sanguinarine, dropsy, glaucoma and cancer. *Indian Pract* 1967; **20**: 129-141 [PMID: 6037511]
103 **Das M**, Ansari KM, Dhawan A, Shukla Y, Khanna SK. Correlation of DNA damage in epidemic dropsy patients to carcinogenic potential of argemone oil and isolated sanguinarine alkaloid in mice. *Int J Cancer* 2005; **117**: 709-717 [PMID: 15981203 DOI: 10.1002/ijc.21234]
104 **Ansari KM**, Dhawan A, Khanna SK, Das M. In vivo DNA damaging potential of sanguinarine alkaloid, isolated from argemone oil, using alkaline Comet assay in mice. *Food Chem Toxicol* 2005; **43**: 147-153 [PMID: 15582207 DOI: 10.1016/j.fct.2004.09.005]
105 **Anderson KM**, Stoner GD, Fields HW, Chacon GE, Dohar AL, Gregg BR, Mallery SR. Immunohistochemical assessment of

Viadent-associated leukoplakia. *Oral Oncol* 2005; **41**: 200-207 [PMID: 15695122 DOI: 10.1016/j.oraloncology.2004.08.008]

106 **Damm DD**, Curran A, White DK, Drummond JF. Leukoplakia of the maxillary vestibule--an association with Viadent? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; **87**: 61-66 [PMID: 9927082 DOI: 10.1016/S1079-2104(99)70296-9]

107 **Karp JM**, Rodrigo KA, Pei P, Pavlick MD, Andersen JD, McTigue DJ, Fields HW, Mallery SR. Sanguinarine activates polycyclic aromatic hydrocarbon associated metabolic pathways in human oral keratinocytes and tissues. *Toxicol Lett* 2005; **158**: 50-60 [PMID: 15993743 DOI: 10.1016/j.toxlet.2005.02.007]

108 **Wang M**, Thanou M. Targeting nanoparticles to cancer. *Pharmacol Res* 2010; **62**: 90-99 [PMID: 20380880 DOI: 10.1016/j.phrs.2010.03.005]

109 **Li W**, Li H, Yao H, Mu Q, Zhao G, Li Y, Hu H, Niu X. Pharmacokinetic and anti-inflammatory effects of sanguinarine solid lipid nanoparticles. *Inflammation* 2014; **37**: 632-638 [PMID: 24272172 DOI: 10.1007/s10753-013-9779-8]

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REVIEW

Helicobacter pylori infection and gastric carcinoma: Not all the strains and patients are alike

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Abstract

Gastric carcinoma (GC) develops in only 1%-3% of *Helicobacter pylori* (*H. pylori*) infected people. The role in GC formation of the bacterial genotypes, gene polymorphisms and host's factors may therefore be important. The risk of GC is enhanced when individuals are infected by strains expressing the oncoprotein CagA, in particular if CagA has a high number of repeats containing the EPIYA sequence in its C'-terminal variable region or particular amino acid sequences flank the EPIYA motifs. *H. pylori* infection triggers an inflammatory response characterised by an increased secretion of some chemokines by immunocytes and colonised gastric epithelial cells; these molecules are especially constituted by proteins composing the interleukin-1beta (IL-1β) group and tumour necrosis factor-alpha (TNF-α). Polymorphisms in the promoter regions of genes encoding these molecules, could account for high concentrations of IL-1β and TNF-α in the gastric mucosa, which may cause hypochlorhydria and eventually GC. Inconsistent results have been attained with other haplotypes of inflammatory and anti-inflammatory cytokines. Genomic mechanisms of GC development are mainly based on chromosomal or microsatellite instability (MSI) and deregulation of signalling transduction pathways. *H. pylori* infection may induce DNA instability and breaks of double-strand DNA in gastric mucocytes. Different *H. pylori* strains seem to differently increase the risk of cancer development run by the host. Certain *H. pylori* genotypes (such as the *cagA* positive) induce high degrees of chronic inflammation and determine an increase of mutagenesis rate, oxidative-stress, mismatch repair mechanisms, down-regulation of base excision and genetic instability, as well as generation of reactive oxygen species that modulate apoptosis; these phenomena may end to trigger or concur to GC development.

Key words: *Helicobacter pylori* infection; CagA; *CagA* gene polymorphism; Haplotype; Human gene mutation;

Gene methylation; Gastric carcinoma; Inflammatory cytokine

Core tip: CagA and the *cagA* types may play different roles in the intestinal and diffuse histotypes of gastric carcinoma (GC); The current criteria of *Helicobacter pylori* (*H. pylori*) strain classification based on their carcinogenic potential gave rise to confusion and should be unified. The possible role of inflammatory cytokine haplotypes in GC development should be reassessed taking into account some host's factors, the most important being different ethnic origin. Infection by the *cagA* positive *H. pylori* genotype may determine an increased inflammatory response and a consequent enhancement of mutagenesis rate, oxidative-stress, reactive oxygen species generation, dysfunction of DNA repair mechanisms, genetic instability and resultant high risk of GC development.

Figura N, Marano L, Moretti E, Ponzetto A. *Helicobacter pylori* infection and gastric carcinoma: Not all the strains and patients are alike. *World J Gastrointest Oncol* 2016; 8(1): 40-54 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i1/40.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i1.40

INTRODUCTION

Gastric carcinoma (GC) is the second most frequent cause of death from cancer worldwide and the most common example of a neoplasia developing on a ground of a chronically inflamed mucosa. GC has also another record: It is the only known malignant tumour that can develop as a consequence of a chronic bacterial infection[1]. In 1994, the International Agency for Research on Cancer classified the organism responsible for the infection, *Helicobacter pylori* (*H. pylori*) - a Gram negative, microaerophilic and spiral-shaped species that finds its *habitat* in human stomachs - as a definite carcinogen to humans (Group 1): The connection of *H. pylori* with gastric cancer was considered similar to that existing between the cigarette smoke and lung cancer[2].

The bacterium H. pylori: Not all the strains are alike

It soon became clear, however, that such comparison was reductive and too simplistic, especially because the ability of these bacteria to trigger a neoplasm is not limited to the inflammatory and immune response to the infection that they cause, but it also resides in a series of bacterial factors capable of prompting and modulating the carcinogenic process[3].

As is the case for all diseases, also GC develops from the concomitance of three factors: The etiological agent, the host and the environment. Of course, many other factors may occur; for example, the cancer histological variant, the degree of differentiation of the neoplasia *etc.*[4]. Regarding the etiological agent, *H. pylori*, there are many indications that not all strains are equivalent in their carcinogenic potential and that those expressing an immunodominant peptide determinant called *CagA* (cytotoxin associated gene A), are endowed with an increased inflammatory and carcinogenic potential[5-9]. A first point has therefore been established: Strain genomic diversity corresponds to different ability to promote cancer. The possibility that a bacterial factor (*CagA*) could trigger or concur to the development of GC is one of the most important scientific achievements following the isolation of *H. pylori*.

The importance of being called CagA positive

It is worthwhile mentioning the steps that paved the way to the discovery of CagA. At the end of the 80ies, Leunk *et al*[10] first proposed that *H. pylori* should not be considered a clonal pathogen, as a relevant proportion of isolates produce a vacuolating toxin, which could account in part for the gastric mucosa damage observed in infected individuals. Afterward, our group suggested that infection by cytotoxic strains increased the risk of developing peptic ulceration[11] and that virtually all cytotoxic isolates also secreted a 120 kDa highly immunogenic protein, later called CagA[12]. In 1992, Crabtree *et al*[13] demonstrated, through *ex vivo* experiments, that such a protein was produced either by the bacteria isolated in culture and also by the organisms colonizing the gastric epithelium: Gastric antral explants of patients with GC and other pathologies were cultured *in vitro* for a few days; the bacteria that colonized the mucosa kept on secreting this peptide, which could lastly be detected in the culture medium by using immunological methods[13]. In 1993, the same team established, for the first time, the existence of a relationship between infection by strains expressing the 120 kDa protein and GC development[14]. Their observations were important also because these researchers found anti-120 kDa protein mucosal IgA antibodies even in the absence of systemic IgG to this protein and, in some patients, also in cases with urease negative biopsies (false negatives). In the same year (1993), the gene encoding for the 120 kDa protein was cloned, sequenced and called *cagA* due to the strict association of protein expression with cytotoxin production[5]. As a result of these findings, the number of studies dealing with the characterisation of CagA and its potential carcinogenicity increased exponentially and results lead to the common conclusion that such peptide is a major factor in gastric carcinogenesis.

CagA is the product of the homonymous gene placed at the end of the so-called pathogenicity island (PAI) *cag*, a fragment of DNA encompassing an approximately 40 kb cluster of genes involved in virulence. In the field of bacteriology there are numerous examples of PAIs harboured by diverse bacterial species or their virulent variants, whether they are human (*Bordetella*

pertussis, *Escherichia coli, Salmonella enterica, etc.*) or plant pathogens (*Agrobacterium tumefaciens*). In some species, PAI genes cooperate to translate effectors (mainly proteins) endowed with carcinogenic potential inside colonised cells. In *H. pylori*, such determinant is CagA. Similarly, *A. tumefaciens* exploits the Type Ⅳ secretion system *vir* to translate a single-stranded form of T-region (T-strand) coated by the ssDNA-binding protein VirE2 (T-Complex) into the host's vegetal cell nuclei. Once inside the nucleus, the T-strand can be converted in a double-stranded form (T-DNA), whose expression causes an uncontrolled host cell proliferation and tumour development[15].

Epidemiological and genomic studies suggest that the development of GC is a possible consequence of infection by strains expressing CagA[1,8,9,14,16]. In effects, using Mongolian gerbils infected experimentally, it was shown that only CagA positive (CagA+) *H. pylori* strains were able to induce stomach tumours[17]. In addition, a study of our group revealed that, while virtually all patients with intestinal histotype of GC had serum antibodies to CagA, the prevalence of anti-CagA antibodies in patients with the diffuse GC variety was similar to that observed in infected controls without neoplasia[16]. These data were confirmed by the results of an epidemiological study: The overall GC risk in infected people lacking anti-CagA antibodies (CagA-) was increased, but in non-significant way; in any case, CagA- *H. pylori* infection was associated with the growth of the diffuse variety of GC (with an OR of 9.0)[8].

It therefore seems that infections by CagA+ strains expose people to an increased risk of GC respect to infections by CagA- *H. pylori* strains, which can only be associated with the diffuse histotype. In effect, things work slightly differently. In a recent study, we examined for the presence of *cagA* up to 25 distinct, well separated colonies per patient with GC; even though only individuals with diffuse histotype GC harboured *cagA* negative (*cagA*-) organisms, in all cases patients were also infected by at least one *cagA* positive (*cagA*+) strain[18]. These observations do not corroborate the supposed propensity of strains lacking *cag* PAI to concur to diffuse GC development and suggest that, for a better comprehension of the role played by *cagA* in the histological variety of GC, many colonies per patient should be examined genomically.

CagA phosphorylation by mucocytes: Like shooting oneself in the foot

H. pylori organisms expressing CagA differ in their carcinogenic potential. Let us have a look at the mechanisms that may influence the ability of such a protein to trigger and/or concur to GC formation. CagA, following colonisation, is translated into the gastric epithelial cells through a conjugative apparatus encoded by the *cag* PAI genes upstream *cagA*; then, a portion of intracellular CagA is phosphorylated by numerous kinases, members of the host cell Src family (such as Yes, Lyn, Fyn and c-Src,) at the EPIYA C'-terminal site (Glu-Pro-Ile-Tyr-Ala) motif of tyrosine[19]. Phosphorylated CagA physically interacts with the oncogenic tyrosine phosphatase SHP-2 (Src homology phosphatase 2), modifying cellular functions and altering mammalian signal transduction machineries. SHP-2, in fact, is implicated in the regulation of cell adhesion, spreading and migration. In this manner, phosphorylated CagA causes deregulation of SHP-2 and induces abnormal proliferation, as well as movement of cells of the gastric epithelial layer, activates mitogenic signalling and disturbs host-signalling routes[20]. All these events may also predispose cells to accumulate multiple genetic and epigenetic alterations involved in gastric tumorigenesis[21].

Unphosphorylated CagA, on the other hand, interacts with the tumour suppressor protein of p53 (ASPP2), which also exert an apoptosis-stimulating activity[22]. In normal conditions, following genotoxic and oncogenic stimuli, ASPP2 associates with tumour suppressor p53, activates it and induces apoptosis. After interaction with CagA, cytosolic p53 is recruited by ASPP2 and subsequently is degraded by an enzyme complex that control cell-cycle and apoptosis, the proteasome. As a consequence, the apoptotic response of host cells is inhibited. In other words, unphosphorylated CagA takes control of ASPP2 and subverts the tumour suppressor pathway of apoptosis-stimulating protein p53 with consequent promotion of cell survival and cell transformation[20-22]. The resultant abnormal proliferation of gastric epithelial cells may contribute to GC development.

Individual CagA proteins have different biological activity and tumorigenic potential: When size matters

H. pylori strains secreting CagA protein endowed with increased biological activity can be considered more virulent and even more closely associated with gastric cancer. At the end of 90's, the group of Graham discovered the ability of distinct CagA proteins to perturb cellular functions might vary in different isolates[23]. The CagA C'-terminal region contains one or more repeats of the same amino acids in sequence (Glu-Pro-Ile-Tyr-Ala, or EPIYA)[5]; the teleonomic significance of such phenomenon reflects the bacterial strategy to generate antigenic diversity, which may protect the organisms from the immune response. The number of EPIYA motifs correlates with the size of the *cagA* variable region. Yamaoka *et al*[23] basing of the amplicon sizes obtained with primers encompassing the entire *cagA* variable region, classified *H. pylori* isolates in *cagA* structural types A, B, C and D (amplicons characterising types B and D have the same size and can be differentiated by sequencing). Strains with the *cagA* structural type C have the highest number of EPIYA phosphorylation motifs and were isolated significantly more often from patients with GC[23], confirming a previous observation that individuals with GC are infected by strains expressing CagA proteins with higher mass[24]. The incr-

eased carcinogenic potential of the *cagA* structural type C was also confirmed by another study of the same group, which showed that patients infected by *H. pylori* with this *cagA* genotype run a higher risk of developing gastric mucosa atrophy, a precancerous condition[25].

Now, it should be highlighted that the primers used in these studies to amplify the *cagA* variable region were designed on oriental (Japanese) strains, which may differ from western strains in the nucleotide sequence encoding the C'-terminal variable region[26]. Probably for this reason not all surveys on the same subject have confirmed the Yamaoka *et al*[23,25]'s findings. In an initial study of our group, in which we used the Yamaoka's primers to amplify the *cagA* variable region of Italian strains[27], we sometime obtained amplicons shorter than the PCR product that characterises the *cagA* structural type A; strains producing such amplicons, which presented a deletion of about 100 bp respect to *cagA* type A, were named type A(I), with (I) standing for Italy, because, as far as we know, similar *cagA* structural variety had not previously been described. In a more recent investigation, we observed similar proportions of the various *cagA* structural types in Italian *H. pylori* organisms isolated from GC cases and from controls (patients without neoplasia, with chronic gastritis only)[28]. In addition, in the control subjects, we frequently detected strains with the *cagA* structural type A(I). The increased prevalence of such a *cagA* type in individuals without GC prompted us to hypothesise that the reduced dimensions of the encoded CagA may decrease the ability of these bacteria to trigger a neoplastic process. As a matter of fact, the capability of CagA of binding SHP-2, and therefore of disturbing the various cellular functions, is regulated by the amounts of tyrosine phosphorylation site sequences, *i.e.*, the CagA size[20]; therefore, the shorter the protein, the less numerous are the EPIYA repeats that undergo phosphorylation and the lower is the carcinogenic potential of strains.

The employment of primers proposed by Yamaoka *et al*[23] to amplify the *cagA* variable region of western strains has sometimes led to results similar to those of the Japanese researchers: South African researchers, for instance, have confirmed that patients with GC have an increased prevalence of strains with type C CagA[29]. Investigations dealing with this important subject, however, are not numerous; in addition, sometimes they attained different conclusions and have even contributed to create confusion in this subject. Malaysian authors, for instance, using the primers designed by Yamaoka *et al*[23], determined the presence and distribution of *cagA* variants among different ethnic groups and various gastroduodenal diseases[30]. They obtained three types of amplicons and named the *cagA* subtypes with capital letters, A, B and C (like did Yamaoka *et al*[23]), but, just to complicate this topic further, they used a different criterion (respect to that of the previous study) and called subtype C the strains with the smallest amplicon size and subtype B those with the greatest one. Specific *cagA* subtype A strains (those yielding amplicons of intermediate size) were predominantly isolated from Chinese compared to Malays and Indians patients. Since Chinese patients have the highest risk of GC disease respect to the other ethnic groups, these investigators concluded that *cagA* subtyping could be used as a clinical biomarker for severe outcome of infection. Such statement, however, was based on indirect observations because they did not examine strains from GC cases[30].

In 2002, Higashi *et al*[20] observed that the ability of CagA secreted by different strains to disturb host-cell functions can be influenced by the strength of SHP-2 binding activity, which was increased in *H. pylori* strains obtained from patients living in East Asian areas (Japan, Chorea and China) respect to those isolated from patients of Western countries (Europe, America, and Australia). In addition to the number of EPIYA repeats, it was also found that polymorphism in the nucleotide sequence flanking the regions that encode EPIYA could affect the potential of different *H. pylori* strains to promote gastric carcinogenesis[31]. According to the geographic regions in which strains are isolated, it is possible to characterise the *cagA* variable region on the basis of the number and the type of sequences. The Western *H. pylori* CagA has two segments of 32 and 40 amino acids flanking EPIYA (EPIYA-A and EPIYA-B types) and one to three 34 amino acid EPIYA-C segments (A-B-C type CagA)[21]. Eastern CagA presents EPIYA-A and EPIYA-B segments, but none of the EPIYA-C fragment; it has instead one copy alone of a segment called EPIYA-D, which represents the main tyrosine phosphorylation site[20,21]. In western strains, the major site of tyrosine phosphorylation of CagA is EPIYA-C; the tyrosine residues that characterize EPIYA-A and EPIYA-B segments are phosphorylated only very weakly. Such a difference in phosphorylation degrees resides in the diverse consensus high-affinity binding sequence for the SH2 domains of SHP-2. Unlike what happens for EPIYA-D type, the western EPIYA-C type of CagA differs by a single amino acid from the consensus SHP-2 binding sequence[21].

In conclusion, the carcinogenic potential of *H. pylori* varies according to the number and the *cagA* structural types of the EPIYA flanking regions. East Asian CagA has an increased virulence and a strong ability to trigger GC, while, among western isolates, more carcinogenic are the helicobacters with two or three CagA EPIYA-C sites. This was also demonstrated by a study in which it was observed that 83.3% of GC strains possessed multiple EPIYA-C sites, *vs* only 5.2% of strains isolated from patients with chronic gastritis only (controls)[32].

Often, *cagA*+ strains are also isolated from patients with duodenal ulcer (NF personal observation). This finding may create confusion, because is common knowledge that patients with duodenal ulcer are like protected from GC development; however, the results of a recent study[33] showed that the increased virulence

of strains with CagA EPIYA-C type augmented the risk of gastric cancer and not peptic ulceration. Some studies diverge from these conclusions: Findings of an investigation carried out in Colombia suggest that polymorphic CagA proteins, based on sequences flanking the EPIYA motifs, are not clearly associated with the outcome of the infection[34]. The absence of association between the CagA polymorphisms and pathogenesis of gastroduodenal diseases could be due to geographic factors and/or the host's genetic features and environmental determinants.

In conclusion, the results of this kind of investigations are potentially useful, but the confusion existing in this field ought to be rectified and the different researchers should use the same criteria for classification. The various groups, however, have reached a common conclusion: Not all the strains are alike in their carcinogenic potential.

NOT ALL PATIENTS ARE ALIKE: THE ROLE OF THE HOST'S INFLAMMATORY CYTOKINE HAPLOTYPES IN GC DEVELOPMENT

Background

The hypothesis that human genetic polymorphisms may affect predisposition to GC has recently been explored. GC develops in only 1%-3% of *H. pylori* infected individuals, which suggests that the host background matters in this neoplasia. Several pro-inflammatory cytokines are produced by the immune system against *H. pylori*; among them, IL-1β is of paramount importance; a second one is tumor necrosis factor-alpha (TNF-α); both of them are closely related to epithelial injury and gastric hypochlorhydria[35,36]. At low concentrations, TNF-α enhances the protective inflammatory response; at high concentrations, it can injure the gastric mucosa and cause severe pathology[37]. IL-1β increases the surface molecule expression on endothelial cells, causing leukocytes to adhere; IL-1β also induces the production of macrophage chemokines leading to neutrophil activation. Recent investigations have revealed that there is a genetic regulation of the host cytokine response to inflammatory stimuli. Genomic variants of *IL-1β* and *TNF-α* were shown to correlate with the clinical outcomes of tumors, including GC[38,39]. The *IL-1β* gene cluster is polymorphic, with some alleles present at relatively high frequencies. Particular *IL-1β* haplotypes enhance the risk of GC because they induce an over expression of its product in the stomach, causing chronic hypochlorhydria, which in turn may produce gastric atrophy and, eventually and in the presence of other risk factors, GC[40]. In addition, patients with a particular haplotype of the gene that encodes IL-RA (receptor antagonist) have an elevated risk of developing GC. IL-RA is an anti-inflammatory cytokine, which is a competitor for IL-1β receptors, thus regulating the possible harmful effects of IL-1β receptors.

The role of the host in GC development could be important because the inflammatory response to infections varies from patient to patient due to the gene polymorphism of inflammatory and anti-inflammatory cytokines. Many studies have been performed in the last 15 years, with the scope of identifying a genetic marker that can determine whether or not people carrying the infection might be at risk of developing GC in *H. pylori* infected patients[41-59]. Such a marker is still lacking, and we shall try to explain some of the reasons underlying this problem.

The association between chronic inflammation and cancer has been known since Virchow, in 1864, wrote that cancer would arise from sites of inflammation: "Chronic irritation which is manifested by a chronic inflammation is a key promoter of cancer" (Quoted by Balkwill and Mantovani[60]). Individual cytokines were specifically examined, in particular the proinflammatory ones. The most well known cytokine is IL-1, along with its receptor (IL-1R) and the antagonist of this receptor (IL-1RA); all of them share the chromosomal location of the *IL-1* gene family (namely 2q13-14). IL-1β has thus been established as an important regulator of carcinogenesis, characteristic of interactions between the host and environment[54].

IL-1β haplotypes

The *IL-1β* gene displays considerable polymorphism[54]; the presence of C to T transition was frequently found either in the promoter region at positions -511 (CT; dbSNP: rs16944), at position -31 (TC; dbSNP: rs1143627) or in the coding region at position +3954 (CT; dbSNP: rs1143634) base pairs from the origin of transcription. The two single nucleotide polymorphisms (SNPs) within promoter region are in linkage disequilibrium. The *IL-1β* -31 TC substitution disrupts a TATA-box motif; this leads to several transcription factors having altered binding affinities, resulting in modified IL-1β transcription. The *IL-1β* +3954 CT substitution is a synonymous SNP. It was demonstrated *in vitro* that the C to T transition at positions -511 and +3954 correlated with elevated IL-1β levels as a result of lipopolysaccharide (LPS)-stimulated IL-1β protein secretion[54].

The paradigm of all subsequent studies regarding GC with respect to different haplotypes in cytokines was the focus of the paper by El-Omar *et al*[38], who noted for the first time that the presence of two polymorphisms (rs16944 and rs1143627) in the promoter region of the *IL-1β* gene, identified an increased risk of hypochlorhydria, as a result of *H. pylori* infection and GC[38]. These polymorphisms determine an increased secretion of IL-1β[61], a conclusion confirmed and generalized by later reports[62]. The discrepancy of results reached in different papers was clarified only after several years, once taking into account the origin of the population and the type of GC. Overall, more than 90 publications dealt with this issue, over half of them from Asia, and a single one

from North America, a clear indication of the perceived relevance of this neoplasia in the different populations.

Since the single studies are extremely inconsistent and, if taken alone, contribute little to the general overview, we opted for reporting from selected meta-analyses in this paper. Meta-analyses accrued from time to time in a number of studies originating from different countries, which is one of the main causes of contradictory results, as well as from different histological variety of stomach malignancies, another cause of strong difference in results[41-59].

The overall findings from the large amount of efforts can be summarized as follows:

(1) *IL-1β receptor antagonist* (*IL-1β RA*) polymorphism: The most credible and consistent association of peculiar genetic variation with GC was found for *IL-1β RA* haplotypes. Four alleles, numbered 1 to 4, are widely present in the general populations. People carrying the homozygous allele 2/2 (*IL-1β RN2*) were found to be at higher risk of developing cancer among non-Asian populations. Moreover, the analysis of GC patients altogether, without stratifying according to histological type, anatomic site or country of origin, showed that patients carrying homozygous allele 2, or *IL-1 RN*2 had an increased risk of developing cancer, which was statistically significant. The risk was found both in cardia and non-cardia types of neoplasia. A possible explanation for the risk stems from the high IL-1β levels circulating among *IL-RA* allele 2/2 carriers[63].

(2) *IL-1β* -31 CT polymorphism: A second plausible association was the decreased risk of GC in Asians carrying the haplotypes in the *IL-1β* -31 CC promoter region. A decreased risk of GC among *IL-1β* -31C carriers was confirmed, but solely for Asian patients.

(3) *IL-1β* -511 CT polymorphism in populations of different ethnic origin: Sub-analysis of various populations revealed a statistically significant association of stomach cancer with the *IL-1β* polymorphism at promoter region -511 CT in case-control studies based on populations (OR = 1.20, 95%CI: 1.00-1.43)[54]. The association is more consistent if only Caucasian populations are analyzed. Nevertheless, if taken together, the studies failed to show the association when stratified by ethnicities; *IL-1β* -511 CT polymorphism according to tumor site: A significant association of *IL-1β* -511 CT promoter region polymorphism was observed for stomach cancers when the tumor site (cardia *vs* non-cardia) was taken into account, as well as for histology subtypes (intestinal or diffuse/mixed). The association was present both in the case of non-cardia GC (OR = 1.57, 95%CI: 1.06-2.31) as well as intestinal GC (respectively OR = 1.57, 95%CI: 1.06-2.31 and OR = 1.24, 95%CI: 1.04-1.49)[54]; *IL-1β* +3954 CT polymorphism: Recently, Xu *et al*[54] performed a meta-analysis that was confirmed by that one by Xue *et al*[49]: There is a lack of association between *IL-1β* +3954 CT and GC risk.

(4) *IL-10* haplotypes: IL-10 - regarded as the major anti-inflammatory cytokine - will bind in form of homodimer its complex receptor, comprising four IL-10 receptor molecules, namely 2 IL-10 R1 and 2 IL-10 R2. The binding induces STAT3 signaling *via* the phosphorylation of the cytoplasmic tails of IL-10 receptor 1. IL-10 can inhibit the synthesis of pro-inflammatory cytokines; moreover it can block the function of nuclear factor-kappa B (NF-κB), and has other regulatory properties, *e.g*., JAK-STAT signaling[64]. The *IL-10* gene is known to possess several SNPs, some in the distal region upstream of the coding gene (-1082 A/G, -819 T/C) and a proximal one (the -592 A/C). Again, the complex signaling and polymorphism of *IL-10* can explain the contradictory results of the investigations.

(5) *IL-10* -1082 AG polymorphism: A clear and curious dichotomy is evident, that is, when the studies were stratified according to Asian and non-Asian populations the observations reached opposite results. The Asian populations had greater risk of GC among *IL-10* -1082 G carriers; conversely, there was a decreased risk among the non-Asian populations. Meta-analysis specific for *IL-10* confirmed for Asian population the increased risk for intestinal type of gastric neoplasia in *IL-10* -1082 GG or GA haplotypes[53]; *IL-10* -592 AC polymorphism: The -592 AC polymorphism failed to show any association, as the odd ratios for GC were 0.93 and 0.94 for homozygous and heterozygous population[55,65]; *IL-10* -819 TC polymorphism: Little data is available for this polymorphism, confirming a protective effect in Asian populations. Nevertheless, it was not found to be associated with the reduced susceptibility to GC in individuals infected with *H. pylori* compared to uninfected controls. The *IL-10* -819 TT genotype was found to be inversely correlated with the risk of the diffuse subtype, but not the intestinal subtype GC[51].

(6) *IL-8* - 251 polymorphisms: Continuous expression of human IL-8 in transgenic mice (whereby IL-8 is under the control of its own regulatory elements) increased tumorigenesis. Therefore, IL-8 may play an important role in gastrointestinal cancers. Elevated IL-8 levels could be linked to a poor prognosis of neoplasia, henceforth its levels may be indicative of more aggressive GCs.

Early data seemed to provide a possible association in GC as well[66]. A recent meta-analysis showed that the *IL-8* -251 AA genotype in the Han population correlates with augmented risk of developing GC and AA genotype carriers appear to be more likely to develop GC in Asian populations. In addition, the *IL-8* -251 AA genotype tended to be related to intestinal GC, but not with *H. pylori* infectious status[52]. There was no link between *IL-8* polymorphisms and *H pylori*-related gastric malignancies in non-Asian populations in all the meta-analyses examined[48,67].

(7) *TNF-α* polymorphism: Experimental studies have implicated TNF-α in processes that are involved in cancer progression, including promotion of metastatic behaviour and cancer associated cachexia[68,69]. The lack of *TNF-α*

in mice makes them resistant to carcinogenesis[70]. Clearly, such observation highlighted the link between genetic haplotypes for *TNF-α* and GC.

TNF-α -308 AG polymorphism: It was surprising to find a lack of association of this polymorphism with increased risk of GC, with only one exception: Non-Asian patients with distal cancer and homozygous for -308 AA alleles; the association, moreover, appeared to exist for cancer of diffuse type only. However, this association was not confirmed when only good quality studies were taken into account, according to Persson *et al*[48]. Opposite conclusions were obtained by Zhu *et al*[59]; they recently analyzed all studies and concluded that, in the Caucasian populations, *TNF-α* rs1800629 (-308 AG) polymorphism indeed posed increased risk of GC. They used several genetic comparison models, *i.e.*, A *vs* G, AA *vs* GG and AA *vs* GG/GA that gave OR respectively of 1.32, 1.76 and 1.62, all highly significant (A *vs* G: OR = 1.32, 95%CI: 1.12-1.56, $P = 0.001$; AA *vs* GG: OR = 1.76, 95%CI: 1.37-2.26, $P < 0.001$; AA *vs* GG/GA: OR = 1.62, 95%CI: 1.27-2.07, $P < 0.001$)[59].

TNF-α -238 polymorphism did not correlate with an increased cancer risk[48,58].

TNF-α 857 CT polymorphism: Reports on this topic are quite controversial. Cen *et al*[57] recently published his analysis of nine studies (all the reported ones); overall, they confirm that the *TNF-α* 857 CT polymorphism posed an elevated risk of GC solely among Asians; all four genetic models considered T *vs* C, TT *vs* CC, CT *vs* CC and TT *vs* CT gave consistent data, respectively with OR of 1.19, 1.44, 1.19 and 1.21 (their statistical significance being $P = 0.002$, $P = 0.032$, $P = 0.008$, $P = 0.003$ respectively).

TNF-β 252 AG polymorphism: A weak association with stomach malignancy was present in Asian populations, according to Xu *et al*[71]. Analysis by ethnicity revealed that the *TNF-β* 252 AG polymorphism correlated with a minor risk of GC (G *vs* A: OR = 1.10, 95%CI: 1.02-1.19, $P = 0.015$) exclusively in Asians, not in Caucasians.

Dutch patients were analyzed for their polymorphism of IL-1β; they were found to carry lower risk of GC when heterozygous for either the IL-1B -511 and for the IL1β -31 TATA-box (genotype T/C)[72]. The EBV status of the patients did not affect this correlation and there could therefore be an early shared molecular mechanism in the progression of EBV-positive and negative GCs[72].

IL-6 polymorphism was not studied in relation to GC.

IL-6 knockout mice develop cancer less frequently[73]. It is therefore plausible that high IL-6 levels will promote tumorigenesis. Today, IL-6 is considered to be a relevant tumor-promoting factor also in humans. Indeed, it was correlated with glioma, lymphoma and melanoma at first, then with solid cancers such as breast and colorectal neoplasia (also ovarian and pancreatic), prostate, renal and colorectal cancers.

IL-6 is a critical factor during chronic inflammation, since it is required for the induction of effector Th17 cells and inhibits the differentiation of regulatory T cells.

Stomach cells, however, lack IL-6 receptors; hence it cannot dimerize with the second receptor (gp 130) and the "classic signaling" is restricted to cells bearing both mIL-6R and gp130 on their surface. The latter is widely expressed, whilst mIL-6R expression is limited to some leukocytes, hepatocytes and cancer cells. It is therefore quite understandable that, as recently reported, a large meta-analysis on 105000 people established the lack of association of cancer risk with *IL-6* polymorphism in Caucasians[74], despite the association which holds true for Africans.

Other cytokines

Gene polymorphism concerning cytokine different from those we have dealt with was recently considered in relation to the risk of developing GC. The studies are not sufficiently large so far; however it is worth reporting that a haplotypes of *IL-17*, *IL-17F* rs763780 TC, was significantly associated with GC development in Asian population[75].

IL-11 was taken into consideration in a single study[76]. A reduced risk for developing cancer at the gastric site was found for a polymorphism in the *IL-4* -590 CT gene in Caucasian but not in Asian populations. *H pylori* status was not taken into consideration in these studies[77].

Different results in different studies: The origin of the problem

IL-1β, TNF-α and the remaining dozens of cytokines are not the final executor of immune signaling or the resulting consequences in cancer promotion and spread. IL-1, TNF-α, together with bacterial antigens, LPS and several other signaling molecules bind their respective receptors on the cell membrane; a cascade of signals ensues upon receptor activation, which depends on the levels of Mg-ATP availability (in turn on Mg^{2+} concentration in cells). Numerous different proteins are involved and regulate the signaling pathway, which finally results in the activation of a large family of DNA-binding proteins, the NF-κB family[78], which is a complex that regulates DNA transcription. NF-κB dimers are formed upon activation, stimulating the transcription of genes that encode cytokines, growth factors, chemokines, and anti-apoptotic factors[79]. However, some NF-κB dimers act by repressing, whilst others activate specific genes.

Cytokine polymorphism and Epstein-Barr virus-associated GC

Worldwide, it was noted that Epstein-Barr virus (EBV) is present in a relevant proportion of malignant tumors of the stomach, with an incidence that is inversely proportional to that of GC. In the USA 16% to 18% of all stomach tumors were found EBV-associated (EBVaGC), in Southern China only 4.3%[80]; a survey of 101 published papers reported that EBVaGC was evident in 7.08% of intestinal type GC, while diffuse type GC had

an incidence of 9.82%[80]. Western and Central Asian countries had significantly more EBV positive cases than South-Eastern countries; in Europe, the frequency of EBV infection ranged from 1.7% in the United Kingdom to 40% in Poland[80].

An *in vitro* model of EBVaGC was used to demonstrate that gastric cells, following EBV infection, have a high IL-1β expression, compared to EBV-negative gastric tumour cells. EBV-positive clones rapidly proliferated and were shown to be anchorage-independent in colony-forming assays[81].

Since EBV infection is highly prevalent in all populations, whilst EBVaGC is quite rare, there were attempts to identify people who run an increased risk of developing GC. Polymorphisms of proinflammatory, as well as anti-inflammatory cytokines were studied, in particular in the promoter regions of *IL-10* and *TNF-α*. For the latter, the allele -308 A (linked to high levels of TNF-α) had significantly higher frequency among EBVaGC individuals (23.3%) when compared to control subjects (12.0%, $P < 0.05$). The opposite was found in the case of the anti-inflammatory *IL-10*: The high-producer allele (-1082 G) was found to be less frequent in EBVaGC patients in comparison to controls (6.3% *vs* 3.0%, $P < 0.05$)[39,72].

The extreme complexities of all these interactions can explain the great variability in data when investigating the possible correlation between cytokine haplotypes and GC.

Gleanings on the usefulness of characterising H. pylori infected individuals for inflammatory haplotype

In addition to the complexity of this subject, the expectations created by the assertion that the host's factors could contribute to the development of GC are disappointing, at least as far as the host's inflammatory response to *H. pylori* infection is concerned. Once we get into details, we realize that in fact, the only determinant that really matters is the infection. The examination of the scientific literature on the cytokine subject has led to contradictory results: For each cytokine, the observations made by studying Caucasian people cannot be applied tout course to Asians; in certain cases, we get opposite results. In the different surveys, one can find association of determined haplotypes of inflammatory cytokines with an increased risk of GC, the opposite, or nil. Even the results of meta-analyses do not agree one another, according to whether studies are carried out by Chines or researchers from other nations. What does it mean? Is it because cytokines are not the final effectors, as they principally work on the long and winding road paved by the broad NF-κB family, which leads to GC (which means that the final response to inflammatory stimuli is far from hitting its target)? And what about the observations that people suffering from diseases far more inflammatory than chronic gastritis, such as rheumatoid arthritis, are likely protected from developing GC[82].

These observations may suggest that, if host factors are important in GC development, they probably have to be sought outside of the genes encoding the inflammatory cytokines.

NOT ALL PATIENTS ARE ALIKE: MOLECULAR BIOLOGY

The recent advances of molecular biological techniques allowed researchers to reach important insights into the oncogenesis mechanisms in gastric cancer. Besides the well-known pathogenic factor, *H. pylori*, several oncogenes and tumour suppressor genes, including cell cycle regulation genes involved in the growth and signal transduction pathways, have been identified[83-85]. In particular, alterations of genes involved in signalling pathways deregulation, patterns of aberrant DNA methylation, and chromosomal imbalances have been evidenced[86,87].

CHROMOSOMAL INSTABILITY

Chromosomal instability (CIN) represents one of the main type of genomic instability observed in several neoplasms and it has been observed in a large cohort of patients with gastric cancer[88]. In particular, it is commonly detected in gastric malignant tumours and has been shown in up to 84% of gastrointestinal cancers[89].

CIN is characterized by chromosomal anomalies, including gain or loss of the complete chromosome (aneuploidy) and segments of chromosomes (loss of heterozygosity, amplifications and translocations)[90]. These abnormalities can impact on the oncogenes expression, tumour suppressor genes and other genes, as well as those involved in digestion, DNA repair, growth regulation, and control of cell cycle checkpoint[91-93]. The genetic mechanisms leading to CIN are not entirely known; *H. pylori* infection, smoking habit and some chemical substances such as nitrates and nitrites probably have an effect on inducing CIN; anyway their influence is actually uncertain[94]. On the other side, defects of chromosome segregation (CS), imperfect DNA damage response (DDR), anomalies in cell cycle regulators and telomere dysfunction have been identified as factors leading to numerical and structural chromosome alterations[95,96]. These carcinogens may alter chromosomes and the cytoskeleton promoting malignant modification[97].

CS alterations

CS represents an important cellular process inducing the gastric epithelial cells division. Alterations of CS regulating mechanisms can cause DNA alterations or mitotic failures, leading to unfixable mutations as well as chromosomal number alterations[98]. In particular, the three recently proposed ways producing CIN are: Altered expression, polymorphisms and/or mutations of mitotic genes implicated in CS and the carcinogen activity upon

susceptible genetic background of individuals[99,100]. Many authors showed an aberrant expression of mitotic genes in CS. Moreover, the altered expression of BUB1 protein (involved in controlling the spindle assembly checkpoint), was significantly increased in patients with diffuse type gastric adenocarcinoma, but not related to DNA ploidy[89]. Furthermore, in another study, BubR1 and AURKB (proteins involved in the mitotic spindle assembly) expression resulted in association with a low risk of GC progression[101-103]. Aurora kinase A (AURKA/STK15), a cell-cycle-regulated kinase with important role in microtubule formation and stabilization during CS, is often overexpressed in adenocarcinomas of the stomach, showing a suggestive new oncogenic pathway in GC[104].

Defective DDR

The mucosa of the stomach is continually subject to several environmental and intracellular mutagens, like ROS, *H. pylori* infection, nitrates, natrium, nitrites, and other water and food contaminants, able to induce DNA damage through different mechanisms[105,106]. Failure of the most important mechanisms of repair [nucleotide excision repair, base excision repair, mismatch repair (MMR) and recombination and/or DDR] may conduce to CIN and genetic aberrations, favouring carcinogenic process[107,108]. Several studies revealed differential mRNA expression of genes implicated in DNA repair process: *ATM* and *HMGB1* (implicated in base excision repair), *RAD23B* (involved in nucleotide excision repair), *UBE2V2*, *MUS81* [involved in resolving Holliday junctions (a branched DNA structure that contains four double-stranded arms joined together, considered the central intermediate in homologous recombination)], *REV3L* (involved in replication post-DNA damage), *TP53, hHR23A* and *DDB1* (implicated in nucleotide excision repair), and *XRCC1* (implicated in single-strand breaks repair) and *MUTYH* (implicated in base excision repair)[109-113].

H. pylori

H. pylori has been shown to be able to induce DDR and double-strand breaks in gastric cancer with a mechanism of adhesion of bacteria that takes place between Lewis epitopes of the host and BabA adhesin[114]. Anyway, gastric mucosa cells can repair the DNA lesions induced by short-term infections. On the other side, prolonged infections induce saturation of repair mechanisms with a consequent ineffective DNA repair and malignant process begin. Moreover, continued infections lead to chronic inflammation, with resulting increase of mutagenesis rate, oxidative-stress, down-regulation of MMR mechanisms, instability of genes and modulation of apoptosis by means of ROS formation[115-119]. Gastric inflammation represents an important host response able to induce *H. pylori*-related carcinogenesis[120]. In fact, in infected patients with *IL-1β*, *TNF-α*, *IL-10* and *IL-8* polymorphisms, has been observed an increased risk of distal gastric cancer progression[120,121]. Furthermore, different *H. pylori* strains seem to differently increase cancer risk by means of host genotypes[122] as these bacteria are able to communicate with their hosts. The equilibrium is determined both by host and bacterial features and may explain the reason why some *H. pylori* strains augment the carcinogenesis risk. For example, CagA positive strains promote severe gastritis and increase the pro-inflammatory cytokines' level. This may lead to an environment favourable to the growth of other bacteria that can support inflammation and continually induce oxidative stress, increasing the risk for GC[1].

MICROSATELLITE INSTABILITY

Microsatellite instability (MSI) represents a genomic instability commonly detected in almost half of patients with GC. It is often observed in the Lynch syndrome (hereditary non-polyposis colorectal cancer) and in several sporadic cancers[123]. MSI phenotype is characterized by a high replication mistake rate leading to insertions and/or deletions of nucleotides within microsatellite repeats in neoplastic areas[123]. The MMR proteins are able to detect and repair these alterations, causing the dysfunction in *MMR* genes (*MLH1* and *MSH2*) a MSI phenotype's establishment, with a consequently power off of cancer suppressor genes' and loss of heterozygosity[124,125]. To this address, genes that are frequently modified induce cell cycle regulation and apoptosis (*TGFβ RII, RIZ, IGFIIR, TCF4, BAX, FAS, CASPASE5, BCL10* and *APAF1*) or are involved in the maintenance of genomic integrity (*MSH6, MED1, MSH3, BLM, RAD50, ATR*, and *MRE11*)[126].

DEREGULATION OF SIGNALLING TRASDUCTION PATHWAYS

The effects of genomic destabilization consist of aneuploidy and gain or loss of the chromosome tracts involved in mRNA transcription. Genomic alterations can modify the normal cellular biology with a consequent neoplastic switch[127]. The clearly explored pathways that probably are involved in gastric pathogenesis are Wnt/betacatenin, extracellular signal-regulated MAPK, Hedgehog, Notch, NF-κB, TGF-β/BMP pathways, COX2/PGE_2, and tyrosine kinase signalling[128-143].

Finally, several studies evidenced that pathway deregulation involved in systemic inflammatory response, such as IL-11/STAT1/gp130/STAT3, can induce a carcinogenic transformation too[144,145].

CONCLUSION

GC is a multifactorial disease. The main determinant, *H. pylori* infection, can be considered a *sine qua non* for GC development; however, despite almost all individuals who get GC are currently, or have been infected, it

is neither a necessary nor a sufficient condition. The intricacy of this topic resides in the proportion of infected people that will never get GC: 97% to 99%, according to the ethnic groups and geographic areas. Other remarks are unraveling the tangle: Almost all *H. pylori* strains from Japan and East Asia, the areas with the highest incidence of GC, are *cagA*+ and can be considered highly carcinogenic; in addition, the infection by certain *cagA* genotypes in western countries increases by far the risk of GC.

At this point, one may wonder why these strains keep on infecting people. Following along with the evolution, only the characteristics that provide a selective advantage continue to be transmitted; this is a basic rule in eukaryotic and prokaryotic worlds. What is the benefit of being infected by carcinogenic strains? Why do they not disappear? People infected by strains that multiply the risk of GC by many times over, cannot be considered advantaged. Possible answers could reside in the following observations: (1) 97% to 99% of people never acquire GC; (2) the development of the sequence gastritis-metaplasia-dysplasia-cancer takes 40 years, or more, after the infection; which means that all women, as well as men, are fertile before the age at which GC occurs (many men also in old age, but they are less important, statistically speaking); hence, fertility is not affected by cancer development; and (3) women, who develop GC far less frequently than men, are the necessary genetic traits holders. Could these answers satisfy the laws of evolution (or distract them)? And how can the occurrence of GC in younger and younger ages be explained?

Apart from the complexity of this subject, the prospect created by the assertion that the host's factors, such as the way the host reacts to infectious stimuli, may be important in the development of GC is discouraging. Despite cytokines involved in the inflammatory response to infection, there are more than 30, just over half a dozen that have been examined in the relationship of *H. pylori* infection with GC and only haplotypes of *IL-1* and *TNF-α* genes were found to possibly increase the GC risk, but only if the ethnicity of patients is not considered. Pursuing this line of inquiry makes us run the risk of sounding racist.

The hypothesis that the dissection of oncogenes and tumour suppressing genes could provide us with an answer to the question whether host factors are important in GC development has only been partly proved. However, the conclusions have led us to a starting point, that is, they ended to indirectly confirm the pathogenic role of strains expressing CagA. The local and systemic levels of substances endowed with an increased mutagenic potential, ROS, generated by immunocytes, and the consequent DNA damage are far higher when the infecting organisms harbour the *cag* PAI.

In conclusion, as regards the development of GC, not all the *H. pylori* strains and patients are alike and not all share the same responsibility, but the only determinant that really matters is the infection.

REFERENCES

1 **Peek RM**, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; **2**: 28-37 [PMID: 11902583 DOI: 10.1038/nrc703]

2 **International Agency for Research on Cancer**. IARC monographs on the evaluation of the carcinogenic risks to humans. Schistosomes, liver flukes and Helicobacter pylori. Lyon: International Agency for Research on Cancer, 1994; **61**: 177

3 **Odenbreit S**, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; **287**: 1497-1500 [PMID: 10688800 DOI: 10.1126/science.287.5457.1497]

4 **Wang X**, Wei M, Sun Z. An association study of histological types of gastric carcinoma with Helicobacter pylori infection. *Cell Biochem Biophys* 2014; **70**: 1283-1287 [PMID: 24898806 DOI: 10.1007/s12013-014-0052-z]

5 **Covacci A**, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N, Rappuoli R. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993; **90**: 5791-5795 [PMID: 8516329 DOI: 10.1073/pnas.90.12.5791]

6 **Censini S**, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996; **93**: 14648-14653 [PMID: 8962108 DOI: 10.1073/pnas.93.25.14648]

7 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115 [PMID: 7743510]

8 **Parsonnet J**, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. *Gut* 1997; **40**: 297-301 [PMID: 9135515 DOI: 10.1136/gut.40.3.297]

9 **Enroth H**, Kraaz W, Engstrand L, Nyrén O, Rohan T. Helicobacter pylori strain types and risk of gastric cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 981-985 [PMID: 11008919]

10 **Leunk RD**, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. *J Med Microbiol* 1988; **26**: 93-99 [PMID: 3385767 DOI: 10.1099/00222615-26-2-93]

11 **Figura N**, Guglielmetti P, Rossolini A, Barberi A, Cusi G, Musmanno RA, Russi M, Quaranta S. Cytotoxin production by Campylobacter pylori strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. *J Clin Microbiol* 1989; **27**: 225-226 [PMID: 2913034]

12 **Figura N**, Bugnoli M, Cusi MG, Pucci AM, Lusini P, Quaranta S, Barberi A, Rossolini A, Di Tommaso A, De Magistris T, Rappuoli R, Marri L, Musmanno RA, Russi M, Guarna M, Losi M. Pathogenic mechanisms of Helicobacter pylori: production of cytotoxin. In: Malfertheiner P, Ditschuneit H. Helicobacter pylori, Gastritis and Peptic Ulcer. Berlin Heidelberg Springer Verlag, 1990: 86-95 [DOI: 10.1007/978-3-642-75315-2_13]

13 **Crabtree JE**, Figura N, Taylor JD, Bugnoli M, Armellini D, Tompkins DS. Expression of 120 kilodalton protein and cytotoxicity in Helicobacter pylori. *J Clin Pathol* 1992; **45**: 733-734 [PMID: 1401190 DOI: 10.1136/jcp.45.8.733]

14 **Crabtree JE**, Wyatt JI, Sobala GM, Miller G, Tompkins DS, Primrose JN, Morgan AG. Systemic and mucosal humoral responses to Helicobacter pylori in gastric cancer. *Gut* 1993; **34**: 1339-1343 [PMID: 8244098 DOI: 10.1136/gut.34.10.1339]

15 **Souza RC**, del Rosario Quispe Saji G, Costa MO, Netto DS, Lima NC, Klein CC, Vasconcelos AT, Nicolás MF. AtlasT4SS: a curated

database for type IV secretion systems. *BMC Microbiol* 2012; **12**: 172 [PMID: 22876890 DOI: 10.1186/1471-2180-12-172]
16 **Figura N**, Vindigni C, Pinto E, Gennari C, Presenti L, Tosi P, Roviello F. Gastric cancer, H. pylori (HP) infection, serum antibodies to CagA. *Gut* 1996; **39** suppl 2; A17
17 **Ogura K**, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M. Virulence factors of Helicobacter pylori responsible for gastric diseases in Mongolian gerbil. *J Exp Med* 2000; **192**: 1601-1610 [PMID: 11104802 DOI: 10.1084/jem.192.11.1601]
18 **Figura N**, Valassina M, Moretti E, Vindigni C, Collodel G, Iacoponi F, Giordano N, Roviello F, Marrelli D. Histological variety of gastric carcinoma and Helicobacter pylori cagA and vacA polymorphism. *Eur J Gastroenterol Hepatol* 2015; **27**: 1017-1021 [PMID: 26067222 DOI: 10.1097/MEG.0000000000000414]
19 **Higashi H**, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci USA* 2002; **99**: 14428-14433 [PMID: 12391297 DOI: 10.1073/pnas.222375399]
20 **Higashi H**, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. *Science* 2002; **295**: 683-686 [PMID: 11743164 DOI: 10.1126/science.1067147]
21 **Hatakeyama M**, Higashi H. Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. *Cancer Sci* 2005; **96**: 835-843 [PMID: 16367902 DOI: 10.1111/j.1349-7006.2005.00130.x]
22 **Buti L**, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL. Helicobacter pylori cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. *Proc Natl Acad Sci USA* 2011; **108**: 9238-9243 [PMID: 21562218 DOI: 10.1073/pnas.1106200108]
23 **Yamaoka Y**, Kodama T, Kashima K, Graham DY, Sepulveda AR. Variants of the 3' region of the cagA gene in Helicobacter pylori isolates from patients with different H. pylori-associated diseases. *J Clin Microbiol* 1998; **36**: 2258-2263 [PMID: 9666002]
24 **Sepulveda AR**, Dore MP, Gutierrez O, Miehlke S, Go MF, Kim JG, Figura N, Graham DY. Helicobacter pylori CagA proteins of higher size are associated with gastric carcinoma. *Gut* 1997; **41** suppl 1: A112
25 **Yamaoka Y**, El-Zimaity HM, Gutierrez O, Figura N, Kim JG, Kodama T, Kashima K, Graham DY. Relationship between the cagA 3' repeat region of Helicobacter pylori, gastric histology, and susceptibility to low pH. *Gastroenterology* 1999; **117**: 342-349 [PMID: 10419915 DOI: 10.1053/gast.1999.0029900342]
26 **Miura M**, Ohnishi N, Tanaka S, Yanagiya K, Hatakeyama M. Differential oncogenic potential of geographically distinct Helicobacter pylori CagA isoforms in mice. *Int J Cancer* 2009; **125**: 2497-2504 [PMID: 19588494 DOI: 10.1002/ijc.24740]
27 **Figura N**, Valassina M, Roviello F, Pinto F, Lenzi C, Giannace R, Marrelli D, Valentini M, Valensin PE. Helicobacter pylori cagA and vacA types and gastric carcinoma. *Dig Liver Dis* 2000; **32** Suppl 3: S182-S183 [PMID: 11245289 DOI: 10.1016/S1590-8658(00)80272-8]
28 **Figura N**, Moretti E, Roviello F, Papini F, Marrelli D. cagA structural types of Helicobacter pylori strains isolated from patients with gastric carcinoma and chronic gastritis only. *Intern Emerg Med* 2012; **7** Suppl 2: S103-S105 [PMID: 22311514 DOI: 10.1007/s11739-012-0759-z]
29 **Kidd M**, Lastovica AJ, Atherton JC, Louw JA. Heterogeneity in the Helicobacter pylori vacA and cagA genes: association with gastroduodenal disease in South Africa? *Gut* 1999; **45**: 499-502 [PMID: 10486355 DOI: 10.1136/gut.45.4.499]
30 **Ramelah M**, Aminuddin A, Alfizah H, Isa MR, Jasmi AY, Tan HJ, Rahman AJ, Rizal AM, Mazlam MZ. cagA gene variants in Malaysian Helicobacter pylori strains isolated from patients of different ethnic groups. *FEMS Immunol Med Microbiol* 2005; **44**: 239-242 [PMID: 15866222 DOI: 10.1016/j.femsim.2005.02.001]
31 **Hatakeyama M**. Oncogenic mechanisms of the Helicobacter pylori CagA protein. *Nat Rev Cancer* 2004; **4**: 688-694 [PMID: 15343275 DOI: 10.1038/nrc1433]
32 **Argent RH**, Kidd M, Owen RJ, Thomas RJ, Limb MC, Atherton JC. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of Helicobacter pylori. *Gastroenterology* 2004; **127**: 514-523 [PMID: 15300584 DOI: 10.1053/j.gastro.2004.06.006]
33 **Batista SA**, Rocha GA, Rocha AM, Saraiva IE, Cabral MM, Oliveira RC, Queiroz DM. Higher number of Helicobacter pylori CagA EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer. *BMC Microbiol* 2011; **11**: 61 [PMID: 21435255 DOI: 10.1186/1471-2180-11-61]
34 **Acosta N**, Quiroga A, Delgado P, Bravo MM, Jaramillo C. Helicobacter pylori CagA protein polymorphisms and their lack of association with pathogenesis. *World J Gastroenterol* 2010; **16**: 3936-3943 [PMID: 20712055 DOI: 10.3748/wjg.v16.i31.3936]
35 **Crabtree JE**. Gastric mucosal inflammatory responses to Helicobacter pylori. *Aliment Pharmacol Ther* 1996; **10** Suppl 1: 29-37 [PMID: 8730257 DOI: 10.1046/j.1365-2036.1996.22164003.x]
36 **Beales IL**, Calam J. Interleukin 1 beta and tumour necrosis factor alpha inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. *Gut* 1998; **42**: 227-234 [PMID: 9536948]
37 **Strieter RM**, Kunkel SL, Bone RC. Role of tumor necrosis factor-alpha in disease states and inflammation. *Crit Care Med* 1993; **21**: S447-S463 [PMID: 8403983]
38 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402 [PMID: 10746728 DOI: 10.1038/35006081]
39 **Wu MS**, Huang SP, Chang YT, Shun CT, Chang MC, Lin MT, Wang HP, Lin JT. Tumor necrosis factor-alpha and interleukin-10 promoter polymorphisms in Epstein-Barr virus-associated gastric carcinoma. *J Infect Dis* 2002; **185**: 106-109 [PMID: 11756988 DOI: 10.1086/324771]
40 **Machado JC**, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simões M. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology* 2003; **125**: 364-371 [PMID: 12891537 DOI: 10.1016/S0016-5085(03)00899-0]
41 **Hamajima N**, Naito M, Kondo T, Goto Y. Genetic factors involved in the development of Helicobacter pylori-related gastric cancer. *Cancer Sci* 2006; **97**: 1129-1138 [PMID: 16879717 DOI: 10.1111/j.1349-7006.2006.00290.x]
42 **Kamangar F**, Cheng C, Abnet CC, Rabkin CS. Interleukin-1B polymorphisms and gastric cancer risk--a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1920-1928 [PMID: 17035400 DOI: 10.1158/1055-9965.EPI-06-0267]
43 **Camargo MC**, Mera R, Correa P, Peek RM, Fontham ET, Goodman KJ, Piazuelo MB, Sicinschi L, Zabaleta J, Schneider BG. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1674-1687 [PMID: 16985030 DOI: 10.1158/1055-9965.EPI-06-0189]
44 **Wang P**, Xia HH, Zhang JY, Dai LP, Xu XQ, Wang KJ. Association of interleukin-1 gene polymorphisms with gastric cancer: a meta-analysis. *Int J Cancer* 2007; **120**: 552-562 [PMID: 17096351 DOI: 10.1002/ijc.22353]
45 **Gorouhi F**, Islami F, Bahrami H, Kamangar F. Tumour-necrosis factor-A polymorphisms and gastric cancer risk: a meta-analysis. *Br J Cancer* 2008; **98**: 1443-1451 [PMID: 18319718 DOI: 10.1038/sj.bjc.6604277]
46 **Vincenzi B**, Patti G, Galluzzo S, Pantano F, Venditti O, Santini D, Ruzzo A, Schiavon G, Caraglia M, Marra M, Graziano F, Tonini G. Interleukin 1beta-511T gene (IL1beta) polymorphism is correlated with gastric cancer in the Caucasian population: results from a

meta-analysis. *Oncol Rep* 2008; **20**: 1213-1220 [PMID: 18949424 DOI: 10.3892/or_00000132]
47 **Peleteiro B**, Lunet N, Carrilho C, Durães C, Machado JC, La Vecchia C, Barros H. Association between cytokine gene polymorphisms and gastric precancerous lesions: systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 762-776 [PMID: 20200422 DOI: 10.1158/1055-9965.EPI-09-0917]
48 **Persson C**, Canedo P, Machado JC, El-Omar EM, Forman D. Polymorphisms in inflammatory response genes and their association with gastric cancer: A HuGE systematic review and meta-analyses. *Am J Epidemiol* 2011; **173**: 259-270 [PMID: 21178102 DOI: 10.1093/aje/kwq370]
49 **Xue H**, Lin B, Ni P, Xu H, Huang G. Interleukin-1B and interleukin-1 RN polymorphisms and gastric carcinoma risk: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 1604-1617 [PMID: 20880168 DOI: 10.1111/j.1440-1746.2010.06428.x]
50 **He B**, Zhang Y, Pan Y, Xu Y, Gu L, Chen L, Wang S. Interleukin 1 beta (IL1B) promoter polymorphism and cancer risk: evidence from 47 published studies. *Mutagenesis* 2011; **26**: 637-642 [PMID: 21653279 DOI: 10.1093/mutage/ger025]
51 **Xue H**, Lin B, An J, Zhu Y, Huang G. Interleukin-10-819 promoter polymorphism in association with gastric cancer risk. *BMC Cancer* 2012; **12**: 102 [PMID: 22436502 DOI: 10.1186/1471-2407-12-102]
52 **Xue H**, Liu J, Lin B, Wang Z, Sun J, Huang G. A meta-analysis of interleukin-8 -251 promoter polymorphism associated with gastric cancer risk. *PLoS One* 2012; **7**: e28083 [PMID: 22279522 DOI: 10.1371/journal.pone.0028083]
53 **Ni P**, Xu H, Xue H, Lin B, Lu Y. A meta-analysis of interleukin-10-1082 promoter polymorphism associated with gastric cancer risk. *DNA Cell Biol* 2012; **31**: 582-591 [PMID: 22335769 DOI: 10.1089/dna.2011.1440]
54 **Xu J**, Yin Z, Cao S, Gao W, Liu L, Yin Y, Liu P, Shu Y. Systematic review and meta-analysis on the association between IL-1B polymorphisms and cancer risk. *PLoS One* 2013; **8**: e63654 [PMID: 23704929 DOI: 10.1371/journal.pone.0063654]
55 **Pan XF**, Yang SJ, Loh M, Xie Y, Wen YY, Tian Z, Huang H, Lan H, Chen F, Soong R, Yang CX. Interleukin-10 gene promoter polymorphisms and risk of gastric cancer in a Chinese population: single nucleotide and haplotype analyses. *Asian Pac J Cancer Prev* 2013; **14**: 2577-2582 [PMID: 23725178]
56 **Rokkas T**. Answer to Professor Kountouras's letter. *Eur J Gastroenterol Hepatol* 2014; **26**: 123-124 [PMID: 24280805 DOI: 10.1097/MEG.0b013e3283657e0f]
57 **Cen G**, Wu W. Association between tumor necrosis factor-alpha 857C/T polymorphism and gastric cancer: a meta-analysis. *Tumour Biol* 2013; **34**: 3383-3388 [PMID: 23821300 DOI: 10.1007/s13277-013-0910-0]
58 **Yu JY**, Li L, Ma H, Liu K, Cheng X, Li YL, Song XL. Tumor necrosis factor-α 238 G/A polymorphism and gastric cancer risk: a meta-analysis. *Tumour Biol* 2013; **34**: 3859-3863 [PMID: 23900678 DOI: 10.1007/s13277-013-0972-z]
59 **Zhu F**, Zhao H, Tian X, Meng X. Association between tumor necrosis factor-α rs1800629 polymorphism and risk of gastric cancer: a meta-analysis. *Tumour Biol* 2014; **35**: 1799-1803 [PMID: 24142527 DOI: 10.1007/s13277-013-1240-y]
60 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545 [PMID: 11229684 DOI: 10.1016/S0140-6736(00)04046-0]
61 **Pociot F**, Mølvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest* 1992; **22**: 396-402 [PMID: 1353022 DOI: 10.1111/j.1365-2362.1992.tb01480.x]
62 **Chen H**, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C, Rogus J, Beck JD, Offenbacher S, Cork MJ, Rafie-Kolpin M, Hsieh CM, Kornman KS, Duff GW. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Hum Mol Genet* 2006; **15**: 519-529 [PMID: 16399797 DOI: 10.1093/hmg/ddi469]
63 **Santtila S**, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 1998; **47**: 195-198 [PMID: 9519856 DOI: 10.1046/j.1365-3083.1998.00300.x]
64 **Mosser DM**, Zhang X. Interleukin-10: new perspectives on an old cytokine. *Immunol Rev* 2008; **226**: 205-218 [PMID: 19161426 DOI: 10.1111/j.1600-065X.2008.00706.x]
65 **Loh M**, Koh KX, Yeo BH, Song CM, Chia KS, Zhu F, Yeoh KG, Hill J, Iacopetta B, Soong R. Meta-analysis of genetic polymorphisms and gastric cancer risk: variability in associations according to race. *Eur J Cancer* 2009; **45**: 2562-2568 [PMID: 19375306 DOI: 10.1016/j.ejca.2009.03.017]
66 **Taguchi A**, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, Niwa Y, Goto H. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 2487-2493 [PMID: 16284368 DOI: 10.1158/1055-9965.epi-05-0326]
67 **Liu L**, Zhuang W, Wang C, Chen Z, Wu XT, Zhou Y. Interleukin-8 -251 A/T gene polymorphism and gastric cancer susceptibility: a meta-analysis of epidemiological studies. *Cytokine* 2010; **50**: 328-334 [PMID: 20363644 DOI: 10.1016/j.cyto.2010.03.008]
68 **Orosz P**, Echtenacher B, Falk W, Rüschoff J, Weber D, Männel DN. Enhancement of experimental metastasis by tumor necrosis factor. *J Exp Med* 1993; **177**: 1391-1398 [PMID: 8478614 DOI: 10.1084/jem.177.5.1391]
69 **Wu S**, Boyer CM, Whitaker RS, Berchuck A, Wiener JR, Weinberg JB, Bast RC. Tumor necrosis factor alpha as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor alpha expression. *Cancer Res* 1993; **53**: 1939-1944 [PMID: 8385577]
70 **Moore RJ**, Owens DM, Stamp G, Arnott C, Burke F, East N, Holdsworth H, Turner L, Rollins B, Pasparakis M, Kollias G, Balkwill F. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat Med* 1999; **5**: 828-831 [PMID: 10395330 DOI: 10.1038/10552]
71 **Xu Z**, Shi R, Zhang R, Zhang D, Wang L. Association between tumor necrosis factor β 252 A/G polymorphism and risk of gastric cancer: a meta-analysis. *Tumour Biol* 2013; **34**: 4001-4005 [PMID: 23904263 DOI: 10.1007/s13277-013-0989-3]
72 **zur Hausen A**, Crusius JB, Murillo LS, Alizadeh BZ, Morré SA, Meijer CJ, van den Brule AJ, Peña AS. IL-1B promoter polymorphism and Epstein-Barr virus in Dutch patients with gastric carcinoma. *Int J Cancer* 2003; **107**: 866-867 [PMID: 14566840 DOI: 10.1002/ijc.11468]
73 **Grivennikov S**, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 2009; **15**: 103-113 [PMID: 19185845 DOI: 10.1016/j.ccr.2009.01.001]
74 **Liu RY**, Song X, Chen P, Lei Z, Miao JC, Yi N, Zhang K, Pasche B, Zhang HT. Association between IL6 -174G/C and cancer: A meta-analysis of 105,482 individuals. *Exp Ther Med* 2012; **3**: 655-664 [PMID: 22969947 DOI: 10.3892/etm.2012.454]
75 **Niu YM**, Yuan H, Zhou Y. Interleukin-17 gene polymorphisms contribute to cancer risk. *Mediators Inflamm* 2014; **2014**: 128490 [PMID: 25147431 DOI: 10.1155/2014/128490]
76 **Jackson CB**, Judd LM, Menheniott TR, Kronborg I, Dow C, Yeomans ND, Boussioutas A, Robb L, Giraud AS. Augmented gp130-mediated cytokine signalling accompanies human gastric cancer progression. *J Pathol* 2007; **213**: 140-151 [PMID: 17724739 DOI: 10.1002/path.2218]
77 **Sun Z**, Cui Y, Jin X, Pei J. Association between IL-4 -590C& gt; T polymorphism and gastric cancer risk. *Tumour Biol* 2014; **35**: 1517-1521 [PMID: 24072495 DOI: 10.1007/s13277-013-1209-x]
78 **Barnes PJ**, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; **336**: 1066-1071 [PMID: 9091804 DOI: 10.1056/NEJM199704103361506]

79 **Ghosh S**, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002; **109** Suppl: S81-S96 [PMID: 11983155 DOI: 10.1016/S0092-8674(02)00703-1]

80 **Iizasa H**, Nanbo A, Nishikawa J, Jinushi M, Yoshiyama H. Epstein-Barr Virus (EBV)-associated gastric carcinoma. *Viruses* 2012; **4**: 3420-3439 [PMID: 23342366 DOI: 10.3390/v4123420]

81 **Sousa H**, Pinto-Correia AL, Medeiros R, Dinis-Ribeiro M. Epstein-Barr virus is associated with gastric carcinoma: the question is what is the significance? *World J Gastroenterol* 2008; **14**: 4347-4351 [PMID: 18666324 DOI: 10.3748/wjg.14.4347]

82 **Gridley G**, McLaughlin JK, Ekbom A, Klareskog L, Adami HO, Hacker DG, Hoover R, Fraumeni JF. Incidence of cancer among patients with rheumatoid arthritis. *J Natl Cancer Inst* 1993; **85**: 307-311 [PMID: 8426374 DOI: 10.1093/jnci/85.4.307]

83 **Correia M**, Machado JC, Ristimäki A. Basic aspects of gastric cancer. *Helicobacter* 2009; **14** Suppl 1: 36-40 [PMID: 19712166 DOI: 10.1111/j.1523-5378.2009.00696.x]

84 **Keller G**, Höfler H, Becker KF. Molecular medicine of gastric adenocarcinomas. *Expert Rev Mol Med* 2005; **7**: 1-13 [PMID: 16156904 DOI: 10.1017/S1462399405009592]

85 **David S**, Meltzer SJ. Stomach - Genetic and epigenetic alterations of preneoplastic and neoplastic lesions. *Cancer Biomark* 2010; **9**: 493-507 [PMID: 22112492 DOI: 10.3233/CBM-2011-0169]

86 **Panani AD**. Cytogenetic and molecular aspects of gastric cancer: clinical implications. *Cancer Lett* 2008; **266**: 99-115 [PMID: 18381231 DOI: 10.1016/j.canlet.2008.02.053]

87 **Milne AN**, Carneiro F, O'Morain C, Offerhaus GJ. Nature meets nurture: molecular genetics of gastric cancer. *Hum Genet* 2009; **126**: 615-628 [PMID: 19657673 DOI: 10.1007/s00439-009-0722-x]

88 **Buffart TE**, Louw M, van Grieken NC, Tijssen M, Carvalho B, Ylstra B, Grabsch H, Mulder CJ, van de Velde CJ, van der Merwe SW, Meijer GA. Gastric cancers of Western European and African patients show different patterns of genomic instability. *BMC Med Genomics* 2011; **4**: 7 [PMID: 21226972 DOI: 10.1186/1755-8794-4-7]

89 **Grabsch HI**, Askham JM, Morrison EE, Pomjanski N, Lickvers K, Parsons WJ, Boecking A, Gabbert HE, Mueller W. Expression of BUB1 protein in gastric cancer correlates with the histological subtype, but not with DNA ploidy or microsatellite instability. *J Pathol* 2004; **202**: 208-214 [PMID: 14743503]

90 **Martin SA**, Hewish M, Lord CJ, Ashworth A. Genomic instability and the selection of treatments for cancer. *J Pathol* 2010; **220**: 281-289 [PMID: 19890832 DOI: 10.1002/path.2631]

91 **Kang JU**, Kang JJ, Kwon KC, Park JW, Jeong TE, Noh SM, Koo SH. Genetic alterations in primary gastric carcinomas correlated with clinicopathological variables by array comparative genomic hybridization. *J Korean Med Sci* 2006; **21**: 656-665 [PMID: 16891809 DOI: 10.3346/jkms.2006.21.4.656]

92 **Morohara K**, Nakao K, Tajima Y, Nishino N, Yamazaki K, Kaetsu T, Suzuki S, Tsunoda A, Kawamura M, Aida T, Tachikawa T, Kusano M. Analysis by comparative genomic hybridization of gastric cancer with peritoneal dissemination and/or positive peritoneal cytology. *Cancer Genet Cytogenet* 2005; **161**: 57-62 [PMID: 16080958 DOI: 10.1016/j.cancergencyto.2005.01.007]

93 **Weiss MM**, Kuipers EJ, Postma C, Snijders AM, Pinkel D, Meuwissen SG, Albertson D, Meijer GA. Genomic alterations in primary gastric adenocarcinomas correlate with clinicopathological characteristics and survival. *Cell Oncol* 2004; **26**: 307-317 [PMID: 15623941]

94 **Matysiak-Budnik T**, Mégraud F. Helicobacter pylori infection and gastric cancer. *Eur J Cancer* 2006; **42**: 708-716 [PMID: 16556496]

95 **Pérez de Castro I**, de Cárcer G, Malumbres M. A census of mitotic cancer genes: new insights into tumor cell biology and cancer therapy. *Carcinogenesis* 2007; **28**: 899-912 [PMID: 17259655 DOI: 10.1093/carcin/bgm019]

96 **Gollin SM**. Mechanisms leading to chromosomal instability. *Semin Cancer Biol* 2005; **15**: 33-42 [PMID: 15613286]

97 **Duesberg P**, Li R, Rasnick D, Rausch C, Willer A, Kraemer A, Yerganian G, Hehlmann R. Aneuploidy precedes and segregates with chemical carcinogenesis. *Cancer Genet Cytogenet* 2000; **119**: 83-93 [PMID: 10867141 DOI: 10.1016/S0165-4608(99)00236-8]

98 **Schmit TL**, Ahmad N. Regulation of mitosis via mitotic kinases: new opportunities for cancer management. *Mol Cancer Ther* 2007; **6**: 1920-1931 [PMID: 17620424 DOI: 10.1158/1535-7163.MCT-06-0781]

99 **Duesberg P**, Li R, Fabarius A, Hehlmann R. The chromosomal basis of cancer. *Cell Oncol* 2005; **27**: 293-318 [PMID: 16373963]

100 **Iovino F**, Lentini L, Amato A, Di Leonardo A. RB acute loss induces centrosome amplification and aneuploidy in murine primary fibroblasts. *Mol Cancer* 2006; **5**: 38 [PMID: 16987420 DOI: 10.1186/1476-4598-5-38]

101 **Enjoji M**, Iida S, Sugita H, Ishikawa T, Uetake H, Inokuchi M, Yamada H, Kojima K, Sugihara K. BubR1 and AURKB overexpression are associated with a favorable prognosis in gastric cancer. *Mol Med Rep* 2009; **2**: 589-596 [PMID: 21475871 DOI: 10.3892/mmr_00000142]

102 **Osaki M**, Inoue T, Yamaguchi S, Inaba A, Tokuyasu N, Jeang KT, Oshimura M, Ito H. MAD1 (mitotic arrest deficiency 1) is a candidate for a tumor suppressor gene in human stomach. *Virchows Arch* 2007; **451**: 771-779 [PMID: 17674037 DOI: 10.1007/s00428-007-0470-z]

103 **Wang L**, Yin F, Du Y, Chen B, Liang S, Zhang Y, Du W, Wu K, Ding J, Fan D. Depression of MAD2 inhibits apoptosis and increases proliferation and multidrug resistance in gastric cancer cells by regulating the activation of phosphorylated survivin. *Tumour Biol* 2010; **31**: 225-232 [PMID: 20440596 DOI: 10.1007/s13277-010-0036-6]

104 **Dar AA**, Zaika A, Piazuelo MB, Correa P, Koyama T, Belkhiri A, Washington K, Castells A, Pera M, El-Rifai W. Frequent overexpression of Aurora Kinase A in upper gastrointestinal adenocarcinomas correlates with potent antiapoptotic functions. *Cancer* 2008; **112**: 1688-1698 [PMID: 18311783 DOI: 10.1002/cncr.23371]

105 **Dar AA**, Belkhiri A, El-Rifai W. The aurora kinase A regulates GSK-3beta in gastric cancer cells. *Oncogene* 2009; **28**: 866-875 [PMID: 19060929 DOI: 10.1038/onc.2008.434]

106 **Zheng L**, Wang L, Ajani J, Xie K. Molecular basis of gastric cancer development and progression. *Gastric Cancer* 2004; **7**: 61-77 [PMID: 15224192]

107 **Guo T**, Lee SS, Ng WH, Zhu Y, Gan CS, Zhu J, Wang H, Huang S, Sze SK, Kon OL. Global molecular dysfunctions in gastric cancer revealed by an integrated analysis of the phosphoproteome and transcriptome. *Cell Mol Life Sci* 2011; **68**: 1983-2002 [PMID: 20953656 DOI: 10.1007/s00018-010-0545-x]

108 **Palli D**, Polidoro S, D'Errico M, Saieva C, Guarrera S, Calcagnile AS, Sera F, Allione A, Gemma S, Zanna I, Filomena A, Testai E, Caini S, Moretti R, Gomez-Miguel MJ, Nesi G, Luzzi I, Ottini L, Masala G, Matullo G, Dogliotti E. Polymorphic DNA repair and metabolic genes: a multigenic study on gastric cancer. *Mutagenesis* 2010; **25**: 569-575 [PMID: 20817763 DOI: 10.1093/mutage/geq042]

109 **Hudler P**, Repše S, Juvan R, Komel R. A genomic approach to investigate expression profiles in Slovenian patients with gastric cancer. *Oncol Lett* 2011; **2**: 1003-1014 [PMID: 22866164 DOI: 10.3892/ol.2011.362]

110 **Kim HK**, Choi IJ, Kim CG, Kim HS, Oshima A, Michalowski A, Green JE. A gene expression signature of acquired chemoresistance to cisplatin and fluorouracil combination chemotherapy in gastric cancer patients. *PLoS One* 2011; **6**: e16694 [PMID: 21364753 DOI: 10.1371/journal.pone.0016694]

111 **Wang P**, Tang JT, Peng YS, Chen XY, Zhang YJ, Fang JY. XRCC1 downregulated through promoter hypermethylation is involved in human gastric carcinogenesis. *J Dig Dis* 2010; **11**: 343-351 [PMID: 21091896 DOI: 10.1111/j.1751-2980.2010.00459.x]

112 **Wu F**, Shirahata A, Sakuraba K, Kitamura Y, Goto T, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K. Down-regulation of Mus81 as a potential marker for the malignancy of gastric cancer. *Anticancer Res* 2010; **30**: 5011-5014 [PMID:

21187482]
113 **Kang B**, Guo RF, Tan XH, Zhao M, Tang ZB, Lu YY. Expression status of ataxia-telangiectasia-mutated gene correlated with prognosis in advanced gastric cancer. *Mutat Res* 2008; **638**: 17-25 [PMID: 17928013 DOI: 10.1016/j.mrfmmm.2007.08.013]
114 **Toller IM**, Neelsen KJ, Steger M, Hartung ML, Hottiger MO, Stucki M, Kalali B, Gerhard M, Sartori AA, Lopes M, Müller A. Carcinogenic bacterial pathogen Helicobacter pylori triggers DNA double-strand breaks and a DNA damage response in its host cells. *Proc Natl Acad Sci USA* 2011; **108**: 14944-14949 [PMID: 21896770 DOI: 10.1073/pnas.1100959108]
115 **Baek HY**, Lim JW, Kim H, Kim JM, Kim JS, Jung HC, Kim KH. Oxidative-stress-related proteome changes in Helicobacter pylori-infected human gastric mucosa. *Biochem J* 2004; **379**: 291-299 [PMID: 14711373 DOI: 10.1042/BJ20031208]
116 **Ding SZ**, O'Hara AM, Denning TL, Dirden-Kramer B, Mifflin RC, Reyes VE, Ryan KA, Elliott SN, Izumi T, Boldogh I, Mitra S, Ernst PB, Crowe SE. Helicobacter pylori and H2O2 increase AP endonuclease-1/redox factor-1 expression in human gastric epithelial cells. *Gastroenterology* 2004; **127**: 845-858 [PMID: 15362040 DOI: 10.1053/j.gastro.2004.06.017]
117 **Farinati F**, Cardin R, Russo VM, Busatto G, Franco M, Rugge M. Helicobacter pylori CagA status, mucosal oxidative damage and gastritis phenotype: a potential pathway to cancer? *Helicobacter* 2003; **8**: 227-234 [PMID: 12752735 DOI: 10.1046/j.1523-5378.2003.00149.x]
118 **Machado AM**, Figueiredo C, Touati E, Máximo V, Sousa S, Michel V, Carneiro F, Nielsen FC, Seruca R, Rasmussen LJ. Helicobacter pylori infection induces genetic instability of nuclear and mitochondrial DNA in gastric cells. *Clin Cancer Res* 2009; **15**: 2995-3002 [PMID: 19383819 DOI: 10.1158/1078-0432.CCR-08-2686]
119 **Slomiany BL**, Slomiany A. Helicobacter pylori Induces Disturbances in Gastric Mucosal Akt Activation through Inducible Nitric Oxide Synthase-Dependent S-Nitrosylation: Effect of Ghrelin. *ISRN Gastroenterol* 2011; **2011**: 308727 [PMID: 21991502 DOI: 10.5402/2011/308727]
120 **El-Omar EM**. The importance of interleukin 1beta in Helicobacter pylori associated disease. *Gut* 2001; **48**: 743-747 [PMID: 11358884 DOI: 10.1136/gut.48.6.743]
121 **Crabtree JE**, Shallcross TM, Heatley RV, Wyatt JI. Mucosal tumour necrosis factor alpha and interleukin-6 in patients with Helicobacter pylori associated gastritis. *Gut* 1991; **32**: 1473-1477 [PMID: 1773951]
122 **Figueiredo C**, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinha AF, Quint W, Caldas C, van Doorn LJ, Carneiro F, Sobrinho-Simões M. Helicobacter pylori and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. *J Natl Cancer Inst* 2002; **94**: 1680-1687 [PMID: 12441323 DOI: 10.1093/jnci/94.22.1680]
123 **Ottini L**, Falchetti M, Lupi R, Rizzolo P, Agnese V, Colucci G, Bazan V, Russo A. Patterns of genomic instability in gastric cancer: clinical implications and perspectives. *Ann Oncol* 2006; **17** Suppl 7: vii97-vi102 [PMID: 16760303 DOI: 10.1093/annonc/mdl960]
124 **Buermeyer AB**, Deschênes SM, Baker SM, Liskay RM. Mammalian DNA mismatch repair. *Annu Rev Genet* 1999; **33**: 533-564 [PMID: 10690417 DOI: 10.1146/annurev.genet.33.1.533]
125 **Ottini L**, Falchetti M, Saieva C, De Marco M, Masala G, Zanna I, Paglierani M, Giannini G, Gulino A, Nesi G, Mariani Costantini R, Palli D. MRE11 expression is impaired in gastric cancer with microsatellite instability. *Carcinogenesis* 2004; **25**: 2337-2343 [PMID: 15319296 DOI: 10.1093/carcin/bgh257]
126 **Yamamoto H**, Perez-Piteira J, Yoshida T, Terada M, Itoh F, Imai K, Perucho M. Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. *Gastroenterology* 1999; **116**: 1348-1357 [PMID: 10348818]
127 **Kang DH**, Han ME, Song MH, Lee YS, Kim EH, Kim HJ, Kim GH, Kim DH, Yoon S, Baek SY, Kim BS, Kim JB, Oh SO. The role of hedgehog signaling during gastric regeneration. *J Gastroenterol* 2009; **44**: 372-379 [PMID: 19291354 DOI: 10.1007/s00535-009-0006-1]
128 **García I**, del Casar JM, Corte MD, Allende MT, García-Muñiz JL, Vizoso F. Epidermal growth factor receptor and c-erbB-2 contents in unresectable (UICC R1 or R2) gastric cancer. *Int J Biol Markers* 2003; **18**: 200-206 [PMID: 14535591]
129 **García I**, Vizoso F, Martín A, Sanz L, Abdel-Lah O, Raigoso P, García-Muñiz JL. Clinical significance of the epidermal growth factor receptor and HER2 receptor in resectable gastric cancer. *Ann Surg Oncol* 2003; **10**: 234-241 [PMID: 12679307]
130 **Gencer S**, Şen G, Doğusoy G, Bellı AK, Paksoy M, Yazicioğlu MB. β-Catenin-independent noncanonical Wnt pathway might be induced in gastric cancers. *Turk J Gastroenterol* 2010; **21**: 224-230 [PMID: 20931424]
131 **Hayashi M**, Inokuchi M, Takagi Y, Yamada H, Kojima K, Kumagai J, Kawano T, Sugihara K. High expression of HER3 is associated with a decreased survival in gastric cancer. *Clin Cancer Res* 2008; **14**: 7843-7849 [PMID: 19047113 DOI: 10.1158/1078-0432.CCR-08-1064]
132 **Katoh M**, Kirikoshi H, Terasaki H, Shiokawa K. WNT2B2 mRNA, up-regulated in primary gastric cancer, is a positive regulator of the WNT- beta-catenin-TCF signaling pathway. *Biochem Biophys Res Commun* 2001; **289**: 1093-1098 [PMID: 11741304 DOI: 10.1006/bbrc.2001.6076]
133 **Kim MA**, Lee HS, Lee HE, Jeon YK, Yang HK, Kim WH. EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. *Histopathology* 2008; **52**: 738-746 [PMID: 18397279 DOI: 10.1111/j.1365-2559.2008.03021.x]
134 **Liang B**, Wang S, Zhu XG, Yu YX, Cui ZR, Yu YZ. Increased expression of mitogen-activated protein kinase and its upstream regulating signal in human gastric cancer. *World J Gastroenterol* 2005; **11**: 623-628 [PMID: 15655810 DOI: 10.3748/wjg.v11.i5.623]
135 **Nabais S**, Machado JC, Lopes C, Seruca R, Carneiro F, Sobrinho-Simões M. Patterns of beta-catenin expression in gastric carcinoma: clinicopathological relevance and mutation analysis. *Int J Surg Pathol* 2003; **11**: 1-9 [PMID: 12598910 DOI: 10.1177/106689690301100102]
136 **Song MS**, Park YK, Lee JH, Park K. Induction of glucose-regulated protein 78 by chronic hypoxia in human gastric tumor cells through a protein kinase C-epsilon/ERK/AP-1 signaling cascade. *Cancer Res* 2001; **61**: 8322-8330 [PMID: 11719466]
137 **Sun Y**, Gao X, Liu J, Kong QY, Wang XW, Chen XY, Wang Q, Cheng YF, Qu XX, Li H. Differential Notch1 and Notch2 expression and frequent activation of Notch signaling in gastric cancers. *Arch Pathol Lab Med* 2011; **135**: 451-458 [PMID: 21466361 DOI: 10.1043/2009-0665-OA.1]
138 **Velho S**, Corso G, Oliveira C, Seruca R. KRAS signaling pathway alterations in microsatellite unstable gastrointestinal cancers. *Adv Cancer Res* 2010; **109**: 123-143 [PMID: 21070916 DOI: 10.1016/B978-0-12-380890-5.00004-1]
139 **Yk W**, Cf G, T Y, Z C, Xw Z, Xx L, Nl M, Wz Z. Assessment of ERBB2 and EGFR gene amplification and protein expression in gastric carcinoma by immunohistochemistry and fluorescence in situ hybridization. *Mol Cytogenet* 2011; **4**: 14 [PMID: 21689422 DOI: 10.1186/1755-8166-4-14]
140 **Soutto M**, Belkhiri A, Piazuelo MB, Schneider BG, Peng D, Jiang A, Washington MK, Kokoye Y, Crowe SE, Zaika A, Correa P, Peek RM, El-Rifai W. Loss of TFF1 is associated with activation of NF-κB-mediated inflammation and gastric neoplasia in mice and humans. *J Clin Invest* 2011; **121**: 1753-1767 [PMID: 21490402 DOI: 10.1172/JCI43922]
141 **Hsu PI**, Hsieh HL, Lee J, Lin LF, Chen HC, Lu PJ, Hsiao M. Loss of RUNX3 expression correlates with differentiation, nodal metastasis, and poor prognosis of gastric cancer. *Ann Surg Oncol* 2009; **16**: 1686-1694 [PMID: 19290488 DOI: 10.1245/s10434-009-0428-2]
142 **Byun DS**, Cho K, Ryu BK, Lee MG, Park JI, Chae KS, Kim HJ, Chi SG. Frequent monoallelic deletion of PTEN and its reciprocal associatioin with PIK3CA amplification in gastric carcinoma. *Int J*

Cancer 2003; **104**: 318-327 [PMID: 12569555]
143 **Deng JY**, Sun D, Liu XY, Pan Y, Liang H. STAT-3 correlates with lymph node metastasis and cell survival in gastric cancer. *World J Gastroenterol* 2010; **16**: 5380-5387 [PMID: 21072904]
144 **Merchant JL**. What lurks beneath: IL-11, via Stat3, promotes inflammation-associated gastric tumorigenesis. *J Clin Invest* 2008; **118**: 1628-1631 [PMID: 18431518 DOI: 10.1172/JCI35344]
145 **Fan XY**, Hu XL, Han TM, Wang NN, Zhu YM, Hu W, Ma ZH, Zhang CJ, Xu X, Ye ZY, Han CM, Pan WS. Association between RUNX3 promoter methylation and gastric cancer: a meta-analysis. *BMC Gastroenterol* 2011; **11**: 92 [PMID: 21867527 DOI: 10.1186/1471-230X-11-92]

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REVIEW

State of the art biological therapies in pancreatic cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with a five-year survival rate of approximately 5%. Several target agents have been tested in PDAC, but almost all have failed to demonstrate efficacy in late phase clinical trials, despite the better understanding of PDAC molecular biology generated by large cancer sequencing initiatives in the past decade. Eroltinib (a small-molecule tyrosine-kinase inhibitor of epidermal growth factor receptor) plus gemcitabine is the only schedule with a biological agent approved for advanced pancreatic cancer, but it has resulted in a very modest survival benefit in unselected patients. In our work, we report a summary of the main clinical trials (closed and ongoing) that refer to biological therapy evaluation in pancreatic cancer treatment.

Key words: Pancreatic cancer; Molecular characterization; Targeted therapy; Epidermal growth factor receptor inhibitors; Embryonic pathway inhibitors; Antiangiogenic therapies

Core tip: Our study aims to give an overview of the progress made in molecular targeted therapy for pancreatic cancer in recent years and the current status of clinical trials in the field. Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with a five-year survival rate of approximately 5%. Several target agents have been tested in PDAC but almost all have failed to demonstrate efficacy in late phase clinical trials, even with a better understanding of PDAC molecular biology generated by large cancer sequencing initiatives in the past decade. Eroltinib (small-molecule tyrosine-kinase inhibitor of epidermal growth factor receptor) plus gemcitabine is actually the only schedule

with a biological agent approved for advanced pancreatic cancer, but it resulted in a very modest survival benefit in unselected patients. In our work, we reported a summary of the main clinical trials (close and ongoing) that refer to biological therapy evaluation in pancreatic cancer treatment.

Di Marco M, Grassi E, Durante S, Vecchiarelli S, Palloni A, Macchini M, Casadei R, Ricci C, Panzacchi R, Santini D, Biasco G. State of the art biological therapies in pancreatic cancer. *World J Gastrointest Oncol* 2016; 8(1): 55-66 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i1/55.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i1.55

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies, representing the fourth leading cause of cancer death. The five-year survival rate is approximately 5%, and surgery remains the most effective treatment[1].

Unfortunately, only 20% of patients are suitable for radical resection, and recurrence of disease occurs in 80% of patients who undergo resection[2].

The most important improvement concerns the conventional chemotherapy, represented by FOLFIRINOX and gemcitabine plus nab-paclitaxel regimens, but it results in a modest outcome advantage[3,4].

No significant progress has been made in the field of targeted therapy. Eroltinib [a small-molecule tyrosine-kinase inhibitor of epidermal growth factor receptor (EGFR)] plus gemcitabine is actually the only schedule with a biological agent approved for pancreatic cancer, but it results in a very modest survival benefit in unselected patients[5].

In recent decades, several combinations of classic chemotherapy and novel biological agents have been studied, but they have not improved overall survival, and furthermore, those trials did not use biomarkers to select responder patients[6].

Our study aims to give an overview of the progress made in molecularly targeted therapy for pancreatic cancer in recent years and the current status of clinical trials in the field, as summarized in Tables 1-3.

MOLECULAR CHARACTERIZATION OF PDAC: HAS A BETTER UNDERSTANDING OF THE TUMOUR'S MOLECULAR BIOLOGY REALLY IMPROVED TARGETED THERAPY APPLICATIONS?

Large cancer sequencing initiatives generated a large quantity of data in the past decades. Those findings showed a complex genomic landscape characterized particularly by inter-tumoural and intra-tumoural heterogeneity involving genomic aberration[7].

With the exception of the well-known KRAS, TP53, CDKN2A and SMAD4 alterations occurring at respective frequencies of 71%, 49%, 22% and 20%, a large number of genomic rearrangements with mutational frequencies less than 2% were found[8,9].

The majority of single gene mutations in pancreatic cancer can be grouped into common cellular pathways. Jones *et al*[10] identified 69 mutated gene sets in most of the 24 samples analysed in their pioneering sequencing study, of which 31 could be grouped into 12 core signalling pathways. These pathways included KRAS signalling, the transforming growth factor β (TGF-β) pathway, DNA damage control, apoptosis, regulation of G1/S cell cycle transition, Hedgehog signalling, the homophilic cell adhesion pathway, integrin signalling, TGF-β signalling, Wnt/Notch signalling, and the invasion pathway[10].

Genomic heterogeneity, a characteristic of PDAC, implies genomic instability, which is due to the acquisition of telomere dysfunction and abnormal cell-cycle control occurring predominantly in early cancer stages, but it persists after cancer dissemination, resulting in parallel evolution among different metastases. Cell clones arranging metastasis may require other driver mutations compared with primary tumour cells implementing genetic variation in pancreatic cancer[11,12].

Given this molecular complexity, it is very difficult to separate passenger from driver mutations, to identify molecular mutations with a crucial role in pancreatic carcinogenesis that can be developed into actionable molecular targets of novel biological agents or to identify patients potentially responsive to existing agents already approved for human use in other cancers (Figure 1), and currently no predictive or prognostic biological factors are employed in clinical practice.

TARGETED THERAPY IN PDAC

EGFR pathway inhibitors

EGFR is a transmembrane receptor member of the ErbB family with a tyrosine kinase domain that is activated by many ligands including epidermal growth factor (EGF), TGF-α, heparin-binding EGF, amphiregulin, epiregulin, betacellulin and neuregulin (an epidermal growth factor). EGFR is involved in cell cycle regulation, cell survival, adhesion and differentiation through activation of the Ras/MAP kinase, phosphatidylinositol 3'-kinase (PI3K)/Akt, Janus kinase/Stat and phospholipase C/protein kinase C pathways. Several trials showed that EGFR is overexpressed in up to 90% of pancreatic cancer samples. Therefore, inhibitors targeting EGFR have been considered a promising therapeutic agent[13].

Eroltinib is a tyrosine kinase inhibitor (TKI) molecule that competes with ATP for binding to the kinase domain, thereby blocking downstream signal transduction. A possible therapeutic role was evaluated in a large phase Ⅲ trial, enrolling 569 chemotherapy naïve patients with locally advanced or metastatic pancreatic

Table 1 Principal phase Ⅲ clinical trials involving targeted therapy in pancreatic cancer

Agent	Target pathway	Treatment	Setting	*n*	mOS (mo)	PFS (mo)	FDA approval	Ref.
Erlotinib	EGFR signaling	GEM plus erlotinib *vs* GEM plus P	M/LA	569	6.24 *vs* 5.91 (*P* = 0.038)	3.75 *vs* 3.55 (*P* = 0.004)	Yes	[5]
Cetuximab	EGFR signaling	GEM plus cetuximab *vs* GEM	M/LA	766	6.5 *vs* 6 (*P* = 0.14)	3.5 *vs* 3 (*P* = 0.058)	No	[17]
Tipifarnib	KRAS pathway	GEM plus tipifarnib *vs* GEM	M/LA	688	6.3 *vs* 6 (*P* = 0.75)	3.7 *vs* 3.6 (*P* = 0.72)	No	[30]
Ganitumab	IGFR pathway	GEM plus ganitumab (12 mg/kg or 20 mg/kg) *vs* GEM plus P	M	800	12 mg/kg arm 7.0 *vs* 7.2 (*P* = 0.494) 60 mg/kg arm 7.1 *vs* 7.2 (*P* = 0.397)	12 mg/kg arm 3.7 *vs* 3.6 (*P* = 0.520) 60 mg/kg arm 3.7 *vs* 3.7 (*P* = 0.403)	No	[35]
Bevacizumab	Angiogenesis	GEM plus bevacizumab *vs* GEM plus P	M/LA	602	5.7 *vs* 6.0 (*P* = 0.40)	4.8 *vs* 4.3 (*P* = 0.99)	No	[36]
Aflibercept	Angiogenesis	GEM plus aflibercept *vs* GEM plus P	M/LA	546	6.5 *vs* 7.8 (*P* = 0.203)	3.7 *vs* 3.7 (*P* = 0.864)	No	[38]
Axitinib	Angiogenesis	GEM plus axitinib *vs* GEM plus P	M/LA	632	8.5 *vs* 8.2 (*P* = 0.543)	4.4 *vs* 4.4 (*P* = 0.520)	No	[41]
Marimastat	Tumor stroma	GEM plus marimastat *vs* GEM	M/LA	239	5.4 *vs* 5.4 (NA)	3 *vs* 3.1 (NA)	No	[75]

GEM: Gemcitabine; P: Placebo; mOS: Median overall survival; PFS: Progression free survival; *n*: Number of patients enrolled; LA: Locally advanced cancer; M: Metastatic cancer; NA: Not available.

Table 2 Principal phase Ⅱ clinical trials involving targeted therapy in pancreatic cancer

Agent	Target pathway	Treatment	Setting	*n*	Ref.
Cetuximab	EGFR signaling	GEM plus cisplatin plus cetuximab *vs* GEM plus cisplatin	M/LA	84	[16]
Gefitinib	EGFR signaling	GEM plus gefitinib (single arm)	M/LA	57	[18]
Trastuzumab	EGFR signaling	GEM plus trastuzumab (single arm)	M/LA 2+/3+ HER-2 expression	34	[20]
Trastuzumab	EGFR signaling	Capecitabine plus trastuzumab (single arm)	M/LA 3+ HER-2 expression or gene amplification	17 (212 screened)	[21]
Nimotuzumab	EGFR signaling	GEM plus nimotuzumab (single arm)	M/LA	18	[23]
Nimotuzumab	EGFR signaling	Nimotuzumab monotherapy (single arm)	Refractory to first line standard chemotherapy M/LA	56	[24]
Selumetinib	KRAS/MEK pathway	Capecitabine plus selumetinib *vs* Capecitabine	Refractory to first line standard chemotherapy M/LA	70	[31]
Trametinib	KRAS/MEK pathway	GEM plus trametinib *vs* GEM plus P	M/LA	160	[32]
Sorafenib	Angiogenesis	GEM plus sorafenib (single arm)	M/LA	70	[40]
RO4929097	Hedgehog signaling	RO4929097 monotherapy (single arm)	Refractory to first line standard chemotherapy M	18	[57]
Everolimus	mTOR pathway	Everolimus plus capecitabine (single arm)	M/LA	31	[67]

GEM: Gemcitabine; P: Placebo; *n*: Number of patients enrolled; LA: Locally advanced cancer; M: Metastatic cancer.

adenocarcinoma randomized to receive gemcitabine plus placebo or gemcitabine plus erlotinib 100-150 mg daily. The median overall survival (mOS) and progression free survival (PFS) were modestly, but statistically significantly, improved in the combination arm, 6.24 mo *vs* 5.91 mo (*P* = 0.038) and 3.75 mo *vs* 3.55 mo (*P* = 0.004), respectively[5].

Neither EGFR status nor KRAS status analysed in the subgroup of patients treated with erlotinib was shown to be predictive of a survival benefit in patients receiving the combination schedule[14].

Erlotinib has been approved by the FDA in combination with gemcitabine as a first-line treatment for advanced pancreatic adenocarcinoma.

Cetuximab is a monoclonal antibody binding the extracellular domain of EGFR. After encouraging results

Table 3 Principal ongoing trials involving targeted therapy in pancreatic cancer

ClinicalTrials.gov identifier	Agent	Target	Status
NCT01728818	Afatinib	EGFR signaling	Recruiting
NCT01659502	TL-118	Angiogenesis	Not yet recruiting
NCT01621243	Necuparanib	Angiogenesis	Recruiting
NCT01088815	Vismodegib	Hedgehog signaling	Recruiting
NCT01096732	Vismodegib	Hedgehog signaling	Terminated
NCT01431794	LDE-225	Hedgehog signaling	Recruiting
NCT00515866	KU-0059436	PARP inhibitor	Completed
NCT01585805	Veliparib	PARP inhibitor	Recruiting
NCT01571024	BKM120	mTOR and PI3K/Akt pathway	Recruiting
NCT01028495	RX-0201	mTOR and PI3K/Akt pathway	Completed
NCT01337765	BEZ235 + MEK162	mTOR and PI3K/Akt pathway	Completed
NCT00560963	Everolimus	mTOR pathway	Completed
NCT00075647	Temsirolimus	mTOR pathway	Completed
NCT01839487	PEGPH20	Tumor stroma	Recruiting

EGFR: Epidermal growth factor receptor; mTOR: Mammalian target of rapamycin.

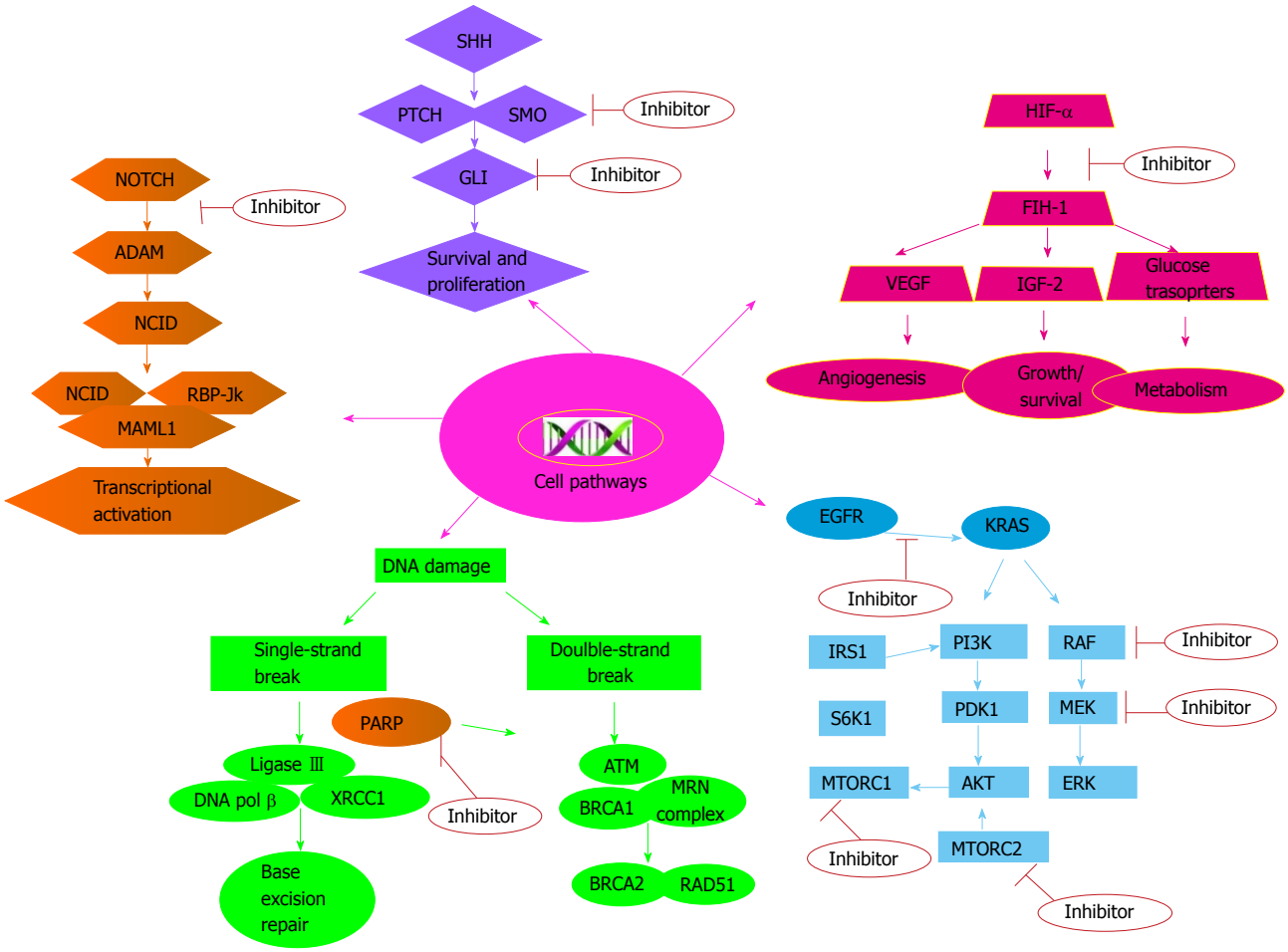

Figure 1 Principal cell signaling pathways involved in pancreatic ductal adenocarcinoma carcinogenesis and actionable molecular targets. SHH: Sonic hedgehog; PARP: Poly ADP-ribose polymerase; VEGF: Vascular endothelial growth factor; IGF: Insulin like growth factor; EGFR: Epidermal growth factor receptor; PI3K: Phosphatidylinositol 3'-kinase.

in a phase Ⅰ trial, subsequent studies in association with gemcitabine-based chemotherapy have failed to demonstrate any survival benefit[15,16].

A phase Ⅱ study has evaluated the possible therapeutic role of gefitinib, a competitive inhibitor of ATP binding to the intracellular kinase domain of EGFR, in combination with gemcitabine in inoperable or metastatic pancreatic cancer patients. The combination demonstrated promising activity with a mOS and PFS in the combination arm of 7.3 and 4.1 mo, respectively, but other evidence supporting a role of gefitinib in PDAC treatment is lacking[17].

Another ErbB family of transmembrane tyrosine kinase receptors is HER-2, which is overexpressed in 11% of pancreatic adenocarcinoma cases. HER2-positive status has also been correlated with shorter survival[18].

Trastuzumab plus gemcitabine was tested in 34 metastatic pancreatic cancer patients with HER-2 overexpression as determined by immunohistochemistry, and partial responses were observed in 6% of cases[19]. Harder *et al*[20] in a multicentre phase Ⅱ study, investigated the efficacy and toxicity of the HER2 antibody, trastuzumab, plus capecitabine in patients with pancreatic cancer and HER2 overexpression, but this treatment did not perform favourably with respect to either PFS or OS compared with standard chemotherapy.

After FDA approval of lapatinib, clinical trials have been initiated to test the effect of this HER-2 inhibitor combined with chemotherapy in pancreatic carcinoma. In particular, lapatinib was tested in combination with capecitabine as a second-line treatment in advanced pancreatic cancer with promising preliminary results. Further studies are needed to evaluate the real effectiveness and role of this molecule in the treatment of PADC[21].

Nimotuzumab, another anti-EGFR monoclonal antibody, showed promising results[22]. In a phase Ⅱ trial where advanced pancreatic cancer patients were randomized to receive second-line monotherapy with nimotuzumab, Strumberg *et al*[23] showed PFS after 1 year of 10.3% and median overall survival of 18.1 wk with a tolerable toxicity profile.

Based on preclinical evidence, afatinib, an inhibitor of EGFR, HER2 and HER4, is under evaluation in an ongoing phase Ⅱ trial[24,25].

The KRAS pathway and downstream signalling cascade inhibitors

KRAS activating mutations are present in 70% to 90% of cases of pancreatic cancer. K-Ras is a GTPase protein belonging to the Ras protein family, which has oncogenic activity, and gain-of-function mutations resulting in constitutive activation promote proliferation and inhibit apoptosis through the RAF/MEK/ERK and PIK3/AKT pathways. K-Ras is very difficult to target, and no inhibitors are actually available to use in clinical practice[26].

Preclinical study has shown that farnesylation is an important post-translational modification required for Ras activation, allowing the protein to be attached to the plasma membrane for signal transduction[27].

After promising results in terms of anti-proliferative activity in pancreatic tumour cell lines, farnesyl-transferase inhibitors, particularly tipifarnib, failed to improve overall survival either as a single agent or in combination with gemcitabine in a phase Ⅲ trial[28,29].

Due to the difficulty of targeting Ras directly, a possible solution could be to block targets downstream of KRAS, such as the protein kinase MEK. Selumetinib is an oral small molecule that inhibits MEK1/2. In a phase Ⅱ trial, patients were randomized to receive single-agent capecitabine or selumetinib as a second-line treatment for advanced pancreatic cancer. The selumetinib arm showed a median overall survival of 5.4 mo *vs* 5.0 mo in the capecitabine arm, but this result was not statistically significant[30].

Another MEK1/2 inhibitor, trametinib, was tested in pancreatic cancer in combination with gemcitabine against a regimen of gemcitabine plus placebo in a phase Ⅱ randomized multicentre study. Nevertheless, no significant advantages were demonstrated in terms of overall survival or PFS[31].

Rigosertib, a first-in-class Ras mimetic and small molecule inhibitor of multiple signalling pathways, including polo-like kinase 1 and phosphoinositide 3-kinase (PI3K), was assessed in combination with gemcitabine in patients with treatment-naïve metastatic pancreatic adenocarcinoma in a phase Ⅱ/Ⅲ randomized study, but the combination regimen did not improve survival or response, as recently presented at the 2015 ASCO Annual Meeting[32].

Research in this field is in development, but the available trials have failed to show any survival benefit.

IGFR pathway inhibitors

Another possible target in ductal pancreatic cancer is represented by insulin like growth factor 1 receptor, which is highly expressed in pancreatic cells, and upon ligand binding activates several pathways involved in cell proliferation and cell survival such as the PIK3/AKT pathway[33].

Monoclonal antibodies against IGFR (cixutumumab, ganitumab) were evaluated in PDAC treatment, but unfortunately, they failed to show a statically significant survival benefit[34].

In particular, the phase Ⅲ trial assessing ganitumab in combination with gemcitabine was closed early based on a pre-planned futility analysis: The median overall survival was 7.1 mo in the maximum dose ganitumab arm *vs* 7.2 mo in the placebo arm (HR, 0.97, P = 0.397)[35].

Angiogenesis pathway inhibitors

Neoangiogenesis is essential for tumour progression and metastatization mechanisms. Vascular endothelial growth factor (VEGF) stimulates the proliferation of endothelial cells and is overexpressed in human pancreatic cancer. Nevertheless, neoangiogenesis inhibitors, particularly VEGF inhibitors, failed to improve overall survival in combination with gemcitabine in advanced pancreatic cancer. After encouraging results, phase Ⅲ trials that tested the efficacy of bevacizumab in association with gemcitabine alone, or gemcitabine plus erlotinib, did not confirm previous findings[36,37].

Aflibercept, a new recombinant fusion protein with extracellular portions of VEGFR-1 and VEGFR-2, which binds VEGF-A, VEGF-B and placental growth factors 1 and 2 thereby inhibiting VEGF-ligand-dependent signalling processes, suppresses tumour growth in pancreatic cell lines and xenografts. Nevertheless, a

phase Ⅲ study aiming to investigate OS in metastatic pancreatic cancer patients receiving standard gemcitabine and either aflibercept or placebo demonstrated that adding aflibercept to gemcitabine did not improve OS in metastatic pancreatic cancer patients[38].

Similarly sorafenib, an oral multikinase inhibitor of Raf-kinase, VEGF-R2/-R3 and PDGFR-β, tested alone or in combination with gemcitabine in small phase Ⅰ and Ⅱ trials, and axitinib, an anti-angiogenesis agent assessed in combination with gemcitabine, showed no statistically significant efficacy in a phase Ⅲ trial in advanced PDAC[39-41].

Phase Ⅱ studies combining chemotherapy with promising new anti-angiogenic molecular agents, such as TL-118, a nonsteroidal anti-inflammatory oral medication, or necuparanib, which is re-engineered from heparin with possible anti-tumour activity, are underway[42,43].

Embryonic pathway inhibitors

Hedgehog signalling has a critical role in cell proliferation and survival during embryonic development. Normal pancreatic cells silence this pathway, but pathological activation is observed in many solid tumours, particularly in PADC. Hedgehog binds to the extracellular receptor Patched, which, in the absence of Hedgehog, suppresses activation of the G-protein-coupled receptor Smoothened and upregulates glioma associated oncogene homolog1 transcriptional activity. Cancer cell lines show both Hedgehog ligand-dependent and -independent mechanisms of aberrant signalling[44].

Bailey *et al*[45] showed how Sonic hedgehog (SHH) and other proteins downstream of the Hedgehog pathway, detected in precursor lesions and in PDAC primary tumour samples, contribute to the formation of the desmoplastic reaction, an important characteristic of pancreatic cancer that limits the effective delivery of anticancer agents to pancreatic cancer cells. Genetically engineered mouse models demonstrated a depletion of tumour matrix from SHH pathway inhibition, which could be a promising strategy in pancreatic cancer therapy[46].

Vismodegib (GDC-0449), an oral small-molecule inhibitor targeting Smoothened[47], is under assessment in open phase Ⅱ trials in combination with gemcitabine in advanced cancer, in combination with gemcitabine and nab-paclitaxel in metastatic settings with promising preliminary data[48], and as a single agent in neoadjuvant settings followed by surgery[49-51].

The Smoothened inhibitor saridegib (IPI-926) was tested in association with gemcitabine against gemcitabine plus placebo in a randomized, double-blind, placebo-controlled phase Ⅱ trial enrolling patients with metastatic disease. Unfortunately, this study was closed ahead of time due to evidence of decreased patient survival in the saridegib arm[52].

Hedgehog inhibitors are an active research field, and several clinical trials are ongoing[53]. Notch signalling is another embryonic pathway crucial for pancreatic organogenesis, but after pancreas development, it is active only in a stem cell subgroup. This pathway is upregulated in PDAC and promotes tumourigenesis. Binding of Notch ligand to its receptor promotes a cascade of proteolytic cleavages, mediated by γ-secretase (presenilin). The activated form ICN (intra cellular notch) forms part of a transcription complex that, after translocating to the nucleus, regulates transcription of several genes involved in proliferation and differentiation of cells, interacting with other pathways such as Hedgehog, KRAS and NF-κB signalling[54,55].

RO4929097 is a selective inhibitor of the γ-secretase enzyme with anti-tumour activity in preclinical studies[56].

A recent phase Ⅱ single-arm trial assessed the possible role of RO4929097, enrolling 18 previously treated advanced PDAC patients. The treatment was well tolerated; the median survival was 4.1 mo, and the median progression-free survival was 1.5 mo[57].

Encouraging clinical results were observed testing demcizumab, an anti- Delta-like ligand 4 antibody, plus gemcitabine and nab-paclitaxel in advanced PDAC in a phase Ⅰb trial. Further evidence is needed to confirm these preliminary data[58].

PARP inhibitors

Mutations affecting BRCA pathway components, especially the tumour suppressor gene BRCA2, which is associated with hereditary predisposition to breast, ovarian and pancreatic cancer, promote deficiency in DNA damage repair mechanisms and genomic instability[11].

Poly ADP-ribose polymerase (PARP) is a nuclear enzyme recruited to repair cell DNA damage, and as recent evidence showed, patients with defects in the homologous DNA recombination pathway may benefit from the use of PARP inhibitors as monotherapy or in combination with radiation or other chemotherapeutic agents. Clinical trials testing those new agents in selected patients are currently in the development phase[59-61].

mTOR and PI3K/Akt pathway inhibitors

After activation, Ras can phosphorylate PI3K, which activates Akt, a serine/threonine kinase. Signal transduction by activated PI3K/Akt plays a role in tumour cell proliferation, survival and metabolism, usually through several downstream targets, including the mammalian target of rapamycin (mTOR)[62].

Several trials testing PI3K/AKT axis inhibitors are currently ongoing in advanced pancreatic cancer patients after encouraging preclinical model results[63]. These trials included the following PI3K/AKT axis inhibitors: BKM120, a PI3K inhibitor tested in combination with the mFOLFOX-6 schedule; RX-0201, an Akt antisense oligonucleotide tested in a phase Ⅱ study plus gemcitabine; and BEZ235, a combined inhibitor of PI3K and mTOR assessed in a phase study in combination with the MEK inhibitor MEK162[64-66].

Wolpin *et al*[67] evaluated a possible role of everolimus, an oral mTOR inhibitor, as monotherapy in 33

gemcitabine-refractory pancreatic cancer patients. The PFS and OS were 1.8 and 4.5 mo, respectively.

Recently, the results of a single arm phase Ⅱ study where everolimus was tested in combination with capecitabine were published. The median OS was 8.9 mo and PFS was 3.6 mo[68].

The results of a phase Ⅰ/Ⅱ study testing everolimus in combination with gemcitabine in advanced settings and the results of a phase Ⅱ trial testing temsirolimus, another mTOR inhibitor, in locally advanced or metastatic settings are anticipated[69,70].

Tumour stroma inhibitors

The stroma is a dynamic compartment of pancreatic tumours that is critically involved in tumour formation, progression and the metastasis process. Therefore, targeting stromal microenvironment elements could be an efficient therapeutic strategy in addition to previously described trials evaluating Hedgehog signalling inhibitors[71].

After promising data derived from a preliminary clinical study on the possible role of PEGPH20, a pegylated formulation of recombinant hyaluronidase, a phase Ⅱ trial is currently in the recruitment phase. The purpose of that study is to enrol untreated patients with metastatic disease to receive a combination of PEGPH20, nab-naclitaxel and gemcitabine or a combination of nab-paclitaxel and gemcitabine[72,73].

Additionally, inhibition of PDGFR, a receptor expressed in stromal cells with a critical role in recruiting pericytes and in interstitial fluid pressure in the tumour stroma, could be an interesting molecular target, as suggested by preclinical studies using an orthotopic pancreatic tumour mouse model[74].

TKI258, a PDGFR inhibitor, is under evaluation in a phase Ⅰ dose assessment for advanced pancreatic cancer patients[75].

In the past, matrix metalloproteinase inhibitors such as marimastat were tested. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes responsible for the degradation of connective tissue proteins, and aberrant MMP expression is observed in PDAC. Nevertheless, the results of a phase Ⅲ trial provided no evidence to support a combination of marimastat with gemcitabine in patients with advanced pancreatic cancer[76].

CONCLUSION

Knowledge of the molecular biology of PDAC has important potential clinical relevance, but current efforts to improve understanding of the mutational profile of this tumour have not provided any significant advantage in the use of targeted therapy. Several agents have been tested in PDAC, but almost all have failed to demonstrate efficacy in late phase clinical trials. Only erlotinib has been approved by the FDA for advanced pancreatic cancer treatment, but the improvement of overall survival is barely 2 wk compared with gemcitabine alone[5].

There could be many reasons for those unsatisfying results. First of all, the extreme genomic heterogeneity of PDAC is an important block to identifying new candidate actionable molecular targets or to testing existing biological therapies already approved for human use for other cancers. In addition, no significant results have been observed by matching targeted agents with patients harbouring the cognate molecular abnormality, such as, for example, the use of trastuzumab in HER2 overexpression cases. Due to poor results derived from targeting a single molecule, new strategies using multitargeted agents or molecular agent combinations are in the development phase in order to block more than one driving genomic aberration and to prevent or evade resistance.

Additionally, the type of chemotherapy used in combination could be a failure factor. Indeed, the majority of trials have combined target agents with gemcitabine, but actually, the first-line schedules are represented by FOLFIRINOX or gemcitabine plus Nab-paclitaxel. Therefore, greater efficacy may be obtained from the combination of target agents with those chemotherapeutic drugs.

Furthermore, most studies in which molecular or chemotherapeutic agents in pancreatic cancer were tested enrolled an unselected population of patients to treat. In the last 3 years, approximately 116 trials specific for PDAC systemic therapy were registered of which only about 8% applied criteria to select a patient subset based upon molecular biomarkers[77].

FUTURE CHALLENGES

Most studies in which molecular or chemotherapeutic agents in pancreatic cancer were tested enrolled an unselected population of patients to treat. In the last 3 years, approximately 116 trials specific for PDAC systemic therapy were registered of which only about the 8% applied criteria to select a patient subset based upon molecular biomarkers[77].

To stratify patients, the Australian Pancreatic Cancer Genome Initiative has started a pilot study to evaluate the feasibility of assessing a more stratified approach in the management of pancreatic cancer through predefined actionable molecular phenotypes. Patients are enrolled in this trial, called IMPaCT (Individualised Molecular Pancreatic Cancer Therapy), after a preliminary phenotype screening in order to compare the use of gemcitabine in an unselected population to a stratified approach. The aim of the study is to create a tailored approach to pancreatic cancer treatment, which seems to be one of the major challenges for the future[78].

Finally, thanks to biotechnology advancement, biological agents can find application in cancer treatment by tumour-targeted delivery of cytotoxic drugs. Particularly, Ahn *et al*[79] developed antibody fragment-installed polymeric micelles *via* maleimide-thiol conjugation

for selective delivery of platinum drugs to pancreatic tumours. This antibody-drug conjugate significantly suppressed the growth of pancreatic tumour xenografts. This technology, with potential activity *in vitro* and in a mouse model, could be a promising future strategy in pancreatic cancer therapy[79].

In conclusion, the lack of efficacy of targeted therapy in PDAC represents a challenge for the future, and more efforts are needed in order to make pancreatic cancer a curable disease.

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REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]

2 **Sohn TA**, Yeo CJ, Cameron JL, Koniaris L, Kaushal S, Abrams RA, Sauter PK, Coleman J, Hruban RH, Lillemoe KD. Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. *J Gastrointest Surg* 2000; **4**: 567-579 [PMID: 11307091]

3 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

4 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

5 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677]

6 **Di Marco M**, Di Cicilia R, Macchini M, Nobili E, Vecchiarelli S, Brandi G, Biasco G. Metastatic pancreatic cancer: is gemcitabine still the best standard treatment? (Review). *Oncol Rep* 2010; **23**: 1183-1192 [PMID: 20372829]

7 **Chang DK**, Grimmond SM, Biankin AV. Pancreatic cancer genomics. *Curr Opin Genet Dev* 2014; **24**: 74-81 [PMID: 24480245 DOI: 10.1016/j.gde.2013.12.001]

8 8 **Hudson TJ**, Anderson W, Artez A, Barker AD, Bell C, Bernabé RR, Bhan MK, Calvo F, Eerola I, Gerhard DS, Guttmacher A, Guyer M, Hemsley FM, Jennings JL, Kerr D, Klatt P, Kolar P, Kusada J, Lane DP, Laplace F, Youyong L, Nettekoven G, Ozenberger B, Peterson J, Rao TS, Remacle J, Schafer AJ, Shibata T, Stratton MR, Vockley JG, Watanabe K, Yang H, Yuen MM, Knoppers BM, Bobrow M, Cambon-Thomsen A, Dressler LG, Dyke SO, Joly Y, Kato K, Kennedy KL, Nicolás P, Parker MJ, Rial-Sebbag E, Romeo-Casabona CM, Shaw KM, Wallace S, Wiesner GL, Zeps N, Lichter P, Biankin AV, Chabannon C, Chin L, Clément B, de Alava E, Degos F, Ferguson ML, Geary P, Hayes DN, Hudson TJ, Johns AL, Kasprzyk A, Nakagawa H, Penny R, Piris MA, Sarin R, Scarpa A, Shibata T, van de Vijver M, Futreal PA, Aburatani H, Bayés M, Botwell DD, Campbell PJ, Estivill X, Gerhard DS, Grimmond SM, Gut I, Hirst M, López-Otín C, Majumder P, Marra M, McPherson JD, Nakagawa H, Ning Z, Puente XS, Ruan Y, Shibata T, Stratton MR, Stunnenberg HG, Swerdlow H, Velculescu VE, Wilson RK, Xue HH, Yang L, Spellman PT, Bader GD, Boutros PC, Campbell PJ, Flicek P, Getz G, Guigó R, Guo G, Haussler D, Heath S, Hubbard TJ, Jiang T, Jones SM, Li Q, López-Bigas N, Luo R, Muthuswamy L, Ouellette BF, Pearson JV, Puente XS, Quesada V, Raphael BJ, Sander C, Shibata T, Speed TP, Stein LD, Stuart JM, Teague JW, Totoki Y, Tsunoda T, Valencia A, Wheeler DA, Wu H, Zhao S, Zhou G, Stein LD, Guigó R, Hubbard TJ, Joly Y, Jones SM, Kasprzyk A, Lathrop M, López-Bigas N, Ouellette BF, Spellman PT, Teague JW, Thomas G, Valencia A, Yoshida T, Kennedy KL, Axton M, Dyke SO, Futreal PA, Gerhard DS, Gunter C, Guyer M, Hudson TJ, McPherson JD, Miller LJ, Ozenberger B, Shaw KM, Kasprzyk A, Stein LD, Zhang J, Haider SA, Wang J, Yung CK, Cros A, Liang Y, Gnaneshan S, Guberman J, Hsu J, Bobrow M, Chalmers DR, Hasel KW, Joly Y, Kaan TS, Kennedy KL, Knoppers BM, Lowrance WW, Masui T, Nicolás P, Rial-Sebbag E, Rodriguez LL, Vergely C, Yoshida T, Grimmond SM, Biankin AV, Bowtell DD, Cloonan N, deFazio A, Eshleman JR, Etemadmoghadam D, Gardiner BB, Kench JG, Scarpa A, Sutherland RL, Tempero MA, Waddell NJ, Wilson PJ, McPherson JD, Gallinger S, Tsao MS, Shaw PA, Petersen GM, Mukhopadhyay D, Chin L, DePinho RA, Thayer S, Muthuswamy L, Shazand K, Beck T, Sam M, Timms L, Ballin V, Lu Y, Ji J, Zhang X, Chen F, Hu X, Zhou G, Yang Q, Tian G, Zhang L, Xing X, Li X, Zhu Z, Yu Y, Yu J, Yang H, Lathrop M, Tost J, Brennan P, Holcatova I, Zaridze D, Brazma A, Egevard L, Prokhortchouk E, Banks RE, Uhlén M, Cambon-Thomsen A, Viksna J, Ponten F, Skryabin K, Stratton MR, Futreal PA, Birney E, Borg A, Børresen-Dale AL, Caldas C, Foekens JA, Martin S, Reis-Filho JS, Richardson AL, Sotiriou C, Stunnenberg HG, Thoms G, van de Vijver M, van't Veer L, Calvo F, Birnbaum D, Blanche H, Boucher P, Boyault S, Chabannon C, Gut I, Masson-Jacquemier JD, Lathrop M, Pauporté I, Pivot X, Vincent-Salomon A, Tabone E, Theillet C, Thomas G, Tost J, Treilleux I, Calvo F, Bioulac-Sage P, Clément B, Decaens T, Degos F, Franco D, Gut I, Gut M, Heath S, Lathrop M, Samuel D, Thomas G, Zucman-Rossi J, Lichter P, Eils R, Brors B, Korbel JO, Korshunov A, Landgraf P, Lehrach H, Pfister S, Radlwimmer B, Reifenberger G, Taylor MD, von Kalle C, Majumder PP, Sarin R, Rao TS, Bhan MK, Scarpa A, Pederzoli P, Lawlor RA, Delledonne M, Bardelli A, Biankin AV, Grimmond SM, Gress T, Klimstra D, Zamboni G, Shibata T, Nakamura Y, Nakagawa H, Kusada J, Tsunoda T, Miyano S, Aburatani H, Kato K, Fujimoto A, Yoshida T, Campo E, López-Otín C, Estivill X, Guigó R, de Sanjosé S, Piris MA, Montserrat E, González-Díaz M, Puente XS, Jares P, Valencia A, Himmelbauer H, Quesada V, Bea S, Stratton MR, Futreal PA, Campbell PJ, Vincent-Salomon A, Richardson AL, Reis-Filho JS, van de Vijver M, Thomas G, Masson-Jacquemier JD, Aparicio S, Borg A, Børresen-Dale AL, Caldas C, Foekens JA, Stunnenberg HG, van't Veer L, Easton DF, Spellman PT, Martin S, Barker AD, Chin L, Collins FS, Compton CC, Ferguson ML, Gerhard DS, Getz G, Gunter C, Guttmacher A, Guyer M, Hayes DN, Lander ES, Ozenberger B, Penny R, Peterson J, Sander C, Shaw KM, Speed TP, Spellman PT, Vockley JG, Wheeler DA, Wilson RK, Hudson TJ, Chin L, Knoppers BM, Lander ES, Lichter P, Stein LD, Stratton MR, Anderson W, Barker AD, Bell C, Bobrow M, Burke W, Collins FS, Compton CC, DePinho RA, Easton DF, Futreal PA, Gerhard DS, Green AR, Guyer M, Hamilton SR, Hubbard TJ, Kallioniemi OP, Kennedy KL, Ley TJ, Liu ET, Lu Y, Majumder P, Marra M, Ozenberger B, Peterson J, Schafer AJ, Spellman PT, Stunnenberg HG, Wainwright BJ, Wilson RK, Yang H. International network of cancer genome projects. *Nature* 2010; **464**: 993-998 [PMID: 20393554 DOI: 10.1038/nature08987]

9 **Biankin AV**, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, Miller DK, Wilson PJ, Patch AM, Wu J, Chang DK, Cowley MJ, Gardiner BB, Song S, Harliwong I, Idrisoglu S, Nourse C, Nourbakhsh E, Manning S, Wani S, Gongora M, Pajic

M, Scarlett CJ, Gill AJ, Pinho AV, Rooman I, Anderson M, Holmes O, Leonard C, Taylor D, Wood S, Xu Q, Nones K, Fink JL, Christ A, Bruxner T, Cloonan N, Kolle G, Newell F, Pinese M, Mead RS, Humphris JL, Kaplan W, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chou A, Chin VT, Chantrill LA, Mawson A, Samra JS, Kench JG, Lovell JA, Daly RJ, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Kakkar N, Zhao F, Wu YQ, Wang M, Muzny DM, Fisher WE, Brunicardi FC, Hodges SE, Reid JG, Drummond J, Chang K, Han Y, Lewis LR, Dinh H, Buhay CJ, Beck T, Timms L, Sam M, Begley K, Brown A, Pai D, Panchal A, Buchner N, De Borja R, Denroche RE, Yung CK, Serra S, Onetto N, Mukhopadhyay D, Tsao MS, Shaw PA, Petersen GM, Gallinger S, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Schulick RD, Wolfgang CL, Morgan RA, Lawlor RT, Capelli P, Corbo V, Scardoni M, Tortora G, Tempero MA, Mann KM, Jenkins NA, Perez-Mancera PA, Adams DJ, Largaespada DA, Wessels LF, Rust AG, Stein LD, Tuveson DA, Copeland NG, Musgrove EA, Scarpa A, Eshleman JR, Hudson TJ, Sutherland RL, Wheeler DA, Pearson JV, McPherson JD, Gibbs RA, Grimmond SM. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature* 2012; **491**: 399-405 [PMID: 23103869 DOI: 10.1038/nature11547]

10 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]

11 **Waddell N**, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, Quinn MC, Robertson AJ, Fadlullah MZ, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Wani S, Wilson PJ, Markham E, Cloonan N, Anderson MJ, Fink JL, Holmes O, Kazakoff SH, Leonard C, Newell F, Poudel B, Song S, Taylor D, Waddell N, Wood S, Xu Q, Wu J, Pinese M, Cowley MJ, Lee HC, Jones MD, Nagrial AM, Humphris J, Chantrill LA, Chin V, Steinmann AM, Mawson A, Humphrey ES, Colvin EK, Chou A, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Pettitt JA, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Jamieson NB, Graham JS, Niclou SP, Bjerkvig R, Grützmann R, Aust D, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Falconi M, Zamboni G, Tortora G, Tempero MA, Gill AJ, Eshleman JR, Pilarsky C, Scarpa A, Musgrove EA, Pearson JV, Biankin AV, Grimmond SM. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 2015; **518**: 495-501 [PMID: 25719666 DOI: 10.1038/nature14169]

12 **Campbell PJ**, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, Morsberger LA, Latimer C, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal SA, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Griffin CA, Burton J, Swerdlow H, Quail MA, Stratton MR, Iacobuzio-Donahue C, Futreal PA. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature* 2010; **467**: 1109-1113 [PMID: 20981101 DOI: 10.1038/nature09460]

13 **Tobita K**, Kijima H, Dowaki S, Kashiwagi H, Ohtani Y, Oida Y, Yamazaki H, Nakamura M, Ueyama Y, Tanaka M, Inokuchi S, Makuuchi H. Epidermal growth factor receptor expression in human pancreatic cancer: Significance for liver metastasis. *Int J Mol Med* 2003; **11**: 305-309 [PMID: 12579331]

14 **da Cunha Santos G**, Dhani N, Tu D, Chin K, Ludkovski O, Kamel-Reid S, Squire J, Parulekar W, Moore MJ, Tsao MS. Molecular predictors of outcome in a phase 3 study of gemcitabine and erlotinib therapy in patients with advanced pancreatic cancer: National Cancer Institute of Canada Clinical Trials Group Study PA.3. *Cancer* 2010; **116**: 5599-5607 [PMID: 20824720 DOI: 10.1002/cncr.25393]

15 **Cascinu S**, Berardi R, Labianca R, Siena S, Falcone A, Aitini E, Barni S, Di Costanzo F, Dapretto E, Tonini G, Pierantoni C, Artale S, Rota S, Floriani I, Scartozzi M, Zaniboni A. Cetuximab plus gemcitabine and cisplatin compared with gemcitabine and cisplatin alone in patients with advanced pancreatic cancer: a randomised, multicentre, phase II trial. *Lancet Oncol* 2008; **9**: 39-44 [PMID: 18077217]

16 **Philip PA**, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL, Blanke CD. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. *J Clin Oncol* 2010; **28**: 3605-3610 [PMID: 20606093 DOI: 10.1200/JCO.2009.25.7550]

17 **Fountzilas G**, Bobos M, Kalogera-Fountzila A, Xiros N, Murray S, Linardou H, Karayannopoulou G, Koutras AK, Bafaloukos D, Samantas E, Christodoulou C, Economopoulos T, Kalogeras KT, Kosmidis P. Gemcitabine combined with gefitinib in patients with inoperable or metastatic pancreatic cancer: a phase II Study of the Hellenic Cooperative Oncology Group with biomarker evaluation. *Cancer Invest* 2008; **26**: 784-793 [PMID: 18798073 DOI: 10.1080/07357900801918611]

18 **Kimura K**, Sawada T, Komatsu M, Inoue M, Muguruma K, Nishihara T, Yamashita Y, Yamada N, Ohira M, Hirakawa K. Antitumor effect of trastuzumab for pancreatic cancer with high HER-2 expression and enhancement of effect by combined therapy with gemcitabine. *Clin Cancer Res* 2006; **12**: 4925-4932 [PMID: 16914581]

19 **Safran H**, Iannitti D, Ramanathan R, Schwartz JD, Steinhoff M, Nauman C, Hesketh P, Rathore R, Wolff R, Tantravahi U, Hughes TM, Maia C, Pasquariello T, Goldstein L, King T, Tsai JY, Kennedy T. Herceptin and gemcitabine for metastatic pancreatic cancers that overexpress HER-2/neu. *Cancer Invest* 2004; **22**: 706-712 [PMID: 15581051]

20 **Harder J**, Ihorst G, Heinemann V, Hofheinz R, Moehler M, Buechler P, Kloeppel G, Röcken C, Bitzer M, Boeck S, Endlicher E, Reinacher-Schick A, Schmoor C, Geissler M. Multicentre phase II trial of trastuzumab and capecitabine in patients with HER2 overexpressing metastatic pancreatic cancer. *Br J Cancer* 2012; **106**: 1033-1038 [PMID: 22374460 DOI: 10.1038/bjc.2012.18]

21 **Safran H**, Miner T, Resnick M, Dipetrillo T, McNulty B, Evans D, Joseph P, Plette A, Millis R, Sears D, Gutman N, Kennedy T. Lapatinib/gemcitabine and lapatinib/gemcitabine/oxaliplatin: a phase I study for advanced pancreaticobiliary cancer. *Am J Clin Oncol* 2008; **31**: 140-144 [PMID: 18391597 DOI: 10.1097/COC.0b013e318145b9a5]

22 **Su D**, Jiao SC, Wang LJ, Shi WW, Long YY, Li J, Bai L. Efficacy of nimotuzumab plus gemcitabine usage as first-line treatment in patients with advanced pancreatic cancer. *Tumour Biol* 2014; **35**: 2313-2318 [PMID: 24142531 DOI: 10.1007/s13277-013-1306-x]

23 **Strumberg D**, Schultheis B, Scheulen ME, Hilger RA, Krauss J, Marschner N, Lordick F, Bach F, Reuter D, Edler L, Mross K. Phase II study of nimotuzumab, a humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody, in patients with locally advanced or metastatic pancreatic cancer. *Invest New Drugs* 2012; **30**: 1138-1143 [PMID: 21170759 DOI: 10.1007/s10637-010-9619-8]

24 **Ioannou N**, Dalgleish AG, Seddon AM, Mackintosh D, Guertler U, Solca F, Modjtahedi H. Anti-tumour activity of afatinib, an irreversible ErbB family blocker, in human pancreatic tumour cells. *Br J Cancer* 2011; **105**: 1554-1562 [PMID: 21970876 DOI: 10.1038/bjc.2011.396]

25 **PD Dr. med. Volker Heinemann**. Afatinib as Cancer Therapy for Exocrine Pancreatic Tumours (ACCEPT). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01728818 NLM Identifier: NCT01728818

26 **di Magliano MP**, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. *Gastroenterology* 2013; **144**:

1220-1229 [PMID: 23622131 DOI: 10.1053/j.gastro.2013.01.071]
27 **Takashima A**, Faller DV. Targeting the RAS oncogene. *Expert Opin Ther Targets* 2013; **17**: 507-531 [PMID: 23360111 DOI: 10.1517/14728222.2013.764990]
28 **Pylayeva-Gupta Y**, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer* 2011; **11**: 761-774 [PMID: 21993244 DOI: 10.1038/nrc3106]
29 **Van Cutsem E**, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y, Von Hoff D. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol* 2004; **22**: 1430-1438 [PMID: 15084616]
30 **Bodoky G**, Timcheva C, Spigel DR, La Stella PJ, Ciuleanu TE, Pover G, Tebbutt NC. A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. *Invest New Drugs* 2012; **30**: 1216-1223 [PMID: 21594619 DOI: 10.1007/s10637-011-9687-4]
31 **Infante JR**, Somer BG, Park JO, Li CP, Scheulen ME, Kasubhai SM, Oh DY, Liu Y, Redhu S, Steplewski K, Le N. A randomised, double-blind, placebo-controlled trial of trametinib, an oral MEK inhibitor, in combination with gemcitabine for patients with untreated metastatic adenocarcinoma of the pancreas. *Eur J Cancer* 2014; **50**: 2072-2081 [PMID: 24915778 DOI: 10.1016/j.ejca.2014.04.024]
32 **Scott AJ,** O'Neil BH, Ma WW, Cohen SJ, Aisner D, Menter AR, Abdulaziz Tejani M. A phase II/III randomized study to compare the efficacy and safety of rigosertib plus gemcitabine versus gemcitabine alone in patients with previously untreated metastatic pancreatic cancer. ASCO Annual Meeting. *J Clin Oncol* 33 2015; abstr 4117
33 **Sachdev D**, Yee D. Disrupting insulin-like growth factor signaling as a potential cancer therapy. *Mol Cancer Ther* 2007; **6**: 1-12 [PMID: 17237261]
34 **Philip PA**, Goldman B, Ramanathan RK, Lenz HJ, Lowy AM, Whitehead RP, Wakatsuki T, Iqbal S, Gaur R, Benedetti JK, Blanke CD. Dual blockade of epidermal growth factor receptor and insulin-like growth factor receptor-1 signaling in metastatic pancreatic cancer: phase Ib and randomized phase II trial of gemcitabine, erlotinib, and cixutumumab versus gemcitabine plus erlotinib (SWOG S0727). *Cancer* 2014; **120**: 2980-2985 [PMID: 25041791 DOI: 10.1002/cncr.28744]
35 **Fuchs CS**, Azevedo S, Okusaka T, Van Laethem JL, Lipton LR, Riess H, Szczylik C, Moore MJ, Peeters M, Bodoky G, Ikeda M, Melichar B, Nemecek R, Ohkawa S, Świeboda-Sadlej A, Tjulandin SA, Van Cutsem E, Loberg R, Haddad V, Gansert JL, Bach BA, Carrato A. A phase 3 randomized, double-blind, placebo-controlled trial of ganitumab or placebo in combination with gemcitabine as first-line therapy for metastatic adenocarcinoma of the pancreas: the GAMMA trial. *Ann Oncol* 2015; **26**: 921-927 [PMID: 25609246 DOI: 10.1093/annonc/mdv027]
36 **Kindler HL**, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, Picus J, Bhargava P, Mayer RJ, Schilsky RL, Goldberg RM. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol* 2010; **28**: 3617-3622 [PMID: 20606091 DOI: 10.1200/JCO.2010.28.1386]
37 **Van Cutsem E**, Vervenne WL, Bennouna J, Humblet Y, Gill S, Van Laethem JL, Verslype C, Scheithauer W, Shang A, Cosaert J, Moore MJ. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 2231-2237 [PMID: 19307500 DOI: 10.1200/JCO.2008.20.0238]
38 **Rougier P**, Riess H, Manges R, Karasek P, Humblet Y, Barone C, Santoro A, Assadourian S, Hatteville L, Philip PA. Randomised, placebo-controlled, double-blind, parallel-group phase III study evaluating aflibercept in patients receiving first-line treatment with gemcitabine for metastatic pancreatic cancer. *Eur J Cancer* 2013; **49**: 2633-2642 [PMID: 23642329 DOI: 10.1016/j.ejca.2013.04.002]
39 **Chiorean EG**, Schneider BP, Akisik FM, Perkins SM, Anderson S, Johnson CS, DeWitt J, Helft P, Clark R, Johnston EL, Spittler AJ, Deluca J, Bu G, Shahda S, Loehrer PJ, Sandrasegaran K, Cardenes HR. Phase 1 pharmacogenetic and pharmacodynamic study of sorafenib with concurrent radiation therapy and gemcitabine in locally advanced unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2014; **89**: 284-291 [PMID: 24726286 DOI: 10.1016/j.ijrobp.2014.02.024]
40 **Kindler HL**, Wroblewski K, Wallace JA, Hall MJ, Locker G, Nattam S, Agamah E, Stadler WM, Vokes EE. Gemcitabine plus sorafenib in patients with advanced pancreatic cancer: a phase II trial of the University of Chicago Phase II Consortium. *Invest New Drugs* 2012; **30**: 382-386 [PMID: 20803052 DOI: 10.1007/s10637-010-9526-z]
41 **Kindler HL**, Ioka T, Richel DJ, Bennouna J, Létourneau R, Okusaka T, Funakoshi A, Furuse J, Park YS, Ohkawa S, Springett GM, Wasan HS, Trask PC, Bycott P, Ricart AD, Kim S, Van Cutsem E. Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: a double-blind randomised phase 3 study. *Lancet Oncol* 2011; **12**: 256-262 [PMID: 21306953 DOI: 10.1016/S1470-2045(11)70004-3]
42 **Tiltan Pharma Ltd**. Investigator's Initiated Phase II Study for Pancreatic Cancer Patients. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01659502 NLM Identifier: NCT01659502
43 **Momenta Pharmaceuticals, Inc**. M402 in Combination With Nab-Paclitaxel and Gemcitabine in Pancreatic Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01621243 NLM Identifier: NCT01621243
44 **Di Marco M**, Macchini M, Vecchiarelli S, Sina S, Biasco G. Hedgehog signaling: from the cuirass to the heart of pancreatic cancer. *Pancreatology* 2012; **12**: 388-393 [PMID: 22898642 DOI: 10.1016/j.pan.2012.06.001]
45 **Bailey JM**, Swanson BJ, Hamada T, Eggers JP, Singh PK, Caffery T, Ouellette MM, Hollingsworth MA. Sonic hedgehog promotes desmoplasia in pancreatic cancer. *Clin Cancer Res* 2008; **14**: 5995-6004 [PMID: 18829478 DOI: 10.1158/1078-0432.CCR-08-0291]
46 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461 [PMID: 19460966 DOI: 10.1126/science.1171362]
47 **Singh BN**, Fu J, Srivastava RK, Shankar S. Hedgehog signaling antagonist GDC-0449 (Vismodegib) inhibits pancreatic cancer stem cell characteristics: molecular mechanisms. *PLoS One* 2011; **6**: e27306 [PMID: 22087285 DOI: 10.1371/journal.pone.0027306]
48 **De Jesus-Acosta A,** O'Dwyer P, Ramanathan R, Von Hoff D, Maitra A, Rasheed Z. A phase II study of vismodegib, a hedgehog (Hh) pathway inhibitor, combined with gemcitabine and nab-paclitaxel (nab-P) in patients (Pts) with untreated metastatic pancreatic ductal adenocarcinoma (PDA). *ASCO Gastrointestinal Cancer Symposium* 2014; Abstract No. 257
49 **National Cancer Institute (NCI)**. Gemcitabine Hydrochloride With or Without Vismodegib in Treating Patients With Recurrent or Metastatic Pancreatic Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01064622 NLM

Identifier: NCT01064622
50 **Sidney Kimmel Comprehensive Cancer Center**. Pancreas, Hedgehog Inhibitors for Metastatic Adenocarcinoma of the Pancreas. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01088815 NLM Identifier: NCT01088815
51 **Lisa Bax**. Hedgehog Inhibition for Pancreatic Ductal Adenocarcinoma (PDAC) in the Preoperative Setting (HIPPoS). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01096732 NLM Identifier: NCT01096732
52 Infinity Reports Update from Phase 2 Study of Saridegib Plus Gemcitabine in Patients with Metastatic Pancreatic Cancer. Available from: URL: http://www.businesswire.com
53 **Sidney Kimmel Comprehensive Cancer Center**. Gemcitabine + Nab-paclitaxel With LDE-225 (Hedgehog Inhibitor) as Neoadjuvant Therapy for Pancreatic Adenocarcinoma. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01431794 NLM Identifier: NCT01431794
54 **Sjölund J**, Manetopoulos C, Stockhausen MT, Axelson H. The Notch pathway in cancer: differentiation gone awry. *Eur J Cancer* 2005; **41**: 2620-2629 [PMID: 16239105]
55 **Ristorcelli E**, Lombardo D. Targeting Notch signaling in pancreatic cancer. *Expert Opin Ther Targets* 2010; **14**: 541-552 [PMID: 20392166 DOI: 10.1517/14728221003769895]
56 **Plentz R**, Park JS, Rhim AD, Abravanel D, Hezel AF, Sharma SV, Gurumurthy S, Deshpande V, Kenific C, Settleman J, Majumder PK, Stanger BZ, Bardeesy N. Inhibition of gamma-secretase activity inhibits tumor progression in a mouse model of pancreatic ductal adenocarcinoma. *Gastroenterology* 2009; **136**: 1741-9.e6 [PMID: 19208345 DOI: 10.1053/j.gastro.2009.01.008]
57 **De Jesus-Acosta A**, Laheru D, Maitra A, Arcaroli J, Rudek MA, Dasari A, Blatchford PJ, Quackenbush K, Messersmith W. A phase II study of the gamma secretase inhibitor RO4929097 in patients with previously treated metastatic pancreatic adenocarcinoma. *Invest New Drugs* 2014; **32**: 739-745 [PMID: 24668033 DOI: 10.1007/s10637-014-0083-8]
58 **Hidalgo M,** Cooray P, Jameson MB, Carrato A, Parnis F, Jeffery M, Grimison PS, Stagg RJ, Kapoun AM, Dupont J. A phase Ib study of the anti-cancer stem cell agent demcizumab (DEM) & gemcitabine (GEM) /- paclitaxel protein bound particles (nab-paclitaxel) in pts with pancreatic cancer. 2015 ASCO Annual Meeting. *J Clin Oncol* 2015; abstr 4118
59 **Tangutoori S**, Baldwin P, Sridhar S. PARP inhibitors: A new era of targeted therapy. *Maturitas* 2015; **81**: 5-9 [PMID: 25708226 DOI: 10.1016/j.maturitas.2015.01.015]
60 **AstraZeneca**. Study to Assess the Safety & Tolerability of a PARP Inhibitor in Combination With Gemcitabine in Pancreatic Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT00515866 NLM Identifier: NCT00515866
61 **National Cancer Institute (NCI)**. Gemcitabine Hydrochloride and Cisplatin With or Without Veliparib or Veliparib Alone in Patients With Locally Advanced or Metastatic Pancreatic Cancer. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01585805 NLM Identifier: NCT01585805
62 **Hezel AF**, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev* 2006; **20**: 1218-1249 [PMID: 16702400]
63 **Ito D**, Fujimoto K, Mori T, Kami K, Koizumi M, Toyoda E, Kawaguchi Y, Doi R. In vivo antitumor effect of the mTOR inhibitor CCI-779 and gemcitabine in xenograft models of human pancreatic cancer. *Int J Cancer* 2006; **118**: 2337-2343 [PMID: 16331623]
64 **UNC Lineberger Comprehensive Cancer Center**. BKM120 + mFOLFOX6 in Advanced Solid Tumors With Expansion Cohort Pancreatic Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01571024 NLM Identifier: NCT01571024
65 **Rexahn Pharmaceuticals, Inc**. A Safety and Efficacy Study of RX-0201 Plus Gemcitabine in Metastatic Pancreatic Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01028495 NLM Identifier: NCT01028495
66 **Novartis Pharmaceuticals**. Safety, Pharmacokinetics and Pharmacodynamics of BEZ235 Plus MEK162 in Selected Advanced Solid Tumor Patients. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01337765 NLM Identifier: NCT01337765
67 **Wolpin BM**, Hezel AF, Abrams T, Blaszkowsky LS, Meyerhardt JA, Chan JA, Enzinger PC, Allen B, Clark JW, Ryan DP, Fuchs CS. Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 193-198 [PMID: 19047305 DOI: 10.1200/JCO.2008]
68 **Kordes S**, Klümpen HJ, Weterman MJ, Schellens JH, Richel DJ, Wilmink JW. Phase II study of capecitabine and the oral mTOR inhibitor everolimus in patients with advanced pancreatic cancer. *Cancer Chemother Pharmacol* 2015; **75**: 1135-1141 [PMID: 25822310 DOI: 10.1007/s00280-015-2730-y]
69 **Novartis Pharmaceuticals**. Treatment of Patients Suffering From a Progressive Pancreas Carcinoma With Everolimus (RAD001) and Gemcitabine. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT00560963 NLM Identifier: NCT00560963
70 **National Cancer Institute (NCI)**. CCI-779 in Treating Patients With Locally Advanced or Metastatic Pancreatic Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT00075647 NLM Identifier: NCT00075647
71 **Heinemann V**, Reni M, Ychou M, Richel DJ, Macarulla T, Ducreux M. Tumour-stroma interactions in pancreatic ductal adenocarcinoma: rationale and current evidence for new therapeutic strategies. *Cancer Treat Rev* 2014; **40**: 118-128 [PMID: 23849556 DOI: 10.1016/j.ctrv.2013.04.004]
72 **Provenzano PP**, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **21**: 418-429 [PMID: 22439937 DOI: 10.1016/j.ccr.2012.01.007]
73 **Halozyme Therapeutics**. PEGPH20 Plus Nab-Paclitaxel Plus Gemcitabine Compared With Nab-Paclitaxel Plus Gemcitabine in Subjects With Stage IV Untreated Pancreatic Cancer (HALO-109-202). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01839487 NLM Identifier: NCT01839487
74 **Hwang RF**, Yokoi K, Bucana CD, Tsan R, Killion JJ, Evans DB, Fidler IJ. Inhibition of platelet-derived growth factor receptor phosphorylation by STI571 (Gleevec) reduces growth and metastasis of human pancreatic carcinoma in an orthotopic nude mouse model. *Clin Cancer Res* 2003; **9**: 6534-6544 [PMID: 14695158]
75 **Roswell Park Cancer Institute**. Dovitinib Lactate, Gemcitabine Hydrochloride, and Capecitabine in Treating Patients With Advanced or Metastatic Solid Tumors, Pancreatic Cancer and Biliary Cancers. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01497392 NLM Identifier: NCT01497392
76 **Bramhall SR**, Schulz J, Nemunaitis J, Brown PD, Baillet M, Buckels JA. A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. *Br J Cancer* 2002; **87**: 161-167 [PMID: 12107836 DOI: 10.1038/sj.bjc.6600446]

77 **ClinicalTrials.gov**. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2015 May]. Available from: URL: http: //www.clinicaltrials.gov

78 **Chantrill LA**, Nagrial AM, Watson C, Johns AL, Martyn-Smith M, Simpson S, Mead S, Jones MD, Samra JS, Gill AJ, Watson N, Chin VT, Humphris JL, Chou A, Brown B, Morey A, Pajic M, Grimmond SM, Chang DK, Thomas D, Sebastian L, Sjoquist K, Yip S, Pavlakis N, Asghari R, Harvey S, Grimison P, Simes J, Biankin AV. Precision Medicine for Advanced Pancreas Cancer: The Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) Trial. *Clin Cancer Res* 2015; **21**: 2029-2037 [PMID: 25896973 DOI: 10.1158/1078-0432.CCR-15-0426]

79 **Ahn J**, Miura Y, Yamada N, Chida T, Liu X, Kim A, Sato R, Tsumura R, Koga Y, Yasunaga M, Nishiyama N, Matsumura Y, Cabral H, Kataoka K. Antibody fragment-conjugated polymeric micelles incorporating platinum drugs for targeted therapy of pancreatic cancer. *Biomaterials* 2015; **39**: 23-30 [PMID: 25477168 DOI: 10.1016/j.biomaterials.2014.10.069]

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REVIEW

Multimodality treatment strategies have changed prognosis of peritoneal metastases

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Abstract

For a long time, treatment of peritoneal metastases (PM) was mostly palliative and thus, this status was link with "terminal status/despair". The current multimodal treatment strategy, consisting of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), has been strenuously achieved over time, but seems to be the best treatment option for PM patients. As we reviewed the literature data, we could emphasize some milestones and also, controversies in the history of proposed multimodal treatment and thus, outline the philosophy of this approach, which seems to be an unusual one indeed. Initially marked by nihilism and fear, but benefiting from a remarkable joint effort of human and material resources (multi-center and -institutional research), over a period of 30 years, CRS and HIPEC found their place in the treatment of PM. The next 4 years were dedicated to the refinement of the multimodal treatment, by launching research pathways. In selected patients, with requires training, it demonstrated a significant survival results (similar to the Hepatic Metastases treatment), with acceptable risks and costs. The main debates regarding CRS and HIPEC treatment were based on the oncologists' perspective and the small number of randomized clinical trials. It is important to statement the PM patient has the right to be informed of the existence of CRS and HIPEC, as a real treatment resource, the decision being made by multidisciplinary teams.

Key words: Hyperthermic intraperitoneal chemotherapy; Peritonectomy procedures; Systemic chemotherapy; Peritoneal metastases; Survival

Core tip: The multimodal treatment of peritoneal metastases (PM), involving cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, has been strenuously achieved over time, but seems to be the best treatment option, for selected cases. This paper addresses data about the multimodal treatment strategy, focused to patient's survival, the key indicator for assessing results, in the case of PM. Also, it were highlighted the treatment key aspects and the controversies, high in the 35 years of treatment implementing. By understanding the philosophy of multimodal treatment, physicians will be able to offer an alternative to the routine systemic chemotherapy.

Lungoci C, Mironiuc AI, Muntean V, Oniu T, Leebmann H, Mayr M, Piso P. Multimodality treatment strategies have changed prognosis of peritoneal metastases. *World J Gastrointest Oncol* 2016; 8(1): 67-82 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i1/67.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i1.67

INTRODUCTION

Peritoneal metastases (PM) were described by Sampson *et al*[1] (1931) in an ovarian cancer patient. For a long time since then, treatment was mostly palliative and thus, PM was linked to "terminal status/despair". The current multimodal treatment consisting of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) has been strenuously achieved over time, but seems to be the best treatment option for selected PM cases.

As we reviewed the literature data, we could emphasize some milestones and also, controversies in the history of PM treatment and thus, outline the philosophy of proposed multimodal radical treatment, which seems to be an unusual one indeed. To understand this radical treatment, we start with the natural evolution of PM and "conventional" systemic chemotherapy approach, in fact the treatment of choice for stage Ⅳ cancer, regardless of the dissemination type and location. Four periods must be considered in the evolution of "dedicated" PM treatment: Before 1980; 1980-2000; 2000-2010 and 2010-present. The first time period, before 1980, was a period of palliative intraperitoneal treatment of ascites. In 1980-2000 were proposed the methods and define the foundation laying for a new multimodal approach of PM by intraperitoneal chemotherapy and CRS. The next periods, until 2010, were those of the progressive development of dedicated multimodal treatment strategy, concluding with the actual CRS-HIPEC. From 2010, the studies were focused about new research pathways. The PM treatment approach related survival was the main issue considered in this review.

NATURAL EVOLUTION OF PM

In the literature concerned with PM, the EVOCAPE Ⅰ study[2] is classical and it reflected the natural evolution of patients with non-gynecological PM. The mean survival (mS) was 6 mo, significantly correlated with the PM stage (Figure 1), according Gilly system[3] (nodules/lumps < 5 mm: 9.8 mo; > 2 cm: 3.1 mo). PM of pancreatic origin had the lowest mS (2.9 mo), followed by PM of gastric origin (6.5 mo) and of colorectal origin (6.9 mo). The degree of differentiation had no influence on survival.

Several other "historical"[4-6] studies confirmed the unfavorable prognosis of PM. Ascites is a negative prognostic factor: In pancreatic cancer, median survival (MS) was < 1 mo, with an important negative impact on the quality of life. Surgery was aimed at palliating gastrointestinal complications, as it was contraindicated in patients with gastric, pancreatic tumors or ascites[4]. In colorectal cancer (marked by a favorable biological pattern), MS was significantly less for synchronous PM than that in metachronous (7 mo *vs* 28 mo, $P < 0.001$)[6].

"CONVENTIONAL" SYSTEMIC CHEMOTHERAPY OF PM

Recent studies on colorectal cancer compared patients having only PM, as a distant metastatic location, with patients having other systemic dissemination. Franko *et al*[7] (2012) revealed a significantly lower ($P < 0.001$) global MS (12.7 mo *vs* 17.6 mo) and disease-free survival (5.8 mo *vs* 7.2 mo) for patients with PM *vs* other metastatic locations. Also, the poor global MS of PM metastasis patient was unchanged by various chemotherapy regimens (Figure 2).

Systemic chemotherapy carried MS significant benefit ($P = 0.026$) in colorectal PM patients only compared to those patients who did not receive chemotherapy (Figure 3): Increasing from 5 mo without chemotherapy (95%CI: 3-7 mo) to 11 mo with the fluorouracil-leucovorin protocol (95%CI: 6-9 mo), and to 12 mo with the oxaliplatin-irinotecan protocol (95%CI: 4-20 mo)[8].

Despite the progressive development of systemic chemotherapy, in a population-based study, Lemmens *et al*[9] confirmed the poor MS in PM patients (1995-2001: 7 mo; 2002-2008: 8 mo), unlike that of patients with liver metastases, which underwent improvement (1995-2001: 8 mo; 2002-2008: 12 mo).

"DEDICATED" INTRAPERITONEAL TREATMENT OF PM

Palliative treatment (< 1980)

The first attempts for a treatment approach of peritoneal malignancies began in 1950, with the sporadic use of intraperitoneal chemotherapy for malignant ascites. For this intraperitoneal treatment, hemisulphur mustard[10],

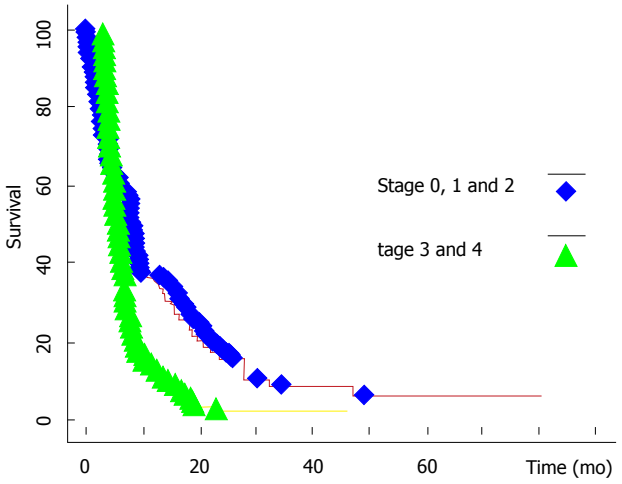

Figure 1 Kaplan-Meier survival curve of non-gynecological peritoneal carcinomatosis, stratified according to the Gilly staging system (Stage 0, 1 and 2 *vs* Stage 3 and 4)[2] (with permission). Kaplan-Meier survival curve according to PM staging[2]: Available from: URL: http://click.info.copyright.com/?qs=e232cb87f594dcec17211f4d00b2929249713825e6b912c104538a1695c335dde91d3fdf5f03fa97. PM: Peritoneal metastases.

thiotepa[11], nitrogen mustard[12], quinacrine[13] and bleomycin[14] were used. Nitrogen mustard had a high digestive toxicity, so it was replaced by thiotepa (considered elective for ascites control in 1964), but even at that time it was foreseen that it would be replaced by 5-flurouracil in digestive cancer palliation[15]. Nevertheless, nitrogen mustard was the basis for developing further drugs: Cyclophosphamide, chlorambucil, uramustine, ifosfamide, melphalan, and bendamustine.

Multimodal treatment - methods and foundation-laying (1980-2000)

In 1978-1980, the first documented data became available, at first referring to the clearance of intraperitoneal cytostatic drugs[16], then to circulating intraperitoneal cytostatic solutions, all thanks to the contributions of Speyer *et al*[17] and Spratt *et al*[18]. They were "the fathers" of regional intraperitoneal chemotherapy. Spratt augmented the cytostatic effect by hyperthermia using a specially designed device (Thermal Infusion Filtration System). Thus, the foundation was laid for a multimodal treatment of PM by normothermic and HIPEC.

The mechanism by which hyperthermia cytotoxic effects associating with and increasing the cytostatic drug effect is due to certain particularities of these drugs; studies regarding this aspect are exhaustive[19-25].

The key merit in developing and implementing this multimodal treatment strategy belongs to Sugarbaker, who outlined and detailed the premises substantiating it. The starting model was that of PM in appendiceal cancer[26]. The most important phase was the adjustment in the existing pathophysiological concept of PM, as a systemic disease and, consecutively, its treatment with systemic chemotherapy. In the new approach, the peritoneum was regarded as an organ (similar to the liver), the pathogeny of PM implying, first and foremost, peritoneal dissemination. Thus, it appears natural to use a regional treatment in PM[27-31]. Sugarbaker's research was regarded mistrustfully, and 25 years had to pass before the "European contributions to the Sugarbaker protocol"[32] appeared: One multicenter retrospective study[33], two randomized prospective phase Ⅲ studies[34,35] and the use of oxaliplatin and irinotecan as new cytostatic drugs in the protocols for intraperitoneal chemotherapy[36,37].

Sugarbaker also has the merit of being the first to have described and implemented the surgical procedures associated with regional chemotherapy, generically named "Peritonectomy"[38]. So, the road lay open for the PM multimodal treatment by CRS and HIPEC.

He then brought in other special aspects regarding the surgical technique of Peritonectomy procedures: Required electrocautery, circumferential skin traction, dissection of subpyloric space and falciform ligament[39-42]. He also described a method for staging PM and assessing the result of CRS, which were subsequently used in the majority of studies: The "Peritoneal Cancer Index" (PCI), based on the extension of peritoneal injury and the size of peritoneal deposits, respectively, the "Completeness of Cytoreduction Score" (CCRS), based on the size of the remaining peritoneal nodules/lumps[43].

PM MULTIMODAL TREATMENT - CONFIRMATION, ASPECTS, PATIENT SELECTION, CONTROVERSIES (2000-2010)

"Confirmation"

Although the number of patients progressively and significantly increased and the so-called "long-term survivors" were identified, it took about 20 years before the "confirmations of a multimodal treatment option for PM" appeared. The initiator was Verwaal (2003)[35], who carried out a randomized clinical trial for patients with colorectal PM. He showed that, during a mean follow-up of 21.6 mo, the MS of patients treated with CRS-HIPEC (22.3 mo) was significantly ($P < 0.032$) improved compared to patients treated with palliative surgery and systemic chemotherapy with fluorouracil-leucovorin (12.6 mo) (Figure 4A).

Verwaal's research was confirmed by two other reference-worthy studies, which had the merit of comparing CRS-HIPEC with modern systemic chemotherapy treatment, in colorectal PM. Elias *et al*[44] (2009) compared systemic irinotecan-oxaliplatin chemotherapy with CRS-HIPEC. Global survival in the CRS-HIPEC group (2 years: 81%, 5 years: 51%) was significantly ($P < 0.05$) improved compared to the systemic chemotherapy (2 years: 65%, 5 years: 13%). Franko *et al*[45] (2010) analyzed systemic chemotherapy with irinotecan, oxalipatin, bevacizumab, and cetuximab. MS in the CRS-HIPEC treatment group (34.7 mo) was significantly ($P < 0.01$) longer than systemic chemotherapy (16.8 mo) (Figure 4B). It was emphasized that the best results

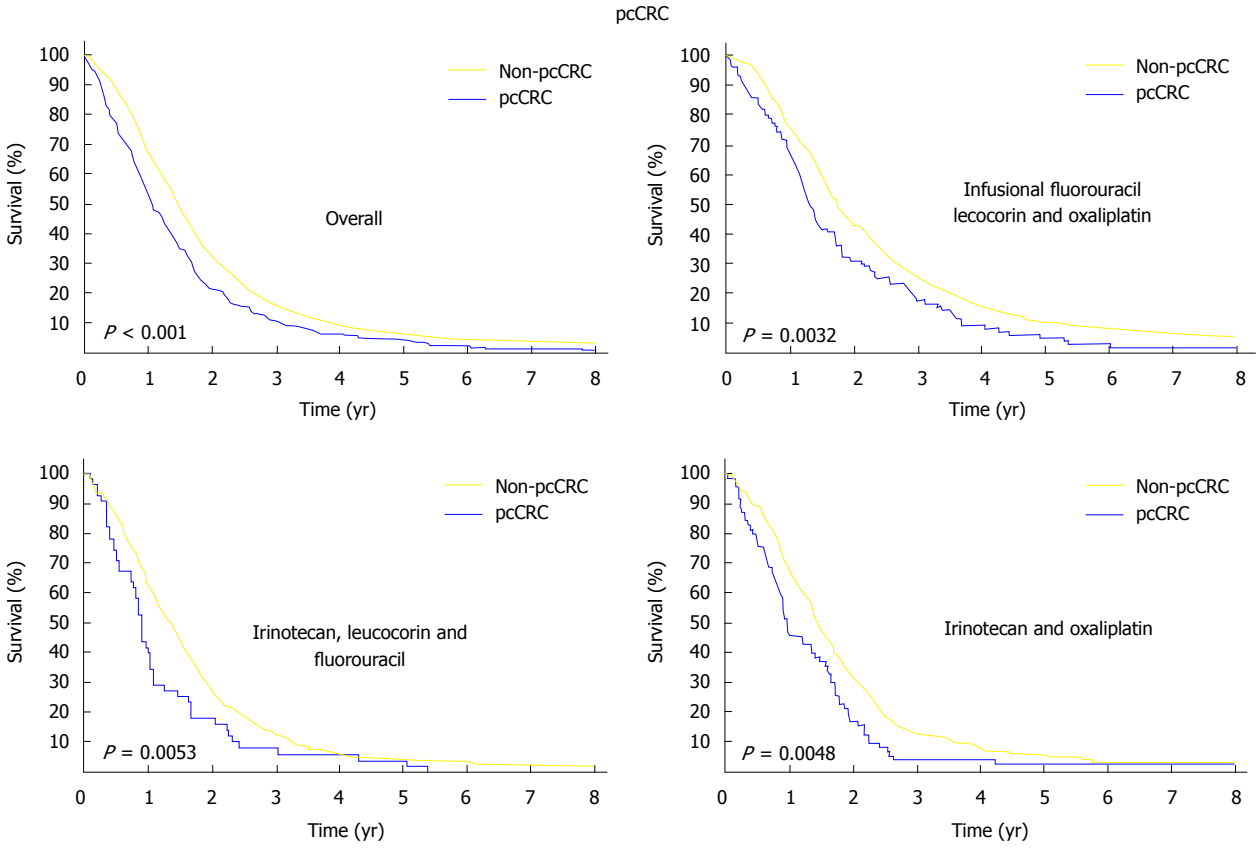

Figure 2 Kaplan-Meier survival curve of metastatic colorectal cancer stratified according to the metastatic locations (peritoneal carcinomatosis *vs* non peritoneal carcinomatosis) and chemotherapy protocols (fluorouracil, leucovorin and oxaliplatin; fluorouracil, leucovorin and irinotecan; irinotecan and oxaliplatin)[7] (with permission). Kaplan-Meier survival curve of Metastatic Colorectal Cancer stratified by peritoneal metastatic status and chemotherapy protocols[7]: Available from: URL: http://click.info.copyright.com/?qs=da4305023350a474912bb7bdbc04210a63a294c209e715b6497fdff613ea3697d486bdb9bffe0470. pcCRC: Peritoneal carcinomatosis from colorectal cancer.

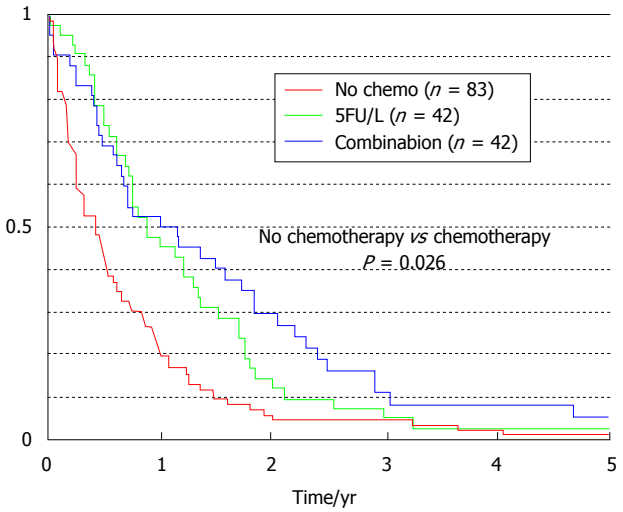

Figure 3 Kaplan-meier survival curve of colorectal peritoneal carcinomatosis stratified by chemotherapy protocols[8] (with permission). Kaplan-Meier survival curve of Colorectal PM stratified by chemotherapy protocols[8]: This is an Open Access article distributed under the terms of the Creative Commons Attribution License (Available from: URL: http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. PM: Peritoneal metastases. 5FU: 5-fluorouracil.

were achieved by associating CRS-HIPEC treatment with systemic chemotherapy.

For gastric PM, studies also showed a benefit in terms of survival. The prospective randomized clinical trial GYMSSA[46] compared survival in patients treated with CRS-HIPEC and systemic chemotherapy *vs* systemic chemotherapy treatment alone. Within the limitation of a small number of patients, it showed a longer MS (11.3 mo *vs* 4.3 mo) for CRS-HIPEC treatment trial arm.

Likewise, Yang *et al*[47] showed in a phase Ⅲ randomized clinical trial the importance of connecting CRS with HIPEC, in the treatment of PM of gastric cancer origin. The CRS-HIPEC association *vs* CRS alone significantly (P = 0.046) increased MS: 11 mo (95%CI: 10-11.9 mo) *vs* 6.5 mo (95%CI: 4.8-8.2 mo) (Figure 5).

A meta-analysis of randomized clinical trials performed by Yan *et al*[48] showed that in advanced gastric cancer, HIPEC associated with surgery led to a significant increase in survival, compared with patients benefiting from surgery alone.

In addition to randomized clinical trials (the gold standard in the treatment implementation), there are a series of multi-center studies showing survival results for patients treated with CRS-HIPEC.

Thus, several multi-center studies, focused on pseudomyxoma peritonei, colorectal and ovarian cancers, were conducted in France (Figure 6). For the treatment of

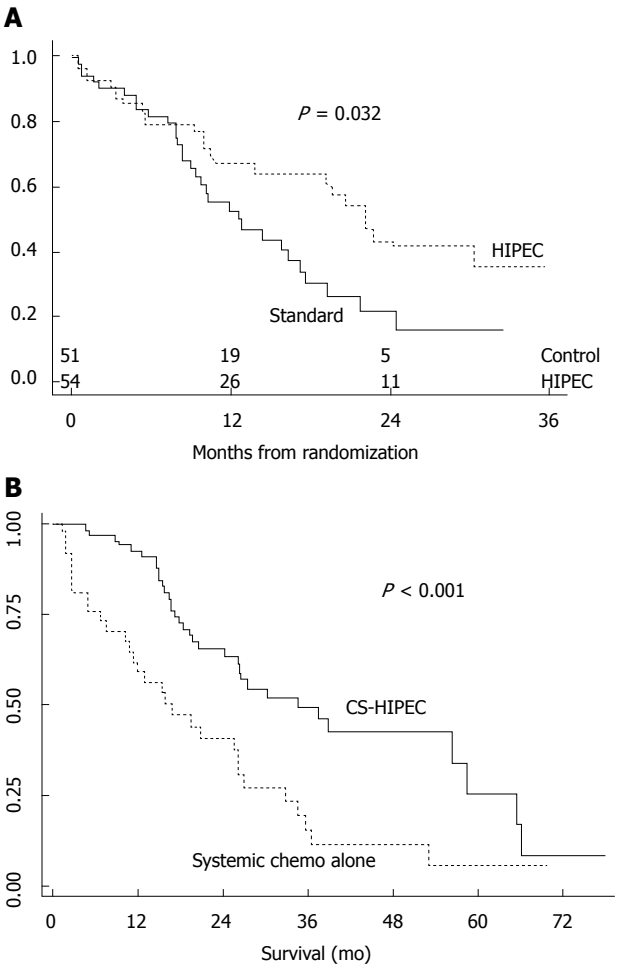

Figure 4 Kaplan-Meier survival curve comparing hyperthermic intraperitoneal chemotherapy to standard treatment, for colorectal peritoneal carcinomatosis[35] (with permission) (A) and comparing cytoreductive surgery and hyperthermic intraperitoneal chemotherapy treatment to systemic chemotherapy alone, for colorectal peritoneal carcinomatosis[45] (with permission) (B). A: Kaplan-Meier survival curve comparing HIPEC to standard treatment, for Colorectal PM[35]: Available from: URL: http://click.info.copyright.com/ ?qs=2b7046b10dd0c5471bbbb8bac0096e00e895f442c0326423c4ec2ccdd7b4701b99fd4bd5b32f0283; B: Kaplan-Meier survival curves comparing CRS-HIPEC treatment to systemic chemotherapy alone, for Colorectal PM[45]: Available from: URL: http://click.info.copyright.com/ ?qs=2b7046b10dd0c5471bbbb8bac0096e00e895f442c03264235cab52a23497a2d7e22ca0949c16bf1f. CRS-HIPEC: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy; PM: Peritoneal metastases.

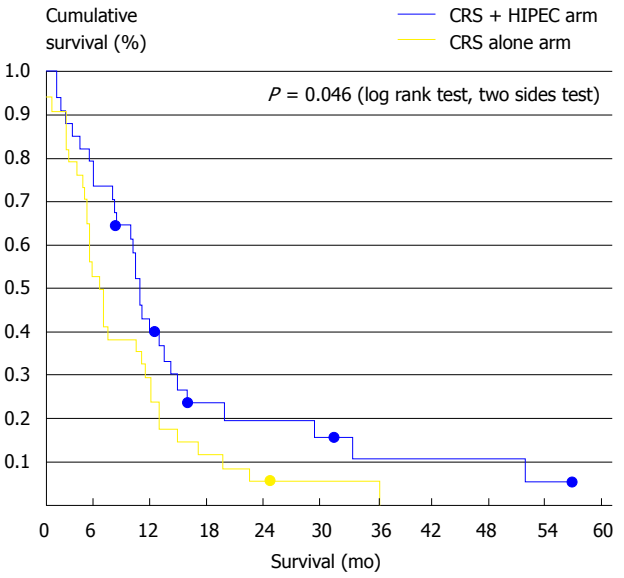

Figure 5 Kaplan-Meier survival curves comparing cytoreductive surgery and hyperthermic intraperitoneal chemotherapy treatment to cytoreductive surgery alone, for gastric peritoneal carcinomatosis[47] (with permission). Kaplan-Meier survival curves comparing CRS-HIPEC treatment to CRS alone, for Gastric PM[47]: This is an open access article distributed under the terms of the Creative Commons Attribution Noncommercial License, which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited. CRS-HIPEC: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy; PM: Peritoneal metastases.

pseudomyxoma peritonei, CRS-HIPEC was designated the "gold standard" due to the yielded results (at 5 years: 73% global survival, 56% disease-free survival)[49]. Favorable results were shown for colorectal cancer (at 5 years: 27% global survival, 10% disease-free survival)[50], and also for ovarian cancer (global survival: Advanced forms 35.4 mo, recurrent forms 45.7 mo)[51].

The results were also confirmed by a long-term data analysis in the Netherlands, after the implementation of the CRS-HIPEC treatment: MS was 33 mo (95%CI: 28-38 mo) in colorectal cancer and 130 mo (95%CI: 98-162 mo) in pseudomyxoma peritonei (Figure 6)[52].

The experience of a reference center for PM treatment (St. George's Hospital, Sydney) sustains the higher results obtained by the use of CRS-HIPEC treatment in pseudomyxoma peritonei (MS 104 mo; 5-year survival 75%) and colorectal cancer (MS 33 mo; 3-year survival 46%)[53].

A series of systematic reviews were conducted under the leadership of Sugarbaker, demonstrating a better survival for CRS-HIPEC treatment compared to conventional systemic chemotherapy. PMs of different origins were analyzed: Colorectal[54], gastric[48], ovarian[55] cancers, and malignant peritoneal mesothelioma[56]. Other systematic review studies also reported higher results for CRS-HIPEC in colorectal[57] and gastric[58] cancers.

All these studies (numerous and enrolling an increased number of patients) shows joint international efforts to identify the role of CRS-HIPEC in multimodal PM treatment. They have allowed the development of an important medical database which, by confirming the higher results in terms of survival and disease-free survival, upholds this treatment strategy. This is also confirmed by the evidence-based medicine approach studies[59].

The MS, as a result of CRS-HIPEC treatment, related to the tumor entities and study type, were presented in Table 1.

As a recognition of the foundation-laying treatment of PM with CRS-HIPEC, this was included in the treatment guidelines in France[60], Germany[61], United Kingdom (http://www.nice.org.uk/guidance/IPG331/PublicInfo), the Netherlands[52,62]. There are a number of ongoing symposia focused on Peritoneal Surface Malignancies, under the patronage of the European Society of Surgical

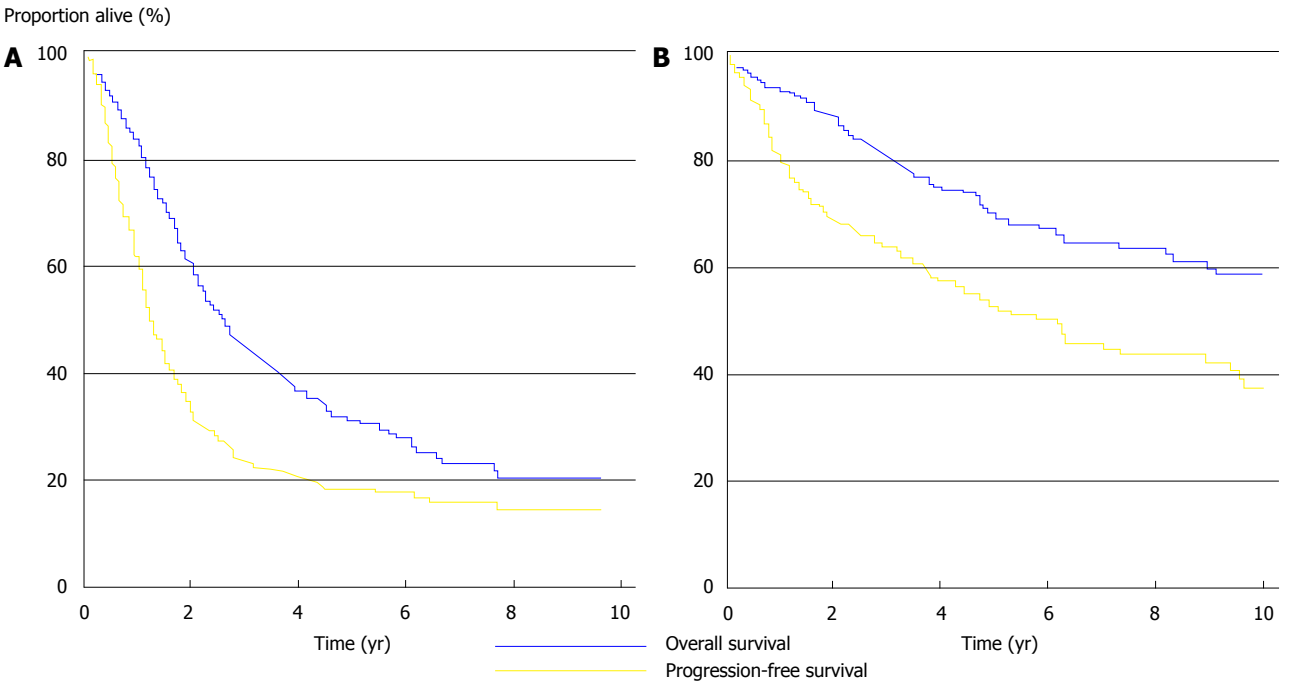

Figure 6 Overall survival compared with disease-free survival for pseudomyxoma peritonei (A) and colorectal peritoneal carcinomatosis (B), treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, from the Netherlands[52] (with permission). Overall and disease-free survival of Pseudomyxoma Peritonei (A) and Colorectal PM (B), treated with CRS-HIPEC, from the Netherlands[52]. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CRS-HIPEC: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy; PM: Peritoneal metastases.

Table 1 Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy median survival, related to the tumor entities and study type

Primitive tumor origin	Study type	Median survival (mo)
Colorectal	Randomized clinical trials	22.3[35]
	Single center experience	33[53], 34.7[45]
	Systematic reviews	13-29[54]
	Multi-institutional studies	33[52]
Gastric	Randomized clinical trials	11.3[46], 11[47]
Pseudomyxoma peritonei	Single center experience	104[53]
	Multi-institutional studies	130[52]
Ovarian	Multi-institutional studies	35.4-45.7[51]
	Systematic reviews	22-54[55]
Malignant peritoneal mesothelioma	Systematic reviews	34-92[56]

Oncology, as well as a world congress.

The view of those who consider that multimodal PM treatment is "more of an experimental kind, based on common-sense evidence rather than on solid data"[63,64] is already obsolete. Even so, the confirmation of CRS-HIPEC, as an effective treatment approach by medical studies, such as randomized clinical trials, is not mandatory[65]. And more than that, the life of patients could be endangered by denying them a treatment resource, since the validation process may take as long as 40 years[66].

Aspects

Unfortunately, there is no single perfect treatment option, valid in any setting, so the problems raised were concerned with treatment risk (morbidity, mortality, medical team risk), costs and with the indication of CRS-HIPEC, in terms of prognostic factors.

In the beginning, morbidity and mortality were the key factors initiating distrust among patients and physicians[67]. The causes of morbidity and mortality may well be suspected to belong to either CRS (surgery proper), or HIPEC (thermal effects of circulating fluid and toxic cytostatic drug effects). In a systematic-review study, morbidity was 21.5% and mortality 4.8%[68], but literature reports data within a large range: Morbidity 12%-67% and mortality 0%-9%. The main complications include digestive fistulae, postoperative bleeding, pleural-pulmonary complications, bone marrow suppression, hemodynamic instability and renal failure. Protective ileostomy, chest drain and postoperative thoracic imaging are routinely used.

However, it was shown that morbidity and mortality were not significantly increased, compared to extensive organ resection surgery (*e.g.*, Whipple's operation)[69]. Treatment complications were significantly correlated with the number of Peritonectomy procedures, left diaphragmatic Peritonectomy, duration of surgery and the number of large bowel anastomoses[70]. The global incidence for the 1th to 4th degree of gastrointestinal toxicities (according to the Common Terminology Criteria for Adverse Events) was 17%, and for symptomatic surgical site infections incidence reached 35.85% (with a global morbidity of 45%)[71].

The learning curve must be respected by any medi-

cal procedure, even more so when it implies new skills for the surgeon. The "breaking points" of the learning curve in achieving complete CRS, of a morbidity less than 3th degree and an absence of treatment-related mortality are evaluated to be 141, 158 and 144 cases, respectively (the Milan experience), or 126, 134 and 60 cases, respectively (the Bentivoglio experience)[72].

As the Dutch model of CRS multimodal treatment implementation was analyzed, it was proven that, unlike in the initial stage (experience gathering), in the stage of treatment becoming standard, the percentage of radical surgeries increased significantly (66% *vs* 86%, $P < 0.001$) and major morbidity (3th-5th degree) decreased significantly (64% *vs* 32%, $P < 0.001$)[73].

If there existed accreditation centers and HIPEC registers, coursing through the learning curve could be faster[74]. Suggestions were made for training in CRS-HIPEC to begin during the residency programmer[75].

As for the risks the medical team are exposed to during HIPEC, it was shown in several pharmacological studies that, in relation to the HIPEC method (closed/open), with required training, these risks may be reduced to a minimum[76-78].

The costs implied by CRS-HIPEC treatment are definitely high, but financial calculations show that it is a better solution in terms of treatment results[53,79-81]. This is why in Germany, HIPEC is adopted, considered a surgical procedure and coded as such[61].

Patient selection

One of today's challenges in treating PM is patient selection. This is why literature studies are focused on factors/variables correlating with survival.

The randomized clinical trial performed by Verwaal *et al*[35] showed that in colorectal cancer, the variables with the highest impact are extension of PM and the radical feature of CRS ($P < 0.0001$) (Figure 7). The GYMSSA randomized clinical trial[46] also showed that essential conditions for a significant increase in survival in gastric cancer are complete CRS and a PCI $\leqslant$ 15.

A single-center experience, the most comprehensive in terms of the number of patients (1000 patients), shows that the prognostic factors significantly correlated with survival ($P < 0.001$) are: The performance index, the location of the primary tumor, the CCRS, and the center experience[82]. Another single-center experience (109 patients) correlated survival to the following factors: Histology of non-adenocarcinoma ($P = 0.001$), appendiceal location ($P = 0.001$), absence of liver metastases ($P = 0.01$), and complete resection of all gross disease ($P < 0.001$)[83].

A multi-center French study shows that in colorectal cancer, CCRS is the most important prognostic factor for MS: 32.4 mo for complete *vs* 8.4 mo for incomplete CCRS ($P < 0.001$) (Figure 8)[33]. The multi-center SITILO study[84] also reveals in colorectal cancer the following independent prognostic variables correlated with survival: PCI, CCRS, and presence of hepatic metastases.

The consensus conference on PM treatment in colon cancer statutes that the indication of CRS-HIPEC treatment should be based on complete CRS[85].

All those prognostic factors are in dynamics, the trend being towards broadening the indication range, except for the radical feature of CRS: It is absolutely necessary that this should be complete, or at least optimal. Traditionally, the treatment approach in PM was reserved for colon or appendicular cancer, pseudomyxoma peritonei and peritoneal malignant mesothelioma, but now the range of indications includes rectal, gastric and ovarian cancers. The importance of PCI is not an absolute one, it must be correlated with primary tumor location (colonic PM origin *vs* gastric), histological grading (well and moderate differentiated *vs* poorly differentiated and undifferentiated), and the anatomical sites involved (Treitz ligament, porta hepatis and suprahepatic veins, coelic axis and mesenteric vessels). The presence of other systemic dissemination (abdominal or extra-abdominal) was a contraindication for the treatment approach in PM, but this is no longer (Figure 9) the case provided that complete CRS can be obtained[86-89].

Except for the patient status (evaluated by the performance index), the main CRS-HIPEC treatment contraindication is small bowel involvement[90], which is regarded as an independent prognostic factor[91-93].

A series of studies have confirmed the prognostic value of tumor grading in colorectal cancer. The "signet-ring cell" *vs* other types of differentiation has a significantly poorer MS (14.1 mo *vs* 35.1 mo; $P < 0.01$) and an increased relapse rate (68.8% *vs* 43.7%; $P = 0.05$)[94]. CRS-HIPEC treatment carries no survival benefit in colorectal PM with signet-ring cell histology, unless complete CRS is obtained[95]. Also, for the aggressive type of pseudomyxoma peritonei, systemic chemotherapy is indicated instead of CRS-HIPEC treatment[96].

In some studies, the prognostic factors were grouped into scores. The Peritoneal Surface Disease Severity Score may be used to stratify patients in clinical trials[97]. Different scores used in PM of colorectal origin are: The Peritoneal Surface Disease Severity Score, the Prognostic Score, and the Colorectal Peritoneal Score. The Colorectal Peritoneal Score (value $\geqslant$ 6) identifies patients with an unfavorable prognosis in terms of survival, and it does so better than PCI (value > 20) or the other two scoring systems[98].

Controversies

At the same time, there are also reserved attitudes regarding the CRS-HIPEC approach of PM. This is mainly the position of oncologists, who are refractory to this treatment option, using the argument of the risk/benefit ratio. The theoretical premise they use is based on the lack of difference (pathophysiology, evolution, and treatment) between PM and other systemic dissemination. The treatment is not adapted, so different protocols of systemic chemotherapy (from the classic de Gramont chemotherapy to oxaliplatin, irinotecan and biological molecular agents) are given in different clinical

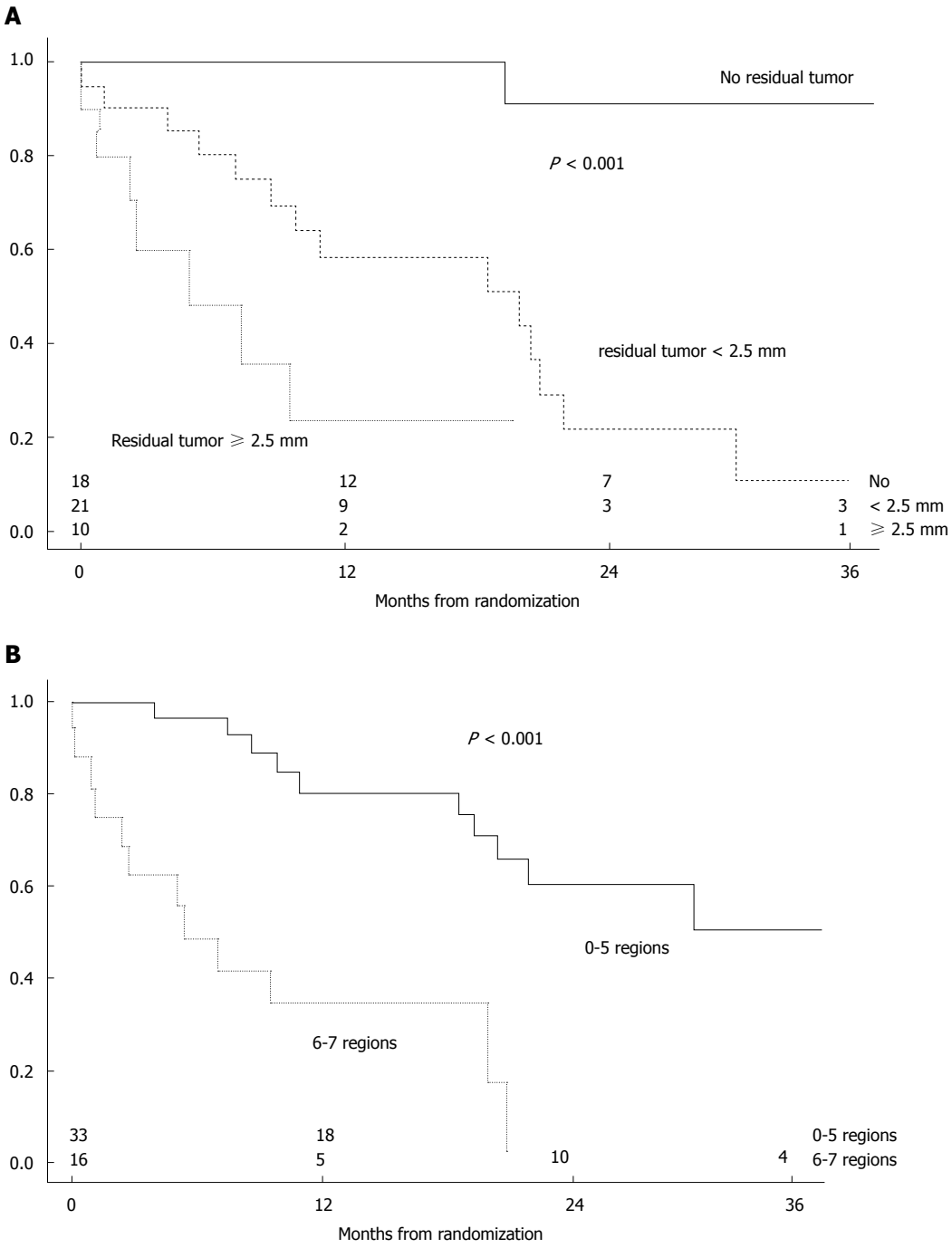

Figure 7 Kaplan-Meier survival curve of colorectal peritoneal carcinomatosis treated by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, stratified according to the size of residual tumors (A) and the number of regions with residual tumors (B)[35] (with permission). Kaplan-Meier survival curve of CRS-HIPEC treatment, for Colorectal PM, stratified by the number of regions affected (A) and the number of regions with residual tumors (B)[35]: http://click.info.copyright.com/?qs=2b7046b10dd0c5471bbbb8bac0096e00e895f442c0326423c4ec2ccdd7b4701b99fd4bd5b32f0283. CRS-HIPEC: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy; PM: Peritoneal metastases.

trials, without considering the variances between the specific dissemination biology[99-101]. The indispensable condition that oncologists require for inclusion in a clinical trial is the presence of measurable lesions. In the case of PM, this condition is almost impossible to meet. Imaging modalities have a low sensitivity in detecting the peritoneal dissemination, and this is true for computer tomography as well as for magnetic resonance imaging. The only parameters based on which the results of CRS-HIPEC treatment may be assessed are disease-free survival and global survival.

Furthermore, concerning the oncologists' perspective, there is at least one more important argument supporting the dedicated multimodal treatment of PM: The studies matching hepatic metastases *vs* PM. These have shown that the pathway of dissemination are different and the treatment results are comparable (Figure 10), if treatment is potentially radical[102-104].

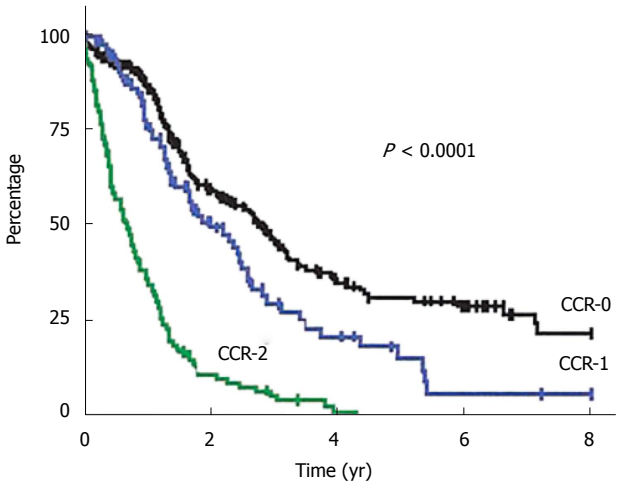

Figure 8 Kaplan-Meier survival curve of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy treatment, for colorectal peritoneal carcinomatosis, stratified by completeness of cytoreduction score[33] (with permission). Kaplan-Meier survival curve of CRS-HIPEC treatment, for Colorectal PM, stratified by CCRS[33]: Available from: URL: http://click.info.copyright.com/ ?qs=2b7046b10dd0c5471bbbb8bac0096e00e895f442c0326423b795894c1892230b84270e7e00c4e014. CCRS: Completeness of cytoreduction score; CRS-HIPEC: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy; PM: Peritoneal metastases.

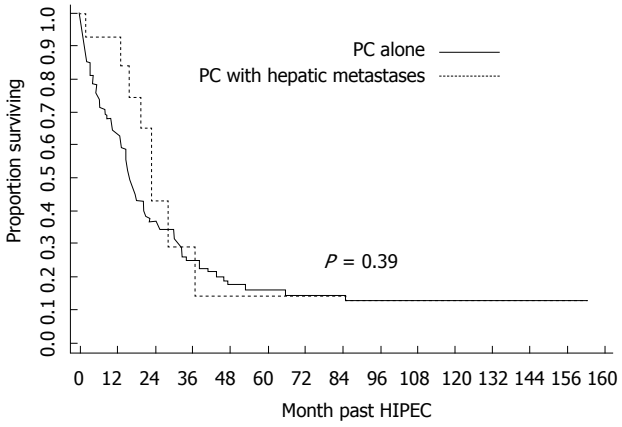

Figure 9 Kaplan-Meier survival curve of colorectal peritoneal carcinomatosis stratified according to hepatic involvement (peritoneal carcinomatosis alone *vs* peritoneal carcinomatosis with hepatic metastases)[86] (with permission). Kaplan-Meier survival curve of Colorectal PM alone and PM with Hepatic Metastases[86]: Available from: URL: http://click.info.copyright.com/ ?qs=2b7046b10dd0c5471bbbb8bac0096e00e895f442c032642364879a25688a50cc82757e6f8509f6e1. PM: Peritoneal metastases.

Nowadays, in colorectal cancer, by analogy with Hepatic Metastases, multimodal CRS-HIPEC treatment has been adopted and is the indicated approach for CP, in most institutions in the United States[31].

PM MULTIMODAL TREATMENT - RESEARCH PATHWAYS (2010-2014)

Currently, there is reason to talk about a higher level, where the problem of timing for CRS-HIPEC is raised. There are studies showing that PM prophylaxis is a valid approach.

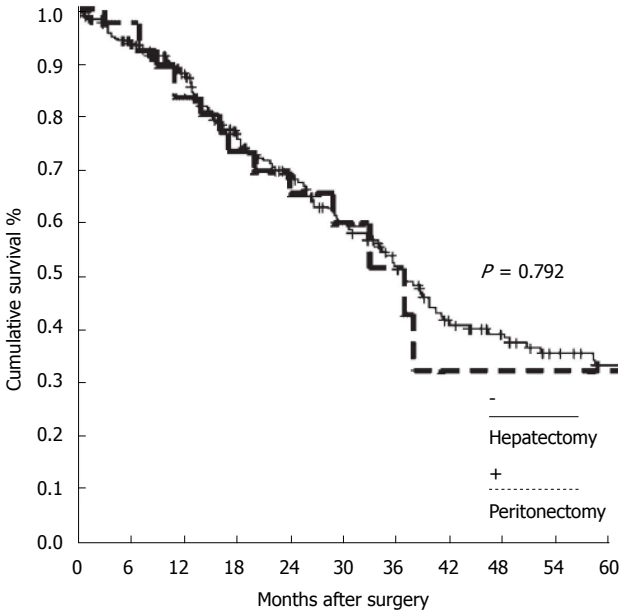

Figure 10 Kaplan-Meier survival curve for colorectal peritoneal metastases and colorectal hepatic metastases, who achieved optimal treatment[102] (with permission). Kaplan-Meier survival curve for Colorectal PM and Colorectal Hepatic Metastases, with optimal treatment[102]: Available from: URL: http://click.info.copyright.com/ ?qs=751ae7c8f8e03ecb0a4741c79a3b341599b5318899e258b3d458148f31158885e37d98de5cb03229. PM: Peritoneal metastases.

"Proactive management" defines a treatment concept, targeting patients with a high risk to develop PM, and prescribes surgery with HIPEC. In colorectal cancer, this has brought about a significant results ($P < 0.03$), related to control group (only surgery), in terms of PM developed and local recurrence (4% *vs* 28%, over a 48-mo follow-up period). Patients had also significant longer MS (59.2 mo *vs* 52 mo; $P < 0.04$) and disease-free survival ($P < 0.05$)[105].

There was a hypothesis of second-look surgery at 1 year in patients at high risk for PM, after the first radical surgery for colorectal cancer. PM might be identified and treated at an earlier stage in about 55% of patients[106]. Such researches were also led for gastric cancer, with promising preliminary results[107,108].

In the same context of colorectal PM prophylaxis, the HIPEC laparoscopic approach was described and indicated after a mean interval of 6 wk (3-9 wk). This approach showed its feasibility[109].

The main issue is selecting high-risk patients for developing PM. The debated risk factors are: Invasion of or beyond the serosa (pT3, pT4), perforated tumors, positive peritoneal cytology (augmented by immunohistochemistry), occurrence of Krukenberg tumors and mucinous type of tumor[110]. Following a systematic review, three situations were identified to be associated with an increased frequency of metachronous PM development: Synchronous PM, ovarian metastases, and perforated tumor[111].

Despite all human and material efforts dedicated to CRS-HIPEC treatment, there are patients in whom evolving disease occurs. The right question is whether

Table 2 Key aspects and peritoneal metastases model in the evolution of multimodal treatment

Period	PM treatment	Key aspects	PM model
All the period	"Conventional" systemic chemotherapy	Significant lower survival for PM *vs* other type of metastases	Colo-rectal
1950-1980	"Dedicated" intraperitoneal treatment - Palliative treatment	The basis for developing further cytostatic drugs	Malignant ascites
1980-2000	"Dedicated" intraperitoneal treatment - Multimodal radical treatment	Regional intraperitoneal normothermic and hyperthermic chemotherapy Peritonectomy procedures Define PCI and CCRS	Appendicular
2000-2010	Multimodal radical treatment - confirmation, aspects, patient selection, controversies	Significant higher survival *vs* palliative surgery and diverse systemic chemotherapy regimes	Colo-rectal
		Acceptable morbidity and mortality, no significant risk for medical team	Appendicular
		Respect de learning curve	Pseudomyxoma peritonei
		High costs	Malignant peritoneal mesothelioma
		Define the prognostic factors	Gastric
		Position of oncologists	Ovarian
		Comparison with hepatic metastases	PM with hepatic metastases
2010-2014	Multimodal treatment – research pathways	PM prophylaxis	High-risk patients for developing PM
		Laparoscopic HIPEC	Recurrent PM
		Integration of chemotherapy with surgery	
		Extension of CRS	

PM: Peritoneal metastases; CCRS: Completeness of cytoreduction score; CRS-HIPEC: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy; PCI: Peritoneal cancer index.

iterative treatment of PM might be a solution. There are at least three problems: Estimate the morbidity/mortality associated with the iterative treatment; documentation the prognostic factors; estimation the treatment results in terms of survival. The only few related studies define no clear attitude, supported by statistically significant data. Morbidity/mortality does not seem to differ significantly[112-114], only one single study reporting increased values[115].

HIPEC remains an important tool in the treatment of recurrent PM. Age (P = 0.049), time lapse between surgeries (P = 0.08), association of HIPEC (P = 0.005), and small bowel resections (P < 0.001) are statistically correlated with survival in PM appendiceal origin and malignant peritoneal mesothelioma[113]. The iterative approach of colorectal PM results in a MS of 22.6 mo, with the following survival percentages: 1 year - 94%; 2 years - 48%; 3 years - 12%[114].

The iterative approach treatment of the patient with PM and Hepatic Metastases has also promising results[116].

An important research pathway is the way by which chemotherapy might be integrated into various treatment approaches: Perioperative neoadjuvant; HIPEC; bidirectional intraoperative; early postoperative intraperitoneal. The multidisciplinary approach of PM is based on treatment with complete CRS. Unfortunately, this cannot be achieved in approximately 70% of patients[31].

The place of systemic chemotherapy in PM multimodal treatment is difficult to assess. There are definite reports regarding the results of perioperative neoadjuvant chemotherapy, demonstrated by histological response in colon cancer[117] and by increased survival in appendix cancer[118]. Likewise, in gastric cancer, survival benefits have been shown for perioperative neoadjuvant and early postoperative intraperitoneal chemotherapy[119].

The complexity of multimodal treatment approach is also certified by the wide range of cytostatic drugs and the occurrence of new principles. It is possible that Mitomycin C, in colorectal PM, in the context of complete CRS, may yield superior survival results compared to oxaliplatin[120]. For gastric PM, catumaxomab seems to confirm positive results[119], but in colorectal PM, bevacizumab leads to a double mortality rate after CRS-HIPEC treatment[121].

Furthermore, as far as the array of CRS treatment is concerned, along with visceral resection and Peritonectomy procedures, surgery on the urinary tract was assessed. Partial cystectomy and ureter segmental resection were the most used. There was no further increase in the recorded morbidity or mortality and survival was comparable to that of patients with CRS-HIPEC without urinary tract surgery[122,123]. The same statute was dedicated to the hepatobiliary procedures[124].

At present, there are over 50 clinical trials underway, aimed at assessing multimodal radical PM treatment (http://clinicaltrialsfeeds.org/clinical-trials/results/term=HIPEC). Out of these, some were reported in the literature: GASTRICHIP (D2 gastric resection and HIPEC

for locally advanced gastric cancer)[125], COMBATAC (multimodal treatment of PM of appendiceal and colorectal origin)[126], NCT01095523 (second-look and CRS-HIPEC treatment for colorectal cancer at risk for PM)[127].

Key aspects and PM model in the evolution of multimodal treatment strategies

We summarized the key aspects of the evolution of multimodal treatment strategies in Table 2.

CONCLUSION

Initially marked by nihilism and fear, but benefiting from a remarkable joint effort of human and material resources (multi-center and -institutional research), over a period of 30 years, CRS-HIPEC found its place in the multimodal treatment of PM. The next 4 years were dedicated to the refinement of multimodal treatment, by launching research pathways. In selected patients, with requires training, it demonstrated a significant survival results (similar to the Hepatic Metastases treatment), with acceptable risks and costs. Also, CRS-HIPEC opens a lot of new opportunities with reference to the patients' selection and adopted methodology of this multimodal treatment.

The main debates regarding CRS-HIPEC were based on the oncologists' perspective and the small number of randomized clinical trials. It is hard to find a common view on different challenges, raised by CRS-HIPEC in the treatment of PM. Probably, Dr. Bernard Fisher met the same mistrust as he revolutionized breast cancer treatment and the same could be said about the surgical approach to metastatic melanoma. Indeed, there is a discrepancy between the great number of multi-center, -institutional studies and the small number of randomized clinical trials.

We may say that there are a series of determining factors, for the long-term assessment of multimodal PM treatment and the bias in medical studies type. Treatment complexity, results from the interaction between different therapeutic principles (surgery, chemotherapy, and hyperthermia), are an essential factor. Also, the peritoneal cavity is a complex anatomical space, and the pathogenesis of PM is indeed multifactorial conditioning (loco-regional and systemic dissemination). Not least, the multidisciplinary approach of PM implies the teamwork of specialists with different training, treatment concepts and results assessment, making it difficult to find a common view.

It is important to statement the patients with colorectal, appendicular, gastric, and ovarian peritoneal carcinomatosis, as well as patients with pseudomyxoma peritonei and peritoneal malignant mesothelioma must be informed about CRS-HIPEC as a valid treatment resource. The eligibility criteria for patients' selection will be assessed by multidisciplinary teams, in high level, dedicated treatment centers, according to the performance index, PCI, histological grading, the perspective to obtaining a complete CRS, and the availability of sustaining chemotherapy protocols.

REFERENCES

1 **Sampson JA**. Implantation Peritoneal Carcinomatosis of Ovarian Origin. *Am J Pathol* 1931; **7**: 423-444.39 [PMID: 19969977]

2 **Sadeghi B**, Arvieux C, Glehen O, Beaujard AC, Rivoire M, Baulieux J, Fontaumard E, Brachet A, Caillot JL, Faure JL, Porcheron J, Peix JL, François Y, Vignal J, Gilly FN. Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. *Cancer* 2000; **88**: 358-363 [PMID: 10640968 DOI: 10.1002/(SICI)1097-0142(20000115)88: 2<358: : AID-CNCR16>3.0.CO; 2-O]

3 **Gilly FN**, Carry PY, Sayag AC, Brachet A, Panteix G, Salle B, Bienvenu J, Burgard G, Guibert B, Banssillon V. Regional chemotherapy (with mitomycin C) and intra-operative hyperthermia for digestive cancers with peritoneal carcinomatosis. *Hepatogastroenterology* 1994; **41**: 124-129 [PMID: 8056398]

4 **Chu DZ**, Lang NP, Thompson C, Osteen PK, Westbrook KC. Peritoneal carcinomatosis in nongynecologic malignancy. A prospective study of prognostic factors. *Cancer* 1989; **63**: 364-367 [PMID: 2910444]

5 **Glehen O**, Osinsky D, Beaujard AC, Gilly FN. Natural history of peritoneal carcinomatosis from nongynecologic malignancies. *Surg Oncol Clin N Am* 2003; **12**: 729-739, xiii [PMID: 14567027 DOI: 10.1016/S1055-3207(03)00044-9]

6 **Jayne DG**, Fook S, Loi C, Seow-Choen F. Peritoneal carcinomatosis from colorectal cancer. *Br J Surg* 2002; **89**: 1545-1550 [PMID: 12445064 DOI: 10.1046/j.1365-2168.2002.02274.x]

7 **Franko J**, Shi Q, Goldman CD, Pockaj BA, Nelson GD, Goldberg RM, Pitot HC, Grothey A, Alberts SR, Sargent DJ. Treatment of colorectal peritoneal carcinomatosis with systemic chemotherapy: a pooled analysis of north central cancer treatment group phase III trials N9741 and N9841. *J Clin Oncol* 2012; **30**: 263-267 [PMID: 22162570 DOI: 10.1200/JCO.2011.37.1039]

8 **Pelz JO**, Chua TC, Esquivel J, Stojadinovic A, Doerfer J, Morris DL, Maeder U, Germer CT, Kerscher AG. Evaluation of best supportive care and systemic chemotherapy as treatment stratified according to the retrospective peritoneal surface disease severity score (PSDSS) for peritoneal carcinomatosis of colorectal origin. *BMC Cancer* 2010; **10**: 689 [PMID: 21176206 DOI: 10.1186/1471-2407-10-689]

9 **Lemmens VE**, Klaver YL, Verwaal VJ, Rutten HJ, Coebergh JW, de Hingh IH. Predictors and survival of synchronous peritoneal carcinomatosis of colorectal origin: a population-based study. *Int J Cancer* 2011; **128**: 2717-2725 [PMID: 20715167 DOI: 10.1002/ijc.25596]

10 **Green TH**. Hemisulfur mustard in the palliation of patients with metastatic ovarian carcinoma. *Obstet Gynecol* 1959; **13**: 383-393 [PMID: 13644835 DOI: 10.1097/00006254-195908000-00045]

11 **Kottmeier HL**. Treatment of ovarian cancer with thiotepa. *Clin Obstet Gynecol* 1968; **11**: 428-438 [PMID: 4176467]

12 **Jordan E**, Karnicka-Mlodkowska H. [Intrapleural and intraperitoneal instillation of nitrogen mustard in palliative treatment of malignant effusions]. *Pol Tyg Lek* 1971; **26**: 676-678 [PMID: 4103813]

13 **Lee M**, Boyes DA. The use of quinacrine hydrochloride for the control of malignant serous effusions. *J Obstet Gynaecol Br Commonw* 1971; **78**: 843-844 [PMID: 5097172]

14 **Baas EU**, Krönig B. [Volume determination of ascites using intraperitoneal bromsulphalein administration]. *Verh Dtsch Ges Inn Med* 1971; **77**: 1374-1376 [PMID: 5156034]

15 **Hart GD**. Palliative management of gastrointestinal cancer. *Can Med Assoc J* 1964; **90**: 1265-1268 [PMID: 14158958]

16 **Dedrick RL**, Myers CE, Bungay PM, DeVita VT. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. *Cancer Treat Rep* 1978; **62**: 1-11 [PMID: 626987]

17 **Speyer JL**, Myers CE. The use of peritoneal dialysis for delivery of chemotherapy to intraperitoneal malignancies. *Recent Results Cancer Res* 1980; **74**: 264-269 [PMID: 7444143]

18 **Spratt JS**, Adcock RA, Muskovin M, Sherrill W, McKeown J. Clinical delivery system for intraperitoneal hyperthermic chemotherapy. *Cancer Res* 1980; **40**: 256-260 [PMID: 6766084]

19 **Hahn GM**, Braun J, Har-Kedar I. Thermochemotherapy: synergism between hyperthermia (42-43 degrees) and adriamycin (of bleomycin) in mammalian cell inactivation. *Proc Natl Acad Sci USA* 1975; **72**: 937-940 [PMID: 48253 DOI: 10.1073/pnas.72.3.937]

20 **Urano M**, Ling CC. Thermal enhancement of melphalan and oxaliplatin cytotoxicity in vitro. *Int J Hyperthermia* 2002; **18**: 307-315 [PMID: 12079586 DOI: 10.1080/02656730210123534]

21 **Mohamed F**, Marchettini P, Stuart OA, Urano M, Sugarbaker PH. Thermal enhancement of new chemotherapeutic agents at moderate hyperthermia. *Ann Surg Oncol* 2003; **10**: 463-468 [PMID: 12734097 DOI: 10.1245/ASO.2003.08.006]

22 **Smythe WR**, Mansfield PF. Hyperthermia: has its time come? *Ann Surg Oncol* 2003; **10**: 210-212 [PMID: 12679303 DOI: 10.1245/ASO.2003.02.011]

23 **Lepock JR**. How do cells respond to their thermal environment? *Int J Hyperthermia* 2005; **21**: 681-687 [PMID: 16338849 DOI: 10.1080/02656730500307298]

24 **González-Moreno S**, González-Bayón LA, Ortega-Pérez G. Hyperthermic intraperitoneal chemotherapy: Rationale and technique. *World J Gastrointest Oncol* 2010; **2**: 68-75 [PMID: 21160924 DOI: 10.4251/wjgo.v2.i2.68]

25 **Pelz JO**, Vetterlein M, Grimmig T, Kerscher AG, Moll E, Lazariotou M, Matthes N, Faber M, Germer CT, Waaga-Gasser AM, Gasser M. Hyperthermic intraperitoneal chemotherapy in patients with peritoneal carcinomatosis: role of heat shock proteins and dissecting effects of hyperthermia. *Ann Surg Oncol* 2013; **20**: 1105-1113 [PMID: 23456378 DOI: 10.1245/s10434-012-2784-6]

26 **Sugarbaker PH**, Chang D, Koslowe P. Peritoneal carcinomatosis from appendiceal cancer: A paradigm for treatment of abdomino-pelvic dissemination of gastrointestinal malignancy. *Eur Surg* 1996; **28**: 4-8 [DOI: 10.1007/BF02625947]

27 **Sugarbaker PH**. Metastatic inefficiency: the scientific basis for resection of liver metastases from colorectal cancer. *J Surg Oncol Suppl* 1993; **3**: 158-160 [PMID: 8503973]

28 **Sugarbaker PH**. It's what the surgeon doesn't see that kills the patient. *J Nippon Med Sch* 2000; **67**: 5-8 [PMID: 10777840 DOI: 10.1272/jnms.67.5]

29 **Sugarbaker PH**. Overview of Peritoneal Carcinomatosis. *Cancerologia* 2008; **3**: 119-124

30 **Sugarbaker PH**. Surgical responsibilities in the management of peritoneal carcinomatosis. *J Surg Oncol* 2010; **101**: 713-724 [PMID: 20512948 DOI: 10.1002/jso.21484]

31 **Sugarbaker PH**. Cytoreductive surgery plus hyperthermic perioperative chemotherapy for selected patients with peritoneal metastases from colorectal cancer: a new standard of care or an experimental approach? *Gastroenterol Res Pract* 2012; **2012**: 309417 [PMID: 22888335 DOI: 10.1155/2012/309417]

32 **Gómez Portilla A**, Cendoya I, Olabarría I, Martínez de Lecea C, Gómez Martínez de Lecea C, Gil A, Martín E, Muriel J, Magrach L, Romero E, Lirola A, Guede N, Moraza N, Fernández E, Kvadatze M, Valdovinos M, Larrabide I, Ruiz de Alegría N, Fernández JL, Castillo C, Rua O, Ulibarrena MA. The European contribution to "Sugarbaker's protocol" for the treatment of colorectal peritoneal carcinomatosis. *Rev Esp Enferm Dig* 2009; **101**: 97-102, 103-106 [PMID: 19335045 DOI: 10.4321/S1130-01082009000200003]

33 **Glehen O**, Kwiatkowski F, Sugarbaker PH, Elias D, Levine EA, De Simone M, Barone R, Yonemura Y, Cavaliere F, Quenet F, Gutman M, Tentes AA, Lorimier G, Bernard JL, Bereder JM, Porcheron J, Gomez-Portilla A, Shen P, Deraco M, Rat P. Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: a multi-institutional study. *J Clin Oncol* 2004; **22**: 3284-3292 [PMID: 15310771 DOI: 10.1200/JCO.2004.10.012]

34 **Elias D**, Delperro JR, Sideris L, Benhamou E, Pocard M, Baton O, Giovannini M, Lasser P. Treatment of peritoneal carcinomatosis from colorectal cancer: impact of complete cytoreductive surgery and difficulties in conducting randomized trials. *Ann Surg Oncol* 2004; **11**: 518-521 [PMID: 15123461 DOI: 10.1245/ASO.2004.09.008]

35 **Verwaal VJ**, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, Zoetmulder FA. Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *J Clin Oncol* 2003; **21**: 3737-3743 [PMID: 14551293 DOI: 10.1200/JCO.2003.04.187]

36 **Elias DM**, Sideris L. Pharmacokinetics of heated intraoperative intraperitoneal oxaliplatin after complete resection of peritoneal carcinomatosis. *Surg Oncol Clin N Am* 2003; **12**: 755-769, xiv [PMID: 14567029 DOI: 10.1016/S1055-3207(03)00051-6]

37 **Elias D**, Matsuhisa T, Sideris L, Liberale G, Drouard-Troalen L, Raynard B, Pocard M, Puizillou JM, Billard V, Bourget P, Ducreux M. Heated intra-operative intraperitoneal oxaliplatin plus irinotecan after complete resection of peritoneal carcinomatosis: pharmacokinetics, tissue distribution and tolerance. *Ann Oncol* 2004; **15**: 1558-1565 [PMID: 15367418 DOI: 10.1093/annonc/mdh398]

38 **Sugarbaker PH**. Peritonectomy procedures. *Ann Surg* 1995; **221**: 29-42 [PMID: 7826158]

39 **Sugarbaker PH**. Dissection by electrocautery with a ball tip. *J Surg Oncol* 1994; **56**: 246-248 [PMID: 8057651 DOI: 10.1002/jso.2930560409]

40 **Sugarbaker PH**. The subpyloric space: an important surgical and radiologic feature in pseudomyxoma peritonei. *Eur J Surg Oncol* 2002; **28**: 443-446 [PMID: 12099657 DOI: 10.1053/ejso.2001.1238]

41 **Sugarbaker PH**. Circumferential cutaneous traction for exposure of the layers of the abdominal wall. *J Surg Oncol* 2008; **98**: 472-475 [PMID: 18770521 DOI: 10.1002/jso.21138]

42 **Sugarbaker PH**. Pont hepatique (hepatic bridge), an important anatomic structure in cytoreductive surgery. *J Surg Oncol* 2010; **101**: 251-252 [PMID: 20082353 DOI: 10.1002/jso.21478]

43 **Sugarbaker PH**. Technical Handbook for the Integration of Cytoreductive Surgery and Perioperative Intraperitoneal Chemotherapy into the Surgical Management of Gastrointestinal and Gynecologic Malignancy. 4th ed. Michigan: The Ludann Company Grand Rapids, 2005: 19-24

44 **Elias D**, Lefevre JH, Chevalier J, Brouquet A, Marchal F, Classe JM, Ferron G, Guilloit JM, Meeus P, Goéré D, Bonastre J. Complete cytoreductive surgery plus intraperitoneal chemohyperthermia with oxaliplatin for peritoneal carcinomatosis of colorectal origin. *J Clin Oncol* 2009; **27**: 681-685 [PMID: 19103728 DOI: 10.1200/JCO.2008.19.7160]

45 **Franko J**, Ibrahim Z, Gusani NJ, Holtzman MP, Bartlett DL, Zeh HJ. Cytoreductive surgery and hyperthermic intraperitoneal chemoperfusion versus systemic chemotherapy alone for colorectal peritoneal carcinomatosis. *Cancer* 2010; **116**: 3756-3762 [PMID: 20564081 DOI: 10.1002/cncr.25116]

46 **Rudloff U**, Langan RC, Mullinax JE, Beane JD, Steinberg SM, Beresnev T, Webb CC, Walker M, Toomey MA, Schrump D, Pandalai P, Stojadinovic A, Avital I. Impact of maximal cytoreductive surgery plus regional heated intraperitoneal chemotherapy (HIPEC) on outcome of patients with peritoneal carcinomatosis of gastric origin: results of the GYMSSA trial. *J Surg Oncol* 2014; **110**: 275-284 [PMID: 25042700 DOI: 10.1002/jso.23633]

47 **Yang XJ**, Huang CQ, Suo T, Mei LJ, Yang GL, Cheng FL, Zhou YF, Xiong B, Yonemura Y, Li Y. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy improves survival of patients with peritoneal carcinomatosis from gastric cancer: final results of a phase III randomized clinical trial. *Ann Surg Oncol* 2011; **18**: 1575-1581 [PMID: 21431408 DOI: 10.1245/s10434-011-1631-5]

48 **Yan TD**, Black D, Sugarbaker PH, Zhu J, Yonemura Y, Petrou G, Morris DL. A systematic review and meta-analysis of the randomized controlled trials on adjuvant intraperitoneal chemotherapy for resectable gastric cancer. *Ann Surg Oncol* 2007; **14**: 2702-2713 [PMID: 17653801 DOI: 10.1245/s10434-007-9487-4]

49 **Elias D**, Gilly F, Quenet F, Bereder JM, Sidéris L, Mansvelt B, Lorimier G, Glehen O. Pseudomyxoma peritonei: a French multicentric study of 301 patients treated with cytoreductive surgery

and intraperitoneal chemotherapy. *Eur J Surg Oncol* 2010; **36**: 456-462 [PMID: 20227231 DOI: 10.1016/j.ejso.2010.01.006]

50 **Elias D**, Gilly F, Boutitie F, Quenet F, Bereder JM, Mansvelt B, Lorimier G, Dubè P, Glehen O. Peritoneal colorectal carcinomatosis treated with surgery and perioperative intraperitoneal chemotherapy: retrospective analysis of 523 patients from a multicentric French study. *J Clin Oncol* 2010; **28**: 63-68 [PMID: 19917863 DOI: 10.1200/JCO.2010.29.0163]

51 **Bakrin N**, Bereder JM, Decullier E, Classe JM, Msika S, Lorimier G, Abboud K, Meeus P, Ferron G, Quenet F, Marchal F, Gouy S, Morice P, Pomel C, Pocard M, Guyon F, Porcheron J, Glehen O. Peritoneal carcinomatosis treated with cytoreductive surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) for advanced ovarian carcinoma: a French multicentre retrospective cohort study of 566 patients. *Eur J Surg Oncol* 2013; **39**: 1435-1443 [PMID: 24209430 DOI: 10.1016/j.ejso.2013.09.030]

52 **Kuijpers AM**, Mirck B, Aalbers AG, Nienhuijs SW, de Hingh IH, Wiezer MJ, van Ramshorst B, van Ginkel RJ, Havenga K, Bremers AJ, de Wilt JH, Te Velde EA, Verwaal VJ. Cytoreduction and HIPEC in the Netherlands: nationwide long-term outcome following the Dutch protocol. *Ann Surg Oncol* 2013; **20**: 4224-4230 [PMID: 23897008 DOI: 10.1245/s10434-013-3145-9]

53 **Chua TC**, Liauw W, Morris DL. The St George Hospital Peritoneal Surface Malignancy Program--where are we now? *ANZ J Surg* 2009; **79**: 416-418 [PMID: 19566860 DOI: 10.1111/j.1445-2197.2009.04940.x]

54 **Yan TD**, Black D, Savady R, Sugarbaker PH. Systematic review on the efficacy of cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for peritoneal carcinomatosis from colorectal carcinoma. *J Clin Oncol* 2006; **24**: 4011-4019 [PMID: 16921055 DOI: 10.1200/JCO.2006.07.1142]

55 **Bijelic L**, Jonson A, Sugarbaker PH. Systematic review of cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy for treatment of peritoneal carcinomatosis in primary and recurrent ovarian cancer. *Ann Oncol* 2007; **18**: 1943-1950 [PMID: 17496308 DOI: 10.1093/annonc/mdm137]

56 **Yan TD**, Welch L, Black D, Sugarbaker PH. A systematic review on the efficacy of cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for diffuse malignancy peritoneal mesothelioma. *Ann Oncol* 2007; **18**: 827-834 [PMID: 17130182 DOI: 10.1093/annonc/mdl428]

57 **Cao C**, Yan TD, Black D, Morris DL. A systematic review and meta-analysis of cytoreductive surgery with perioperative intraperitoneal chemotherapy for peritoneal carcinomatosis of colorectal origin. *Ann Surg Oncol* 2009; **16**: 2152-2165 [PMID: 19434455 DOI: 10.1245/s10434-009-0487-4]

58 **Matharu G**, Tucker O, Alderson D. Systematic review of intraperitoneal chemotherapy for gastric cancer. *Br J Surg* 2011; **98**: 1225-1235 [PMID: 21644239 DOI: 10.1002/bjs.7586]

59 **Nissan A**, Stojadinovic A, Garofalo A, Esquivel J, Piso P. Evidence-based medicine in the treatment of peritoneal carcinomatosis: Past, present, and future. *J Surg Oncol* 2009; **100**: 335-344 [PMID: 19697442 DOI: 10.1002/jso.21323]

60 **Kavanagh M**, Ouellet JF. [Clinical practice guideline on peritoneal carcinomatosis treatment using surgical cytoreduction and hyperthermic intraoperative intraperitoneal chemotherapy]. *Bull Cancer* 2006; **93**: 867-874 [PMID: 16980229]

61 **Piso P**, Leebmann H, März L, Mayr M. [Cytoreductive surgery for malignant peritoneal tumors]. *Chirurg* 2015; **86**: 38-46 [PMID: 24722868 DOI: 10.1007/s00104-014-2724-z]

62 **Verwaal VJ**, Bruin S, Boot H, van Slooten G, van Tinteren H. 8-year follow-up of randomized trial: cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy in patients with peritoneal carcinomatosis of colorectal cancer. *Ann Surg Oncol* 2008; **15**: 2426-2432 [PMID: 18521686 DOI: 10.1245/s10434-008-9966-2]

63 **Macrì A**. New approach to peritoneal surface malignancies. *World J Gastrointest Oncol* 2010; **2**: 9-11 [PMID: 21160811 DOI: 10.4251/wjgo.v2.i1.World]

64 **Evrard S**, Mazière C, Désolneux G. HIPEC: standard of care or an experimental approach? *Lancet Oncol* 2012; **13**: e462-e463 [PMID: 23117000 DOI: 10.1016/S1470-2045(12)70448-5]

65 **Glasziou P**, Chalmers I, Rawlins M, McCulloch P. When are randomised trials unnecessary? Picking signal from noise. *BMJ* 2007; **334**: 349-351 [PMID: 17303884 DOI: 10.1136/bmj.39070.527986.68]

66 **Sugarbaker PH**. Evolution of cytoreductive surgery and perioperative intraperitoneal chemotherapy for peritoneal carcinomatosis: are there treatment alternatives? *Am J Surg* 2011; **201**: 157-159 [PMID: 20870209 DOI: 10.1016/j.amjsurg.2010.04.010]

67 **Spiegle G**, Schmocker S, Huang H, Victor JC, Law C, McCart JA, Kennedy ED. Physicians' awareness of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for colorectal cancer carcinomatosis. *Can J Surg* 2013; **56**: 237-242 [PMID: 23883493 DOI: 10.1503/cjs.003912]

68 **Gill RS**, Al-Adra DP, Nagendran J, Campbell S, Shi X, Haase E, Schiller D. Treatment of gastric cancer with peritoneal carcinomatosis by cytoreductive surgery and HIPEC: a systematic review of survival, mortality, and morbidity. *J Surg Oncol* 2011; **104**: 692-698 [PMID: 21713780 DOI: 10.1002/jso.22017]

69 **Chua TC**, Yan TD, Saxena A, Morris DL. Should the treatment of peritoneal carcinomatosis by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy still be regarded as a highly morbid procedure?: a systematic review of morbidity and mortality. *Ann Surg* 2009; **249**: 900-907 [PMID: 19474692 DOI: 10.1097/SLA.0b013e3181a45d86]

70 **Saxena A**, Yan TD, Morris DL. A critical evaluation of risk factors for complications after cytoreductive surgery and perioperative intraperitoneal chemotherapy for colorectal peritoneal carcinomatosis. *World J Surg* 2010; **34**: 70-78 [PMID: 19760317 DOI: 10.1007/s00268-009-0206-0]

71 **Valle M**, Federici O, Carboni F, Toma L, Gallo MT, Prignano G, Giannarelli D, Cenci L, Garofalo A. Postoperative infections after cytoreductive surgery and HIPEC for peritoneal carcinomatosis: proposal and results from a prospective protocol study of prevention, surveillance and treatment. *Eur J Surg Oncol* 2014; **40**: 950-956 [PMID: 24246609 DOI: 10.1016/j.ejso.2013.10.015]

72 **Kusamura S**, Baratti D, Virzì S, Bonomi S, Iusco DR, Grassi A, Hutanu I, Deraco M. Learning curve for cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in peritoneal surface malignancies: analysis of two centres. *J Surg Oncol* 2013; **107**: 312-319 [PMID: 22926536 DOI: 10.1002/jso.23231]

73 **Kuijpers AM**, Aalbers AG, Nienhuijs SW, de Hingh IH, Wiezer MJ, van Ramshorst B, van Ginkel RJ, Havenga K, Heemsbergen WD, Hauptmann M, Verwaal VJ. Implementation of a standardized HIPEC protocol improves outcome for peritoneal malignancy. *World J Surg* 2015; **39**: 453-460 [PMID: 25245434 DOI: 10.1007/s00268-014-2801-y]

74 **Pelz JO**, Germer CT. [Morbidity and mortality of hyperthermic intraperitoneal chemoperfusion]. *Chirurg* 2013; **84**: 957-961 [PMID: 24218093 DOI: 10.1007/s00104-013-2513-0]

75 **Small T**. Introduction of hyperthermic intraoperative intraperitoneal chemotherapy (HIPEC) to the surgery program. *ORNAC J* 2013; **31**: 12-17, 28-33 [PMID: 23957107]

76 **Stuart OA**, Stephens AD, Welch L, Sugarbaker PH. Safety monitoring of the coliseum technique for heated intraoperative intraperitoneal chemotherapy with mitomycin C. *Ann Surg Oncol* 2002; **9**: 186-191 [PMID: 11888877 DOI: 10.1245/aso.2002.9.2.186]

77 **Schmid K**, Boettcher MI, Pelz JO, Meyer T, Korinth G, Angerer J, Drexler H. Investigations on safety of hyperthermic intraoperative intraperitoneal chemotherapy (HIPEC) with Mitomycin C. *Eur J Surg Oncol* 2006; **32**: 1222-1225 [PMID: 16784832 DOI: 10.1016/j.ejso.2006.05.011]

78 **Guerbet M**, Goullé JP, Lubrano J. Evaluation of the risk of contamination of surgical personnel by vaporization of oxaliplatin during the intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC). *Eur J Surg Oncol* 2007; **33**: 623-626 [PMID: 17408909 DOI: 10.1016/j.ejso.2007.02.027]

79 **Bonastre J**, Chevalier J, Elias D, Classe JM, Ferron G, Guilloit

JM, Marchal F, Meeus P, De Pouvourville G. Cost-effectiveness of intraperitoneal chemohyperthermia in the treatment of peritoneal carcinomatosis from colorectal cancer. *Value Health* 2008; **11**: 347-353 [PMID: 18489663 DOI: 10.1111/j.1524-4733.2007.00249.x]

80 **Hultman B**, Lundkvist J, Glimelius B, Nygren P, Mahteme H. Costs and clinical outcome of neoadjuvant systemic chemotherapy followed by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in peritoneal carcinomatosis from gastric cancer. *Acta Oncol* 2012; **51**: 112-121 [PMID: 22070666 DOI: 10.3109/0284186X.2011.594809]

81 **Mirnezami R**, Mehta AM, Chandrakumaran K, Cecil T, Moran BJ, Carr N, Verwaal VJ, Mohamed F, Mirnezami AH. Cytoreductive surgery in combination with hyperthermic intraperitoneal chemotherapy improves survival in patients with colorectal peritoneal metastases compared with systemic chemotherapy alone. *Br J Cancer* 2014; **111**: 1500-1508 [PMID: 25225906 DOI: 10.1038/bjc.2014.419]

82 **Levine EA**, Stewart JH, Shen P, Russell GB, Loggie BL, Votanopoulos KI. Intraperitoneal chemotherapy for peritoneal surface malignancy: experience with 1,000 patients. *J Am Coll Surg* 2014; **218**: 573-585 [PMID: 24491244 DOI: 10.1016/j.jamcollsurg.2013.12.013]

83 **Shen P**, Levine EA, Hall J, Case D, Russell G, Fleming R, McQuellon R, Geisinger KR, Loggie BW. Factors predicting survival after intraperitoneal hyperthermic chemotherapy with mitomycin C after cytoreductive surgery for patients with peritoneal carcinomatosis. *Arch Surg* 2003; **138**: 26-33 [PMID: 12511145]

84 **Cavaliere F**, De Simone M, Virzì S, Deraco M, Rossi CR, Garofalo A, Di Filippo F, Giannarelli D, Vaira M, Valle M, Pilati P, Perri P, La Pinta M, Monsellato I, Guadagni F. Prognostic factors and oncologic outcome in 146 patients with colorectal peritoneal carcinomatosis treated with cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy: Italian multicenter study S.I.T.I.L.O. *Eur J Surg Oncol* 2011; **37**: 148-154 [PMID: 21093205 DOI: 10.1016/j.ejso.2010.10.014]

85 **Esquivel J**, Sticca R, Sugarbaker P, Levine E, Yan TD, Alexander R, Baratti D, Bartlett D, Barone R, Barrios P, Bieligk S, Bretcha-Boix P, Chang CK, Chu F, Chu Q, Daniel S, de Bree E, Deraco M, Dominguez-Parra L, Elias D, Flynn R, Foster J, Garofalo A, Gilly FN, Glehen O, Gomez-Portilla A, Gonzalez-Bayon L, Gonzalez-Moreno S, Goodman M, Gushchin V, Hanna N, Hartmann J, Harrison L, Hoefer R, Kane J, Kecmanovic D, Kelley S, Kuhn J, Lamont J, Lange J, Li B, Loggie B, Mahteme H, Mann G, Martin R, Misih RA, Moran B, Morris D, Onate-Ocana L, Petrelli N, Philippe G, Pingpank J, Pitroff A, Piso P, Quinones M, Riley L, Rutstein L, Saha S, Alrawi S, Sardi A, Schneebaum S, Shen P, Shibata D, Spellman J, Stojadinovic A, Stewart J, Torres-Melero J, Tuttle T, Verwaal V, Villar J, Wilkinson N, Younan R, Zeh H, Zoetmulder F, Sebbag G. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in the management of peritoneal surface malignancies of colonic origin: a consensus statement. Society of Surgical Oncology. *Ann Surg Oncol* 2007; **14**: 128-133 [PMID: 17072675 DOI: 10.1245/s10434-007-9599-x]

86 **Varban O**, Levine EA, Stewart JH, McCoy TP, Shen P. Outcomes associated with cytoreductive surgery and intraperitoneal hyperthermic chemotherapy in colorectal cancer patients with peritoneal surface disease and hepatic metastases. *Cancer* 2009; **115**: 3427-3436 [PMID: 19499577 DOI: 10.1002/cncr.24385]

87 **Randle RW**, Doud AN, Levine EA, Clark CJ, Swett KR, Shen P, Stewart JH, Votanopoulos KI. Peritoneal surface disease with synchronous hepatic involvement treated with Cytoreductive Surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC). *Ann Surg Oncol* 2015; **22**: 1634-1638 [PMID: 25120252 DOI: 10.1245/s10434-014-3987-9]

88 **Izzo F**, Piccirillo M, Palaia R, Albino V, Di Giacomo R, Mastro AA. Management of colorectal liver metastases in patients with peritoneal carcinomatosis. *J Surg Oncol* 2009; **100**: 345-347 [PMID: 19697443 DOI: 10.1002/jso.21331]

89 **de Cuba EM**, Kwakman R, Knol DL, Bonjer HJ, Meijer GA, Te Velde EA. Cytoreductive surgery and HIPEC for peritoneal metastases combined with curative treatment of colorectal liver metastases: Systematic review of all literature and meta-analysis of observational studies. *Cancer Treat Rev* 2013; **39**: 321-327 [PMID: 23244778 DOI: 10.1016/j.ctrv.2012.11.003]

90 **Benizri EI**, Bernard JL, Rahili A, Benchimol D, Bereder JM. Small bowel involvement is a prognostic factor in colorectal carcinomatosis treated with complete cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy. *World J Surg Oncol* 2012; **10**: 56 [PMID: 22494563 DOI: 10.1186/1477-7819-10-56]

91 **Chua TC**, Koh JL, Yan TD, Liauw W, Morris DL. Cytoreductive surgery and perioperative intraperitoneal chemotherapy for peritoneal carcinomatosis from small bowel adenocarcinoma. *J Surg Oncol* 2009; **100**: 139-143 [PMID: 19544356 DOI: 10.1002/jso.21315]

92 **Braam HJ**, Boerma D, Wiezer MJ, van Ramshorst B. Hyperthermic intraperitoneal chemotherapy during primary tumour resection limits extent of bowel resection compared to two-stage treatment. *Eur J Surg Oncol* 2013; **39**: 988-993 [PMID: 23810334 DOI: 10.1016/j.ejso.2013.06.002]

93 **Elias D**, Mariani A, Cloutier AS, Blot F, Goéré D, Dumont F, Honoré C, Billard V, Dartigues P, Ducreux M. Modified selection criteria for complete cytoreductive surgery plus HIPEC based on peritoneal cancer index and small bowel involvement for peritoneal carcinomatosis of colorectal origin. *Eur J Surg Oncol* 2014; **40**: 1467-1473 [PMID: 25086990 DOI: 10.1016/j.ejso.2014.06.006]

94 **van Oudheusden TR**, Braam HJ, Nienhuijs SW, Wiezer MJ, van Ramshorst B, Luyer P, de Hingh IH. Poor outcome after cytoreductive surgery and HIPEC for colorectal peritoneal carcinomatosis with signet ring cell histology. *J Surg Oncol* 2015; **111**: 237-242 [PMID: 25195780 DOI: 10.1002/jso.23784]

95 **Winer J**, Zenati M, Ramalingam L, Jones H, Zureikat A, Holtzman M, Lee K, Ahrendt S, Pingpank J, Zeh HJ, Bartlett DL, Choudry HA. Impact of aggressive histology and location of primary tumor on the efficacy of surgical therapy for peritoneal carcinomatosis of colorectal origin. *Ann Surg Oncol* 2014; **21**: 1456-1462 [PMID: 24201745 DOI: 10.1245/s10434-013-3328-4]

96 **Faris JE**, Ryan DP. Controversy and consensus on the management of patients with pseudomyxoma peritonei. *Curr Treat Options Oncol* 2013; **14**: 365-373 [PMID: 23934509 DOI: 10.1007/s11864-013-0240-x]

97 **Esquivel J**, Lowy AM, Markman M, Chua T, Pelz J, Baratti D, Baumgartner JM, Berri R, Bretcha-Boix P, Deraco M, Flores-Ayala G, Glehen O, Gomez-Portilla A, González-Moreno S, Goodman M, Halkia E, Kusamura S, Moller M, Passot G, Pocard M, Salti G, Sardi A, Senthil M, Spilioitis J, Torres-Melero J, Turaga K, Trout R. The American Society of Peritoneal Surface Malignancies (ASPSM) Multiinstitution Evaluation of the Peritoneal Surface Disease Severity Score (PSDSS) in 1,013 Patients with Colorectal Cancer with Peritoneal Carcinomatosis. *Ann Surg Oncol* 2014; **21**: 4195-4201 [PMID: 24854493 DOI: 10.1245/s10434-014-3798-z]

98 **Cashin PH**, Graf W, Nygren P, Mahteme H. Comparison of prognostic scores for patients with colorectal cancer peritoneal metastases treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Ann Surg Oncol* 2013; **20**: 4183-4189 [PMID: 23975316 DOI: 10.1245/s10434-013-3204-2]

99 **de Gramont A**, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000; **18**: 2938-2947 [PMID: 10944126]

100 **Douillard JY**, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L, Rougier P. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000; **355**: 1041-1047 [PMID: 10744089 DOI: 10.1016/S0140-6736(00)02034-1]

101 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth

J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435 DOI: 10.1056/NEJMoa032691]

102 **Cao CQ**, Yan TD, Liauw W, Morris DL. Comparison of optimally resected hepatectomy and peritonectomy patients with colorectal cancer metastasis. *J Surg Oncol* 2009; **100**: 529-533 [PMID: 19697395 DOI: 10.1002/jso.21369]

103 **Tan GH**, Teo MC, Chen W, Lee SY, Ng DW, Tham CK, Soo KC. Surgical management of colorectal peritoneal metastases: treatment and outcomes compared with hepatic metastases. *J Gastrointest Cancer* 2013; **44**: 170-176 [PMID: 23138597 DOI: 10.1007/s12029-012-9452-8]

104 **Blackham AU**, Russell GB, Stewart JH, Votanopoulos K, Levine EA, Shen P. Metastatic colorectal cancer: survival comparison of hepatic resection versus cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Ann Surg Oncol* 2014; **21**: 2667-2674 [PMID: 24615177 DOI: 10.1245/s10434-014-3563-3]

105 **Sammartino P**, Sibio S, Biacchi D, Cardi M, Mingazzini P, Rosati MS, Cornali T, Sollazzo B, Atta JM, Di Giorgio A. Long-term results after proactive management for locoregional control in patients with colonic cancer at high risk of peritoneal metastases. *Int J Colorectal Dis* 2014; **29**: 1081-1089 [PMID: 24980687 DOI: 10.1007/s00384-014-1929-4]

106 **Elias D**, Goéré D, Di Pietrantonio D, Boige V, Malka D, Kohneh-Shahri N, Dromain C, Ducreux M. Results of systematic second-look surgery in patients at high risk of developing colorectal peritoneal carcinomatosis. *Ann Surg* 2008; **247**: 445-450 [PMID: 18376188 DOI: 10.1097/SLA.0b013e31815f0113]

107 **Roviello F**, Caruso S, Neri A, Marrelli D. Treatment and prevention of peritoneal carcinomatosis from gastric cancer by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy: overview and rationale. *Eur J Surg Oncol* 2013; **39**: 1309-1316 [PMID: 24183797 DOI: 10.1016/j.ejso.2013.10.010]

108 **Saladino E**, Fleres F, Mazzeo C, Pruiti V, Scollica M, Rossitto M, Cucinotta E, Macrì A. The role of prophylactic hyperthermic intraperitoneal chemotherapy in the management of serosal involved gastric cancer. *Anticancer Res* 2014; **34**: 2019-2022 [PMID: 24692741]

109 **Sloothaak DA**, Gardenbroek TJ, Crezee J, Bemelman WA, Punt CJ, Buskens CJ, Tanis PJ. Feasibility of adjuvant laparoscopic hyperthermic intraperitoneal chemotherapy in a short stay setting in patients with colorectal cancer at high risk of peritoneal carcinomatosis. *Eur J Surg Oncol* 2014; **40**: 1453-1458 [PMID: 25073662 DOI: 10.1016/j.ejso.2014.06.012]

110 **Sammartino P**, Sibio S, Biacchi D, Cardi M, Accarpio F, Mingazzini P, Rosati MS, Cornali T, Di Giorgio A. Prevention of Peritoneal Metastases from Colon Cancer in High-Risk Patients: Preliminary Results of Surgery plus Prophylactic HIPEC. *Gastroenterol Res Pract* 2012; **2012**: 141585 [PMID: 22645605 DOI: 10.1155/2012/141585]

111 **Honoré C**, Goéré D, Souadka A, Dumont F, Elias D. Definition of patients presenting a high risk of developing peritoneal carcinomatosis after curative surgery for colorectal cancer: a systematic review. *Ann Surg Oncol* 2013; **20**: 183-192 [PMID: 23090572 DOI: 10.1245/s10434-012-2473-5]

112 **Votanopoulos KI**, Ihemelandu C, Shen P, Stewart JH, Russell GB, Levine EA. Outcomes of repeat cytoreductive surgery with hyperthermic intraperitoneal chemotherapy for the treatment of peritoneal surface malignancy. *J Am Coll Surg* 2012; **215**: 412-417 [PMID: 22634117 DOI: 10.1016/j.jamcollsurg.2012.04.023]

113 **Chua TC**, Quinn LE, Zhao J, Morris DL. Iterative cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for recurrent peritoneal metastases. *J Surg Oncol* 2013; **108**: 81-88 [PMID: 23737041 DOI: 10.1002/jso.23356]

114 **Williams BH**, Alzahrani NA, Chan DL, Chua TC, Morris DL. Repeat cytoreductive surgery (CRS) for recurrent colorectal peritoneal metastases: yes or no? *Eur J Surg Oncol* 2014; **40**: 943-949 [PMID: 24378009 DOI: 10.1016/j.ejso.2013.10.022]

115 **Golse N**, Bakrin N, Passot G, Mohamed F, Vaudoyer D, Gilly FN, Glehen O, Cotte E. Iterative procedures combining cytoreductive surgery with hyperthermic intraperitoneal chemotherapy for peritoneal recurrence: postoperative and long-term results. *J Surg Oncol* 2012; **106**: 197-203 [PMID: 22331814 DOI: 10.1002/jso.23062]

116 **Kianmanesh R**, Scaringi S, Sabate JM, Castel B, Pons-Kerjean N, Coffin B, Hay JM, Flamant Y, Msika S. Iterative cytoreductive surgery associated with hyperthermic intraperitoneal chemotherapy for treatment of peritoneal carcinomatosis of colorectal origin with or without liver metastases. *Ann Surg* 2007; **245**: 597-603 [PMID: 17414609 DOI: 10.1097/01.sla.0000255561.87771.11]

117 **Passot G**, You B, Boschetti G, Fontaine J, Isaac S, Decullier E, Maurice C, Vaudoyer D, Gilly FN, Cotte E, Glehen O. Pathological response to neoadjuvant chemotherapy: a new prognosis tool for the curative management of peritoneal colorectal carcinomatosis. *Ann Surg Oncol* 2014; **21**: 2608-2614 [PMID: 24668148 DOI: 10.1245/s10434-014-3647-0]

118 **Bijelic L**, Kumar AS, Stuart OA, Sugarbaker PH. Systemic Chemotherapy prior to Cytoreductive Surgery and HIPEC for Carcinomatosis from Appendix Cancer: Impact on Perioperative Outcomes and Short-Term Survival. *Gastroenterol Res Pract* 2012; **2012**: 163284 [PMID: 22899903 DOI: 10.1155/2012/163284]

119 **Montori G**, Coccolini F, Ceresoli M, Catena F, Colaianni N, Poletti E, Ansaloni L. The treatment of peritoneal carcinomatosis in advanced gastric cancer: state of the art. *Int J Surg Oncol* 2014; **2014**: 912418 [PMID: 24693422 DOI: 10.1155/2014/912418]

120 **Prada-Villaverde A**, Esquivel J, Lowy AM, Markman M, Chua T, Pelz J, Baratti D, Baumgartner JM, Berri R, Bretcha-Boix P, Deraco M, Flores-Ayala G, Glehen O, Gomez-Portilla A, González-Moreno S, Goodman M, Halkia E, Kusamura S, Moller M, Passot G, Pocard M, Salti G, Sardi A, Senthil M, Spiliotis J, Torres-Melero J, Turaga K, Trout R. The American Society of Peritoneal Surface Malignancies evaluation of HIPEC with Mitomycin C versus Oxaliplatin in 539 patients with colon cancer undergoing a complete cytoreductive surgery. *J Surg Oncol* 2014; **110**: 779-785 [PMID: 25088304 DOI: 10.1002/jso.23728]

121 **Eveno C**, Passot G, Goéré D, Soyer P, Gayat E, Glehen O, Elias D, Pocard M. Bevacizumab doubles the early postoperative complication rate after cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (HIPEC) for peritoneal carcinomatosis of colorectal origin. *Ann Surg Oncol* 2014; **21**: 1792-1800 [PMID: 24337648 DOI: 10.1245/s10434-013-3442-3]

122 **Leapman MS**, Jibara G, Tabrizian P, Franssen B, Yang MJ, Romanoff A, Hall SJ, Palese M, Sarpel U, Hiotis S, Labow D. Genitourinary resection at the time of cytoreductive surgery and heated intraperitoneal chemotherapy for peritoneal carcinomatosis is not associated with increased morbidity or worsened oncologic outcomes: a case-matched study. *Ann Surg Oncol* 2014; **21**: 1153-1158 [PMID: 24322531 DOI: 10.1245/s10434-013-3393-8]

123 **Votanopoulos KI**, Randle RW, Craven B, Swett KR, Levine EA, Shen P, Stewart JH, Mirzazadeh M. Significance of urinary tract involvement in patients treated with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC). *Ann Surg Oncol* 2014; **21**: 868-874 [PMID: 24217789 DOI: 10.1245/s10434-013-3354-2]

124 **Glockzin G**, Renner P, Popp FC, Dahlke MH, von Breitenbuch P, Schlitt HJ, Piso P. Hepatobiliary procedures in patients undergoing cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Ann Surg Oncol* 2011; **18**: 1052-1059 [PMID: 21046262 DOI: 10.1245/s10434-010-1415-3]

125 **Glehen O**, Passot G, Villeneuve L, Vaudoyer D, Bin-Dorel S, Boschetti G, Piaton E, Garofalo A. GASTRICHIP: D2 resection and hyperthermic intraperitoneal chemotherapy in locally advanced gastric carcinoma: a randomized and multicenter phase III study. *BMC Cancer* 2014; **14**: 183 [PMID: 24628950 DOI: 10.1186/1471-2407-14-183]

126 **Glockzin G**, Rochon J, Arnold D, Lang SA, Klebl F, Zeman F, Koller M, Schlitt HJ, Piso P. A prospective multicenter phase II study evaluating multimodality treatment of patients with peritoneal

carcinomatosis arising from appendiceal and colorectal cancer: the COMBATAC trial. *BMC Cancer* 2013; **13**: 67 [PMID: 23391248 DOI: 10.1186/1471-2407-13-67]

127 **Ripley RT**, Davis JL, Kemp CD, Steinberg SM, Toomey MA, Avital I. Prospective randomized trial evaluating mandatory second look surgery with HIPEC and CRS vs. standard of care in patients at high risk of developing colorectal peritoneal metastases. *Trials* 2010; **11**: 62 [PMID: 20500867 DOI: 10.1186/1745-6215-11-62]

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REVIEW

Molecular approach to genetic and epigenetic pathogenesis of early-onset colorectal cancer

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Abstract

Colorectal cancer (CRC) is the third most frequent cancer type and the incidence of this disease is increasing gradually per year in individuals younger than 50 years old. The current knowledge is that early-onset CRC (EOCRC) cases are heterogeneous population that includes both hereditary and sporadic forms of the CRC. Although EOCRC cases have some distinguishing clinical and pathological features than elder age CRC, the molecular mechanism underlying the EOCRC is poorly clarified. Given the significance of CRC in the world of medicine, the present review will focus on the recent knowledge in the molecular basis of genetic and epigenetic mechanism of the hereditary forms of EOCRC, which includes Lynch syndrome, Familial CRC type X, Familial adenomatous polyposis, MutYH-associated polyposis, Juvenile polyposis syndrome, Peutz-Jeghers Syndrome and sporadic forms of EOCRC. Recent findings about molecular genetics and epigenetic basis of EOCRC gave rise to new alternative therapy protocols. Although exact diagnosis of these cases still remains complicated, the present review paves way for better predictions and contributes to more accurate diagnostic and therapeutic strategies into clinical approach.

Key words: Early-onset; Colorectal cancer; Epigenetic mechanism; Genetic mechanism; Clinical outcome

Core tip: Early-onset colorectal cancer (EOCRC) cases are heterogeneous population that include both hereditary and sporadic forms of the colorectal cancer (CRC). EOCRC cases have some distinguishing clinical and pathological features than elder age CRC. Recent findings about molecular genetics and epigenetic basis of EOCRC gave rise to new alternative therapy protocols. We herein discuss the latest findings about genetic and epigenetic features of EOCRC.

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INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer type and despite improvements in diagnosis and treatment, this disease is the second leading cause of cancer death in developed countries[1]. The highest incidence of CRC is observed in Western Europe, North America and Australia in western populations. It is notable that although the rate of this disease is relatively lower in the communities of the sub-Saharan Africa, South America and Asia, the rate is gradually increasing depending on assimilating life-style and dietary habits of the western countries[2]. In more developed countries, screening programs for 50 years and elder people leads to early detection of CRC and opportunity for more satisfactory treatments; thus, death rates reduced approximately 2% per year[3-5]. However, CRC screening is not common for young adults (between 20-40 ages), the incidence of this disease is increasing gradually per year in individuals younger than 50 years[6]. The tumors of early-onset patients are more aggressive than elder cases[7,8].

Because of the advances in our understanding concerning the molecular mechanism of elder age CRC, we can describe the presenting phenotype depending on the molecular characteristics of the tumor in majority of the cases[9]. This vital knowledge contributes to the available studies in the literature for individual-specific and targeted therapies for CRC patients related to their drug responses. However, the molecular mechanism underlying the early-onset CRC (EOCRC) is poorly clarified in the relevant literature. Recent studies have revealed that EOCRC might evolve in a different pathway and the molecular basis of these cases may be unique for individuals[10]. Therefore, determining identifiable markers of this disease for early diagnoses is required to develop unique treatment protocols and increase the survival of the patients. However, to date, little knowledge has been gained about the molecular basis of young age. Given this gap to be highlighted, the aim of our review is to synthesize and evaluate the current literature regarding the genetic and epigenetic pathogenesis of EOCRC at molecular level.

MOLECULAR PATHOGENESIS OF EOCRC

In comparison to elder CRC, EOCRC cases have some distinguishing clinical and pathological features[11,12]. These tumors are pathologically recognized with low-grade tumor differentiation, mucinous component and high signet ring cells frequency[11,13]. Polyp development is contently observed during the follow-up period of EOCRC[10]. The majority of early-onset tumors occur in the distal colon and the rectum[14]. Previous studies underlined the significance of heritance as an indicator of EOCRC[11,12]. Supporting these views, early-onset and hereditary forms of CRC demonstrate similar well-known pathological features[11,13]. Nevertheless, the current knowledge is that EOCRC is a heterogeneous disease with both familial and sporadic cases. The molecular basis of this heterogeneity has not yet been fully clarified in the literature, however, the severe histopathological properties and a possible genetic feature of the tumor may predispose to expedited tumor growth in young age patients as reported[15,16].

HEREDITARY FORMS OF EOCRC

In the hereditary forms of CRC, the disease can be observed in one or more first and/or second degree relatives of the patient. Thus, familial CRC counts approximately 20% of all CRC patients[17]. With almost 3% observation rate, the most frequently occurring familial CRC is Lynch syndrome (LS)[18]. On the other hand, polyposis syndromes, such as familial adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), Juvenile polyposis syndrome (JPS) and Peutz-Jeghers syndrome (PJS), are less often observed familial colorectal syndromes[17].

LS

LS is frequently right-sided, an autosomal dominant cancer predisposition. The majority of these tumors are synchronous and metachronous. Extracolonic sites of patients, such as brain, ovary, endometrium, renal pelvis, ureter, stomach, small intestine and skin, are also among a high cancer risk[18]. LS is caused by various germline DNA mismatch repair (*MMR*) gene mutations[19-21]. Approximately 90% of the identified LS mutations are observed in *MLH1* or *MSH2* genes and approximately 10% of the LS mutations were identified in *MSH6* and *PMS2* genes[17,22]. The prevalence and characteristics of these mutations vary widely among populations. In 2010, we defined two frame-shift mutations (*MLH1* c.1843dupC and *MLH1* c.1743delG) and three missense mutations (*MLH*1 c.293G < C, *MLH1* c.954_955delinsTA and *MSH2* c.2210G < A) uniquely in Turkish LS cases[23]. In a study of Italian LS families, c.643_648 dupA, c.2156_2157 dupT, c.684_685 dupC and c.1701_1704 delT frameshift mutations andc.2206 G < T, that cause a truncating protein were first time determined in *MLH1* gene. Other truncating protein causing mutations, c.1089 G < T andc.2634-2 A < G, that results with a splice defect was originally reported in *MSH2* gene[24]. In Malaysia population, two novel mutations, c.3341_3342insC and c.3885_3891delTAAAAGC were characterized in *MSH6* and c.2395C > T mutation was defined in *PMS2* gene[25]. Recently, an unidentified mutation of *MLH1*, c.2044_2045del was linked to LS in a Caribbean Hispanic family[26].

Deficient MMR function of LS cases usually promotes microsatellite instability (MSI)[27,28]. MSI is characterized by length alterations within simple repeated sequences that are called microsatellites[28]. MSI is essential for deregulation of cell growth, differentiation and death[29]. MSI also plays roles in modulating the response of patients to various chemotherapeutic agents[27]. Losing the expression of MMR proteins *via* inactivation of MMR-

Table 1 MiRNA profile of lynch syndrome patients

MiRNA	Expression status	Function	Ref.
miR-155	Up	MMR deficiency	Valeri *et al*[46]
		MSI	Earle *et al*[48]
miR-26b	Down	MSS	Earle *et al*[48]
miR-31	Up	MSI	Earle *et al*[48]
miR-223	Up	MSI	Earle *et al*[48]
miR-486-5p	Down	MSI	Balaguer *et al*[47]
miR-622	Up	MSI	Balaguer *et al*[47]
miR-1238	Up	MSI	Balaguer *et al*[47]
miR-1362-5p	Down	MSI	Balaguer *et al*[47]
miR-132	Down	MMR deficiency	Kaur *et al*[49]
miR-345	Down	MMR deficiency	Kaur *et al*[49]

MSS: Microsatellite stabile; MSI: Microsatellite instability; MMR: Mismatch repair.

deficient crypt foci genes causes an MSI phenotype[30]. In these patients, the mutation rates of *ACVR2*, *TAF1B* and *ASTE1*, microsatellite-bearing target genes are higher than 80%[29-33]. Recent studies indicated that in MSI cases, frameshift mutations of apoptotic genes, such as *APAF1*, *BAX* and *FLASH,* lead to intratumoral heterogeneity[28]. The study of Markowitz *et al*[34] demonstrated the relation with DNA repair defects with a specific pathway of CRC progression and three different mutation of *TGFBR2* gene in 1995[34]. However, the latest study of de Miranda *et al*[35] showed the transcription and translation of *TGFBR2* with a 1 nucleotide deletion at its microsatellite sequence still produced a functional *TGFBR2* protein. This protein is required for phosphorylation of *SMAD2*, which is phosphorylated in most of the MSI CRC tissues[35].

The *MMR* gene modifications of LS occur by two hit usually point mutations or large rearrangements may give rise to the first hit. Accordingly, gene conversion or loss of the wild-type allele evokes the second hit[36]. However, recent observations demonstrated the high rate of promoter methylation occurrence as the second hit[37,38]. These findings emphasize the role of epigenetic events in formation of LS[37,38]. Indeed, depending on the studies, germ line hemiallelic methylation of *MLH1* and epimutations of *MSH2* lead to LS with insufficient MLH1 or MSH2 protein expression in mutation negative families[39-42]. Ligtenberg *et al*[43] state that germ line 3′ end deletions of *EPCAM* gene that is located upstream of *MSH2*, correlate with MSI and a loss of *MSH2* protein even though there was no identifiable mutation in *MSH2* gene[44]. Kuiper *et al*[44] found *EPCAM* deletions in approximately 2.3% of *MSH2*-deficient families. This study affirms the epigenetic transgenerational inheritance and the possibility of aberrant promoter methylation occurrence in neighbouring tumor suppressor genes by loosing of polyadenylation signals[28]. In addition, current evidence in the empirical studies supports the role of miRNAs that is responsible for translational rearrangement of proteins, in regulation of MMR genes expressions[45]. In comparison to sporadic MSI tumors, LS patients have a typical miRNA profile. Valeri *et al*[46] demonstrated the association of reduced expression of *MSH2*, *MLH1* and *MSH6* and induction of a mutator phenotype and MSI with over expression of miR-155 in LS. In another study, Balaguer *et al*[47] determined the up-regulation of miR-622 and miR-1238 in these patients. MSI status modulates the miRNA expression levels[48]. Earle *et al*[48] defined the increased expression of miR-155, miR-31, miR-223 and miR-26b in MSI tumors. In addition, Earle *et al*[48] linked over expression of miR-31 and miR-223 to LS. Not only miRNA regulates gene expression in an epigenetic way but also miRNA expressions may be regulated epigenetically. With containing a CpG island in the promoter region, most of the miRNAs are favorable for aberrant methylation which can give rise to dysregulation of miRNA[49,50]. Kaur *et al*[49] identified a correlation between miR-345 and miR-132 hypermethylation and MMR deficiency (Table 1).

Familial CRC type X

MMR germline mutations are observed in approximately 60% of the families, fulfilling clinical criteria for LS[51]. Although familial colorectal cancer type X (FCCTX) accomplish the same clinical criteria with LS, the morphological features, such as right-sided tumor location, poor differentiation, expansive growth pattern, tumor-infiltrating lymphocytes, peritumorous lymphocytes, Crohn-like reactions and lack of dirty necrosis, are not common in FCCTX as LS[52]. In addition, despite these, families demonstrate clinical features in which CRCs with MSI, FCCTX is not related to germline MMR gene mutations[51,53]. The age onset of FCCTX is relatively older than LS cases and this disease differ from LS with the tumorigenic pathways[54,55]. Basically, two individual molecular pathways involve in these families. One of these pathways is loosing of tumor suppressor gene loci genes, such as *TP53*, *APC*, *SMAD4* and *DCC*, somatic mutations of *APC* and *KRAS* and *MGMT* promoter methylation. At the second partway, there is no loosing of tumor suppressor gene loci genes and rarely presenting promoter methylation[56]. Therkildsen *et al*[57] linked to FCCTX tumors with gain of genetic material in two separate regions encompassing, 20q12-13.12 and 20q13.2-13.32. This study revealed that gain of material on chromosome 20q and loss on chromosome 18 differentiate FCCTX from LS. Findings of Dominguez-Valentin *et al*[58] showed that gaining mutations of *GNAS* gene which is located in 20q13.32 and encodes for the Gα-subunit may cause FCCTX *via* activation of the Wnt and ERK1/2 MAPK signalling pathways. Moreover, other 20q located genes, *CDH26*, *SRC* and *ASIP* that play role in proliferation and migration may have a potential to cause FCCTX[58]. Dominguez-Valentin *et al*[58] defined the up-regulation of *PTGER1* in these tumors which can cause tumor growth through altered prostaglandin E2 function[59,60]. Recently, an *SEMA4A* gene variant c.232G > A was determined in Austrian kindred with FCCTX. This study revealed that *SEMA4A* (V78M) lead to activation of MAPK/Erk and PI3K/Akt signaling. Moreover, *SEMA4A* mutations, c.1451G > C and c.977C > T and the single-nucleotide polymorphism c.2044C

Table 2 Molecular characterization of familial colorectal cancer type X patients

Molecular features		Ref.
Germline *MMR* gene mutations	-	Lindor *et al*[51] Klarskov *et al*[52] Sánchez-Tomé *et al*[53]
Tumor supressor gene loci loss		
APC mutations	77%	Francisco *et al*[56]
KRAS mutations	46%	Francisco *et al*[56]
MGMT methylation	36%	Francisco *et al*[56]
Chromosome gains	20q, 19 and 17	Therkildsen *et al*[57]
Chromosome loss	8p, 15, 18	Therkildsen *et al*[57]
Signaling by G protein coupled receptor (*GNAS, F2R, F2RL2, EDN1, EDNRA, GRM8, GNA2, GNG11, GNG12, HCRT, PTGER1, P2RY2, RAMP2, MC1R, TUBB3, VIP*)	up-regulated	Dominguez-Valentin *et al*[58]
SEMA4A variants		Schulz *et al*[61]

> T were determined as associated with the FCCTX phenotype[61]. Recent knowledge about the molecular characterization of FCCTX is summarized in Table 2.

FAP

FAP is an autosomal dominant cancer syndrome[62]. FAP is diagnosed with 100 or more adenomatous polyps in colon or rectum in patients with younger than 40 age[62]. Patients with FAP carry germline mutations of the adenomatous polyposis coli (*APC*) gene located on chromosome 5q21-q22[63]. APC protein is a large scaffolding protein which involves in Wnt signaling pathway. In this protein complex, APC leads to down regulation of b-catenin activity and play a central role in a destruction complex of Axin, GSK-3β and casein kinase 1. This complex directs a series of phosphorylation events on β-catenin that target it for ubiquitination and subsequent proteolysis[64]. In the absence of APC protein, b-catenin binds to several transcription factors of the TCF/LEF and initiates the altered expression of genes associated with proliferation, differentiation, migration and apoptosis. Moreover, the depletion of APC can lead to abnormal chromosome segregation and aberrant mitosis[65,66]. FAP occurs when there are mutations between codons 168-1580 and with severe disease between codons 1250-1464 of *APC* gene[67,68]. The majority of *APC* mutations are either frameshift or nonsense mutations resulting in a truncated protein[69]. The two most frequently described germline mutations are located at codon 1309 (c3927_3931delAAAGA) and codon 1061 (c.3183_87delACAAA)[70]. Although two-thirds of FAP patient disease is inherited, the rest of the cases have no family history and carry unique mutations. Almost all APC mutations results with a colonic phenotype but variable for extra-colonic manifestations, such as desmoid tumor, hepatoblastoma, thyroid carcinoma, medulloblastoma, a litany of benign lesions and brain tumors, particularly medulloblastomas[71-73]. Lamberti *et al*[74] found that *GSTT1* polymorphism showed an uncertain association with extra-intestinal manifestations in a study of 411 FAP patients. Recent studies demonstrated the enrichment of pyloric gland adenomas of the stomach, in addition to fundic gland polyps and foveolar-type adenomas in patients with FAP[75,76]. Hashimoto *et al*[75] analyzed the genetic alterations in these FAP-associated gastric lesions and they demonstrated that, as well as *APC* mutations, these cases had *GNAS* and *KRAS* mutations.

KRAS mutations have been observed in the early development of approximately 40% of colon cancers. Simultaneous *APC* depletion and *KRAS* mutation results with an augmentation in adenomas[76] and induce the spread of stem cell marker carrying cells within the tumor epithelium[77]. Phelps *et al*[78] stated that in FAP adenomas, intestinal differentiation is required two consecutive steps. In the first step, after APC loss, CtBP1 contributes to adenoma initiation and in the following step, KRAS activation and β-catenin nuclear localization promote adenoma progression to carcinomas. On the other hand, Obrador-Hevia *et al*[79] analysed somatic *APC* and *KRAS* mutations, beta-catenin immunostaining, and qRT-PCR of *APC*, *MYC*, *AXIN2* and *SFRP1* genes in sixty adenomas from six FAP patients with known pathogenic *APC* mutations. Based on this study, the Wnt pathway was constitutively activated in all APC-FAP tumors, with alterations occurring both upstream and downstream of APC. Thus, Obrador-Hevia *et al*[79] suggest that for Wnt signalling activation in APC-associated FAP adenomas, oncogenic *KRAS* is not essential.

FAP may also pursue a different way to Wnt signalling pathway alterations though epigenetic mechanisms. Although epigenetic alterations of Wnt signalling are an effective factor for FAP formation, APC mutations exist in almost all FAP patients. Romero-Giménez *et al*[80] evaluated the possible role of germline hypermethylation of the *APC* promoter in FAP families that were negative for *APC* mutations in 21 FAP families and they did not identify signs of abnormal promoter methylation, indicating that this form of epigenetic silencing is not a common cause of FAP. However, Kámory *et al*[81] observed promoter hypermethylation that causes somatic inactivation of *APC* in 21 sporadic cases (30%). In the study of Zhang *et al*[82] within FAP families, although methylation was not present in normal tissues, hypermethylation was determined in tumor tissues of one proband and her son. In addition, loss of heterozygosity was observed in another patient from the same FAP family. Segditsas *et al*[83] declared similar findings with Zhang *et al*[82] They detected *APC* promoter methylation in 27%-45% of colorectal tumors and cell lines but did not detect in normal colorectum. However, they substantially observed that methylation was independent of the *APC* mutations and was not associated with the CpG island methylator phenotype. Although methylation caused the loosing of 1A isoform mRNA and a reduction in total *APC* transcript levels, *APC* gene expression was retained from promoter 1B[83]. Moreover, a recent study of Pavicic demonstrated that promoter 1B deletions of *APC* are not very common[84]. Thus, all these studies imply that

Table 3 Genetic and epigenetic alterations of familial adenomatous polyposis patients besides adenomatous polyposis coli

		Alteration	Ref.
Gene			
	GNAS	Mutation	Hashimoto *et al*[75]
	MYC	Gene activation	Obrador-Hevia *et al*[79]
	AXIN2	Gene activation	Obrador-Hevia *et al*[79]
	SFRP1	Gene activation	Obrador-Hevia *et al*[79]
	GSTT1	Polymorphism	Lamberti *et al*[74]
	MGMT	Methylation	Wynter *et al*[86]
	p14ARF	Methylation	Wynter *et al*[86]
	p16INK4	Methylation	Wynter *et al*[86]
	IGSF4	Methylation	Berkhout *et al*[85]
	TIMP3	Methylation	Berkhout *et al*[85]
	ESR1	Methylation	Berkhout *et al*[85]
	CDH13	Methylation	Berkhout *et al*[85]
miRNA			
	miR-143	Down regulation	Kamatani *et al*[87]
	miR-145	Down regulation	Kamatani *et al*[87]
	miR-126	Down regulation	Yamaguchi *et al*[88]
	miR-20b	Down regulation	Yamaguchi *et al*[88]

even though *APC* promoter methylation occurs in early during colon neoplasia progression, it does not result in complete gene inactivation or act as a "second hit"[84] and promoter-specific alterations of *APC* rarely leads to mutation-negative FAP[84].

In addition to *APC*, hypermethylation of other genes are usually observed in both FAP-related and sporadic duodenal carcinomas[85]. Wynter *et al*[86] study showed that the methylation of *MGMT*, *p14ARF* and *p16INK4* genes promoter regions are frequently observed in both sporadic and familial adenomas. Berkhout *et al*[85] defined the high methylation rate of the *IGSF4*, *TIMP3*, *ESR1*, *APC* and *CDH13,* in both of these cases, however, in the same study, *PAX6* gene was determined as hypermethylated only in FAP-related carcinomas. Recently, the role of altered miRNA expression in Wnt signalling regulation and FAP development has also been evaluated. Lately, the studies indicated the decreased expression of miR-143, miR-145, miR-126 and miR-20b as an early event of colorectal carcinogenesis in FAP tumors[87,88]. Specifically, miR-126 and miR-20b play role in angiogenesis[88]. Thus, downregulation of these miRNAs is an important genetic event for the initiation step in colorectal tumor development[87]. Besides APC alterations, other genetic and epigenetic events determined in FAP patients were summarized in Table 3.

MAP

MAP is an autosomal recessive polyposis syndrome. Approximately 0.3%-1% of all CRCs is associated with MAP[89,90]. Cases with MAP typically present multiple colon adenomas, thus at the first glance, these cases may be diagnosed as FAP. However, because they also can have *MMR* gene mutations, it can reverberate to phenotype as LS[91]. Although existing of multiple colon adenomas, there is not any alteration in *APC* gene of these cases, but further analyses identified mutations in *MUTYH* gene which is a component of a base excision repair system and involves in protecting DNA from oxidative damage[92]. Farrington *et al*[93] reported that mutations of both *MUTYH* gene alleles increase the risk of endometrial tumors. These cases are rare and well known *MUTYH* mutations are linked to this disease are c.494A > G and c.1145G > A[94-96]. However, *MUTYH* mutations can vary with ethnicity[97]. c.536A > G and c.1187G > A in Caucasians, c.231 C > T and c.934-2A > C in Japan, c.1227_1228dup in Portugal, c.1376C > A in Finland were determined as the most frequent *MUTYH* mutations[98-100]. In the North-Eastern Italy, c.933+3A > C (IVS10 + 3A > C), accounts for nearly 1/5 of all *MUTYH* mutations[101]. In addition, because this mutation is also common in Germany, it is supposed to have a common origin in Western Europe[101]. MUTYH mutations that vary with ethnicity are summarized in Table 4.

Table 4 *MUTYH* mutations that vary with ethnicity

MUTYH mutation	Etnicity	Ref.
c.231 C > T	Japan	Miyaki *et al*[99]
c.934-2A > C	Japan	Miyaki *et al*[99]
c.1376C > A	Finland	Alhopuro *et al*[100]
c.933 + 3A > C	North-Eastern Italy, Germany	Pin *et al*[101]
c.536A > G	Caucasians	Yamaguchi *et al*[92]
c.1187 G > A	Caucasians	Yamaguchi *et al*[92]

Germline *MUTYH* mutations may also lead to the mutation of cancer-related genes, such as the *APC* and/or the *KRAS* genes, *via* G to T transversion[92]. In the study of Venesio *et al*[102]. mutated *MUTYH*-associated-polyposis adenomas exhibited only c.34G > T transversion in codon 12, or mutations in codon 13. They affirm that neither of these mutations was found in classical/attenuated familial polyposis adenomas.

JPS

JPS is a rare autosomal dominant disorder. JPS is diagnosed with numerous colon and rectum polyps or polyps with family history or juvenile polyps inside and outside of the intestine[103]. 20%-50% of JPS demonstrates familial pattern and the average disease onset of cases are 16 to 18[103]. JPS may coexist with Osler-Weber-Rendu syndrome [hereditary hemorrhagic telangiectasia (HHT)]. The most frequently encountered symptoms of HHT are Skin telangiectasia, epistaxis, intracranial haemorrhage, development of pulmonary arteriovenous fistulas, brain cavernous angioma and haemangioma[104]. Almost 60% of JPS cases demonstrate mutations in *SMADH4* and *BMPR1A* genes that are connected with TGF-β/BMP signal pathway[105].

To date, a number of mutations leading JPS and/or HHT have been described in *SMAD4* gene. These mutations include point mutations that are resulting with a stop codon or a change in the coded amino acid into another one, codons 361, 533 and 534 mutations, small deletions and insertions[103]. Specifically, Howe *et al*[106] determined the mutation, c.1244-1247delAGAC, in the hot spot of the *SMAD4* gene which leads to a

Table 5 Pathogenic germline mutations of juvenile polyposis syndrome

Mutation	Effect	Ref.
SMAD4		
c.1244-1247delAGAC	Hotspot mutation serious course of JPS with numerous cases of polyps, tumors located in the stomach and intestines	Howe *et al*[106]
BMPR1A		
c.230+452_333+441dup 1995	Frameshift mutation producing a truncated protein (p.D112NfsX2)	Yamaguchi *et al*[107]

JPS: Juvenile polyposis syndrome.

serious course of JPS with numerous cases of polyps and tumors located in the stomach and intestines. In addition, a considerable proportion of mutations in the *BMPR1A* gene are nucleotide changes generating a stop codon (nonsense) or leading to amino acid changes (missense). These mutations are distributed evenly in the entire gene sequence, intronic mutations (intron 1, 3, 4 and 5) and deletions between codon 224 and 359[103]. Yamaguchi *et al*[107] identified a *BMPR1A* mutation, which involves a duplication of coding exon 3 (c.230þ452_333þ441dup1995) that causes a frameshift mutation, producing a truncated protein (p.D112NfsX2) in a patient with JPS (Table 5).

In addition to mutations in *SMAD4* and *BMPR1A* genes, Juvenile polyps also was observed in Cowden, Bannayan-Zonana, and Gorlin syndromes. Cowden and Bannayan-Zonana syndromes is occurred by *PTEN* mutations and Gorlin syndrome develops *via* germline *PTCH* mutations. *PTEN* and *PTCH* mutations have been excluded as the causative mutations in almost all JPS patients[108-110].

To the best of our knowledge, there is a lack of knowledge about epigenetic regulation of JPS so far. However, recently, Ling *et al*[111] defined *SMAD4* as a miR-224 target as a metastasis factor, yet the relation of miR-224 and *SMAD4* expression in formation of juvenile polyps has not been clarified.

PJS

PJS is a rare (approximately 1 in 200000 observation rate) autosomal dominant disease[112,113]. PJS is characterized by occurrence of benign hamartomatous, Peutz-Jeghers-type polyps in the gastrointestinal tract in association with mucocutaneous pigmentation on the lips and oral mucosa[114]. PJS is diagnosed with presence of a hamartoma associated with two of the following three signs: Family history of PJS, mucocutaneous lentiginosis or polyposis of the small-bowel[115]. PJS patients face with abdominal symptoms during the first 10 years of life, almost 50% of patients experience the symptoms before the age of 20 years and they have an increased risk of developing gastrointestinal and extradigestive cancers[115-117]. Cancer development localized in small intestine, stomach, pancreas and colon in most of the cases[116]. In 93% of the affected individuals, there is a risk of developing complicating cancers between aged 15-64 years[116].

Percent of eighty to 90% of patients with PJS have family history[118] and according to genetic analysis, 40%-60% of these cases have germline STK11 (also known as LKB1) mutations as the major cause of this disease[112]. Because the downstream signalling pathway of STK11 has not been fully clarified, the knowledge about the mechanism of hamartomatous polyp formation and mucocutaneous pigmentation also insufficient at present. Studies demonstrated that induced *COX-2* gene expression has also been involved in the promotion of tumor formation from PJS polyps[119,120]. On the other hand, PJS cases with wild-type STK11 demonstrates multiple causative loci such as chromosome1p, a pericentric inversion in chromosome 6, a second PJS locus at 19q13.4 and a heterozygous germline mutation in the MYH11 gene[121-125]. Lately, Wang *et al*[121] performed sequence analysing in three Chinese individuals with PJS and identified 2 variants,OR4C45 c.767-768insAG and ZAN c.5767insG, which occur in PJS cases independently of STK11.

More than 145 different STK11 germline mutations have been reported in the literature result in a truncated premature protein or in transcriptional splicing errors[121,126-129] (Table 6). On the other hand, transcriptional silencing of this tumor suppressor gene by promoter hypermethylation has been shown as an alternative inactivation mechanism[130-133]. In addition to germline mutations, and promoter methylation, Wang *et al*[121] discovered four mutations in pre-microRNAs, MI0003131, MI0003530, MI0014206, and MI0005525, of which the corresponding mature miRNA, hsa-mir-492, hsa-mir-487b, hsa-mir-323b, hsa-mir-300 respectively.

SPORADIC FORMS OF EOCRC

The most well-defined hereditary form of CRC, LS, account for 2%-4% of total CRC and one-third of EOCRC cases[134,135]. FAP cases are observed in less than 1% frequency in total CRC cases. Thus, 70% of all CRC and the majority of EOCRC cases are introduced in sporadic form[136-138]. Sporadic EOCRCs are classified into two major groups. Chromosome unstable CRC (CIN) is characterized by gross chromosomal abnormalities and MSI[135]. Although MSI tumors behave less aggressively compared to CIN, CIN or MSI tumors do not always appear separately[139-141].

Sporadic EOCRC are morphologically characterized with poor cell differentiation, colloid component and lymphocytic stromal reaction[8,12,142]. Therefore, these cases are likely to be confused with LS patients. However, while MMR defects are observed *via* MSI pathway in LS, in sporadic cases MSI is not frequent. Studies to date imply that colorectal tumors characterized by MSI may be distinct from microsatellite stabile (MSS) tumors in many molecular aspects, such as an association with the methylator phenotype, a higher frequency of *BRAF* mutations and a lower frequency of *KRAS*, *APC*, and *TP53* mutations. Thus, MSI and MSS colon tumors

Table 6 *STK11* mutations associated with colorectal cancer caused by peutz-jeghers syndrome

STK11 mutation	Mutation type	Effect on protein	Ref.
c.511 G > A	Missense mutation	G171S	Dong *et al*[127]
c. 595 G > A	Missense mutation	E199K	Dong *et al*[127]
c.622 G > A	Missense mutation	D208N	Dong *et al*[127]
c.644 G > A	Missense mutation	G215D	Dong *et al*[127]
c.941 C > A	Missense mutation	P314H	Resta *et al*[128]
c.1062 C > G	Missense mutation	F354L	Dong *et al*[127]
c.1100 C > T	Missense mutation	T367M	Dong *et al*[127]
c.842delC	Frameshift mutation	truncates	Dong *et al*[127], Bartosova *et al*[129]
IVS2 + 1A > G	Intronic splice site mutation		Bartosova *et al*[129]
OR4C45 c.767-768insAG	Frameshift mutation	truncates	Wang *et al*[121]
ZAN c.5767insG	Frameshift mutation	truncates	Wang *et al*[121]

originate from different molecular backgrounds[143]. In sporadic cases, MMR deficiency occurs mainly through epigenetic inactivation of the *MLH1* gene through biallelic promoter methylation instead of MSI[136]. Both genetic and epigenetic inactivation of MMR genes result in a mutator phenotype, mutations in cancer related genes and CRC development[144]. Kirzin *et al*[137] identified *CTNNB1* as one of the most over-expressed genes in MSS-young patients compared to MSS-old patients and this leads to an over-activation of beta catenin in sporadic EOCRC. In addition, Fernandez-Rozadilla *et al*[145] determined a heterozygous deletion in the 10q22-q23 region involving *BMPR1A* gene of EOCRC cases with MMR proficiency. According to Luo *et al*[146] CDC42, TEX11, QKI, CAV1 and FN1 proteins are representative elements of EOCRC specific networks. Moreover, we defined *REG1A*, *CK20* and *MAP3K8* gene expressions strongly upregulated (more than twofold) in early-onset MSS CRC compared with MSI CRC tumors[147]. CK20 expression is observed in the majority of colorectal tumors[148,149], however, a limited number of studies have evaluated the relationship between *CK20* expression levels and MSI status[147]. In one study, it was suggested that reduced or absent *CK20* expression in CRC is associated with both sporadic and hereditary MSI[150]. In another study associated with EOCRC, *CK20* expression levels were also identified as relatively reduced in MSI tumors[10]. It was determined that *CK20* expression levels are inversely correlated with numbers of aberrant microsatellite locus[150]. We determined the upregulation of *CK20* expression levels in MSS tumors compared with MSI-low (MSI-L) and MSI-high (MSI-H) tumors[150]. According to McGregor *et al*[150] regulation of *CK20* gene expression involves molecular pathways that are altered by MSI-H. We defined 3.98-fold high *CK20* gene expression levels in MSS tumors with lymph nodes metastases than in MSI tumors with lymph nodes metastases[147]. In addition, 17.5-fold upregulation was identified in *CK20* expression levels in low-grade MSS tumors of patients with recurrence and distant metastases[147]. These results indicate that upregulation of *CK20* expression, specifically, is related to poor prognosis in patients with MSS tumors. Therefore, the results of our study indicate that *CK20* expression in MSS tumors allows for the determination of the biological characteristics of EOCRC tissues[147]. The encoded protein by *MAP3K8* gene is a member of the serine/threonine protein kinase family. In one of our study, *MAP3K8* expression in CRC was determined significantly elevated compared with normal mucosa[149]. In addition, we determined *MAP3K8* expression levels more than two fold upregulated in early-onset MSS CRC compared with MSI CRC tumors[147]. *MAP3K8* expression levels were significantly higher in the MSS tumors of patients with a short median survival. Thus, our observations revealed that upregulated *MAP3K8* expression was associated with a poor prognosis in patients with MSS tumors[147]. Human REG1A belongs to the superfamily of calcium-dependent lectins. In several previous studies, *REG1A* was found to be upregulated in CRC[151-153]. We also found that *REG1A* is upregulated in the tumors of early-onset sporadic CRC patients. Furthermore, 25.8-fold high *REG1A* gene expression levels were observed in MSS tumors with lymph nodes metastases. In addition, median survival and disease-free survival were significantly longer only for patients with MSI tumors with low *REG1A* expression compared with those with high expression of this gene. This result indicates that upregulated *REG1A* expression may be related to sporadic EOCRC tumor formation and characterization[147]. Additionally, a recent study from Sengupta *et al*[154] defined a relation with MSS CRC tumors and deletion in *RBFOX1* gene which encodes a highly conserved RNA-binding protein that regulates tissue-specific alternative splicing indicating important basic functions in development and differentiation in a British Bangladeshi MSS CRC population. This study showed that loss of RBFOX1 activity may lead to aberrations in the splicing of genes associated with CRC[154].

Different from MSS tumors, some sporadic EOCRC tumors belong to the MSI pathway[28]. Sporadic EOCRC with MSI is likely to arise from sessile serrated polyps through the serrated neoplastic pathway[155]. The *BRAF* gene, which plays an important role in the mitogen-activated protein kinase signalling pathway, is frequently mutated in these cases. *BRAF* V600E mutation is widely accepted as a prognostic factor of sporadic CRC with MSI and methylated *MLH1*[156]. Although the frequency of *BRAF* V600E mutation is high in MSI tumors, this mut-

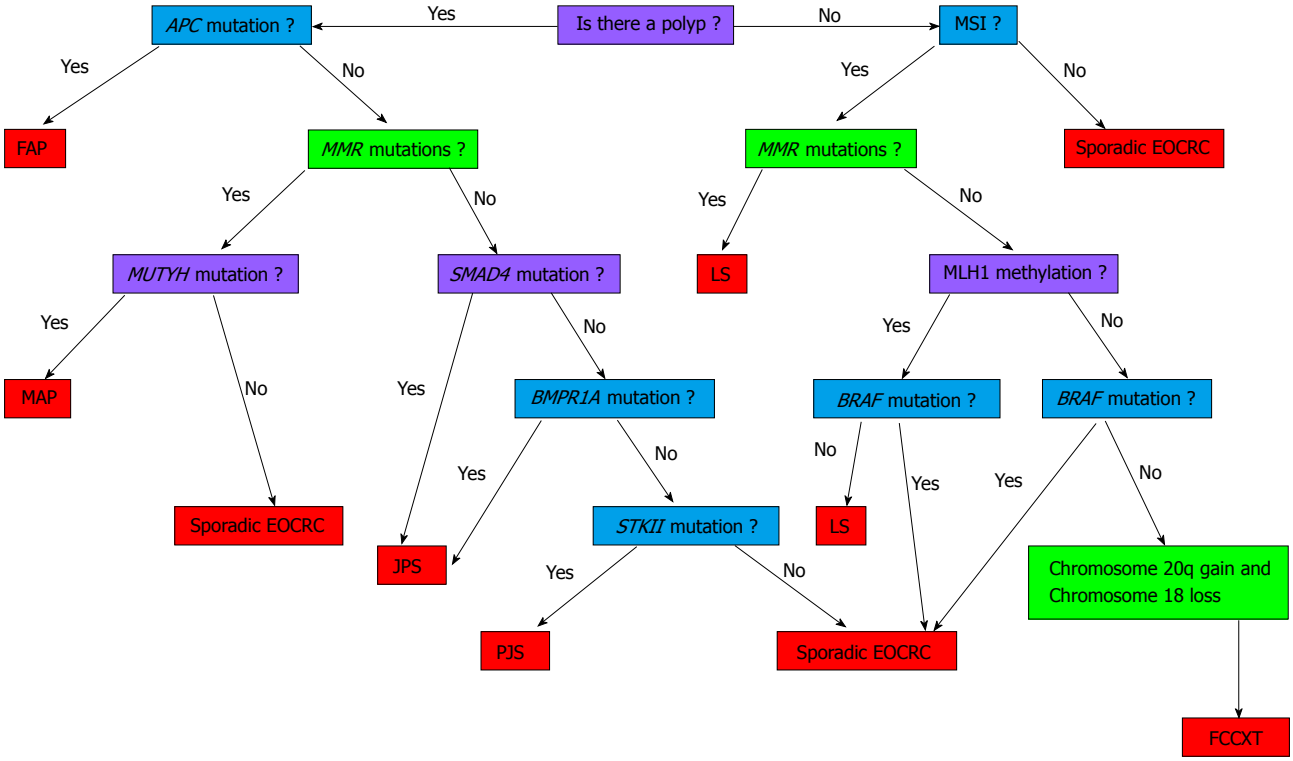

Figure 1 Genetic algorithm of early-onset colorectal cancer. MMR: Mismatch repair; EOCRC: Early-onset colorectal cancer; MSI: Microsatellite instability; APC: Adenomatous polyposis coli; FAP: Familial adenomatous polyposis; MAP: MUTYH-associated polyposis; LS: Lynch syndrome; JPS: Juvenile polyposis syndrome; PJS: Peutz-jeghers syndrome.

ation is not observed in LS cases, thus, this discrepancy between sporadic MSI cancer and LS might be used in a strategy for the detection of LS[156].

The different attitude of sporadic and hereditary forms of EOCRC may also be caused by epigenetic modifications, such as miRNA expressions and their methylation patterns[47,157]. Balaguer *et al*[47] demonstrated that miR-622, miR-362-5p and miR-486-5p could accurately classify the LS and sporadic MSI cases. The similarity of miRNA expression status of LS and sporadic MSI cases may be explained with occurrence of frameshift mutations in *TARBP2*, a miRNA processing gene, in both of these diseases[158]. Moreover, in one of our study, using miRNA polymerase chain reaction arrays, the expression profiles of 38 different miRNAs associated with CRC were evaluated in 40 sporadic Turkish EOCRC patients[157]. The expression of miR-106a was found to be upregulated, and miR-143 and miR-125b levels were found to be downregulated in sporadic EOCRC tissues compared with the normal tissues. In addition, 2.42-fold high expression level of miR-106a and 2.42-fold low expression level of miR-125b were observed in tumors with lymph node metastases compared with the normal colorectal mucosa samples[157]. On the other hand, epigenetic regulations of sporadic EOCRC tumors also differ between each other depend on MSI status. Earle *et al*[48] described the different expression profile of miR-223, miR-155, and miR-92 between MSI and MSS CRCs. So far, Kaur *et al*[49] have investigated the association of miR-132 methylation and sporadic MSI CRC tumors located in the proximal colon in a comparative study of Finnish and Australian population. In addition, different from MSS tumors, hypermethylation of miR-345 had a significant association with sporadic MSI in Finnish CRCs[49].

CLINICAL OUTCOME OF GENETIC AND EPIGENETIC FEATURES OF EOCRC

A major challenge in CRC therapy is drug resistance. The current knowledge of CRC genetics has increased the sufficiency of applied conventional cytotoxic chemotherapy and targeted therapy. Genetic screening of EOCRC patients for hereditary cancer syndrome is determinative not only for the rate of cancer risk of relatives but also for appropriate treatment. A pyrimidine analogue, 5-fluorouracil (5-FU) which is widely used in CRC therapy, involves in induction of DNA replication stress response in cells through inhibiting thymidylate synthase. However, studies showed that *APC* mutations reduces the sensitivity to 5-FU[159]. On the other hand, performance of MSI test is advisable for patients with strongly suspected on the basis of a known family history of colorectal and extracolonic cancers in the case of LS (Figure 1). Studies revealed that while adjuvant chemotherapy with a fluoropyrimidine does not have a beneficial effect on MSI cases and may even worsen the clinical picture, combination of oxaliplatin and infusional 5-FU/leucovorin regarded as more beneficial for these cases[160,161]. According to Violette *et al*[162] increased expression of Reg genes caused *in vitro* resistance to

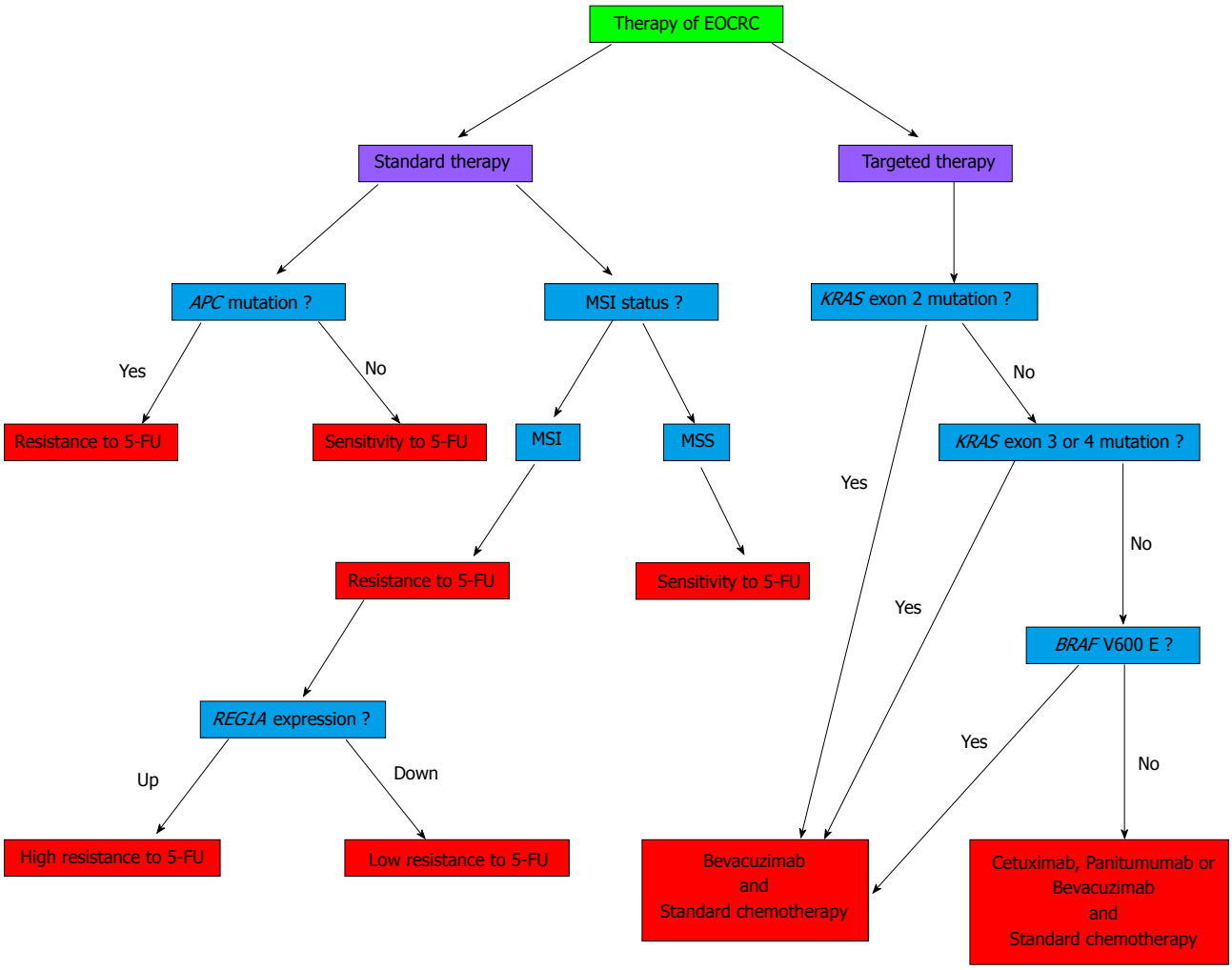

Figure 2 Therapy of early-onset colorectal cancer. EOCRC: Early-onset colorectal cancer; MSI: Microsatellite instability; MSS: Microsatellite stabile; 5-FU: 5-fluorouracil.

the 5-FU. Bishnupuri *et al*[163] observed a mitogenic effect of the Reg Ⅳ protein, with subsequent changes in the expression of genes associated with apoptosis and metastasis. The Reg proteins are previously unappreciated regulators of antiapoptotic proteins in early tumorigenesis and may contribute to increased resistance to apoptotic death during therapy[163]. As another mechanism of resistance to therapy, the result of our study of the poor prognosis of MSI tumors supports the hypothesis that high *REG1A* expression may contribute to increased resistance to apoptotic death during therapy in MSI tumors[147]. Because of the important role of REG1A in tumorigenesis and development of metastasis in MSI tumors, the use of *REG1A*-specific inhibitors in CRC patients have MSI that may represent a novel significant approach to the treatment of cancer. In addition, according to recent studies, alterations in epigenetic regulation of these genes may also lead to resistance to chemotherapeutic agents. For example, Deng *et al*[164] found out that reduced expression of miR-21 plays role in resistance to 5-FU therapy *via* targeting *MSH2*. However, miRNA studies have been performing in *in vitro* conditions and to prove the decisive importance of these markers further advanced studies required.

Nowadays, the application of targeted therapy for CRC has been increasing. The goals of these therapies are interrupting the survival and proliferation of cancer cells[165]. To date, United States Food and Drug Administration has approved several targeted drugs, such as cetuximab and panitumumab, the anti-EGFR antibodies that suppress the tumor angiogenesis and bevacizumab, an anti-VEGF antibody. Recently, different from bevacizumab, aflibercept and regorafenib have been used as new antiangiogenic agents[166-168]. Although EGFR is overexpressed in most of the CRC cases, because of the down-stream modifications of EGFR signalling pathway, patients demonstrated different response to this therapy[169]. Particularly, *KRAS* activating mutations in exon 2 avoid the sufficient therapy with EGFR inhibitors[170,171]. A small number of patients with wild type *KRAS* exon 2 were demonstrated to have mutations exons 3 and 4 that are also caused KRAS activation[172]. Activating mutations in the other genes that play role in downstream pathway of EGFR signalling, NRAS, BRAF, PIK3CA and PTEN are able to lead to resistance to anti-EGFR therapies[173]. Thus, to predict the success of anti-EGFR monoclonal antibody

therapy, examination of downstream mutations of EGFR signalling pathway should be required before receiving an EGFR inhibitor[170] (Figure 2). Second targeted signal pathway for CRC therapy is angiogenesis pathway. Bevacizumab is a monoclonal antibody that binds to VEGF-A preventing its interaction with VEGFR-2[174]. Regorafenib demonstrated a multikinase inhibitor activity against VEGFR-2, VEGFR-3, TIE-2, PDGFR, FGFR, RET, c-Kit and RAF/MEK/ERK pathway[175]. Aflibercept is a recombinant fusion protein and play a role in the inhibition of interactions between VEGF-A, VEGFB proteins and their specific receptors by acting as a trap receptor binding to VEGF-A and VEGFB[176]. Thus, the blockage of the genes that encoded these proteins enhances the success of the therapy.

CONCLUSION

Genetic predispositions have been identified in EOCRC clearly distinct from the other types of CRC. The current knowledge about the molecular and genetic basis of EOCRC provides information regarding prognosis of this disease and response to therapies. A proportion of EOCRCs are hereditary forms. Hence, cases should be evaluated for existing of a germline mutation in one of the several MMR genes for suspicion of LS, in the *APC* gene for suspicion of FAP, or in one of the genes associated with a more uncommon syndrome. Identification of a hereditary syndrome in individuals also provides predictive mutational testing for non-symptomatic relatives. They are found to be positive for the mutation can take precaution for reduction of the risk of cancer-associated morbidity and mortality in this way. In addition, a better understanding of the genetic mechanism of EOCRC is highly likely to lead to develop more beneficial targeted therapies. To date, specifically, studies on MSI CRC, such as LS, herald new diagnostic and therapeutic strategies into clinical approach. It is notable that further research remains to be conducted to more finely characterize the underlying mechanism of sporadic EOCRC, which could allow improved prevention, diagnosis, and treatment of these cases.

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REFERENCES

1 **Sameer AS**. Colorectal cancer: molecular mutations and polymorphisms. *Front Oncol* 2013; **3**: 114 [PMID: 23717813 DOI: 10.3389/fonc.2013.00114]
2 **Vainio H**, Miller AB. Primary and secondary prevention in colorectal cancer. *Acta Oncol* 2003; **42**: 809-815 [PMID: 14968941]
3 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]
4 **Nishihara R**, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, Inamura K, Kim SA, Kuchiba A, Yamauchi M, Imamura Y, Willett WC, Rosner BA, Fuchs CS, Giovannucci E, Ogino S, Chan AT. Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N Engl J Med* 2013; **369**: 1095-1105 [PMID: 24047059 DOI: 10.1056/NEJMoa1301969]
5 **Shaukat A**, Mongin SJ, Geisser MS, Lederle FA, Bond JH, Mandel JS, Church TR. Long-term mortality after screening for colorectal cancer. *N Engl J Med* 2013; **369**: 1106-1114 [PMID: 24047060 DOI: 10.1056/NEJMoa1300720]
6 **Quirke P**, Risio M, Lambert R, von Karsa L, Vieth M. European guidelines for quality assurance in colorectal cancer screening and diagnosis. First Edition--Quality assurance in pathology in colorectal cancer screening and diagnosis. *Endoscopy* 2012; **44** Suppl 3: SE116-SE130 [PMID: 23012115]
7 **Minardi AJ**, Sittig KM, Zibari GB, McDonald JC. Colorectal cancer in the young patient. *Am Surg* 1998; **64**: 849-853 [PMID: 9731812]
8 **Parramore JB**, Wei JP, Yeh KA. Colorectal cancer in patients under forty: presentation and outcome. *Am Surg* 1998; **64**: 563-567; discussion 567-568 [PMID: 9619179]
9 **Ogino S**, Goel A. Molecular classification and correlates in colorectal cancer. *J Mol Diagn* 2008; **10**: 13-27 [PMID: 18165277 DOI: 10.2353/jmoldx.2008.070082]
10 **Perea J**, Alvaro E, Rodríguez Y, Gravalos C, Sánchez-Tomé E, Rivera B, Colina F, Carbonell P, González-Sarmiento R, Hidalgo M, Urioste M. Approach to early-onset colorectal cancer: clinicopathological, familial, molecular and immunohistochemical characteristics. *World J Gastroenterol* 2010; **16**: 3697-3703 [PMID: 20677343]
11 **Losi L**, Di Gregorio C, Pedroni M, Ponti G, Roncucci L, Scarselli A, Genuardi M, Baglioni S, Marino M, Rossi G, Benatti P, Maffei S, Menigatti M, Roncari B, Ponz de Leon M. Molecular genetic alterations and clinical features in early-onset colorectal carcinomas and their role for the recognition of hereditary cancer syndromes. *Am J Gastroenterol* 2005; **100**: 2280-2287 [PMID: 16181381]
12 **Fairley TL**, Cardinez CJ, Martin J, Alley L, Friedman C, Edwards B, Jamison P. Colorectal cancer in U.S. adults younger than 50 years of age, 1998-2001. *Cancer* 2006; **107**: 1153-1161 [PMID: 16862554]
13 **Savas N**, Dagli U, Akbulut S, Yuksel O, Sahin B. Colorectal cancer localization in young patients: should we expand the screening program? *Dig Dis Sci* 2007; **52**: 798-802 [PMID: 17245629]
14 **You YN**, Xing Y, Feig BW, Chang GJ, Cormier JN. Young-onset colorectal cancer: is it time to pay attention? *Arch Intern Med* 2012; **172**: 287-289 [PMID: 22157065 DOI: 10.1001/archinternmed.2011.602]
15 **Calin GA**, Gafà R, Tibiletti MG, Herlea V, Becheanu G, Cavazzini L, Barbanti-Brodano G, Nenci I, Negrini M, Lanza G. Genetic progression in microsatellite instability high (MSI-H) colon cancers correlates with clinico-pathological parameters: A study of the TGRbetaRII, BAX, hMSH3, hMSH6, IGFIIR and BLM genes. *Int J Cancer* 2000; **89**: 230-235 [PMID: 10861498]
16 **Silla IO**, Rueda D, Rodríguez Y, García JL, de la Cruz Vigo F, Perea J. Early-onset colorectal cancer: a separate subset of colorectal cancer. *World J Gastroenterol* 2014; **20**: 17288-17296 [PMID: 25516639 DOI: 10.3748/wjg.v20.i46.17288]
17 **Lynch HT**, Shaw TG. Practical genetics of colorectal cancer. *Chin Clin Oncol* 2013; **2**: 12 [PMID: 25841492 DOI: 10.3978/j.issn.2304-3865.2013.03.04]
18 **Hampel H**, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Clendenning M, Sotamaa K, Prior T, Westman JA, Panescu J, Fix D, Lockman J, LaJeunesse J, Comeras I, de la Chapelle A. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol* 2008; **26**: 5783-5788 [PMID: 18809606 DOI: 10.1200/JCO.2008.17.5950]
19 **Rodriguez-Bigas MA**, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, Lynch H, Perucho M, Smyrk T, Sobin L, Srivastava S. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst* 1997; **89**: 1758-1762 [PMID: 9392616]
20 **Vasen HF**, Watson P, Mecklin JP, Lynch HT. New clinical criteria

for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology* 1999; **116**: 1453-1456 [PMID: 10348829]

21 **Umar A**, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004; **96**: 261-268 [PMID: 14970275]

22 **Bellizzi AM**, Frankel WL. Colorectal cancer due to deficiency in DNA mismatch repair function: a review. *Adv Anat Pathol* 2009; **16**: 405-417 [PMID: 19851131 DOI: 10.1097/PAP.0b013e3181bb6bdc]

23 **Tunca B**, Pedroni M, Cecener G, Egeli U, Borsi E, Zorluoglu A, Di Gregorio C, Yilmazlar T, Yerci O, de Leon MP. Analysis of mismatch repair gene mutations in Turkish HNPCC patients. *Fam Cancer* 2010; **9**: 365-376 [PMID: 20373145 DOI: 10.1007/s10689-010-9336-7]

24 **Lastella P**, Patruno M, Forte G, Montanaro A, Di Gregorio C, Sabbà C, Suppressa P, Piepoli A, Panza A, Andriulli A, Resta N, Stella A. Identification and surveillance of 19 Lynch syndrome families in southern Italy: report of six novel germline mutations and a common founder mutation. *Fam Cancer* 2011; **10**: 285-295 [PMID: 21286823 DOI: 10.1007/s10689-011-9419-0]

25 **Zahary MN**, Kaur G, Hassan MR, Sidek AS, Singh H, Yeh LY, Ankathil R. Germline mutation and protein expression analysis of mismatch repair genes MSH6 and PMS2 in Malaysian Lynch syndrome patients. *Int J Colorectal Dis* 2014; **29**: 261-262 [PMID: 24072394 DOI: 10.1007/s00384-013-1770-1]

26 **Cruz-Correa M**, Diaz-Algorri Y, Pérez-Mayoral J, Suleiman-Suleiman W, Del Mar Gonzalez-Pons M, Bertrán C, Casellas N, Rodríguez N, Pardo S, Rivera K, Mosquera R, Rodriguez-Quilichini S. Clinical characterization and mutation spectrum in Caribbean Hispanic families with Lynch syndrome. *Fam Cancer* 2015; **14**: 415-425 [PMID: 25782445 DOI: 10.1007/s10689-015-9795-y]

27 **Bertagnolli MM**, Niedzwiecki D, Compton CC, Hahn HP, Hall M, Damas B, Jewell SD, Mayer RJ, Goldberg RM, Saltz LB, Warren RS, Redston M. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. *J Clin Oncol* 2009; **27**: 1814-1821 [PMID: 19273709 DOI: 10.1200/JCO.2008.18.2071]

28 **Yamamoto H**, Imai K. Microsatellite instability: an update. *Arch Toxicol* 2015; **89**: 899-921 [PMID: 25701956 DOI: 10.1007/s00204-015-1474-0]

29 **Woerner SM**, Yuan YP, Benner A, Korff S, von Knebel Doeberitz M, Bork P. SelTarbase, a database of human mononucleotide-microsatellite mutations and their potential impact to tumorigenesis and immunology. *Nucleic Acids Res* 2010; **38**: D682-D689 [PMID: 19820113 DOI: 10.1093/nar/gkp839]

30 **Staffa L**, Echterdiek F, Nelius N, Benner A, Werft W, Lahrmann B, Grabe N, Schneider M, Tariverdian M, von Knebel Doeberitz M, Bläker H, Kloor M. Mismatch repair-deficient crypt foci in Lynch syndrome--molecular alterations and association with clinical parameters. *PLoS One* 2015; **10**: e0121980 [PMID: 25816162 DOI: 10.1371/journal.pone.0121980]

31 **Tougeron D**, Fauquembergue E, Rouquette A, Le Pessot F, Sesboüé R, Laurent M, Berthet P, Mauillon J, Di Fiore F, Sabourin JC, Michel P, Tosi M, Frébourg T, Latouche JB. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. *Mod Pathol* 2009; **22**: 1186-1195 [PMID: 19503063 DOI: 10.1038/modpathol.2009.80]

32 **Schwitalle Y**, Linnebacher M, Ripberger E, Gebert J, von Knebel Doeberitz M. Immunogenic peptides generated by frameshift mutations in DNA mismatch repair-deficient cancer cells. *Cancer Immun* 2004; **4**: 14 [PMID: 15563124]

33 **Kim TM**, Laird PW, Park PJ. The landscape of microsatellite instability in colorectal and endometrial cancer genomes. *Cell* 2013; **155**: 858-868 [PMID: 24209623 DOI: 10.1016/j.cell.2013.10.015]

34 **Markowitz S**, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 1995; **268**: 1336-1338 [PMID: 7761852]

35 **de Miranda NF**, van Dinther M, van den Akker BE, van Wezel T, ten Dijke P, Morreau H. Transforming Growth Factor β Signaling in Colorectal Cancer Cells With Microsatellite Instability Despite Biallelic Mutations in TGFBR2. *Gastroenterology* 2015; **148**: 1427-1437.e8 [PMID: 25736321 DOI: 10.1053/j.gastro.2015.02.052]

36 **Peltomäki P**. Epigenetic mechanisms in the pathogenesis of Lynch syndrome. *Clin Genet* 2014; **85**: 403-412 [PMID: 24443998 DOI: 10.1111/cge.12349]

37 **Ollikainen M**, Hannelius U, Lindgren CM, Abdel-Rahman WM, Kere J, Peltomäki P. Mechanisms of inactivation of MLH1 in hereditary nonpolyposis colorectal carcinoma: a novel approach. *Oncogene* 2007; **26**: 4541-4549 [PMID: 17260015]

38 **Nagasaka T**, Rhees J, Kloor M, Gebert J, Naomoto Y, Boland CR, Goel A. Somatic hypermethylation of MSH2 is a frequent event in Lynch Syndrome colorectal cancers. *Cancer Res* 2010; **70**: 3098-3108 [PMID: 20388775 DOI: 10.1158/0008-5472.CAN-09-3290]

39 **Gazzoli I**, Loda M, Garber J, Syngal S, Kolodner RD. A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the MLH1 gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor. *Cancer Res* 2002; **62**: 3925-3928 [PMID: 12124320]

40 **Miyakura Y**, Sugano K, Akasu T, Yoshida T, Maekawa M, Saitoh S, Sasaki H, Nomizu T, Konishi F, Fujita S, Moriya Y, Nagai H. Extensive but hemiallelic methylation of the hMLH1 promoter region in early-onset sporadic colon cancers with microsatellite instability. *Clin Gastroenterol Hepatol* 2004; **2**: 147-156 [PMID: 15017620]

41 **Hitchins MP**, Wong JJ, Suthers G, Suter CM, Martin DI, Hawkins NJ, Ward RL. Inheritance of a cancer-associated MLH1 germ-line epimutation. *N Engl J Med* 2007; **356**: 697-705 [PMID: 17301300]

42 **Chan TL**, Yuen ST, Kong CK, Chan YW, Chan AS, Ng WF, Tsui WY, Lo MW, Tam WY, Li VS, Leung SY. Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. *Nat Genet* 2006; **38**: 1178-1183 [PMID: 16951683]

43 **Ligtenberg MJ**, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, Lee TY, Bodmer D, Hoenselaar E, Hendriks-Cornelissen SJ, Tsui WY, Kong CK, Brunner HG, van Kessel AG, Yuen ST, van Krieken JH, Leung SY, Hoogerbrugge N. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet* 2009; **41**: 112-117 [PMID: 19098912 DOI: 10.1038/ng.283]

44 **Kuiper RP**, Vissers LE, Venkatachalam R, Bodmer D, Hoenselaar E, Goossens M, Haufe A, Kamping E, Niessen RC, Hogervorst FB, Gille JJ, Redeker B, Tops CM, van Gijn ME, van den Ouweland AM, Rahner N, Steinke V, Kahl P, Holinski-Feder E, Morak M, Kloor M, Stemmler S, Betz B, Hutter P, Bunyan DJ, Syngal S, Culver JO, Graham T, Chan TL, Nagtegaal ID, van Krieken JH, Schackert HK, Hoogerbrugge N, van Kessel AG, Ligtenberg MJ. Recurrence and variability of germline EPCAM deletions in Lynch syndrome. *Hum Mutat* 2011; **32**: 407-414 [PMID: 21309036 DOI: 10.1002/humu.21446]

45 **Hutchison J**, Cohen Z, Onyeagucha BC, Funk J, Nelson MA. How microRNAs influence both hereditary and inflammatory-mediated colon cancers. *Cancer Genet* 2013; **206**: 309-316 [PMID: 24042167 DOI: 10.1016/j.cancergen.2013.06.005]

46 **Valeri N**, Gasparini P, Fabbri M, Braconi C, Veronese A, Lovat F, Adair B, Vannini I, Fanini F, Bottoni A, Costinean S, Sandhu SK, Nuovo GJ, Alder H, Gafa R, Calore F, Ferracin M, Lanza G, Volinia S, Negrini M, McIlhatton MA, Amadori D, Fishel R, Croce CM. Modulation of mismatch repair and genomic stability by miR-155.

Proc Natl Acad Sci USA 2010; **107**: 6982-6987 [PMID: 20351277 DOI: 10.1073/pnas.1002472107]
47 **Balaguer F**, Moreira L, Lozano JJ, Link A, Ramirez G, Shen Y, Cuatrecasas M, Arnold M, Meltzer SJ, Syngal S, Stoffel E, Jover R, Llor X, Castells A, Boland CR, Gironella M, Goel A. Colorectal cancers with microsatellite instability display unique miRNA profiles. *Clin Cancer Res* 2011; **17**: 6239-6249 [PMID: 21844009 DOI: 10.1158/1078-0432.CCR-11-1424]
48 **Earle JS**, Luthra R, Romans A, Abraham R, Ensor J, Yao H, Hamilton SR. Association of microRNA expression with microsatellite instability status in colorectal adenocarcinoma. *J Mol Diagn* 2010; **12**: 433-440 [PMID: 20413677 DOI: 10.2353/jmoldx.2010.090154]
49 **Kaur S**, Lotsari JE, Al-Sohaily S, Warusavitarne J, Kohonen-Corish MR, Peltomäki P. Identification of subgroup-specific miRNA patterns by epigenetic profiling of sporadic and Lynch syndrome-associated colorectal and endometrial carcinoma. *Clin Epigenetics* 2015; **7**: 20 [PMID: 25767621 DOI: 10.1186/s13148-015-0059-3]
50 **Ozsolak F**, Poling LL, Wang Z, Liu H, Liu XS, Roeder RG, Zhang X, Song JS, Fisher DE. Chromatin structure analyses identify miRNA promoters. *Genes Dev* 2008; **22**: 3172-3183 [PMID: 19056895 DOI: 10.1101/gad.1706508]
51 **Lindor NM**, Rabe K, Petersen GM, Haile R, Casey G, Baron J, Gallinger S, Bapat B, Aronson M, Hopper J, Jass J, LeMarchand L, Grove J, Potter J, Newcomb P, Terdiman JP, Conrad P, Moslein G, Goldberg R, Ziogas A, Anton-Culver H, de Andrade M, Siegmund K, Thibodeau SN, Boardman LA, Seminara D. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. *JAMA* 2005; **293**: 1979-1985 [PMID: 15855431]
52 **Klarskov L**, Holck S, Bernstein I, Nilbert M. Hereditary colorectal cancer diagnostics: morphological features of familial colorectal cancer type X versus Lynch syndrome. *J Clin Pathol* 2012; **65**: 352-356 [PMID: 22287689 DOI: 10.1136/jclinpath-2011-200535]
53 **Sánchez-Tomé E**, Rivera B, Perea J, Pita G, Rueda D, Mercadillo F, Canal A, Gonzalez-Neira A, Benitez J, Urioste M. Genome-wide linkage analysis and tumoral characterization reveal heterogeneity in familial colorectal cancer type X. *J Gastroenterol* 2015; **50**: 657-666 [PMID: 25381643 DOI: 10.1007/s00535-014-1009-0]
54 **Dunican DS**, McWilliam P, Tighe O, Parle-McDermott A, Croke DT. Gene expression differences between the microsatellite instability (MIN) and chromosomal instability (CIN) phenotypes in colorectal cancer revealed by high-density cDNA array hybridization. *Oncogene* 2002; **21**: 3253-3257 [PMID: 12082642]
55 **Bertucci F**, Salas S, Eysteries S, Nasser V, Finetti P, Ginestier C, Charafe-Jauffret E, Loriod B, Bachelart L, Montfort J, Victorero G, Viret F, Ollendorff V, Fert V, Giovaninni M, Delpero JR, Nguyen C, Viens P, Monges G, Birnbaum D, Houlgatte R. Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters. *Oncogene* 2004; **23**: 1377-1391 [PMID: 14973550]
56 **Francisco I**, Albuquerque C, Lage P, Belo H, Vitoriano I, Filipe B, Claro I, Ferreira S, Rodrigues P, Chaves P, Leitão CN, Pereira AD. Familial colorectal cancer type X syndrome: two distinct molecular entities? *Fam Cancer* 2011; **10**: 623-631 [PMID: 21837511 DOI: 10.1007/s10689-011-9473-7]
57 **Therkildsen C**, Jönsson G, Dominguez-Valentin M, Nissen A, Rambech E, Halvarsson B, Bernstein I, Borg K, Nilbert M. Gain of chromosomal region 20q and loss of 18 discriminates between Lynch syndrome and familial colorectal cancer. *Eur J Cancer* 2013; **49**: 1226-1235 [PMID: 23245329 DOI: 10.1016/j.ejca.2012.11.011]
58 **Dominguez-Valentin M**, Therkildsen C, Veerla S, Jönsson M, Bernstein I, Borg A, Nilbert M. Distinct gene expression signatures in lynch syndrome and familial colorectal cancer type x. *PLoS One* 2013; **8**: e71755 [PMID: 23951239 DOI: 10.1371/journal.pone.0071755]
59 **Watanabe K**, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Ushikubi F, Narumiya S, Sugimura T, Wakabayashi K. Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. *Cancer Res* 1999; **59**: 5093-5096 [PMID: 10537280]
60 **Kim SH**, Park YY, Kim SW, Lee JS, Wang D, DuBois RN. ANGPTL4 induction by prostaglandin E2 under hypoxic conditions promotes colorectal cancer progression. *Cancer Res* 2011; **71**: 7010-7020 [PMID: 21937683 DOI: 10.1158/0008-5472.CAN-11-1262]
61 **Schulz E**, Klampfl P, Holzapfel S, Janecke AR, Ulz P, Renner W, Kashofer K, Nojima S, Leitner A, Zebisch A, Wölfler A, Hofer S, Gerger A, Lax S, Beham-Schmid C, Steinke V, Heitzer E, Geigl JB, Windpassinger C, Hoefler G, Speicher MR, Boland CR, Kumanogoh A, Sill H. Germline variants in the SEMA4A gene predispose to familial colorectal cancer type X. *Nat Commun* 2014; **5**: 5191 [PMID: 25307848 DOI: 10.1038/ncomms6191]
62 **Jarry J**, Brunet JS, Laframboise R, Drouin R, Latreille J, Richard C, Gekas J, Maranda B, Monczak Y, Wong N, Pouchet C, Zaor S, Kasprzak L, Palma L, Wu MK, Tischkowitz M, Foulkes WD, Chong G. A survey of APC mutations in Quebec. *Fam Cancer* 2011; **10**: 659-665 [PMID: 21779980 DOI: 10.1007/s10689-011-9468-4]
63 **Kinzler KW**, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hedge P, McKechnie D. Identification of FAP locus genes from chromosome 5q21. *Science* 1991; **253**: 661-665 [PMID: 1651562]
64 **Polakis P**. Casein kinase 1: a Wnt'er of disconnect. *Curr Biol* 2002; **12**: R499-R501 [PMID: 12176352]
65 **Fodde R**, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C, van Es JH, Breukel C, Wiegant J, Giles RH, Clevers H. Mutations in the APC tumour suppressor gene cause chromosomal instability. *Nat Cell Biol* 2001; **3**: 433-438 [PMID: 11283620]
66 **Kaplan KB**, Burds AA, Swedlow JR, Bekir SS, Sorger PK, Näthke IS. A role for the Adenomatous Polyposis Coli protein in chromosome segregation. *Nat Cell Biol* 2001; **3**: 429-432 [PMID: 11283619]
67 **Rozen P**, Macrae F. Familial adenomatous polyposis: The practical applications of clinical and molecular screening. *Fam Cancer* 2006; **5**: 227-235 [PMID: 16998668]
68 **Schlussel AT**, Gagliano RA, Seto-Donlon S, Eggerding F, Donlon T, Berenberg J, Lynch HT. The evolution of colorectal cancer genetics-Part 2: clinical implications and applications. *J Gastrointest Oncol* 2014; **5**: 336-344 [PMID: 25276406 DOI: 10.3978/j.issn.2078-6891.2014.068]
69 **Béroud C**, Soussi T. APC gene: database of germline and somatic mutations in human tumors and cell lines. *Nucleic Acids Res* 1996; **24**: 121-124 [PMID: 8594558]
70 **Martayan A**, Sanchez-Mete L, Baldelli R, Falvo E, Barnabei A, Conti L, Giacomini P, Appetecchia M, Stigliano V. Gene variants associated to malignant thyroid disease in familial adenomatous polyposis: a novel APC germline mutation. *J Endocrinol Invest* 2010; **33**: 603-606 [PMID: 20935450 DOI: 10.3275/7289]
71 **Hamilton SR**, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, Krush AJ, Berk T, Cohen Z, Tetu B. The molecular basis of Turcot's syndrome. *N Engl J Med* 1995; **332**: 839-847 [PMID: 7661930]
72 **Groen EJ**, Roos A, Muntinghe FL, Enting RH, de Vries J, Kleibeuker JH, Witjes MJ, Links TP, van Beek AP. Extra-intestinal manifestations of familial adenomatous polyposis. *Ann Surg Oncol* 2008; **15**: 2439-2450 [PMID: 18612695 DOI: 10.1245/s10434-008-9981-3]
73 **Septer S**, Slowik V, Morgan R, Dai H, Attard T. Thyroid cancer complicating familial adenomatous polyposis: mutation spectrum of at-risk individuals. *Hered Cancer Clin Pract* 2013; **11**: 13 [PMID: 24093640 DOI: 10.1186/1897-4287-11-13]
74 **Lamberti C**, Jungck M, Laarmann M, Knapp M, Caspari R, Friedl W, Sauerbruch T, Propping P, Kruse R. Arylamine N-acetyltransferase type 2 and glutathione S-transferases M1 and T1 polymorphisms in familial adenomatous polyposis. *Pharmacogenetics* 2002; **12**: 49-54 [PMID: 11773864]
75 **Hashimoto T**, Ogawa R, Matsubara A, Taniguchi H, Sugano K, Ushiama M, Yoshida T, Kanai Y, Sekine S. Familial adenomatous polyposis-associated and sporadic pyloric gland adenomas of the upper gastrointestinal tract share common genetic features.

Histopathology 2015; **67**: 689-698 [PMID: 25832318 DOI: 10.1111/his.12705]
76 **Janssen KP**, Alberici P, Fsihi H, Gaspar C, Breukel C, Franken P, Rosty C, Abal M, El Marjou F, Smits R, Louvard D, Fodde R, Robine S. APC and oncogenic KRAS are synergistic in enhancing Wnt signaling in intestinal tumor formation and progression. *Gastroenterology* 2006; **131**: 1096-1109 [PMID: 17030180]
77 **Haigis KM**, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, Settleman J, Giovannini M, Jacks T. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet* 2008; **40**: 600-608 [PMID: 18372904 DOI: 10.1038/ng.115]
78 **Phelps RA**, Chidester S, Dehghanizadeh S, Phelps J, Sandoval IT, Rai K, Broadbent T, Sarkar S, Burt RW, Jones DA. A two-step model for colon adenoma initiation and progression caused by APC loss. *Cell* 2009; **137**: 623-634 [PMID: 19450512 DOI: 10.1016/j.cell.2009.02.037]
79 **Obrador-Hevia A**, Chin SF, González S, Rees J, Vilardell F, Greenson JK, Cordero D, Moreno V, Caldas C, Capellá G. Oncogenic KRAS is not necessary for Wnt signalling activation in APC-associated FAP adenomas. *J Pathol* 2010; **221**: 57-67 [PMID: 20196079 DOI: 10.1002/path.2685]
80 **Romero-Giménez J**, Dopeso H, Blanco I, Guerra-Moreno A, Gonzalez S, Vogt S, Aretz S, Schwartz S, Capella G, Arango D. Germline hypermethylation of the APC promoter is not a frequent cause of familial adenomatous polyposis in APC/MUTYH mutation negative families. *Int J Cancer* 2008; **122**: 1422-1425 [PMID: 18027849]
81 **Kámory E**, Olasz J, Csuka O. Somatic APC inactivation mechanisms in sporadic colorectal cancer cases in Hungary. *Pathol Oncol Res* 2008; **14**: 51-56 [PMID: 18369740 DOI: 10.1007/s12253-008-9019-y]
82 **Zhang YY**, Chen SQ, Zhu M, Li JT, Ma GJ, Zhang XM, Zhou JN. [Promoter hypermethylation and loss of heterozygosity of the APC gene in patients with familial adenomatous polyposis]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2008; **25**: 378-381 [PMID: 18683131]
83 **Segditsas S**, Sieber OM, Rowan A, Setien F, Neale K, Phillips RK, Ward R, Esteller M, Tomlinson IP. Promoter hypermethylation leads to decreased APC mRNA expression in familial polyposis and sporadic colorectal tumours, but does not substitute for truncating mutations. *Exp Mol Pathol* 2008; **85**: 201-206 [PMID: 18977219 DOI: 10.1016/j.yexmp.2008.09.006]
84 **Pavicic W**, Nieminen TT, Gylling A, Pursiheimo JP, Laiho A, Gyenesei A, Järvinen HJ, Peltomäki P. Promoter-specific alterations of APC are a rare cause for mutation-negative familial adenomatous polyposis. *Genes Chromosomes Cancer* 2014; **53**: 857-864 [PMID: 24946964 DOI: 10.1002/gcc.22197]
85 **Berkhout M**, Nagtegaal ID, Cornelissen SJ, Dekkers MM, van de Molengraft FJ, Peters WH, Nagengast FM, van Krieken JH, Jeuken JW. Chromosomal and methylation alterations in sporadic and familial adenomatous polyposis-related duodenal carcinomas. *Mod Pathol* 2007; **20**: 1253-1262 [PMID: 17873900]
86 **Wynter CV**, Kambara T, Walsh MD, Leggett BA, Young J, Jass JR. DNA methylation patterns in adenomas from FAP, multiple adenoma and sporadic colorectal carcinoma patients. *Int J Cancer* 2006; **118**: 907-915 [PMID: 16152625]
87 **Kamatani A**, Nakagawa Y, Akao Y, Maruyama N, Nagasaka M, Shibata T, Tahara T, Hirata I. Downregulation of anti-oncomirs miR-143/145 cluster occurs before APC gene aberration in the development of colorectal tumors. *Med Mol Morphol* 2013; **46**: 166-171 [PMID: 23397547 DOI: 10.1007/s00795-013-0020-5]
88 **Yamaguchi T**, Iijima T, Wakaume R, Takahashi K, Matsumoto H, Nakano D, Nakayama Y, Mori T, Horiguchi S, Miyaki M. Underexpression of miR-126 and miR-20b in hereditary and nonhereditary colorectal tumors. *Oncology* 2014; **87**: 58-66 [PMID: 24994098 DOI: 10.1159/000363303]
89 **Croitoru ME**, Cleary SP, Di Nicola N, Manno M, Selander T, Aronson M, Redston M, Cotterchio M, Knight J, Gryfe R, Gallinger S. Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. *J Natl Cancer Inst* 2004; **96**: 1631-1634 [PMID: 15523092]
90 **Lubbe SJ**, Di Bernardo MC, Chandler IP, Houlston RS. Clinical implications of the colorectal cancer risk associated with MUTYH mutation. *J Clin Oncol* 2009; **27**: 3975-3980 [PMID: 19620482 DOI: 10.1200/JCO.2008.21.6853]
91 **Church J**, Heald B, Burke C, Kalady M. Understanding MYH-associated neoplasia. *Dis Colon Rectum* 2012; **55**: 359-362 [PMID: 22469805 DOI: 10.1097/DCR.0b013e31823a9392]
92 **Yamaguchi S**, Ogata H, Katsumata D, Nakajima M, Fujii T, Tsutsumi S, Asao T, Sasaki K, Kuwano H, Kato H. MUTYH-associated colorectal cancer and adenomatous polyposis. *Surg Today* 2014; **44**: 593-600 [PMID: 23605219 DOI: 10.1007/s00595-013-0592-7]
93 **Farrington SM**, Tenesa A, Barnetson R, Wiltshire A, Prendergast J, Porteous M, Campbell H, Dunlop MG. Germline susceptibility to colorectal cancer due to base-excision repair gene defects. *Am J Hum Genet* 2005; **77**: 112-119 [PMID: 15931596]
94 **Al-Tassan N**, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, Hodges AK, Davies DR, David SS, Sampson JR, Cheadle JP. Inherited variants of MYH associated with somatic G: C--> T: A mutations in colorectal tumors. *Nat Genet* 2002; **30**: 227-232 [PMID: 11818965]
95 **Knudson AG**. Hereditary cancers: from discovery to intervention. *J Natl Cancer Inst Monogr* 1995; **(17)**: 5-7 [PMID: 8573454]
96 **Jones S**, Emmerson P, Maynard J, Best JM, Jordan S, Williams GT, Sampson JR, Cheadle JP. Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G: C--> T: A mutations. *Hum Mol Genet* 2002; **11**: 2961-2967 [PMID: 12393807]
97 **Sieber OM**, Lipton L, Crabtree M, Heinimann K, Fidalgo P, Phillips RK, Bisgaard ML, Orntoft TF, Aaltonen LA, Hodgson SV, Thomas HJ, Tomlinson IP. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N Engl J Med* 2003; **348**: 791-799 [PMID: 12606733]
98 **Nielsen M**, Joerink-van de Beld MC, Jones N, Vogt S, Tops CM, Vasen HF, Sampson JR, Aretz S, Hes FJ. Analysis of MUTYH genotypes and colorectal phenotypes in patients With MUTYH-associated polyposis. *Gastroenterology* 2009; **136**: 471-476 [PMID: 19032956 DOI: 10.1053/j.gastro.2008.10.056]
99 **Miyaki M**, Iijima T, Yamaguchi T, Hishima T, Tamura K, Utsunomiya J, Mori T. Germline mutations of the MYH gene in Japanese patients with multiple colorectal adenomas. *Mutat Res* 2005; **578**: 430-433 [PMID: 15890374]
100 **Alhopuro P**, Parker AR, Lehtonen R, Enholm S, Järvinen HJ, Mecklin JP, Karhu A, Eshleman JR, Aaltonen LA. A novel functionally deficient MYH variant in individuals with colorectal adenomatous polyposis. *Hum Mutat* 2005; **26**: 393 [PMID: 16134146]
101 **Pin E**, Pastrello C, Tricarico R, Papi L, Quaia M, Fornasarig M, Carnevali I, Oliani C, Fornasin A, Agostini M, Maestro R, Barana D, Aretz S, Genuardi M, Viel A. MUTYH c.933+3A& gt; C, associated with a severely impaired gene expression, is the first Italian founder mutation in MUTYH-Associated Polyposis. *Int J Cancer* 2013; **132**: 1060-1069 [PMID: 22865608 DOI: 10.1002/ijc.27761]
102 **Venesio T**, Balsamo A, Errichiello E, Ranzani GN, Risio M. Oxidative DNA damage drives carcinogenesis in MUTYH-associated-polyposis by specific mutations of mitochondrial and MAPK genes. *Mod Pathol* 2013; **26**: 1371-1381 [PMID: 23599153 DOI: 10.1038/modpathol.2013.66]
103 **Cichy W**, Klincewicz B, Plawski A. Juvenile polyposis syndrome. *Arch Med Sci* 2014; **10**: 570-577 [PMID: 25097590 DOI: 10.5114/aoms.2014.43750]
104 **Gallione CJ**, Repetto GM, Legius E, Rustgi AK, Schelley SL, Tejpar S, Mitchell G, Drouin E, Westermann CJ, Marchuk DA. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet* 2004; **363**: 852-859 [PMID: 15031030]
105 **Brosens LA**, Langeveld D, van Hattem WA, Giardiello FM,

Offerhaus GJ. Juvenile polyposis syndrome. *World J Gastroenterol* 2011; **17**: 4839-4844 [PMID: 22171123 DOI: 10.3748/wjg.v17.i44.4839]
106 **Howe JR**, Shellnut J, Wagner B, Ringold JC, Sayed MG, Ahmed AF, Lynch PM, Amos CI, Sistonen P, Aaltonen LA. Common deletion of SMAD4 in juvenile polyposis is a mutational hotspot. *Am J Hum Genet* 2002; **70**: 1357-1362 [PMID: 11920286]
107 **Yamaguchi J**, Nagayama S, Chino A, Sakata A, Yamamoto N, Sato Y, Ashihara Y, Kita M, Nomura S, Ishikawa Y, Igarashi M, Ueno M, Arai M. Identification of coding exon 3 duplication in the BMPR1A gene in a patient with juvenile polyposis syndrome. *Jpn J Clin Oncol* 2014; **44**: 1004-1008 [PMID: 25129392 DOI: 10.1093/jjco/hyu111]
108 **Woodford-Richens K**, Bevan S, Churchman M, Dowling B, Jones D, Norbury CG, Hodgson SV, Desai D, Neale K, Phillips RK, Young J, Leggett B, Dunlop M, Rozen P, Eng C, Markie D, Rodriguez-Bigas MA, Sheridan E, Iwama T, Eccles D, Smith GT, Kim JC, Kim KM, Sampson JR, Evans G, Tejpar S, Bodmer WF, Tomlinson IP, Houlston RS. Analysis of genetic and phenotypic heterogeneity in juvenile polyposis. *Gut* 2000; **46**: 656-660 [PMID: 10764709]
109 **Woodford-Richens KL**, Rowan AJ, Poulsom R, Bevan S, Salovaara R, Aaltonen LA, Houlston RS, Wright NA, Tomlinson IP. Comprehensive analysis of SMAD4 mutations and protein expression in juvenile polyposis: evidence for a distinct genetic pathway and polyp morphology in SMAD4 mutation carriers. *Am J Pathol* 2001; **159**: 1293-1300 [PMID: 11583957]
110 **Marsh DJ**, Roth S, Lunetta KL, Hemminki A, Dahia PL, Sistonen P, Zheng Z, Caron S, van Orsouw NJ, Bodmer WF, Cottrell SE, Dunlop MG, Eccles D, Hodgson SV, Järvinen H, Kellokumpu I, Markie D, Neale K, Phillips R, Rozen P, Syngal S, Vijg J, Tomlinson IP, Aaltonen LA, Eng C. Exclusion of PTEN and 10q22-24 as the susceptibility locus for juvenile polyposis syndrome. *Cancer Res* 1997; **57**: 5017-5021 [PMID: 9371495]
111 **Ling H,** Pickard K, Ivan C, Isella C, Ikuo M, Mitter R, Spizzo R, Bullock MD, Braicu C, Pileczki V, Vincent K, Pichler M, Stiegelbauer V, Hoefler G, Almeida MI, Hsiao A, Zhang X, Primrose JN, Packham GK, Liu K, Bojja K, Gafà R, Xiao L, Rossi S, Song JH, Vannini I, Fanini F, Kopetz S, Zweidler-McKay P, Wang X, Ionescu C, Irimie A, Fabbri M, Lanza G, Hamilton SR, Berindan-Neagoe I, Medico E, Mirnezami AH, Calin GA, Nicoloso MS. The clinical and biological significance of MIR-224 expression in colorectal cancer metastasis. *Gut* 2015; Epub ahead of print [PMID: 25804630 DOI: 10.1136/gutjnl-2015-309372]
112 **Jenne DE**, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, Müller O, Back W, Zimmer M. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat Genet* 1998; **18**: 38-43 [PMID: 9425897]
113 **Schreibman IR**, Baker M, Amos C, McGarrity TJ. The hamartomatous polyposis syndromes: a clinical and molecular review. *Am J Gastroenterol* 2005; **100**: 476-490 [PMID: 15667510]
114 **Tomas C**, Soyer P, Dohan A, Dray X, Boudiaf M, Hoeffel C. Update on imaging of Peutz-Jeghers syndrome. *World J Gastroenterol* 2014; **20**: 10864-10875 [PMID: 25152588 DOI: 10.3748/wjg.v20.i31]
115 **Giardiello FM**, Trimbath JD. Peutz-Jeghers syndrome and management recommendations. *Clin Gastroenterol Hepatol* 2006; **4**: 408-415 [PMID: 16616343]
116 **Giardiello FM**, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, Cruz-Correa M, Offerhaus JA. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* 2000; **119**: 1447-1453 [PMID: 11113065]
117 **van Lier MG**, Westerman AM, Wagner A, Looman CW, Wilson JH, de Rooij FW, Lemmens VE, Kuipers EJ, Mathus-Vliegen EM, van Leerdam ME. High cancer risk and increased mortality in patients with Peutz-Jeghers syndrome. *Gut* 2011; **60**: 141-147 [PMID: 21205875 DOI: 10.1136/gut.2010.223750]
118 **Kopacova M**, Tacheci I, Rejchrt S, Bures J. Peutz-Jeghers syndrome: diagnostic and therapeutic approach. *World J Gastroenterol* 2009; **15**: 5397-5408 [PMID: 19916169]
119 **Rossi DJ**, Ylikorkala A, Korsisaari N, Salovaara R, Luukko K, Launonen V, Henkemeyer M, Ristimaki A, Aaltonen LA, Makela TP. Induction of cyclooxygenase-2 in a mouse model of Peutz-Jeghers polyposis. *Proc Natl Acad Sci USA* 2002; **99**: 12327-12332 [PMID: 12218179]
120 **Takeda H**, Miyoshi H, Tamai Y, Oshima M, Taketo MM. Simultaneous expression of COX-2 and mPGES-1 in mouse gastrointestinal hamartomas. *Br J Cancer* 2004; **90**: 701-704 [PMID: 14760387]
121 **Wang HH**, Xie NN, Li QY, Hu YQ, Ren JL, Guleng B. Exome sequencing revealed novel germline mutations in Chinese Peutz-Jeghers syndrome patients. *Dig Dis Sci* 2014; **59**: 64-71 [PMID: 24154639 DOI: 10.1007/s10620-013-2875-7]
122 **Mehenni H**, Blouin JL, Radhakrishna U, Bhardwaj SS, Bhardwaj K, Dixit VB, Richards KF, Bermejo-Fenoll A, Leal AS, Raval RC, Antonarakis SE. Peutz-Jeghers syndrome: confirmation of linkage to chromosome 19p13.3 and identification of a potential second locus, on 19q13.4. *Am J Hum Genet* 1997; **61**: 1327-1334 [PMID: 9399902]
123 **Amos CI**, Bali D, Thiel TJ, Anderson JP, Gourley I, Frazier ML, Lynch PM, Luchtefeld MA, Young A, McGarrity TJ, Seldin MF. Fine mapping of a genetic locus for Peutz-Jeghers syndrome on chromosome 19p. *Cancer Res* 1997; **57**: 3653-3656 [PMID: 9288765]
124 **Markie D**, Huson S, Maher E, Davies A, Tomlinson I, Bodmer WF. A pericentric inversion of chromosome six in a patient with Peutz-Jeghers' syndrome and the use of FISH to localise the breakpoints on a genetic map. *Hum Genet* 1996; **98**: 125-128 [PMID: 8698325]
125 **Alhopuro P**, Phichith D, Tuupanen S, Sammalkorpi H, Nybondas M, Saharinen J, Robinson JP, Yang Z, Chen LQ, Orntoft T, Mecklin JP, Järvinen H, Eng C, Moeslein G, Shibata D, Houlston RS, Lucassen A, Tomlinson IP, Launonen V, Ristimäki A, Arango D, Karhu A, Sweeney HL, Aaltonen LA. Unregulated smooth-muscle myosin in human intestinal neoplasia. *Proc Natl Acad Sci USA* 2008; **105**: 5513-5518 [PMID: 18391202 DOI: 10.1073/pnas.0801213105]
126 **Launonen V**. Mutations in the human LKB1/STK11 gene. *Hum Mutat* 2005; **26**: 291-297 [PMID: 16110486]
127 **Dong SM**, Kim KM, Kim SY, Shin MS, Na EY, Lee SH, Park WS, Yoo NJ, Jang JJ, Yoon CY, Kim JW, Kim SY, Yang YM, Kim SH, Kim CS, Lee JY. Frequent somatic mutations in serine/threonine kinase 11/Peutz-Jeghers syndrome gene in left-sided colon cancer. *Cancer Res* 1998; **58**:3787-3790 [PMID: 9731485]
128 **Resta N**, Simone C, Mareni C, Montera M, Gentile M, Susca F, Gristina R, Pozzi S, Bertario L, Bufo P, Carlomagno N, Ingrosso M, Rossini FP, Tenconi R, Guanti G. STK11 mutations in Peutz-Jeghers syndrome and sporadic colon cancer. *Cancer Res* 1998; **58**: 4799-4801 [PMID: 9809980]
129 **Bartosova Z**, Zavodna K, Krivulcik T, Usak J, Mlkva I, Kruzliak T, Hromec J, Usakova V, Kopecka I, Veres P, Bartosova Z, Bujalkova M. STK11/LKB1 germline mutations in the first Peutz-Jeghers syndrome patients identified in Slovakia. *Neoplasma* 2007; **54**: 101-107 [PMID: 17319781]
130 **Esteller M**, Avizienyte E, Corn PG, Lothe RA, Baylin SB, Aaltonen LA, Herman JG. Epigenetic inactivation of LKB1 in primary tumors associated with the Peutz-Jeghers syndrome. *Oncogene* 2000; **19**: 164-168 [PMID: 10644993]
131 **Miyoshi H**, Nakau M, Ishikawa TO, Seldin MF, Oshima M, Taketo MM. Gastrointestinal hamartomatous polyposis in Lkb1 heterozygous knockout mice. *Cancer Res* 2002; **62**: 2261-2266 [PMID: 11956081]
132 **Bardeesy N**, Sinha M, Hezel AF, Signoretti S, Hathaway NA, Sharpless NE, Loda M, Carrasco DR, DePinho RA. Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. *Nature* 2002; **419**: 162-167 [PMID: 12226664]
133 **Hosogi H**, Nagayama S, Kawamura J, Koshiba Y, Nomura A, Itami A, Okabe H, Satoh S, Watanabe G, Sakai Y. Molecular insights into Peutz-Jeghers syndrome: two probands with a germline mutation of LKB1. *J Gastroenterol* 2008; **43**: 492-497 [PMID: 18600394 DOI: 10.1007/s00535-008-2185-6]

134 **Lynch HT**, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003; **348**: 919-932 [PMID: 12621137]

135 **Samowitz WS**, Curtin K, Lin HH, Robertson MA, Schaffer D, Nichols M, Gruenthal K, Leppert MF, Slattery ML. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. *Gastroenterology* 2001; **121**: 830-838 [PMID: 11606497]

136 **Herman JG**, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson JK, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD, Vogelstein B, Kunkel TA, Baylin SB. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 1998; **95**: 6870-6875 [PMID: 9618505]

137 **Kirzin S**, Marisa L, Guimbaud R, De Reynies A, Legrain M, Laurent-Puig P, Cordelier P, Pradère B, Bonnet D, Meggetto F, Portier G, Brousset P, Selves J. Sporadic early-onset colorectal cancer is a specific sub-type of cancer: a morphological, molecular and genetics study. *PLoS One* 2014; **9**: e103159 [PMID: 25083765 DOI: 10.1371/journal.pone.0103159]

138 **Boland CR**, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; **138**: 2073-2087.e3 [PMID: 20420947 DOI: 10.1053/j.gastro.2009.12.064]

139 **Walther A**, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. *Nat Rev Cancer* 2009; **9**: 489-499 [PMID: 19536109 DOI: 10.1038/nrc2645]

140 **Cai G**, Xu Y, Lu H, Shi Y, Lian P, Peng J, Du X, Zhou X, Guan Z, Shi D, Cai S. Clinicopathologic and molecular features of sporadic microsatellite- and chromosomal-stable colorectal cancers. *Int J Colorectal Dis* 2008; **23**: 365-373 [PMID: 18193434 DOI: 10.1007/s00384-007-0423-7]

141 **Silver A**, Sengupta N, Propper D, Wilson P, Hagemann T, Patel A, Parker A, Ghosh A, Feakins R, Dorudi S, Suraweera N. A distinct DNA methylation profile associated with microsatellite and chromosomal stable sporadic colorectal cancers. *Int J Cancer* 2012; **130**: 1082-1092 [PMID: 21455990 DOI: 10.1002/ijc.26104]

142 **Hill DA**, Furman WL, Billups CA, Riedley SE, Cain AM, Rao BN, Pratt CB, Spunt SL. Colorectal carcinoma in childhood and adolescence: a clinicopathologic review. *J Clin Oncol* 2007; **25**: 5808-5814 [PMID: 18089879]

143 **Lanza G**, Ferracin M, Gafà R, Veronese A, Spizzo R, Pichiorri F, Liu CG, Calin GA, Ctabel 6roce CM, Negrini M. mRNA/microRNA gene expression profile in microsatellite unstable colorectal cancer. *Mol Cancer* 2007; **6**: 54 [PMID: 17716371 DOI: 10.1186/1476-4598-6-54]

144 **Yamamoto H**, Adachi Y, Taniguchi H, Kunimoto H, Nosho K, Suzuki H, Shinomura Y. Interrelationship between microsatellite instability and microRNA in gastrointestinal cancer. *World J Gastroenterol* 2012; **18**: 2745-2755 [PMID: 22719182 DOI: 10.3748/wjg.v18.i22.2745]

145 **Fernandez-Rozadilla C**, Brea-Fernández A, Bessa X, Alvarez-Urturi C, Abulí A, Clofent J, Payá A, Jover R, Xicola R, Llor X, Andreu M, Castells A, Carracedo A, Castellví-Bel S, Ruiz-Ponte C. BMPR1A mutations in early-onset colorectal cancer with mismatch repair proficiency. *Clin Genet* 2013; **84**: 94-96 [PMID: 23057600 DOI: 10.1111/cge.12023]

146 **Luo T**, Wu S, Shen X, Li L. Network cluster analysis of protein-protein interaction network identified biomarker for early onset colorectal cancer. *Mol Biol Rep* 2013; **40**: 6561-6568 [PMID: 24197691 DOI: 10.1007/s11033-013-2694-0]

147 **Ak S**, Tunca B, Yilmazlar T, Tezcan G, Cecener G, Egeli U, Ozturk E, Yerci O, Ertürk E, Zorluoglu A. Microsatellite instability status affects gene expression profiles in early onset colorectal cancer patients. *J Surg Res* 2013; **185**: 626-637 [PMID: 23992855 DOI: 10.1016/j.jss.2013.07.014]

148 **Moll R**, Löwe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol* 1992; **140**: 427-447 [PMID: 1371204]

149 **Tunca B**, Tezcan G, Cecener G, Egeli U, Zorluoglu A, Yilmazlar T, Ak S, Yerci O, Ozturk E, Umut G, Evrensel T. Overexpression of CK20, MAP3K8 and EIF5A correlates with poor prognosis in early-onset colorectal cancer patients. *J Cancer Res Clin Oncol* 2013; **139**: 691-702 [PMID: 23322277 DOI: 10.1007/s00432-013-1372-x]

150 **McGregor DK**, Wu TT, Rashid A, Luthra R, Hamilton SR. Reduced expression of cytokeratin 20 in colorectal carcinomas with high levels of microsatellite instability. *Am J Surg Pathol* 2004; **28**: 712-718 [PMID: 15166663]

151 **Astrosini C**, Roeefzaad C, Dai YY, Dieckgraefe BK, Jöns T, Kemmner W. REG1A expression is a prognostic marker in colorectal cancer and associated with peritoneal carcinomatosis. *Int J Cancer* 2008; **123**: 409-413 [PMID: 18452172 DOI: 10.1002/ijc.23466]

152 **Zenilman ME**, Kim S, Levine BA, Lee C, Steinberg JJ. Ectopic expression of reg protein: A marker of colorectal mucosa at risk for neoplasia. *J Gastrointest Surg* 1997; **1**: 194-201; discussion 201-202 [PMID: 9834348]

153 **Rechreche H**, Montalto G, Mallo GV, Vasseur S, Marasa L, Soubeyran P, Dagorn JC, Iovanna JL. pap, reg Ialpha and reg Ibeta mRNAs are concomitantly up-regulated during human colorectal carcinogenesis. *Int J Cancer* 1999; **81**: 688-694 [PMID: 10328217]

154 **Sengupta N**, Yau C, Sakthianandeswaren A, Mouradov D, Gibbs P, Suraweera N, Cazier JB, Polanco-Echeverry G, Ghosh A, Thaha M, Ahmed S, Feakins R, Propper D, Dorudi S, Sieber O, Silver A, Lai C. Analysis of colorectal cancers in British Bangladeshi identifies early onset, frequent mucinous histotype and a high prevalence of RBFOX1 deletion. *Mol Cancer* 2013; **12**: 1 [PMID: 23286373 DOI: 10.1186/1476-4598-12-1]

155 **Panarelli NC**, Vaughn CP, Samowitz WS, Yantiss RK. Sporadic microsatellite instability-high colon cancers rarely display immunohistochemical evidence of Wnt signaling activation. *Am J Surg Pathol* 2015; **39**: 313-317 [PMID: 25602793 DOI: 10.1097/PAS.0000000000000380]

156 **Loughrey MB**, Waring PM, Tan A, Trivett M, Kovalenko S, Beshay V, Young MA, McArthur G, Boussioutas A, Dobrovic A. Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer. *Fam Cancer* 2007; **6**: 301-310 [PMID: 17453358]

157 **Ak S**, Tunca B, Tezcan G, Cecener G, Egeli U, Yilmazlar T, Ozturk E, Yerci O. MicroRNA expression patterns of tumors in early-onset colorectal cancer patients. *J Surg Res* 2014; **191**: 113-122 [PMID: 24746948 DOI: 10.1016/j.jss.2014.03.057]

158 **Melo SA**, Ropero S, Moutinho C, Aaltonen LA, Yamamoto H, Calin GA, Rossi S, Fernandez AF, Carneiro F, Oliveira C, Ferreira B, Liu CG, Villanueva A, Capella G, Schwartz S, Shiekhattar R, Esteller M. A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet* 2009; **41**: 365-370 [PMID: 19219043 DOI: 10.1038/ng.317]

159 **Martino-Echarri E**, Henderson BR, Brocardo MG. Targeting the DNA replication checkpoint by pharmacologic inhibition of Chk1 kinase: a strategy to sensitize APC mutant colon cancer cells to 5-fluorouracil chemotherapy. *Oncotarget* 2014; **5**: 9889-9900 [PMID: 25301724]

160 **Popat S**, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609-618 [PMID: 15659508]

161 **Merok MA**, Ahlquist T, Røyrvik EC, Tufteland KF, Hektoen M, Sjo OH, Mala T, Svindland A, Lothe RA, Nesbakken A. Microsatellite instability has a positive prognostic impact on stage II colorectal cancer after complete resection: results from a large, consecutive Norwegian series. *Ann Oncol* 2013; **24**: 1274-1282 [PMID: 23235802 DOI: 10.1093/annonc/mds614]

162 **Violette S**, Poulain L, Dussaulx E, Pepin D, Faussat AM, Chambaz J, Lacorte JM, Staedel C, Lesuffleur T. Resistance of colon cancer cells to long-term 5-fluorouracil exposure is correlated to the relative level of Bcl-2 and Bcl-X(L) in addition to Bax and p53 status. *Int J Cancer* 2002; **98**: 498-504 [PMID: 11920608]

163 **Bishnupuri KS**, Luo Q, Murmu N, Houchen CW, Anant S, Dieckgraefe BK. Reg IV activates the epidermal growth factor receptor/Akt/AP-1 signaling pathway in colon adenocarcinomas. *Gastroenterology* 2006; **130**: 137-149 [PMID: 16401477]

164 **Deng J**, Lei W, Fu JC, Zhang L, Li JH, Xiong JP. Targeting miR-21

enhances the sensitivity of human colon cancer HT-29 cells to chemoradiotherapy in vitro. *Biochem Biophys Res Commun* 2014; **443**: 789-795 [PMID: 24275137 DOI: 10.1016/j.bbrc.2013.11.064]

165 **Institute NC.** Targeted Cancer Therapies. [updated 2014 Apr 25]. Rockville, MD: National Cancer Institute, 2014

166 **Grothey A**, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 303-312 [PMID: 23177514 DOI: 10.1016/S0140-6736(12)61900-X]

167 **Van Cutsem E**, Tabernero J, Lakomy R, Prenen H, Prausová J, Macarulla T, Ruff P, van Hazel GA, Moiseyenko V, Ferry D, McKendrick J, Polikoff J, Tellier A, Castan R, Allegra C. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol* 2012; **30**: 3499-3506 [PMID: 22949147]

168 **El Zouhairi M**, Charabaty A, Pishvaian MJ. Molecularly targeted therapy for metastatic colon cancer: proven treatments and promising new agents. *Gastrointest Cancer Res* 2011; **4**: 15-21 [PMID: 21464866]

169 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313]

170 **Allegra CJ**, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, McAllister PK, Morton RF, Schilsky RL. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009; **27**: 2091-2096 [PMID: 19188670 DOI: 10.1200/JCO.2009.21.9170]

171 **Knickelbein K,** Zhang L. Mutant KRAS as a critical determinant of the therapeutic response of colorectal cancer. *Genes Dis* 2015; **2**: 4-12 [PMID: 25815366]

172 **Therkildsen C**, Bergmann TK, Henrichsen-Schnack T, Ladelund S, Nilbert M. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. *Acta Oncol* 2014; **53**: 852-864 [PMID: 24666267 DOI: 10.3109/0284186X.2014.895036]

173 **French AJ**, Sargent DJ, Burgart LJ, Foster NR, Kabat BF, Goldberg R, Shepherd L, Windschitl HE, Thibodeau SN. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. *Clin Cancer Res* 2008; **14**: 3408-3415 [PMID: 18519771 DOI: 10.1158/1078-0432.CCR-07-1489]

174 **Hess GP**, Wang PF, Quach D, Barber B, Zhao Z. Systemic Therapy for Metastatic Colorectal Cancer: Patterns of Chemotherapy and Biologic Therapy Use in US Medical Oncology Practice. *J Oncol Pract* 2010; **6**: 301-307 [PMID: 21358960 DOI: 10.1200/JOP.2010.000072]

175 **Davis SL**, Eckhardt SG, Messersmith WA, Jimeno A. The development of regorafenib and its current and potential future role in cancer therapy. *Drugs Today* (Barc) 2013; **49**: 105-115 [PMID: 23462625 DOI: 10.1358/dot.2013.49.2.1930525]

176 **Economides AN**, Carpenter LR, Rudge JS, Wong V, Koehler-Stec EM, Hartnett C, Pyles EA, Xu X, Daly TJ, Young MR, Fandl JP, Lee F, Carver S, McNay J, Bailey K, Ramakanth S, Hutabarat R, Huang TT, Radziejewski C, Yancopoulos GD, Stahl N. Cytokine traps: multi-component, high-affinity blockers of cytokine action. *Nat Med* 2003; **9**: 47-52 [PMID: 12483208]

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MINIREVIEWS

Novel therapeutic agents in the treatment of metastatic colorectal cancer

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Abstract

Over the past couple of decades considerable progress has been made in the management of metastatic colorectal cancers (mCRC) leading to a significant improvement in five-year survival. Although part of this success has been rightly attributed to aggressive surgical management and advances in other adjunct treatments, our understanding of the pathogenesis of cancer and emergence of newer molecular targets for colon cancer has created a powerful impact. In this review article we will discuss various targeted therapies in the management of mCRC. Newer agents on the horizon soon to be incorporated in clinical practice will be briefly reviewed as well.

Key words: Metastatic colorectal cancer; Molecular targeted drugs; Anti-angiogenesis inhibitors; Epidermal growth factor receptor inhibitors; Novel therapeutic agents

Core tip: This article reviews the novel agents in the management of metastatic colorectal cancer. The core principles and the evidence behind the use of these agents are discussed. Clinically relevant features are highlighted to help the health care provider involved in the care of metastatic colorectal cancer patients.

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INTRODUCTION

In 2015, a total of 132700 new cases of colorectal cancer are expected to be diagnosed in the United States accounting for about 8% of all new cancer diagnoses. In this same year 49700 patients will die of metastatic colorectal cancers (mCRC), which will contribute to 8.4%

of all cancer related mortality[1]. With the widespread use of screening colonoscopy and newer modalities like Stool DNA based screening, we can expect early diagnosis and curative treatments in patients diagnosed with early disease and hence better survival. However up to > 25% of patients will present with metastatic disease, where systemic treatment options will be desired. Widespread use of genetic screening and sharing platforms like the cancer Genome Atlas has led to a better understanding of carcinogenesis and as a consequence newer molecular targets for colon cancer have been discovered[2]. In this review article we will discuss some of the well-known targetable pathways as well as shed light on some of the novel pathways where we can expect newer therapies to emerge.

ANTI-ANGIOGENESIS AGENTS

Anti-angiogenesis was proposed as an anticancer therapy over four decades ago[3]. We know that angiogenesis is required for invasive tumor growth and metastasis and is an integral part of cancer progression[4]. Angiogenesis is mediated through vascular endothelial growth factor (VEGF), the altered regulation of which is associated with several diseases including malignancy. VEGF is a heparin-binding growth factor specific for vascular endothelial cells that is able to induce angiogenesis *in vivo*[5]. Three notable anti-VEGF agents have been approved by United States Food and Drug Administration (USFDA) for treating mCRC and will be reviewed here.

Bevacizumab

Bevacizumab is a recombinant humanized IgG-1 antibody against soluble VEGF-A which has a high binding specificity with VEGF-A. Once bound, Bevacizumab prevents its interaction with receptors on vascular endothelial cells and thereby truncates the abnormal downstream signaling. After success in early phase trials, this agent was tested in phase 3 clinical trials[6]. In the pivotal trial which had 813 previously untreated patients with mCRC randomized to the two arms, the median duration of survival was 20.3 mo in the Irinotecan, 5-Fluorouracil and Leucovorin (IFL) plus Bevacizumab group, as compared with 15.6 mo in the IFL plus placebo group, corresponding to a hazard ratio for death of 0.66 ($P < 0.001$)[7]. An Eastern Cooperative Oncology Group Study (E3200) showed median duration of survival for the group treated with FOLFOX4 and Bevacizumab was 12.9 mo compared with 10.8 mo for the group treated with FOLFOX4 alone (corresponding hazard ratio for death 0.75, $P < 0.001$), and 10.2 mo for those treated with Bevacizumab alone. Bevacizumab is approved by the USFDA in combination with either an Irinotecan or Oxaliplatin based regimen for the treatment of mCRC[8,9].

Bevacizumab is generally well tolerated when administered in combination with chemotherapy for mCRC. Hypertension, proteinuria, epistaxis and thrombosis are some of the common adverse events associated with its use[6]. No clear guidelines exist on the management of hypertension but in most patients it is usually possible to control hypertension with standard antihypertensive medications. On occasion, it may be necessary to temporarily or permanently discontinue Bevacizumab if hypertension is severe or persistent[10].

Routine use of Bevacizumab as maintenance therapy is controversial. A recent study found no clear benefits of continuing Bevacizumab after 4-6 mo of standard first-line chemotherapy plus Bevacizumab and given the cost and lack of clear benefit, it was not recommended[11]. Whether a certain subgroup with high-risk disease such as high metastatic burden would benefit from this approach needs further investigation[12].

Ziv-Aflibercept

Ziv-Aflibercept is a fusion protein consisting of human VEGF receptor extracellular domains fused to the Fc portion of human immunoglobulin G1, and works by inhibiting VEGF receptor. Aflibercept was used in a large phase 3 trial in combination with 5-Fluorouracil, Irinotecan and Leucovorin (FOLFIRI) and was found to confer a statistically significant survival benefit over FOLFIRI combined with placebo in patients with mCRC previously treated with an Oxaliplatin based regimen[13]. Adding Aflibercept to FOLFIRI showed an improved overall survival relative to placebo plus FOLFIRI (HR = 0.817, 95%CI: 0.713-0.937, $P = 0.0032$) with median survival times of 13.50 mo *vs* 12.06 mo, respectively. Efficacy was maintained across demographic and baseline characteristics and stratification factors at randomization, irrespective of prior treatment with Bevacizumab, with a similar safety profile[14].

Ramucirumab

Ramucirumab is a recombinant human monoclonal anti vascular endothelial growth factor-receptor 2 antibody which was recently approved by USFDA for use in combination with FOLFIRI for the treatment of patients with mCRC whose disease has progressed on first line Bevacizumab, Oxaliplatin- and Fluoropyrimidine-containing regimen. Approval was based on a study that enrolled 1072 patients (536 in each group) and patients were randomized either to receive Ramucirumab or placebo[15]. PFS was significantly improved in patients who received Ramucirumab in combination with FOLFIRI compared to placebo [Median PFS was 5.7 and 4.5 mo; HR = 0.79 (95%CI: 0.70-0.90, $P < 0.001$]. Median overall survival was 13.3 mo (95%CI: 12.4-14.5) for patients in the Ramucirumab group *vs* 11.7 mo (10.8-12.7) for the placebo group (HR = 0.844, 95%CI: 0.730-0.976, log-rank $P = 0.0219$). Diarrhea, hypertension and fatigue were the common adverse events with the use of Ramucirumab, consistent with the previously known safety profile established in previously approved indications.

EPIDERMAL GROWTH FACTOR RECEPTOR AND OTHER KINASES

The epidermal growth factor receptor (EGFR) autocrine pathway has been known to affect a number of processes important to carcinogenesis including cell proliferation, apoptosis and angiogenesis. This has been the rationale for developing EGFR inhibitors, both monoclonal antibodies to prevent ligand binding as well as small molecule inhibitors of the tyrosine kinase enzymatic activity to inhibit auto-phosphorylation and downstream intracellular signaling[16]. Although monoclonal antibodies like cetuximab were initially developed to treat head and neck cancer, traditionally known to highly express EGFR on immunohistochemistry, their use was extended to treating colorectal cancer.

Cetuximab: Cetuximab is a chimeric (mouse/human) monoclonal antibody used in the management of mCRC, which was initially approved by USFDA as a third line single agent in patients who have failed Oxaliplatin- or Irinotecan- based chemotherapy and who are intolerant to Irinotecan. In the pivotal trial which compared FOLFIRI plus Cetuximab *vs* FOLFIRI plus Bevacizumab as first-line treatment for patients with mCRC, 592 patients with KRAS exon 2 wild-type tumors were randomly assigned and received treatment. Median progression-free survival was 10.0 mo (95%CI: 8.8-10.8) in the Cetuximab group and 10.3 mo (95%CI: 9.8-11.3) in the Bevacizumab group (HR = 1.06, 95%CI: 0.88-1.26, *P* = 0.55); however, median overall survival was 28.7 mo (95%CI: 24.0-36.6) in the Cetuximab group compared with 25.0 mo (22.7-27.6) in the Bevacizumab group (HR = 0.77, 95%CI: 0.62-0.96, *P* = 0.017). Anti-EGFR monoclonal antibodies are well tolerated, the most important adverse event being cutaneous reaction including rash, pruritus, and nail changes. These adverse reactions can usually be medically managed and patients tend to continue on the drugs. Occasionally the drug may need to be discontinued due to intolerable side effects.

Panitumumab

Panitumumab is a fully humanized monoclonal antibody specific to EGFR. The efficacy of Panitumumab was established in the PRIME study which showed that in the wild-type KRAS stratum, Panitumumab-FOLFOX4 significantly improved PFS compared with FOLFOX4 (median PFS, 9.6 mo *vs* 8.0 mo, respectively; HR = 0.80; 95%CI: 0.66-0.97, *P* = 0.02). Also noted was a nonsignificant increase in OS for Panitumumab-FOLFOX4 *vs* FOLFOX4 (median OS, 23.9 mo *vs* 19.7 mo, respectively; HR = 0.83, 95%CI: 0.67-1.02, *P* = 0.072)[17]. In an open-label, phase 3 head-to-head study of Panitumumab *vs* Cetuximab which enrolled patients with chemotherapy-refractory mCRC Panitumumab was non-inferior to Cetuximab. Median overall survival was 10.4 mo (95%CI: 9.4-11.6) with Panitumumab and 10.0 mo (9.3-11.0) with Cetuximab (HR = 0.97, 95%CI: 0.84-1.11)[18]. Panitumumab has been shown to induce pathological near complete response or complete response when given along with neoadjuvant concurrent radiation therapy in patients with KRAS wild-type locally advanced rectal cancer[19]. Panitumumab is generally well tolerated and has a similar side effect profile as Cetuximab.

Ras testing and use of egfr antibodies

EGFR expression as measured by immunohistochemistry on many occasions does not predict clinical benefit with the use of EGFR inhibitors[20,21]. It has also been shown that mutations in the KRAS exon 2 (codons 12 and 13), which was down stream to EGFR, dictated the response to EGFR antibodies. Additional mutations like KRAS exon 3 (at codons 59 and 61) and exon 4 (at codons 117 and 146), NRAS exon 2 (at codons 12 and 13), exon 3 (at codons 59 and 61), and exon 4 (at codons 117 and 146), have been demonstrated to be negative predictive biomarkers for EGFR antibody treatment. These additional mutations now account for approximately 17% of patients with wild-type KRAS exon 2 status who harbor a mutation in other RAS exons[22]. Testing for extended EGFR mutation is highly recommended and if truly wild type then use of EGFR antibodies is justified in those cases.

TYROSINE KINASE INHIBITORS

Regorafenib (BAY 73-4506) is a novel oral diphenylurea based multikinase inhibitor, shown to be a potent inhibitor of a wide variety of Tyrosine kinases which include several angiogenic, stromal receptor and oncogenic tyrosine kinases as well as intracellular signaling kinases in preclinical studies[23]. A phase Ⅲ trial in refractory mCRC, (CORRECT) randomized 760 patients between Regorafenib (*n* = 505) and placebo (*n* = 255). It showed a small but statistically significant improvement in OS (median 6.4 mo *vs* 5 mo, one-sided *P* value 0.005) and progression-free survival (median 1.9 mo *vs* 1.7 mo, one-sided *P* value < 0.000001) for Regorafenib[24]. The most common side effects of Regorafenib are fatigue, hand-foot skin reaction (palmar-plantar erythrodysesthesia), diarrhea, mucositis and weight loss for which the patients need to be monitored closely[25]. A novel germline mutation of PDGFR-beta might be associated with clinical response of colorectal cancer to Regorafenib[26].

EMERGING AGENTS

Targeting cancer stem cells

Human cancers have been shown to harbor cancer stem cells which are thought to play an important role in cancer recurrence and metastasis. With the recent discoveries of small molecules that target highly conserved cell homeostasis pathways which have been implicated in the pathogenesis of colorectal cancer, gives us an

Table 1 Ongoing clinical trials in Immunotherapy in colorectal cancer

Drug name	Class	Phase	ClinicalTrials.gov Identifier	Sponsor	Remarks
AMP-224	PD-1 inhibitor	1	NCT02298946	NCI	Combination with stereotactic body radiation therapy
MPDL3280A	Engineered anti-PDL1 antibody	1	NCT01375842	Genentech	Administered as single agent
Varlilumab and nivolumab	Monoclonal antibodies that binds to CD27 and PD-1	1/2	NCT02335918	Celldex therapeutics/ bristol-myers squibb	Phase Ⅱ to determine objective response rate
MPDL3280A and bevacizumab	Engineered anti-PDL1 antibody	1b	NCT01633970	Genentech	Assess the safety, pharmacology and preliminary efficacy of the combination
Avelumab	Antibody targeting PDL-1	1	NCT01772004	EMD serono	Open-label, dose-escalation trial
MEDI4736	Anti PDL-1	2	NCT02227667	Memorial sloan Kettering cancer center	Study to evaluate the efficacy of MEDI4736

Available from: URL: http://www.clinicaltrials.gov, accessed on 4/25/2015.

exciting avenue in treating mCRC. BBI608, an orally-administered first-in-class cancer stem cell inhibitor, has been tried in a Phase 1 study after excellent preclinical evidence. This has shown some promising anticancer activity in patients with CRC[27]. An open label, multi-center, Phase 2 study of BBI608 in combination with cetuximab, Panitumumab or Capecitabine in patients with advanced colorectal cancer is ongoing (ClinicalTrials.gov Identifier: NCT01776307). Another phase 1 dose escalation study with LGK974 is currently ongoing and recruiting patients with special emphasis on those with B-RAF mutant colorectal cancer with documented Wnt pathway alteration (ClinicalTrials.gov Identifier: NCT01351103).

BRAF

BRAF mutations have been shown to be the cause of sporadic CRCs through altered mismatch repair pathway and occur mutually exclusive of *KRAS* mutations[28]. At this time *BRAF* mutation is known to confer a poor prognosis in mCRC, but is not a validated target for anti-cancer therapy[29,30]. Although this mutation is found in a relatively small proportion of CRC (5%-8%), targeting BRAF has been unsuccessful as feedback stimulation of EGFR pathway has been suggested as the reason for the treatment failure[31]. Current studies are focused on dual blockade of BRAF and EGFR or of the subsequent downstream pathway. Initial experience of combining BRAF inhibitor Vemurafenib with EGFR inhibitor Panitumumab has been safe, although the response has been modest[32]. Another Phase Ⅱ Study of Irinotecan and Cetuximab with or without Vemurafenib in BRAF mCRC is still recruiting patients (ClinicalTrials.gov Identifier: NCT02164916). Another potential strategy is the use of ERK inhibitor that is thought to suppress MAPK activity, which is usually upregulated in patients on RAF inhibitors and may overcome resistance. ERK inhibitors are currently in early phase clinical trials[33].

Immunotherapy

The advent of immune check point blockade has been an exciting field in cancer immunotherapy. Already of considerable success in other types of cancers like melanoma and squamous cell lung cancer where Anti PD-1 drugs are approved by USFDA, various groups are studying the efficacy in colorectal cancer. Mismatch-repair status has been useful in predicting clinical benefit of immune checkpoint blockade with Pembrolizumab, with higher response in Microsatellite Instability High (MSI-High) tumors[34]. The table summarizes the current ongoing trials mainly targeting PD-1 - PDL-1 immune checkpoint pathway (Table 1).

Targeting kras with reolysin

Biological strategies like Reovirus Serotype 3 - Dearing Strain (Reolysin), a naturally occurring ubiquitous, non-enveloped human Reovirus, have been explored in mCRC for targeting KRAS. Reovirus has been shown to replicate selectively in RAS-transformed cells causing cell lysis. Activating mutations in RAS or mutations in oncogenes signaling through the RAS pathway may occur in as many as 80% of human tumors and can be targeted by this approach. A multicenter phase 1 study Reolysin in combination with FOLFIRI and Bevacizumab in FOLFIRI naive patients with KRAS mCRC is ongoing (ClinicalTrials.gov Identifier: NCT01274624).

TAS-102

TAS-102 is a novel oral nucleoside and works as an antimetabolite. TAS-102 is a combination of trifluridine, a nucleoside analog, and tipiracil hydrochloride, a thymidine phosphorylase inhibitor. In a double-blind, randomized, placebo-controlled phase 2 trial, 112 patients were allocated to TAS-102 and 57 allocated to placebo. Median overall survival was 9.0 mo (95%CI: 7.3-11.3) in the TAS-102 group and 6.6 mo (4.9-8.0) in the placebo group (hazard ratio for death 0.56, 80%CI: 0.44-0.71, 95%CI: 0.39-0.81, P = 0.0011) on a median follow up of 11.3 mo (interquartile range 10.7-14.0 mo). Hematological toxicities were the important side effects to consider in patients on TAS- 108 arm, 57 (50%) neutropenia of grade 3 or 4, 32 (28%) leucopenia and 19 (17%) experiencing anemia. Serious adverse events were reported in 21 (19%) patients in the TAS-102 group. Recent data from RECOURSE study has shown that median overall survival improved from 5.3 mo with placebo to 7.1 mo with TAS-102. Hazard ratio for death

in the TAS-102 group *vs* the placebo group was 0.68 (95%CI: 0.58-0.81, $P < 0.001$), and this data led to its FDA approval[35].

CONCLUSION

In conclusion, mCRC treatment is a rapidly evolving field with many novel agents under investigation. Although many targeted drugs have been approved and are already in clinical use, there is a clear need for further research and development of more effective treatments. Over the coming years, as understanding of the biology of the disease improves, newer treatment modalities will be investigated. The optimum use and sequencing of these agents, especially in combination with chemotherapy and other targeted agents will need to be better defined.

REFERENCES

1 **National Cancer Institute.** SEER stat fact sheets: colon and rectum cancer. [accessed 2015 April 23]. Available from: URL: http://seer.cancer.gov/statfacts/html/colorect.html

2 **The Cancer Genome Atlas.** Available from: URL: http://www.cancergenome.nih.gov

3 **Folkman J**. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**: 1182-1186 [PMID: 4938153 DOI: 10.1056/nejm197111182852108]

4 **Folkman J**. Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 2002; **29**: 15-18 [PMID: 12516034 DOI: 10.1053/sonc.2002.37263]

5 **Leung DW**, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; **246**: 1306-1309 [PMID: 2479986]

6 **Kabbinavar F**, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; **21**: 60-65 [PMID: 12506171]

7 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435 DOI: 10.1056/NEJMoa032691]

8 **Giantonio BJ**, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, Schwartz MA, Benson AB. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* 2007; **25**: 1539-1544 [PMID: 17442997 DOI: 10.1200/jco.2006.09.6305]

9 **Cohen MH**, Gootenberg J, Keegan P, Pazdur R. FDA drug approval summary: bevacizumab plus FOLFOX4 as second-line treatment of colorectal cancer. *Oncologist* 2007; **12**: 356-361 [PMID: 17405901 DOI: 10.1634/theoncologist.12-3-356]

10 **Izzedine H**, Ederhy S, Goldwasser F, Soria JC, Milano G, Cohen A, Khayat D, Spano JP. Management of hypertension in angiogenesis inhibitor-treated patients. *Ann Oncol* 2009; **20**: 807-815 [PMID: 19150949 DOI: 10.1093/annonc/mdn713]

11 **Koeberle D**, Betticher DC, von Moos R, Dietrich D, Brauchli P, Baertschi D, Matter K, Winterhalder R, Borner M, Anchisi S, Moosmann P, Kollar A, Saletti P, Roth A, Frueh M, Kueng M, Popescu RA, Schacher S, Hess V, Herrmann R. Bevacizumab continuation versus no continuation after first-line chemotherapy plus bevacizumab in patients with metastatic colorectal cancer: a randomized phase III non-inferiority trial (SAKK 41/06). *Ann Oncol* 2015; **26**: 709-714 [PMID: 25605741 DOI: 10.1093/annonc/mdv011]

12 **Fedyanin M**, Tryakin A, Vybarava A, Chekini D, Pokataev I, Sekhina O, Gordeev S, Aliev V, Tjulandin S. Maintenance therapy following first-line chemotherapy in metastatic colorectal cancer: toxicity and efficacy-single-institution experience. *Med Oncol* 2015; **32**: 429 [PMID: 25491142 DOI: 10.1007/s12032-014-0429-2]

13 **Van Cutsem E**, Tabernero J, Lakomy R, Prenen H, Prausová J, Macarulla T, Ruff P, van Hazel GA, Moiseyenko V, Ferry D, McKendrick J, Polikoff J, Tellier A, Castan R, Allegra C. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol* 2012; **30**: 3499-3506 [PMID: 22949147 DOI: 10.1200/jco.2012.42.8201]

14 **Tabernero J**, Van Cutsem E, Lakomý R, Prausová J, Ruff P, van Hazel GA, Moiseyenko VM, Ferry DR, McKendrick JJ, Soussan-Lazard K, Chevalier S, Allegra CJ. Aflibercept versus placebo in combination with fluorouracil, leucovorin and irinotecan in the treatment of previously treated metastatic colorectal cancer: prespecified subgroup analyses from the VELOUR trial. *Eur J Cancer* 2014; **50**: 320-331 [PMID: 24140268 DOI: 10.1016/j.ejca.2013.09.013]

15 **Tabernero J,** Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, Ciuleanu TE, Portnoy DC, Van Cutsem E, Grothey A, Prausova J, Garcia-Alfonso P, Yamazaki K, Clingan PR, Lonardi S, Kim TW, Simms L, Chang SC, Nasroulah F. Ramucirumab versus placebo in combination with second-line folfiri in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (raise): A randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol* 2015; **16:** 499-508 [DOI: 10.1016/s1470-2045(15)70127-0]

16 **Ciardiello F**, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001; **7**: 2958-2970 [PMID: 11595683]

17 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010; **28**: 4697-4705 [PMID: 20921465 DOI: 10.1200/jco.2009.27.4860]

18 **Price TJ**, Peeters M, Kim TW, Li J, Cascinu S, Ruff P, Suresh AS, Thomas A, Tjulandin S, Zhang K, Murugappan S, Sidhu R. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. *Lancet Oncol* 2014; **15**: 569-579 [PMID: 24739896 DOI: 10.1016/s1470-2045(14)70118-4]

19 **Helbling D**, Bodoky G, Gautschi O, Sun H, Bosman F, Gloor B, Burkhard R, Winterhalder R, Madlung A, Rauch D, Saletti P, Widmer L, Borner M, Baertschi D, Yan P, Benhattar J, Leibundgut EO, Bougel S, Koeberle D. Neoadjuvant chemoradiotherapy with or without panitumumab in patients with wild-type KRAS, locally advanced rectal cancer (LARC): a randomized, multicenter, phase II trial SAKK 41/07. *Ann Oncol* 2013; **24**: 718-725 [PMID: 23139259 DOI: 10.1093/annonc/mds519]

20 **Hecht JR**, Mitchell E, Neubauer MA, Burris HA, Swanson P, Lopez T, Buchanan G, Reiner M, Gansert J, Berlin J. Lack of correlation between epidermal growth factor receptor status and response to Panitumumab monotherapy in metastatic colorectal cancer. *Clin Cancer Res* 2010; **16**: 2205-2213 [PMID: 20332321 DOI: 10.1158/1078-0432.ccr-09-2017]

21 **Piene H**, Myhre ES, Winnem B. Optimal and actual end systolic pressure-volume relations in dogs and rabbits. Including new

method of Emax determination. *Acta Physiol Scand* 1988; **133**: 501-511 [PMID: 3227936 DOI: 10.1634/theoncologist.2009-0221]

22 **Douillard JY**, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; **369**: 1023-1034 [PMID: 24024839 DOI: 10.1056/NEJMoa1305275]

23 **Hendlisz A**, Deleporte A, Vandeputte C, Charette N, Paesmans M, Guiot T, Garcia C, Flamen P. Regorafenib assessment in refractory advanced colorectal cancer: RegARd-C study protocol. *BMJ Open* 2015; **5**: e007189 [PMID: 25753361 DOI: 10.1136/bmjopen-2014-007189]

24 **Grothey A**, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 303-312 [PMID: 23177514 DOI: 10.1016/s0140-6736(12)61900-x]

25 **Belum VR**, Wu S, Lacouture ME. Risk of hand-foot skin reaction with the novel multikinase inhibitor regorafenib: a meta-analysis. *Invest New Drugs* 2013; **31**: 1078-1086 [PMID: 23700287 DOI: 10.1007/s10637-013-9977-0]

26 **Rechsteiner M**, Wild P, Kiessling MK, Bohnert A, Zhong Q, Stahel RA, Moch H, Curioni-Fontecedro A. A novel germline mutation of PDGFR-β might be associated with clinical response of colorectal cancer to regorafenib. *Ann Oncol* 2015; **26**: 246-248 [PMID: 25336117 DOI: 10.1093/annonc/mdu471]

27 **Langleben A,** Supko JG, Hotte SJ, Batist G, Hirte HW, Rogoff H, Li Y, Li W, Kerstein D, Leggett D, Hitron MJ, Li C. A dose-escalation phase I study of a first-in-class cancer stemness inhibitor in patients with advanced malignancies. 2013 American Society of Clinical Oncology; 2013 May 30-June 3; Chicago, IL

28 **Rajagopalan H**, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature* 2002; **418**: 934 [PMID: 12198537 DOI: 10.1038/418934a]

29 **Borges LF**. Historical development of neural transplantation. *Appl Neurophysiol* 1988; **51**: 265-277 [PMID: 3048210 DOI: 10.1038/bjc.2011.19]

30 **Benatar SR**, Hönig P. Maximal expiratory flow and lung volume changes associated with exercise-induced asthma in children and the effect of breathing a low-density gas mixture. *Clin Sci Mol Med* 1974; **46**: 317-329 [PMID: 4274188 DOI: 10.1053/j.gastro.2014.09.041]

31 **Prahallad A**, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012; **483**: 100-103 [PMID: 22281684 DOI: 10.1038/nature10868]

32 **Yaeger R**, Cercek A, O'Reilly EM, Reidy DL, Kemeny N, Wolinsky T, Capanu M, Gollub MJ, Rosen N, Berger MF, Lacouture ME, Vakiani E, Saltz LB. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. *Clin Cancer Res* 2015; **21**: 1313-1320 [PMID: 25589621 DOI: 10.1158/1078-0432.ccr-14-2779]

33 **Ahronian LG**, Sennott EM, Van Allen EM, Wagle N, Kwak EL, Faris JE, Godfrey JT, Nishimura K, Lynch KD, Mermel CH, Lockerman EL, Kalsy A, Gurski JM, Bahl S, Anderka K, Green LM, Lennon NJ, Huynh TG, Mino-Kenudson M, Getz G, Dias-Santagata D, Iafrate AJ, Engelman JA, Garraway LA, Corcoran RB. Clinical Acquired Resistance to RAF Inhibitor Combinations in BRAF-Mutant Colorectal Cancer through MAPK Pathway Alterations. *Cancer Discov* 2015; **5**: 358-367 [PMID: 25673644 DOI: 10.1158/2159-8290.cd-14-1518]

34 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]

35 **Mayer RJ**, Van Cutsem E, Falcone A, Yoshino T, Garcia-Carbonero R, Mizunuma N, Yamazaki K, Shimada Y, Tabernero J, Komatsu Y, Sobrero A, Boucher E, Peeters M, Tran B, Lenz HJ, Zaniboni A, Hochster H, Cleary JM, Prenen H, Benedetti F, Mizuguchi H, Makris L, Ito M, Ohtsu A. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med* 2015; **372**: 1909-1919 [PMID: 25970050 DOI: 10.1056/NEJMoa1414325]

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MINIREVIEWS

Long-term outcomes after stenting as a "bridge to surgery" for the management of acute obstruction secondary to colorectal cancer

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Abstract

Obstructive symptoms are present in 8% of cases at the time of initial diagnosis in cases of colorectal cancer. Emergency surgery has been classically considered the treatment of choice in these patients. However, in the majority of studies, emergency colorectal surgery is burdened with higher morbidity and mortality rates than elective surgery, and many patients require temporal colostomy which deteriorates their quality of life and becomes permanent in 10%-40% of cases. The aim of stenting by-pass to surgery is to transform emergency surgery into elective surgery in order to improve surgical results, obtain an accurate tumoral staging and detection of synchronous lesions, stabilization of comorbidities and performance of laparoscopic surgery. Immediate results were more favourable in patients who were stented concerning primary anastomosis, permanent stoma, wound infection and overall morbidity, having the higher surgical risk patients the greater benefit. However, some findings laid out the possible implication of stenting in long-term results of oncologic treatment. Perforation after stenting is related to tumoral recurrence. In studies with perforation rates above 8%, higher recurrences rates in young patients and lower disease free survival have been shown. On the other hand, after stenting the number of removed lymph nodes in the surgical specimen is larger, patients can receive adjuvant chemotherapy earlier and in a greater percentage and the number of patients who can be surgically treated with laparoscopic surgery is larger. Finally, there are no consistent studies able to demonstrate that one strategy is superior to the other in terms of oncologic benefits. At present, it would seem wise to assume a higher initial complication rate in young patients without relevant comorbidities and to accept the risk of local recurrence in old patients (> 70 years) or with high surgical risk (ASA Ⅲ/Ⅳ).

Key words: Self-expanding metallic stent; Colorectal cancer; Obstructive colorectal cancer; Colorectal cancer chemotherapy; Colorectal cancer surgery

Core tip: Self-expanding metal stents placement as a bridge to surgery in patients with obstructive left-colon cancer is controversial. Stent insertion is beneficial regarding perioperative morbidity, being patients with advanced age or with important comorbidity the ones who could obtain more benefit of transforming emergency surgery into elective surgery. But, on the other hand, an increase of local recurrence rate has been shown after stent placement when compared with emergency surgery, compromising oncologic outcome of these patients. Without definitive data, it seems cautious to consider emergency surgery and assume a higher initial complication rate in young patients without relevant co-morbidities avoiding the risk of local recurrence and stenting, accepting the risk of local recurrence but with a lesser perioperative complications rate, in old patients with high surgical risk.

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INTRODUCTION

Colorectal cancer is one of the most frequently diagnosed cancer in developed countries[1], with over 400000 new cases and more than 200000 cancer related deaths per year in Europe[2]. Some patients present colorectal obstruction at the time of diagnosis. Although in previous studies this situation was reported in up to 30% of patients[3], recent papers conclude that obstructive symptoms are present in 8% of cases at the time of initial diagnosis in cases of metastatic tumors[4] and also independently of the tumoral stage[5]. Emergency surgery has been classically considered the treatment of choice in these patients, although patients operated on emergency basis have poorer prognosis than those undergoing elective surgery[6]. Ascanelli *et al*[7] found a 5-year survival rate of 59% in patients electively operated in contrast with 39% in patients surgically treated on emergency basis. For some authors, this worse prognosis correlates with a lower quality surgery due to the emergency situation[8,9]. However, other studies suggest that poorer long-term prognosis in patients undergoing emergency surgery is due to a more advanced tumoral stage[10].

Some studies have been recently published supporting the possibility of performing colonic segmental resection with primary anastomosis in emergency surgery with a complication rate comparable to that of elective surgery. Zorcolo *et al*[11] analysed surgical outcomes in 323 patients and found that primary anastomosis can be performed in emergency surgery with low morbidity and mortality rates in selected patients. However, in the majority of studies, emergency colorectal surgery is burdened with higher morbidity and mortality rates than elective surgery. In a series of 989 patients, Tekkis *et al*[12] proved, after multivariate analysis, that emergency surgery is significantly associated with a higher postoperative mortality (20% *vs* 12.8%) as well as ASA classification and patient age. In another recent study comparing 171 surgically treated patients with obstructive left colon cancer by means of resection and primary anastomosis after intraoperative lavage and 1053 patients operated on elective basis, emergency surgery patients were older and with a more advanced tumoral stage. Besides, both postoperative mortality (4.1% *vs* 0.9%: $P = 0.001$) and morbidity (11.7% *vs* 7.6%: $P = 0.07$) rates were higher in obstructed patients[13].

In this clinical scenario, not all patients are candidates for surgery with primary anastomosis and so, many patients require temporal colostomy which deteriorates their quality of life and becomes permanent in 10%-40% of cases[3,14].

BENEFITS OF SELF-EXPANDABLE METAL STENTS

Self-expandable metal stents can restore large bowel transit achieving colonic decompression. Initially used in patients with non resectable malignant tumors, stents were then indicated in patients with resectable colorectal tumors and obstructive symptoms as a bridge to surgery procedure. The aim of stenting is to transform, in left colon cancer, emergency surgery into elective surgery in order to allow, with lower morbidity, mortality and stoma requirements, accurate tumoral staging and detection of synchronous lesions with CT-colonoscopy or conventional colonoscopy[15,16], stabilization of comorbidities and improvement of the nutritional status before surgery and performance of laparoscopic surgery[17]. Tejero *et al*[18] reported the outcomes of the first two patients treated with this strategy in 1994.

Although the definition of clinical success can be different in published papers, the most commonly used is to consider clinical success as the resolution of obstructive symptoms within the first 72 h after stent placement. In a systematic review including 1785 patients and 1845 stents, Watt *et al*[19] reported a clinical success rate of 92% (46%-100%). Concerning technical success, defined as the passage of the guide wire and the stent across the stricture with further appropriate stent release and expansion, the same authors reported a 96.2% success rate. A multicenter European prospective study, including 182 stented patients under the bridge to surgery indication, reported similar results for both technical (98%) and clinical success (94%) rates[20].

The advantages of stenting were confirmed in retrospective studies. Watt *et al*[19] found that the

rate of primary anastomosis performance in patients treated with elective surgery was two-fold higher than in patients operated on emergency basis. Patients electively operated presented lower stoma requirements, lower complication rate and shorter hospital stay. However, results were not so consistent in randomized control trials. Pirlet *et al*[21] randomized 60 patients with obstructive left colon cancer into two groups, emergency surgery *vs* stenting plus elective surgery. No differences were found concerning stoma performance (56% *vs* 43.3%; *P* = 0.30), mortality, morbidity or hospital stay. However, stenting technical success rate was as low as 46.7% with a perforation rate of 6.7%.

In a Dutch study, 98 patients with obstructive left colon tumors were randomized for emergency surgery or emergency stenting. No differences were found regarding 30-d mortality, overall mortality, morbidity and permanent stoma at the end of follow-up. However, patients included in the emergency surgery arm, presented a higher rate of initial stoma confection (absolute risk difference: 0.23, 95%CI: 0.04-0.40, *P* = 0.016) as well as a reduced rate of stoma related complications (between-group difference: -12.0, 95%CI: -23.7-0.2, *P* = 0.046). Stenting technical success rate was 70.2% and perforation rate 12.8%[22].

The low rates of technical success at the time of stenting in both studies and the high perforation rate of the Dutch publication are surprising, worrisome, and, to a certain extent, question the results of both studies considering that in most published papers reported technical success rates are higher than 85% and perforation rate does not exceed 5%. There is no comment in the French paper about the expertise of participant endoscopists concerning stenting, while the Dutch study mentions that colonic stenting was done by endoscopists who had placed at least 10 colonic stents. According to the recently published clinical guideline of the European Society of Gastrointestinal Endoscopy regarding stenting for obstructive colonic and extracolonic cancer, one of the recommendations is that colonic stent placement should be performed or directly supervised by an experienced operator who has performed al least 20 colonic stent placement procedures[23]. These data might have influenced the study results.

Nevertheless, perioperative results of SEMS insertion are actually better known. In a recent meta-analysis published by Huang *et al*[24] including 7 randomized control trials comparing emergency surgery and stenting plus further elective surgery (382 patients), results were more favourable in patients who were stented concerning primary anastomosis (OR = 0.28; 95%CI: 0.12-0.62; *P* = 0.002), permanent stoma (OR = 2.01; 95%CI: 1.21-3.31; *P* = 0.007), wound infection (OR = 0.31; 95%CI: 0.14-0.68; *P* = 0.004) and overall morbidity (OR = 0.30; 95%CI: 0.11-0.86; *P* = 0.03). No differences were found regarding mortality, anastomosis dehiscence and intra-abdominal infection.

Uncovered SEMS has lesser tendency to migrate than covered SEMS but showed higher tumor in growth rates. Globally, both types are equally effective and safe. Surgery might be performed 5 to 10 d after stent placement[23].

This benefit may not be the same in all groups of patients and, in old patients these benefits can be greater. Gorissen *et al*[25] demonstrated that in-hospital mortality of patients older than 75 was higher in patients undergoing emergency surgery than in those who received a stent as a bridge to surgery procedure (21% *vs* 8%; *P* = 0.228). In a study published in 2007 and based on a decision model (Markov Chain Monte Carlo), authors conclude that stenting is cheaper and more effective than emergency surgery due to a lower mortality and lower permanent stoma requirements. A low perforation rate with stenting and a high surgical risk were determinant factors to obtain these beneficial results with stenting, having the higher risk patient the greater benefit[26].

STENTING AND LONG-TERM ONCOLOGIC OUTCOMES

Although initial studies were focused on short-term results of bridge to surgery stenting, some results laid out the possible implication of stenting in long-term results of oncologic treatment. Maruthachalam *et al*[27] could demonstrate that peripheral blood levels of a tumoral marker, CK20 mRNA, increased after stent placement while did not modify after performing a diagnostic colonoscopy in patients with colorectal cancer. The consequence of this finding on tumoral behaviour is unknown. In a recent prospective multicenter study including 519 patients with stage Ⅲ colonic cancer and receiving adjuvant therapy with FOLFOX, the presence of circulating tumoral cells after surgery did not correlate with a poorer disease-free survival or overall survival[28].

Another study reported an increased perineural tumoral invasion in patients with obstructive left colon cancer and treated with a stent under the bridge to surgery indication in comparison with patients surgically treated on emergency basis. In spite of this finding, no significant differences were found regarding overall survival or disease-free survival between the two groups of patients. Even more, perineural invasion did not correlate with tumoral recurrence or 5-year survival[29]. Anyhow, the finding of an increased perineural invasion and lymph node involvement after stenting has been confirmed by other authors[30].

Kim *et al*[31] reported a shorter overall survival (38.4% *vs* 65.6%; *P* = 0.025) and 5-year disease free survival (48.3% *vs* 75.5%; *P* = 0.024) in patients with obstructive left colon cancer treated with a stent plus elective surgery than in patients with non-obstructive tumors surgically treated on elective basis. Very likely, this poor prognosis associated with stenting is not due to the stent but to the fact that stented patients presented with a large bowel obstruction.

Table 1 Data of recurrence and survival in studies comparing self-expandable metallic stents by-pass to elective surgery and emergency operation for obstructive colorectal cancer

Ref.	Perforation rate	Recurrence SEMS *vs* EO	Survival SEMS *vs* EO
Ghazal *et al*[43]	0	RR: 17.2% *vs* 13.3%; *P* = 0.228	
Saida *et al*[45]	-	RR of Dukes B: 23% *vs* 14%; *P* = 0.51)	3 yr-OS: 48% *vs* 50% 5 yr-OS: 40% *vs* 44%. Log-rank test: *P* = 0.84 DFS of Dukes B: Log-rank test: *P* = 0.71
Alcántara *et al*[46]	0	RR: 53.3% *vs* 15.3%; *P* = 0.055	DFS: 25.4 m *vs* 27 m; *P* = 0.096 OS: Log-rank test: *P* = 0.843
Tung *et al*[34]	0		5 yr-OS: 48% *vs* 27%; *P* = 0.076 5 yr-DFS: 52% *vs* 48%; *P* = 0.63
Pessione *et al*[47]	0		2 yr-OS: 66.6% *vs* 28.5%
Gianotti *et al*[40]	1.2%		HR: 0.412 *P* = 0.007 OS: Log-rank test: *P* = 0.004
van den Berg *et al*[42]	1.7%	5 yr-RR of stage Ⅰ-Ⅱ: 33% *vs* 26%; *P* = 0.81 5 yr-RR of stage Ⅲ: 35% *vs* 51%; *P* = 0.24 3 yr-RR of stage Ⅳ: 32% *vs* 58%; *P* = 0.30	5 yr-OS of stage Ⅰ-Ⅱ: Log-rank test: *P* = 0.85 5 yr-OS of stage Ⅲ: Log-rank test: *P* = 0.48 5 yr-OS of stage Ⅳ: Log-rank test: *P* = 0.08
Kim *et al*[29]	3.3%	RR: 35% *vs* 35%; *P* = 1.000 LR: 0% *vs* 1.6%	5 yr-OSR: 67.2% *vs* 61.6%; *P* = 0.386 5 yr-DFSR: 61.2% *vs* 60%; *P* = 0.932 5 yr-CRSR: 77% *vs* 65%; *P* = 0.233
Sabbagh *et al*[33]	4.2%	Patients with no perforation or metastases 34% *vs* 28 %	Patients with no perforation or metastases 5 yr-OSR: 30% *vs* 67%; *P* = 0.001 5 yr-DFSR: 27% *vs* 43%; *P* = 0.16 5 yr-CSMR: 29% *vs* 22%; *P* = 0.62
Kavanagh *et al*[44]	4.3%	RR 17.3% *vs* 23%	OS: Log-rank test: *P* = 0.13 CSM: Log-rank test: *P* = 0.21 CSMR: 13% *vs* 15.3%
Dastur *et al*[48]	5.2%		3 yr-OS: 48% *vs* 46%; *P* = 0.54
Gorissen *et al*[25]	8%	RR: 31.6 *vs* 28.2; *P* = 0.824 LRR: 23% *vs* 15%; *P* = 0.443 LRR in young patients: 32% *vs* 8%; Log-rank test: *P* = 0.038	CSMR: 24.1% *vs* 37.2%; *P* = 0.180
Sloothaak *et al*[32]	11.5%		4 yr-DFS: 30% *vs* 49%: Log-rank test: *P* = 0.149 4 yr-DSS: 66% *vs* 87%: Log-rank test: *P* = 0.061 4 yr-OS: 58% *vs* 67%: Log-rank test: *P* = 0.468 Stent-related perforation *vs* no perforation 4 yr-DFS: 0% *vs* 45%: Log-rank test: *P* = 0.007 4 yr-DSS: 60% *vs* 69%: Log-rank test: *P* = 0.099 4 yr-OS: 50% *vs* 62%: Log-rank test: *P* = 0.478
Erichsen *et al*[49]	Non-reported	5 yr-RR: 38% *vs* 29%; OR: 1.12; 95%CI: 0.99-1.28	5yOSR: 49% *vs* 40%; OR: 0.98; 95%CI 0.9-1.07
Choi *et al*[50]	Non-reported		5yOSR: 97.8% *vs* 94.3%; *P* = 0.469

RR: Recurrence rate; LRR: Local recurrence rate; OS: Overall survival; OSR: Overall survival rate; DFS: Disease-free survival; DFSR: Disease-free survival rate; CRSR: Cancer related survival rate; CSM: Cancer-specific mortality; CSMR: Cancer-specific mortality rate; DSS: Disease-specific survival; EO: Emergency operation; SEMS: Self-expandable metallic stents.

Going beyond these findings with unclear significance, more relevant data are available now.

Perforation after stenting and tumoral recurrence

Results of stent-in 2 trial showed that, although no significant statistical differences were found regarding disease free survival, cancer related survival and overall survival when comparing patients treated with a stent and further elective surgery and patients who underwent emergency surgery, tumoral recurrence was significantly higher in patients who had been stented and presented a colonic perforation than in those also stented but without any secondary complication (4 year disease free survival: 0% *vs* 45%; *P* = 0.007). However, this fact had no influence on overall survival (4 year overall survival: 50% *vs* 62%; *P* = 0.478)[32]. Gorissen *et al*[25] also reported a slightly higher recurrence rate in the group of stented patients (31.6% *vs* 28.2%; *P* = 0.824). This difference was due to an increased local recurrence in these patients (23% *vs* 15%; *P* = 0.443). Patients younger than 75 years had a significantly higher local recurrence rate (32% *vs* 8%; *P* = 0.038) and, after multivariate analysis, stenting almost reached statistical significance as a risk factor for local recurrence (OR = 12.45, 95%CI: 0.99-156.08; *P* = 0.051). However, it is paramount to remark that the perforation rate in these two studies was 11.5% and 8% respectively (Table 1).

Oncologic benefits of stenting and further elective surgery

In addition to colonic perforation, other factors can affect oncologic evolution of these patients. Quality of surgery could be better in previously stented patients. Sabbagh *et al*[33] reported a significant higher lymph node retrieval

Table 2 Data of lymph node count, administration of adjuvant chemotherapy and laparoscopic surgery in studies comparing self-expandable metallic stents by-pass to elective surgery and emergency operation for obstructive colorectal cancer

Ref.	Lymph node count SEMS *vs* EO	Adjuvant chemotherapy SEMS *vs* EO	Laparoscopic surgery SEMS *vs* EO
Ghazal *et al*[43]		80% *vs* 76.7%	
Saida *et al*[45]		66% *vs* 53%; *P* = 0.54	
Alcántara *et al*[46]	17.7 *vs* 24.2; *P* = 0.099		
Tung *et al*[34]	23 *vs* 11; *P* = 0.005	75% *vs* 54%; *P* = 0.2	
Gianotti *et al*[40]	23 *vs* 18; *P* = 0.08	46.7% *vs* 34%; *P* = 0.28	38.7% *vs* 0%; *P* = 0.000
van den Berg *et al*[42]	Lymph node harvest > 12 62.7% *vs* 60.7%; *P* = NS	39 *vs* 39; *P* = NS	
Kim *et al*[29]	28.9 *vs* 24.4; *P* = 0.25	84% *vs* 65.7%; *P* = 0.085	
Sabbagh *et al*[33]	22 *vs* 15; *P* = 0.002	56.2% *vs* 43.6%; *P* = 0.28	
Kavanagh *et al*[44]	17 *vs* 17; *P* = 0.29	36% *vs* 46%; *P* = 0.29	27% *vs* 12%; *P* = 0.1
Gorissen *et al*[25]		41.6 *vs* 25.6%; *P* = 0.13	59.6% *vs* 23%; *P* = 0.001
Sloothaak *et al*[32]	15 *vs* 13; *P* = 0.180	13 *vs* 15; *P* = 1.000	

SEMS: Self-expandable metallic stents; EO: Emergency operation.

in the surgical specimen of patients electively operated after initial bridge to surgery stenting, reaching statistical significance in some published papers. In a French study, the number of removed lymph nodes was 22 in the stenting group and 15 in the emergency surgery group (*P* = 0.002). Results were similar in an Asian publication (23 *vs* 11; *P* = 0.005)[34]. Significant differences were not reached in other reports (Table 2). In this sense, several studies have correlated the number of removed lymph nodes with survival[35,36]. Furthermore, Tung *et al*[34] reported a higher percentage of curative resection surgery in patients previously stented (91.6% *vs* 54.1%; *P* = 0.01).

Moreover, stent placement is associated with a decreased postoperative complication rate, which is relevant regarding survival[24]. In a recent analysis including 12075 patients, it has been shown that post-operative complications are associated with shorter survival (HR = 1.24; 95%CI: 1.15-1.34; *P* = 0.001). Analysing complications, infectious complications had a significant influence on long-term survival (HR = 1.31; 95%CI: 1.21-1.42; *P* = 0.001)[37].

Another potential benefit could be the percentage of patients receiving adjuvant chemotherapy. A non-statistically significant higher percentage of patients received adjuvant chemotherapy after SEMS placement in seven of ten studies (Table 2).

Finally, the number of patients who can be surgically treated with laparoscopic surgery is larger in patients operated on elective basis after bridge to surgery stenting than in the group of patients undergoing emergency surgery. Laparoscopic surgery could have a beneficial effect on long-term survival. In a randomized study published by Lacy *et al*[38] including 219 patients with colonic cancer, laparoscopic surgery was significantly related to lower recurrence rate (HR = 0.47; 95%CI: 0.23-0.94, *P* = 0.03), cancer-related mortality (HR = 0.44; 95%CI: 0.21-0.92; *P* =0.03) and overall mortality (HR = 0.59; 95%CI: 0.35-0.98; *P* = 0.04) when compared with open surgery. A similar finding has been reported from COLOR Ⅱ trial; in patients with stage-Ⅲ rectal cancer disease-free survival rate was 64.9% in the laparoscopic surgery group and 52% in the open surgery group (difference 12.9 percentage points, 95%CI: 2.2-23.6)[39]. In Gorissen *et al*[25] publication, 59.6% of stented patients and 23.2% of patients who underwent emergency surgery were operated by means of laparoscopic surgery (*P* < 0.001). Gianotti *et al*[40] also found significant differences concerning laparoscopic surgery performance when comparing stented patients and emergency surgery patients (63.3% *vs* 0%; *P* = 0.001) (Table 2).

Stenting vs emergency surgery: Which strategy is more beneficial regarding oncologic outcomes?

At present, there are no consistent studies able to demonstrate that one strategy is superior to the other in terms of oncologic benefits.

In a multicenter French study, 5-year overall survival was lower in the group of stented patients than in the emergency surgery group after excluding patients with colonic perforation or metastases at the time of hospital admission (30% *vs* 67%; *P* = 0.001)[33]. However, the type of patient (more stage Ⅳ patients in one center) and the type of treatment (stenting only in one center) was different in each participating hospital, fact which was not taken into account in multivariate analysis. Moreover, it really attracts attention that with a similar 5-year cancer related mortality (29% *vs* 22%; *P* = 0.62), overall survival differences are considered attributable to one therapeutic strategy.

In stent-in 2 trial, there was a non significant benefit in the emergency surgery group concerning 4-year disease free survival (Stenting: 30% *vs* Emergency Surgery: 49%; *P* = 0.149) and 4-year overall survival (Stenting: 58% *vs* Emergency Surgery: 67%; *P* = 0.468) in relation to colonic perforation after stenting[32] and, a higher rate of local recurrence in young patients was reported by Gorissen[25].

However, these results have not been reproduced in other studies with lower stent-related perforation rates. Kim *et al*[29] reported a similar overall recurrence rate

in both groups of patients (Stenting: 35%; Emergency Surgery: 35%; *P* = 1), with non-significant better results concerning 5-year disease free survival (66.7% *vs* 54.8%; *P* = 0.948) and 5-years overall survival (100% *vs* 77.9%; *P* = 0.103) in the stenting group. In this study no case of local recurrence was registered in the stenting group. Tung *et al*[34] also reported an almost significant benefit in the stenting group regarding 5-year overall survival (48% *vs* 27%: *P* = 0.076) and Gianotti *et al*[40] demonstrated that stenting was the only parameter related to long-term survival (HR = 0.412; 95%CI: 0.217-0.785; *P* = 0.007). Stent related perforation rate in these three studies was 3.3%, 0% and 1.2% respectively. In a recent meta-analysis including 8 clinical trials, four of them reporting long-term results, no significant differences were found regarding 1-year survival (HR = 1.07; 95%CI: 0.87-1.31; *P* = 0.51), 2-year survival (HR = 1.14; 95%CI: 0.98-1.34; *P* = 0.10) and 3-year survival (HR = 1.08; 95%CI: 0.90-1.31; *P* = 0.39) although it was always better in the stenting group[41]. Other studies which evaluate long-term results comparing stenting plus elective surgery *vs* emergency surgery do not find statistical differences in favour of any of the two strategies. Table 1 includes data regarding stent-related perforation, recurrence and survival. Oncologic evolution seems to be better in stented patients while the perforation rate is lower than 8% (Table 1).

In summary, we can't assure that stenting has a deleterious or beneficial effect on oncologic prognosis unless in those cases in which the patient presents a stent-related perforation.

Quality of life

The relevance of choosing one treatment strategy or the other concerning its influence on patient's quality of life has been seldom studied. In the Dutch study, quality of life was assessed with EORTC QLQ-C30 and QLQ-C38 questionnaires and no differences were found comparing stenting with emergency surgery, in spite of the more frequent stoma-related complications in the stenting group[22].

Other studies have described different parameters directly related with quality of life. Permanent stoma performance is significantly higher in patients undergoing emergency surgery according to Tung *et al*[34] (25% *vs* 0%; *P* = 0.03) and Gianotti (26% *vs* 6.3%: *P* = 0.01)[40] publications. In another paper it was also described that stented patients presented milder abdominal pain (4 *vs* 5; *P* = 0.02) and lower postoperative requirements of acetaminophen (8 tablets *vs* 16 tablets; *P* = 0.04) or morphine (40 mg *vs* 60 mg; *P* = 001)[17]. On the other hand, other studies did not find differences regarding permanent stoma performance[22,42].

Another interesting aspect to be assessed is the quality of bowel movements, as it is clearly related with the surgical technique. Ghazal *et al*[43] showed that patients operated on emergency basis performing a subtotal colectomy had a significantly larger number of bowel movements than patients treated with a stent and elective surgery (6 *vs* 2; *P* = 0.013). In this sense, total colectomy was less common in surgically treated patients after bridge to surgery stenting in both Kavanagh *et al*[44] (4.3% *vs* 23%; *P* = 0.027) and Saida *et al*[45] (2% *vs* 30%; *P* value is not reported) studies.

CONCLUSION

Placement of a bridge to surgery self-expandable metal stent is beneficial for the surgical treatment of patients with an obstructive colorectal cancer. This benefit is not identical for every patient, being those patients with an advanced age or with important comorbidity the ones who would obtain more benefit of transforming emergency surgery into elective surgery.

Stenting has no demonstrated influence on survival although patients who present a stent related perforation have a higher risk of tumor recurrence and shorter disease free survival. In studies with perforation rates above 8%, higher recurrences rates in young patients[25] and lower disease free survival[32] have been shown. Each medical team must be well aware of their perforation rate in order to implement improvement measures if needed.

According to the literature, in these clinical setting, we have to choose between a treatment with more perioperative complications and another therapeutic strategy which might increase the risk of tumor recurrence. It seems cautious, as it has been suggested by others[23,32], to consider emergency surgery and assume a higher initial complication rate in young patients without relevant co-morbidities avoiding the risk of local recurrence and stenting, accepting the risk of local recurrence but with a lesser perioperative complications rate, in old patients (> 70 years) with high surgical risk (ASA Ⅲ/Ⅳ).

REFERENCES

1 **Jemal A,** Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Ferlay J**, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* 2007; **18**: 581-592 [PMID: 17287242 DOI: 10.1093/annonc/mdl498]

3 **Deans GT**, Krukowski ZH, Irwin ST. Malignant obstruction of the left colon. *Br J Surg* 1994; **81**: 1270-1276 [PMID: 7953385 DOI: 10.1002/bjs.1800810905]

4 **Winner M**, Mooney SJ, Hershman DL, Feingold DL, Allendorf JD, Wright JD, Neugut AI. Incidence and predictors of bowel obstruction in elderly patients with stage IV colon cancer: a population-based cohort study. *JAMA Surg* 2013; **148**: 715-722 [PMID: 23740130 DOI: 10.1001/jamasurg.2013.1]

5 **Cheynel N**, Cortet M, Lepage C, Benoit L, Faivre J, Bouvier AM. Trends in frequency and management of obstructing colorectal cancers in a well-defined population. *Dis Colon Rectum* 2007; **50**: 1568-1575 [PMID: 17687610 DOI: 10.1007/s10350-007-9007-4]

6 **Korenaga D**, Ueo H, Mochida K, Kusumoto T, Baba H, Tamura S, Moriguchi S, Sugimachi K. Prognostic factors in Japanese patients with colorectal cancer: the significance of large bowel obstruction--univariate and multivariate analyses. *J Surg Oncol* 1991; **47**: 188-192 [PMID: 2072703 DOI: 10.1002/jso.2930470310]

7 **Ascanelli S**, Navarra G, Tonini G, Feo C, Zerbinati A, Pozza E, Carcoforo P. Early and late outcome after surgery for colorectal cancer: elective versus emergency surgery. *Tumori* 2003; **89**: 36-41 [PMID: 12729359]

8 **Serpell JW**, McDermott FT, Katrivessis H, Hughes ES. Obstructing carcinomas of the colon. *Br J Surg* 1989; **76**: 965-969 [PMID: 2804601 DOI: 10.1002/bjs.1800760932]

9 **Anderson JH**, Hole D, McArdle CS. Elective versus emergency surgery for patients with colorectal cancer. *Br J Surg* 1992; **79**: 706-709 [PMID: 1379508 DOI: 10.1002/bjs.1800790739]

10 **Wong SK**, Jalaludin BB, Morgan MJ, Berthelsen AS, Morgan A, Gatenby AH, Fulham SB. Tumor pathology and long-term survival in emergency colorectal cancer. *Dis Colon Rectum* 2008; **51**: 223-230 [PMID: 18097722 DOI: 10.1007/s10350-007-9094-2]

11 **Zorcolo L**, Covotta L, Carlomagno N, Bartolo DC. Safety of primary anastomosis in emergency colo-rectal surgery. *Colorectal Dis* 2003; **5**: 262-269 [PMID: 12780890 DOI: 10.1046/j.1463-1318.2003.00432.x]

12 **Tekkis PP**, Kinsman R, Thompson MR, Stamatakis JD. The Association of Coloproctology of Great Britain and Ireland study of large bowel obstruction caused by colorectal cancer. *Ann Surg* 2004; **240**: 76-81 [PMID: 15213621 DOI: 10.1097/01.sla.0000130723.81866.75]

13 **Jung SH**, Kim JH. Comparative study of postoperative complications in patients with and without an obstruction who had left-sided colorectal cancer and underwent a single-stage operation after mechanical bowel preparation. *Ann Coloproctol* 2014; **30**: 251-258 [PMID: 25580411 DOI: 10.3393/ac.2014.30.6.251]

14 **Leong QM**, Koh DC, Ho CK. Emergency Hartmann's procedure: morbidity, mortality and reversal rates among Asians. *Tech Coloproctol* 2008; **12**: 21-25 [PMID: 18512008 DOI: 10.1007/s10151-008-0393-y]

15 **Cha EY**, Park SH, Lee SS, Kim JC, Yu CS, Lim SB, Yoon SN, Shin YM, Kim AY, Ha HK. CT colonography after metallic stent placement for acute malignant colonic obstruction. *Radiology* 2010; **254**: 774-782 [PMID: 20177092 DOI: 10.1148/radiol.09090842]

16 **Lim SG**, Lee KJ, Suh KW, Oh SY, Kim SS, Yoo JH, Wi JO. Preoperative colonoscopy for detection of synchronous neoplasms after insertion of self-expandable metal stents in occlusive colorectal cancer: comparison of covered and uncovered stents. *Gut Liver* 2013; **7**: 311-316 [PMID: 23710312 DOI: 10.5009/gnl.2013.7.3.311]

17 **Cheung HY**, Chung CC, Tsang WW, Wong JC, Yau KK, Li MK. Endolaparoscopic approach vs conventional open surgery in the treatment of obstructing left-sided colon cancer: a randomized controlled trial. *Arch Surg* 2009; **144**: 1127-1132 [PMID: 20026830 DOI: 10.1001/archsurg.2009.216]

18 **Tejero E**, Mainar A, Fernández L, Tobío R, De Gregorio MA. New procedure for the treatment of colorectal neoplastic obstructions. *Dis Colon Rectum* 1994; **37**: 1158-1159 [PMID: 7956588 DOI: 10.1007/BF02049822]

19 **Watt AM**, Faragher IG, Griffin TT, Rieger NA, Maddern GJ. Self-expanding metallic stents for relieving malignant colorectal obstruction: a systematic review. *Ann Surg* 2007; **246**: 24-30 [PMID: 17592286 DOI: 10.1097/01.sla.0000261124.72687.72]

20 **Jiménez-Pérez J**, Casellas J, García-Cano J, Vandervoort J, García-Escribano OR, Barcenilla J, Delgado AA, Goldberg P, Gonzalez-Huix F, Vázquez-Astray E, Meisner S. Colonic stenting as a bridge to surgery in malignant large-bowel obstruction: a report from two large multinational registries. *Am J Gastroenterol* 2011; **106**: 2174-2180 [PMID: 22085816]

21 **Pirlet IA**, Slim K, Kwiatkowski F, Michot F, Millat BL. Emergency preoperative stenting versus surgery for acute left-sided malignant colonic obstruction: a multicenter randomized controlled trial. *Surg Endosc* 2011; **25**: 1814-1821 [PMID: 21170659 DOI: 10.1007/s00464-010-1471-6]

22 **van Hooft JE**, Bemelman WA, Oldenburg B, Marinelli AW, Lutke Holzik MF, Grubben MJ, Sprangers MA, Dijkgraaf MG, Fockens P. Colonic stenting versus emergency surgery for acute left-sided malignant colonic obstruction: a multicentre randomised trial. *Lancet Oncol* 2011; **12**: 344-352 [PMID: 21398178 DOI: 10.1016/S1470-2045(11)70035-3]

23 **van Hooft JE**, van Halsema EE, Vanbiervliet G, Beets-Tan RG, DeWitt JM, Donnellan F, Dumonceau JM, Glynne-Jones RG, Hassan C, Jiménez-Perez J, Meisner S, Muthusamy VR, Parker MC, Regimbeau JM, Sabbagh C, Sagar J, Tanis PJ, Vandervoort J, Webster GJ, Manes G, Barthet MA, Repici A. Self-expandable metal stents for obstructing colonic and extracolonic cancer: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2014; **46**: 990-1053 [PMID: 25325682 DOI: 10.1055/s-0034-1390700]

24 **Huang X**, Lv B, Zhang S, Meng L. Preoperative colonic stents versus emergency surgery for acute left-sided malignant colonic obstruction: a meta-analysis. *J Gastrointest Surg* 2014; **18**: 584-591 [PMID: 24170606 DOI: 10.1007/s11605-013-2344-9]

25 **Gorissen KJ**, Tuynman JB, Fryer E, Wang L, Uberoi R, Jones OM, Cunningham C, Lindsey I. Local recurrence after stenting for obstructing left-sided colonic cancer. *Br J Surg* 2013; **100**: 1805-1809 [PMID: 24227368 DOI: 10.1002/bjs.9297]

26 **Govindarajan A**, Naimark D, Coburn NG, Smith AJ, Law CH. Use of colonic stents in emergent malignant left colonic obstruction: a Markov chain Monte Carlo decision analysis. *Dis Colon Rectum* 2007; **50**: 1811-1824 [PMID: 17899279 DOI: 10.1007/s10350-007-9047-9]

27 **Maruthachalam K**, Lash GE, Shenton BK, Horgan AF. Tumour cell dissemination following endoscopic stent insertion. *Br J Surg* 2007; **94**: 1151-1154 [PMID: 17541987 DOI: 10.1002/bjs.5790]

28 **Sotelo MJ**, Sastre J, Maestro ML, Veganzones S, Viéitez JM, Alonso V, Grávalos C, Escudero P, Vera R, Aranda E, García-Alfonso P, Gallego-Plazas J, Lopez C, Pericay C, Arrivi A, Vicente P, Ballesteros P, Elez E, López-Ladrón A, Díaz-Rubio E. Role of circulating tumor cells as prognostic marker in resected stage III colorectal cancer. *Ann Oncol* 2015; **26**: 535-541 [PMID: 25515656 DOI: 10.1093/annonc/mdu568]

29 **Kim HJ**, Choi GS, Park JS, Park SY, Jun SH. Higher rate of perineural invasion in stent-laparoscopic approach in comparison to emergent open resection for obstructing left-sided colon cancer. *Int J Colorectal Dis* 2013; **28**: 407-414 [PMID: 22885839 DOI: 10.1007/s00384-012-1556-x]

30 **Sabbagh C**, Chatelain D, Trouillet N, Mauvais F, Bendjaballah S, Browet F, Regimbeau JM. Does use of a metallic colon stent as a bridge to surgery modify the pathology data in patients with colonic obstruction? A case-matched study. *Surg Endosc* 2013; **27**: 3622-3631 [PMID: 23572218 DOI: 10.1007/s00464-013-2934-3]

31 **Kim JS**, Hur H, Min BS, Sohn SK, Cho CH, Kim NK. Oncologic outcomes of self-expanding metallic stent insertion as a bridge to surgery in the management of left-sided colon cancer obstruction: comparison with nonobstructing elective surgery. *World J Surg* 2009; **33**: 1281-1286 [PMID: 19363580 DOI: 10.1007/s00268-009-0007-5]

32 **Sloothaak DA**, van den Berg MW, Dijkgraaf MG, Fockens P, Tanis PJ, van Hooft JE, Bemelman WA. Oncological outcome of malignant colonic obstruction in the Dutch Stent-In 2 trial. *Br J Surg* 2014; **101**: 1751-1757 [PMID: 25298250 DOI: 10.1002/bjs.9645]

33 **Sabbagh C**, Browet F, Diouf M, Cosse C, Brehant O, Bartoli E, Mauvais F, Chauffert B, Dupas JL, Nguyen-Khac E, Regimbeau JM. Is stenting as "a bridge to surgery" an oncologically safe strategy for the management of acute, left-sided, malignant, colonic obstruction? A comparative study with a propensity score analysis. *Ann Surg* 2013; **258**: 107-115 [PMID: 23324856 DOI: 10.1097/SLA.0b013e31827e30ce]

34 **Tung KL**, Cheung HY, Ng LW, Chung CC, Li MK. Endolaparoscopic approach versus conventional open surgery in the treatment of obstructing left-sided colon cancer: long-term follow-up of a randomized trial. *Asian J Endosc Surg* 2013; **6**: 78-81 [PMID: 23601995 DOI: 10.1111/ases.12030]

35 **Chen SL**, Bilchik AJ. More extensive nodal dissection improves survival for stages I to III of colon cancer: a population-based study. *Ann Surg* 2006; **244**: 602-610 [PMID: 16998369 DOI: 10.1097/01.

sla.0000237655.11717.50]
36 **Le Voyer TE**, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, Haller DG. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. *J Clin Oncol* 2003; **21**: 2912-2919 [PMID: 12885809 DOI: 10.1200/JCO.2003.05.062]
37 **Artinyan A**, Orcutt ST, Anaya DA, Richardson P, Chen GJ, Berger DH. Infectious postoperative complications decrease long-term survival in patients undergoing curative surgery for colorectal cancer: a study of 12,075 patients. *Ann Surg* 2015; **261**: 497-505 [PMID: 25185465 DOI: 10.1097/SLA.0000000000000854]
38 **Lacy AM**, Delgado S, Castells A, Prins HA, Arroyo V, Ibarzabal A, Pique JM. The long-term results of a randomized clinical trial of laparoscopy-assisted versus open surgery for colon cancer. *Ann Surg* 2008; **248**: 1-7 [PMID: 18580199 DOI: 10.1097/SLA.0b013e31816a9d65]
39 **Bonjer HJ**, Deijen CL, Abis GA, Cuesta MA, van der Pas MH, de Lange-de Klerk ES, Lacy AM, Bemelman WA, Andersson J, Angenete E, Rosenberg J, Fuerst A, Haglind E. A randomized trial of laparoscopic versus open surgery for rectal cancer. *N Engl J Med* 2015; **372**: 1324-1332 [PMID: 25830422 DOI: 10.1056/NEJMoa1414882]
40 **Gianotti L**, Tamini N, Nespoli L, Rota M, Bolzonaro E, Frego R, Redaelli A, Antolini L, Ardito A, Nespoli A, Dinelli M. A prospective evaluation of short-term and long-term results from colonic stenting for palliation or as a bridge to elective operation versus immediate surgery for large-bowel obstruction. *Surg Endosc* 2013; **27**: 832-842 [PMID: 23052501 DOI: 10.1007/s00464-012-2520-0]
41 **Zhang Y**, Shi J, Shi B, Song CY, Xie WF, Chen YX. Self-expanding metallic stent as a bridge to surgery versus emergency surgery for obstructive colorectal cancer: a meta-analysis. *Surg Endosc* 2012; **26**: 110-119 [PMID: 21789642 DOI: 10.1007/s00464-011-1835-6]
42 **van den Berg MW**, Sloothaak DA, Dijkgraaf MG, van der Zaag ES, Bemelman WA, Tanis PJ, Bosker RJ, Fockens P, ter Borg F, van Hooft JE. Bridge-to-surgery stent placement versus emergency surgery for acute malignant colonic obstruction. *Br J Surg* 2014; **101**: 867-873 [PMID: 24740753 DOI: 10.1002/bjs.9521]
43 **Ghazal AH**, El-Shazly WG, Bessa SS, El-Riwini MT, Hussein AM. Colonic endolumenal stenting devices and elective surgery versus emergency subtotal/total colectomy in the management of malignant obstructed left colon carcinoma. *J Gastrointest Surg* 2013; **17**: 1123-1129 [PMID: 23358847 DOI: 10.1007/s11605-013-2152-2]
44 **Kavanagh DO**, Nolan B, Judge C, Hyland JM, Mulcahy HE, O'Connell PR, Winter DC, Doherty GA. A comparative study of short- and medium-term outcomes comparing emergent surgery and stenting as a bridge to surgery in patients with acute malignant colonic obstruction. *Dis Colon Rectum* 2013; **56**: 433-440 [PMID: 23478610 DOI: 10.1097/DCR.0b013e3182760506]
45 **Saida Y**, Sumiyama Y, Nagao J, Uramatsu M. Long-term prognosis of preoperative "bridge to surgery" expandable metallic stent insertion for obstructive colorectal cancer: comparison with emergency operation. *Dis Colon Rectum* 2003; **46**: S44-S49 [PMID: 14530657]
46 **Alcántara M**, Serra-Aracil X, Falcó J, Mora L, Bombardó J, Navarro S. Prospective, controlled, randomized study of intraoperative colonic lavage versus stent placement in obstructive left-sided colonic cancer. *World J Surg* 2011; **35**: 1904-1910 [PMID: 21559998 DOI: 10.1007/s00268-011-1139y]
47 **Pessione S**, Petruzzelli L, Gentilli S, Mioli P. [Treatment of neoplastic stenosis of the left colon: presurgical expandable metal stent vs emergency surgery. Comparison of results and survival rates]. *Chir Ital* 2007; **59**: 661-669 [PMID: 18019638]
48 **Dastur JK**, Forshaw MJ, Modarai B, Solkar MM, Raymond T, Parker MC. Comparison of short-and long-term outcomes following either insertion of self-expanding metallic stents or emergency surgery in malignant large bowel obstruction. *Tech Coloproctol* 2008; **12**: 51-55 [PMID: 18512013 DOI: 10.1007/s10151-008-0399-5]
49 **Erichsen R**, Horváth-Puhó E, Jacobsen JB, Nilsson T, Baron JA, Sørensen HT. Long-term mortality and recurrence after colorectal cancer surgery with preoperative stenting: a Danish nationwide cohort study. *Endoscopy* 2015; **47**: 517-524 [PMID: 25590181 DOI: 10.1055/s-0034-1391333]
50 **Choi JM**, Lee C, Han YM, Lee M, Choi YH, Jang DK, Im JP, Kim SG, Kim JS, Jung HC. Long-term oncologic outcomes of endoscopic stenting as a bridge to surgery for malignant colonic obstruction: comparison with emergency surgery. *Surg Endosc* 2014; **28**: 2649-2655 [PMID: 24789126 DOI: 10.1007/s00464-014-3517-7]

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MINIREVIEWS

Role of self expandable stents in management of colorectal cancers

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Abstract

Acute malignant colorectal obstruction is a complication of colorectal cancer that can occur in 7%-29% of patients. Self-expanding metallic stent placement for malignant colorectal obstruction has gained popularity as a safe and effective procedure for relieving obstruction. This technique can be used in the palliation of malignant colorectal obstruction, as a bridge to elective surgery for resectable colorectal cancers, palliation of extracolonic malignant obstruction, and for nonmalignant etiologies such as anastomotic strictures, Crohn's disease, radiation therapy, and diverticular diseases. Self-expanding metallic stent has its own advantages and disadvantages over the surgery in these indications. During the insertion of the self-expanding metallic stent, and in the follow-up, short term and long term morbidities should be kept in mind. The most important complications of the stents are perforation, stent obstruction, stent migration, and bleeding. Additionally, given the high risk of perforation, if a patient is treated or being considered fortreatmen-twith antiangiogenic agents such as bevacizumab, it is not recommended to use self-expanding metallic stent as a palliative treatment for obstruction. Therefore, there is a need for careful clinical evaluation for each patient who is a candidate for this procedure. The purpose of this review was to evaluate self-expanding metallic stent in the management of the obstruction of the colon due to the colorectal and extracolonic obstruction.

Key words: Colorectal cancer; Obstruction; Metallic stents; Self-expandable; Extracolonic obstruction

Core tip: Self-expanding metallic stent placement for malignant colorectal obstruction has gained popularity as a safe and effective procedure for relieving obstruction. This technique can be used in the palliation of malignant colorectal obstruction, as a bridge to elective surgery for resectable colorectal cancers, palliation of extracolonic malignant obstruction, and for nonmalignant etiologies such as anastomotic strictures, Crohn's disease, radiation therapy, and diverticular diseases. In this review we

aimed to evaluate the placement technique, indications and complications of self-expanding metallic stent in colorectal obstructions.

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INTRODUCTION

Colorectal cancers are one of the most common cancers worldwide, and it is the second most common diagnosed cancer in women and third in men[1]. Acute malignant colorectal obstruction is a complication of colorectal cancer that can occur in 7%-29% of patients[2]. It is a life-threatening condition that needs prompt evaluation. Large bowel obstruction causes colonic dilatation, bacterial translocation, and electrolyte and fluid imbalance, and has an increased risk of colonic necrosis and perforation[3]. The main treatment of malignant colonic obstruction is resection of the tumor; however, in the past two decades, the use of self-expanding metallic stents (SEMS) has drawn interest since it was first reported in 1991 by Dohmoto[4] for palliation of malignant colonic obstruction.

The major indications of SEMS for colonic stenting are palliation of malignant obstruction and preoperative decompression[5]. Additionally, extracolonic obstructions due to other malignancies and some benign diseases have been shown to be treated by SEMS[3]. Although SEMS placement for treating malignant obstruction seems safe and effective and has some advantages over surgery, short term and long term morbidities should be kept in mind. In this review, we aimed to evaluate SEMS in the treatment of colorectal and extracolonic cancers with advantages and disadvantages of this technique over surgery.

STENT PLACEMENT TECHNIQUE

SEMS placement can be performed by using endoscopic guidance with or without the use of fluoroscopy. It can be inserted through the scope (TTS) or over the guidewire[5]. Most of the SEMS are inserted endoscopically with TTS with the use of fluoroscopy[6]. SEMS placement with endoscope has advantages over the radiologic placement when the obstruction is proximal to the rectosigmoid region or in the presence of a tortuous colon. Kim *et al*[7] evaluated the technical feasibility and clinical effectiveness of fluoroscopically guided placement of SEMS in 42 patients for acute malignant colorectal obstruction; clinical success was achieved in 98% of the patients. They stated that fluoroscopically guided placement was feasible without endoscopic assistance, even in lesions proximal to the splenic flexure and transverse colon. In a multicenter retrospective study, Geraghty *et al*[8] aimed to determine the outcomes after SEMS; TTS endoscopy technique was found to be more successful than radiological placement alone (90.3% *vs* 74.8%, $P < 0.001$) for large bowel obstruction. In another retrospective study, Kim *et al*[9] compared the SEMS placement technique in 111 patients; while the technical success rate was significantly higher in the endoscopic method than in the radiologic method (100% *vs* 92.1%, respectively, $P = 0.038$), the clinical success rate did not differ significantly between the two groups (91.8% *vs* 97.1%, respectively, $P = 0.424$). They concluded that endoscopic and radiologic placement technique have their own advantages and disadvantages, but when an obstructive lesion is located in the tortuous, curved angulation of the sigmoid or descending colons, it is more difficult to pass the stenotic lesion using the radiologic method alone.

Bowel preparation before stent placement is not necessary, and oral bowel cleansing is contraindicated in symptomatic bowel obstruction, but enema can be used for facilitating the stent placement by preparing the bowel distal to the stenosis[5].

Antibiotic prophylaxis before SEMS placement is not recommended routinely because of the low risk of fever and bacteremia after the insertion[5]. However, antibiotic prophylaxis should be considered especially in patients with complete obstruction who have dilated colon and a risk of microperforation during insertion[10].

Operator experience is an important matter in the placement of the stents. In a retrospective study, SEMS placement was performed in 334 patients, and technical and clinical success was higher for operators who had performed more than 10 procedures[8]. In another study, Small *et al*[11] reported that the complication rate was higher when stents were placed by endoscopists who were not experienced in pancreaticobiliary endoscopy.

Technical success is usually defined as stent placement appropriately across the entire length of the stenosis, and clinical success is defined as resolution of colonic obstruction within the first days after the stent placement[6]. Technical and clinical success rates vary between the studies. In a systematic review focusing on 88 studies published in 2007 by Watt *et al*[12] the median rate of technical success was 96.2%, ranging from 66.6% to 100%, and clinical success was achieved in 92% of the cases, ranging from 46% to 100%. It was stated that the etiology of the primary obstruction and indication for the stent placement appeared to have little effect on the rates of technical and clinical success. In a recent meta-analysis that included seven randomized clinical trials, pooled data showed a mean success rate of 76.9% (range: 46.7%-100%)[13]. In another meta-analysis, Cennamo *et al*[14] compared randomized trials in terms of endoscopic stenting and surgical decompression for colorectal cancer obstruction; the stents were successfully inserted in 73.5% of patients, with

clinical relief of obstruction in 72% of patients.

Covered and uncovered SEMS can be used for colonic stenting. In a meta-analysis, in which Zhang *et al*[15] compared covered and uncovered stents, uncovered stents were found to be associated with a lower late migration rate, a higher tumor ingrowth rate, and a prolonged stent patency. No significant difference was found in technical success, clinical success, tumor overgrowth, early migration, perforation and overall complications between type of stents. In another meta-analysis including a total of 1376 patients, Yang *et al*[16] compared covered and uncovered SEMS in terms of technical success, clinical success and stent patency, and no significant difference was found between the two groups. Uncovered stents were found to be more prone to tumor ingrowth, but covered stents had the higher risk of stent migration over uncovered stents. Each type of stent have their own advantages and disadvantages. The main advantage of covered stents is a reduction in the risk of tissue ingrowth, whereas they are more prone to migrate.

INDICATONS OF SEMS

Palliation of malignant obstruction

Acute malignant colorectal obstruction is a complication of colorectal cancer that can occur in 7%-29% of patients[2]. Bowel obstruction can be caused by intrinsic disease or extrinsic compression. Large bowel obstruction causes colonic dilatation, bacterial translocation, electrolyte and fluid imbalance, and has an increased risk of colonic necrosis and perforation, so this gastrointestinal emergency needs urgent evaluation. The main treatment modalities for malignant colorectal obstruction are surgical resection or diverting colostomy. Resection is a suitable procedure in patients with less advanced cancer. Permanent stoma creation is a procedure for relieving symptoms of obstruction in patients with nonresectable tumors[12]. Emergent surgery should be performed for the patients with colonic perforation and ischemia/necrosis. If there are no signs of systemic toxicity, SEMS can be performed in patients with a partial obstruction or with complete obstruction. SEMS is an alternative procedure for relieving the obstruction of the colon. In the literature, several studies have been published showing the feasibility and safety of SEMS in the management of acute malignant obstruction. In 2007, Watt *et al*[12] compared the safety and efficacy of SEMS with surgery through a systematic review. SEMS was found to be effective and safe in overcoming left-sided malignant colorectal obstructions, with high levels of technical and clinical success, shorter hospital stay, and lower rate of serious adverse events than surgery. Zhao *et al*[17] compared surgery with SEMS in the relief of obstruction in a meta-analysis, and the SEMS group showed a lower clinical success rate (99.8% *vs* 93.1%, $P = 0.0009$) but shorter length of hospital stay (18.84 d *vs* 9.55, $P < 0.00001$) and time to initiation of chemotherapy (33.36 d *vs* 15.53 d, $P < 0.00001$), and lower rate of stoma formation (54.0% *vs* 12.7%, $P < 0.00001$). Hospital mortality was significantly lower in the SEMS group, and no difference was found in overall complications between the two groups. Surgery was found to be associated with short term complications and SEMS with late term complications[17]. Liang *et al*[18] compared SEMS with surgery in the same indication mentioned above in a meta-analysis; the success rate of SEMS was found to be 93.9%, and no significant difference was found in mortality between the groups. The hospitalization time was shorter in the SEMS group ($P < 0.01$); however, long term complications were higher than surgery ($P = 0.03$). However, it was mentioned that all of the studies reported only the complications of colostomy or Hartmann's procedure, and none of them considered the complications of the stoma. They stated that morbidity and mortality would be much higher in the multi-stage surgery than with SEMS. In a recent study Young *et al*[19] compared the stent insertion and surgical decompression in patients with incurable large bowel obstruction in terms of improving quality of life. Stent related perforations or deaths were not reported. They found that surgery group had significantly reduced quality of life compared with the stent group. The patients in the stent group was found to have significantly lower permanent stoma rates, reductions in post procedure stay, earlier return of bowel function and shorter hospital stay. Thirty-day mortality for the stent group was 8% and for the surgery was 15% ($P = 0.67$). No significant difference was found in survival rates between treatment groups ($P = 0.61$)[19].

Placement of SEMS as a bridge to elective surgery

SEMS have been suggested to relieve colon obstruction and act as a "bridge to surgery" for resectable colon cancers. There are conflicting results on this subject. In the literature, systematic reviews with meta-analysis have been published in order to evaluate preoperative SEMS placement as a bridge to elective surgery with emergency resection for acute malignant left-sided colonic obstruction. In the most recent meta-analysis that included seven randomized clinical trials, Huang compared emergency surgery and SEMS group. The pooled data showed a mean success rate of colonic stent placement of 76.9% (range: 46.7%-100%). Compared with the emergency surgery group, the SEMS group achieved significantly lower rates of permanent stoma (9% *vs* 27.4%, $P < 0.01$), primary anastomosis (67.2% *vs* 55.1%, $P < 0.01$), lower overall complications (33.1% *vs* 53.9%, $P = 0.03$) and lower wound infections (6.7% *vs* 18.1%, $P < 0.01$). No significant difference was found between the two groups in anastomotic leakage, mortality, or intra-abdominal infection. In this setting, SEMS placement for relieving obstructive symptoms allows time for the optimization of the medical condition, bowel preparation, and staging the disease[13]. Although there are some advantages of SEMS placement preoperatively compared to the emergency surgery, long term oncological outcome, especially in patients with

resectable colon cancer, should be kept in mind.

There has been a major concern about the oncological outcome of the patients with resectable colon cancer who received SEMS placement as a bridge to surgery. In the literature, it was shown that placement of SEMS preoperatively in patients with resectable colon cancer impairs the oncological outcome, because of the dissemination of cancer cells during the procedure[20], and because stent placement will be complicated by perforation and associated with ulceration as well as perineural and lymph node invasion of the tissues[21]. Alcántara *et al*[22] compared short-term results and long-term outcomes of patients who underwent stent placement preoperatively with intraoperative colonic lavage with primary anastomosis. More relapses occured in the SEMS group, but this finding was not significant, and no differences were found in survival. In another study, Sloothaak *et al*[23] compared 5-year overall recurrence rates in the SEMS placement as a bridge to surgery group with the emergency surgery group; the SEMS group was found to have higher recurrence rate (42% *vs* 25%, $P = 0.027$). In a larger prospective study that evaluated the long-term oncological outcome between the same groups, in patients aged $\leqslant$ 75 years, stent as a bridge to surgery was associated with a higher local recurrence rate compared with emergency surgery (32% *vs* 8%, $P = 0.038$) without a difference in the overall survival rates[24]. These findings suggest that use of SEMS in the treatment of curable patients with left-sided malignant colonic obstruction will impair the oncologic outcome. In the recent guidelines, as stent seems to impact the oncological safety with no reduction in postoperative mortality, SEMS as a bridge to elective surgery in curable patients with left-sided malignant colonic obstruction is not recommended. However, this procedure may be a good option for selected patients with a high risk of postoperative mortality, and patients over 70 years old and/or with American Society of Anesthesiologists score $\geqslant$ Ⅲ[5].

Palliation of extracolonic malignant obstruction

Colonic obstructions also can occur due to tumor invasion, peritoneal seeding, or extraluminal compression resulting from advanced extracolonic malignancy[25]. Outcomes of SEMS placement in the treatment of extracolonic malignancies are unclear. There have been published studies that compared the clinical outcomes of SEMS between patients with colon cancer and with extracolonic malignancies. Kim *et al*[25] performed SEMS placement for colorectal cancer in 149 patients and for extracolonic malignancy in 60 patients. Advanced gastric cancer, pancreatic cancer, and ovarian cancer were the most common causes of obstruction in that study. The clinical success rates, complications, and stent patency were similar between the two groups. In another study, Kim *et al*[26] evaluated the clinical outcomes and complications of SEMS compared with emergency surgery for relieving obstruction; technical and clinical success rates were higher in the emergency surgery group. SEMS related complications occurred in 64.5% of the patients, including reobstruction (36.8%), stent migration (10.5%), perforation (13.2%), and bleeding (3.9%). In a retrospective study of palliative stent placement for extracolonic malignancies, clinical success was significantly higher in patients with colorectal cancer than in those with extracolonic malignancies (94.1% *vs* 20%, $P < 0.0001$). Two procedure related deaths occured in the extracolonic malignancy group. Colon stenting for this purpose was found to be less successful in comparison with patients with colorectal cancer[27].

SEMS for nonmalignant etiologies

Colonic stents have been used in avariety of nonmalignant conditions as colonic strictures, including anastomotic strictures, Crohn's disease, radiation therapy, and diverticular disease[3]. In a retrospective study, Keränen *et al*[28] evaluated a total of 21 patients with 23 SEMS procedures for benign colorectal obstruction; eight of the patients had an obstruction in the surgical anastomosis, two patients had anastomotic strictures due to Crohn's disease, 10 patients had the obstruction due to diverticular disease, and one patient had a stricture after radiation therapy. Technical success was achieved in all patients, and clinical success was achieved in 76% of the patients; complications occurred for 9 patients in 10 out of 23 procedures. They concluded that SEMS placement for benign colon strictures may be a good option for the patients who are not fit for surgery. Pommergaard *et al*[29] performed a retrospective study that included 45 patients with benign and malignant colonic obstruction: Technical and clinical success was 97.4% of the patients with malignant etiology, complications occurred in 21%, and mortality rate was 2.6%. For benign etiology, technical success was 85.7%, and clinical success was 71.4%, and complications occurred in 71.4% in this group with a mortality rate of 28.6%.

COMPLICATIONS OF SEMS

Stent related complications can occur in patients with malignant colon obstruction in the palliative or bridge to surgery setting. The most important complications of the stents are perforation, stent obstruction, stent migration, and bleeding. The most seen complication is the stent obstruction because of the tumor ingrowth or overgrowth[30]. The main stent related complications are discussed above.

Perforation

The incidence of colonic perforation after SEMS placement varies between the studies. Perforation is the most feared complication of SEMS. In a review that included a total of 2287 patients, Datye *et al*[31] found overall perforation rate was 4.9%. No significant difference was found in the perforation rates for palliation and bridge to surgery (4.8% *vs* 5.4%, $P = 0.66$). The mortality rate after perforation was 16.2%. Most of the perforations

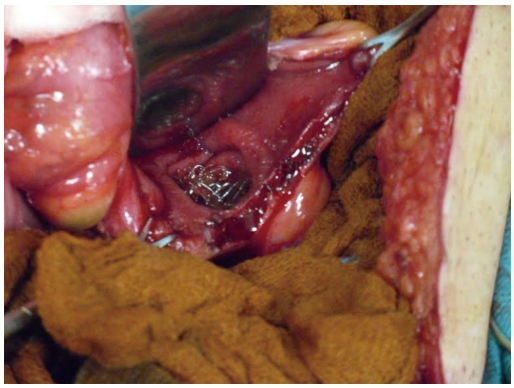

Figure 1 Perforation of the rectum due to the self-expanding metallic stent detected intraoperatively.

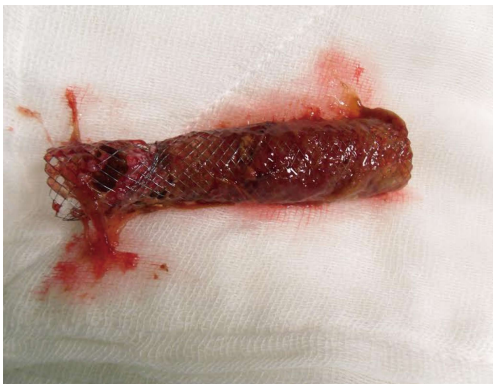

Figure 2 Occluded self-expanding metallic stent due to the tumor ingrowth.

(over 80%) occured within 30 d of stent placement, and almost half of these were noted during or within one day of the procedure; the majority of them were related to dilation, the guidewire, or the stent. In another systematic review, Watt *et al*[12] reported the median rate of perforation caused by either the guidewire or stent was 4.5% (range: 0%-83%). The perforation rate was not found to be affected by the indication for stent placement. Colon perforation can be immediate or delayed, and is more likely to occur in the distal colon where sharp angulation and redundancy make stent deployment challenging[32]. Baron *et al*[10] identified the reasons that can cause perforation after stent placement in four different types: (1) guidewire or catheter malpositioning; (2) dilation of the stricture before or after stent placement; (3) stent-induced perforation; and (4) caused by proximal colonic distention away from the site of stent placement because of inadequate colonic decompression or excessive air insufflation. Delayed perforation can be tumor related, drug related (bevacizumab), and stent related[33]. Figure 1 shows the perforation of the rectum due to the SEMS intraoperatively.

In the literature, published results have shown that patients who have undergone palliative stenting can be treated with chemotherapy without antiangiogenic agents[34]. Safety of SEMS in the colon or rectum of patients who are receiving the anti-angiogenic agent bevacizumab as a component of chemotherapy has been studied in the literature. Radiotherapy and bevacizumab may increase the risk of perforation. In a retrospective study that includes 201 patients undergoing stenting for incurable malignant obstruction, bevacizumab therapy was found to increase the risk of perforation by 19.6-fold over patients who did not receive bevacizumab[35]. In another review, Small *et al*[11] determined long-term efficacy, incidence of complications, and risk factors of SEMS placement for colonic obstruction; the incidence of perforation was higher in patients with bevacizumab treatment compared with untreated patients (15.4% *vs* 6.8%). The complication rate was found to be associated with SEMS placement in men, completely obstructed bowel, with balloon-dilated strictures, and with post-stent bevacizumab treatment. Given the high risk of perforation, if a patient is treated or considered to be treated with antiangiogenic agents like bevacizumab, it is not recommended to use SEMS as a palliative treatment for obstruction[34]. However, there is no strong evidence for the newer antiangiogenic agents like aflibercept and regorafenib, and because of the similar mechanisms, perforation risk should be kept in mind[5]. Based upon these data, it is suggested that colonic stent placement be avoided if possible in patients who are or who will be receiving bevacizumab.

Stent obstruction

The most common complication is stent obstruction because of the tumor ingrowth or overgrowth[31]. Figure 2 shows the occluded SEMS due to the tumor ingrowth. In a meta-analysis that included 13 studies, 11 of them reported stent related complications, rate of perforation was 10.1%, stent migration was 9.2%, and stent obstruction was 18.3%[17]. It is believed that covered stents provide resistance to tumor ingrowth, thus helping to reduce reconstruction events, while uncovered stents are believed to minimize stent migration[12,36,37]. For the uncovered stents, the main disadvantage is the tumor ingrowth, but the membrane of the covered stents can provide a barrier to prevent this. In a systematic review that compared covered SEMS with uncovered SEMS, uncovered SEMS showed a higher tumour ingrowth rate (RR = 5.99; 95%CI: 2.23-16.10, P = 0.0004)[15]. The factors that are associated with the stent obstruction are: Demographic factors, underlying malignancy, length of stent, site of stricture, degree of stent expansion, and chemotherapy after stent insertion[30]. However, Im *et al*[38] performed a study in order to evaluate clinical outcomes, long term complications, and patency of SEMS in patients with malignant colorectal obstruction, and stent patency was not found to be associated with demographic characteristics of patients, site of obstruction, or palliative chemotherapy. In a retrospective study, Suh *et al*[30] analyzed the predictive factors for stent occlusion, and insufficient stent expansion (< 70%) 48 h after stent insertion was significantly associated with stent occlusion during the follow-up.

If SEMS is inserted for the relief of obstruction in advanced and incurable colorectal cancer, the stent patency should be maintained until the death of the patients[30]. but if it is used in the preoperative setting, after 1-2 wk, the stent will be removed en bloc at the time of surgical resection[6]. In a systematic review, 14 studies reported duration of patency; the median of reported study mean durations was 106 d (range: 68-288 d) in the palliative stent population[12]. Suh *et al*[30] reported stent patency mean and median 184 and 141 d respectively.

There have been no accurate data in the management of occluded SEMS in malignant colorectal obstruction. In most of the patients with stent obstruction, this can be treated by stent-in-stent placement. In a retrospective case series, Yoon *et al*[39] determined the effectiveness of stent-in-stent SEMS insertion for the treatment of SEMS obstruction in cases with malignant colorectal obstruction. In this study the clinical success was reported in 75% of the cases; 9 of them had persistant symptoms, 8 of them underwent palliative surgery, but at the end of the follow-up, 16 of 36 patients (44.4%) remained free of obstruction symptoms until death. The success rate was found to be slightly lower than that of primary SEMS placement[40]. In another study, Yoon *et al*[39] compared the clinical outcomes of the patients who underwent second intervention because of the obstruction of the first successful SEMS placement for colorectal obstruction with second SEMS insertion or palliative surgery. No significant difference was found in the median overall survival (8.2 mo *vs* 15.5 mo) and progression-free survival (4.0 mo *vs* 2.7 mo) between the stent and surgery groups. However, the median lumen patency in the stent group was 3.4 mo and 7.9 mo in the surgery (P = 0.003). Male gender and having an obstruction in the right colon were identified as prognostic factors of lumen patency in second SEMS; additional chemotherapy after a second intervention was found to be a prognostic factor with a longer lumen patency in the palliative surgery group.

Stent migration

In a systematic review including 54 studies, Watt *et al*[12] evaluated stent for all indications reported that median rate of migration was 11%, ranging from 0% to 50%. Stent migration can occur at any time following the insertion, but is usually detected within one week of insertion. Migrations tend to occur with stents which are too narrow in diameter and/or too short in relation to the stricture they are placed in[41]. In another systematic review, Khot *et al*[42] reported stent migration in 54 (10%) of 551 technically successful cases; 26% of the stent migration occured within 3 d, and the remaining occured after 3 d. Factors that were associated with the migration were laser pretreatment, chemotherapy, and benign tumor. Covered and small diameter (< 24 mm) SEMS were also found to be associated with stent migration[15,35,36]. Chemotherapy with the mechanism of tumor shrinkage increases the stent migration[43-45].

Others

After the SEMS placement, abdominal pain and bleeding can occur in the follow-up. Bleeding is usually minor after the procedure, and generally no intervention will be required. Abdominal or rectal pain is common and varies between 7.4% and up to 62.5% in patients with SEMS placement within 5 cm of the anal verge[46,47]. Mild abdominal pain generally requires no specific treatment; if needed, use of analgesics will be enough for relieving the pain.

CONCLUSION

SEMS placement can be an alternative method in the treatment of patients with colorectal cancer who have acute malignant colorectal obstruction. SEMS offers favourable results compared to surgery in the setting of colorectal obstruction in advanced disease. Use of SEMS in the treatment of curable patients with left-sided malignant colonic obstruction will impair the oncologic outcome; therefore, SEMS as a bridge to elective surgery in curable patients with left-sided malignant colonic obstruction is not recommended. Although SEMS placement seems to be safe and effective and has some advantages over surgery, short term and long term morbidities should be kept in mind, and it will be preferred for patients who are at increased risk for complications of emergency surgery.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Frago R**, Ramirez E, Millan M, Kreisler E, del Valle E, Biondo S. Current management of acute malignant large bowel obstruction: a systematic review. *Am J Surg* 2014; **207**: 127-138 [PMID: 24124659 DOI: 10.1016/j.amjsurg.2013.07.027]

3 **Bonin EA**, Baron TH. Update on the indications and use of colonic stents. *Curr Gastroenterol Rep* 2010; **12**: 374-382 [PMID: 20703837 DOI: 10.1007/s11894-010-0136-x]

4 **Dohmoto M.** New method: endoscopic implantation of rectal stent in palliative treatment of malignant stenosis. *Endosc Dig* 1991; **3:** 1507-1512

5 **van Hooft JE**, van Halsema EE, Vanbiervliet G, Beets-Tan RG, DeWitt JM, Donnellan F, Dumonceau JM, Glynne-Jones RG, Hassan C, Jiménez-Perez J, Meisner S, Muthusamy VR, Parker MC, Regimbeau JM, Sabbagh C, Sagar J, Tanis PJ, Vandervoort J, Webster GJ, Manes G, Barthet MA, Repici A. Self-expandable metal stents for obstructing colonic and extracolonic cancer: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2014; **46**: 990-1053 [PMID: 25325682 DOI: 10.1055/s-0034-1390700]

6 **Van Halsema EE,** van Hooft JE. Outcome and complications of stenting for malignant obstruction. *Tech Gastrointest Endosc* 2014; **16**: 129-134 [DOI: 10.1016/j.tgie.2014.08.002]

7 **Kim H**, Kim SH, Choi SY, Lee KH, Won JY, Lee do Y, Lee JT. Fluoroscopically guided placement of self-expandable metallic stents and stent-grafts in the treatment of acute malignant colorectal obstruction. *J Vasc Interv Radiol* 2008; **19**: 1709-1716 [PMID: 18845454 DOI: 10.1016/j.jvir.2008.08.020]

8 **Geraghty J**, Sarkar S, Cox T, Lal S, Willert R, Ramesh J, Bodger K, Carlson GL. Management of large bowel obstruction with self-expanding metal stents. A multicentre retrospective study of factors determining outcome. *Colorectal Dis* 2014; **16**: 476-483 [PMID:

24506142 DOI: 10.1111/codi.12582]
9 **Kim JW**, Jeong JB, Lee KL, Kim BG, Jung YJ, Kim W, Kim HY, Ahn DW, Koh SJ, Lee JK.Comparison of clinical outcomes between endoscopic and radiologic placement of self-expandable metal stentin patients with malignant colorectal obstruction. *Korean J Gastroenterol* 2013; **61**: 22-29
10 **Baron TH**, Dean PA, Yates MR, Canon C, Koehler RE. Expandable metal stents for the treatment of colonic obstruction: techniques and outcomes. *Gastrointest Endosc* 1998; **47**: 277-286 [PMID: 9540883 DOI: 10.1016/S0016-5107(98)70327-X]
11 **Small AJ**, Coelho-Prabhu N, Baron TH. Endoscopic placement of self-expandable metal stents for malignant colonic obstruction: long-term outcomes and complication factors. *Gastrointest Endosc* 2010; **71**: 560-572 [PMID: 20189515 DOI: 10.1016/j.gie.2009.10.012]
12 **Watt AM**, Faragher IG, Griffin TT, Rieger NA, Maddern GJ. Self-expanding metallic stents for relieving malignant colorectal obstruction: a systematic review. *Ann Surg* 2007; **246**: 24-30 [PMID: 17592286]
13 **Huang X**, Lv B, Zhang S, Meng L. Preoperative colonic stents versus emergency surgery for acute left-sided malignant colonic obstruction: a meta-analysis. *J Gastrointest Surg* 2014; **18**: 584-591 [PMID: 24170606 DOI: 10.1007/s11605-013-2344-9]
14 **Cennamo V**, Luigiano C, Coccolini F, Fabbri C, Bassi M, De Caro G, Ceroni L, Maimone A, Ravelli P, Ansaloni L. Meta-analysis of randomized trials comparing endoscopic stenting and surgical decompression for colorectal cancer obstruction. *Int J Colorectal Dis* 2013; **28**: 855-863 [PMID: 23151813 DOI: 10.1007/s00384-012-1599-z]
15 **Zhang Y**, Shi J, Shi B, Song CY, Xie WF, Chen YX. Comparison of efficacy between uncovered and covered self-expanding metallic stents in malignant large bowel obstruction: a systematic review and meta-analysis. *Colorectal Dis* 2012; **14**: e367-e374 [PMID: 22540666]
16 **Yang Z**, Wu Q, Wang F, Ye X, Qi X, Fan D. A systematic review and meta-analysis of randomized trials and prospective studies comparing covered and bare self-expandable metal stents for the treatment of malignant obstruction in the digestive tract. *Int J Med Sci* 2013; **10**: 825-835 [PMID: 23794946 DOI: 10.7150/ijms.5969]
17 **Zhao XD**, Cai BB, Cao RS, Shi RH. Palliative treatment for incurable malignant colorectal obstructions: a meta-analysis. *World J Gastroenterol* 2013; **19**: 5565-5574 [PMID: 24023502 DOI: 10.3748/wjg.v19.i33.5565]
18 **Liang TW**, Sun Y, Wei YC, Yang DX. Palliative treatment of malignant colorectal obstruction caused by advanced malignancy: a self-expanding metallic stent or surgery? A system review and meta-analysis. *Surg Today* 2014; **44**: 22-33 [PMID: 23893158 DOI: 10.1007/s00595-013-0665-7]
19 **Young CJ**, De-Loyde KJ, Young JM, Solomon MJ, Chew EH, Byrne CM, Salkeld G, Faragher IG. Improving Quality of Life for People with Incurable Large-Bowel Obstruction: Randomized Control Trial of Colonic Stent Insertion. *Dis Colon Rectum* 2015; **58**: 838-849 [PMID: 26252845 DOI: 10.1097/DCR.0000000000000431]
20 **Maruthachalam K**, Lash GE, Shenton BK, Horgan AF. Tumour cell dissemination following endoscopic stent insertion. *Br J Surg* 2007; **94**: 1151-1154 [PMID: 17541987 DOI: 10.1002/bjs.5790]
21 **Sabbagh C**, Chatelain D, Trouillet N, Mauvais F, Bendjaballah S, Browet F, Regimbeau JM. Does use of a metallic colon stent as a bridge to surgery modify the pathology data in patients with colonic obstruction? A case-matched study. *Surg Endosc* 2013; **27**: 3622-3631 [PMID: 23572218 DOI: 10.1007/s00464-013-2934-3]
22 **Alcántara M**, Serra-Aracil X, Falcó J, Mora L, Bombardó J, Navarro S. Prospective, controlled, randomized study of intraoperative colonic lavage versus stent placement in obstructive left-sided colonic cancer. *World J Surg* 2011; **35**: 1904-1910 [PMID: 21559998 DOI: 10.1007/s00268-011-1139-y]
23 **Sloothaak DA**, van den Berg MW, Dijkgraaf MG, Fockens P, Tanis PJ, van Hooft JE, Bemelman WA. Oncological outcome of malignant colonic obstruction in the Dutch Stent-In 2 trial. *Br J Surg* 2014; **101**: 1751-1757 [PMID: 25298250]
24 **Gorissen KJ**, Tuynman JB, Fryer E, Wang L, Uberoi R, Jones OM, Cunningham C, Lindsey I. Local recurrence after stenting for obstructing left-sided colonic cancer. *Br J Surg* 2013; **100**: 1805-1809 [PMID: 24227368 DOI: 10.1002/bjs.9297]
25 **Kim JY**, Kim SG, Im JP, Kim JS, Jung HC. Comparison of treatment outcomes of endoscopic stenting for colonic and extracolonic malignant obstruction. *Surg Endosc* 2013; **27**: 272-277 [PMID: 22773238 DOI: 10.1007/s00464-012-2439-5]
26 **Kim BK**, Hong SP, Heo HM, Kim JY, Hur H, Lee KY, Cheon JH, Kim TI, Kim WH. Endoscopic stenting is not as effective for palliation of colorectal obstruction in patients with advanced gastric cancer as emergency surgery. *Gastrointest Endosc* 2012; **75**: 294-301 [PMID: 22154416 DOI: 10.1016/j.gie.2011.09.026]
27 **Keswani RN**, Azar RR, Edmundowicz SA, Zhang Q, Ammar T, Banerjee B, Early DS, Jonnalagadda SS. Stenting for malignant colonic obstruction: a comparison of efficacy and complications in colonic versus extracolonic malignancy. *Gastrointest Endosc* 2009; **69**: 675-680 [PMID: 19251009 DOI: 10.1016/j.gie.2008.09.009]
28 **Keränen I**, Lepistö A, Udd M, Halttunen J, Kylänpää L. Outcome of patients after endoluminal stent placement for benign colorectal obstruction. *Scand J Gastroenterol* 2010; **45**: 725-731 [PMID: 20205505 DOI: 10.3109/00365521003663696]
29 **Pommergaard HC**, Vilmann P, Jakobsen HL, Achiam MP. A clinical evaluation of endoscopically placed self-expanding metallic stents in patients with acute large bowel obstruction. *Scand J Surg* 2009; **98**: 143-147 [PMID: 19919918]
30 **Suh JP**, Kim SW, Cho YK, Park JM, Lee IS, Choi MG, Chung IS, Kim HJ, Kang WK, Oh ST. Effectiveness of stent placement for palliative treatment in malignant colorectal obstruction and predictive factors for stent occlusion. *Surg Endosc* 2010; **24**: 400-406 [PMID: 19551432 DOI: 10.1007/s00464-009-0589-x]
31 **Datye A**, Hersh J. Colonic perforation after stent placement for malignant colorectal obstruction--causes and contributing factors. *Minim Invasive Ther Allied Technol* 2011; **20**: 133-140 [PMID: 20929424 DOI: 10.3109/13645706.2010.518787]
32 **Lo SK**. Metallic stenting for colorectal obstruction. *Gastrointest Endosc Clin N Am* 1999; **9**: 459-477 [PMID: 10388861]
33 **Baron TH**, Wong Kee Song LM, Repici A. Role of self-expandable stents for patients with colon cancer (with videos). *Gastrointest Endosc* 2012; **75**: 653-662 [PMID: 22341111 DOI: 10.1016/j.gie.2011.12.020]
34 **Meisner S.** Stent for palliation of advanced colorectal cancer. *Tech Gastrointest Endosc* 2014; **16**: 125-128 [DOI: 10.1016/j.tgie.2014.09.002]
35 **Manes G**, de Bellis M, Fuccio L, Repici A, Masci E, Ardizzone S, Mangiavillano B, Carlino A, Rossi GB, Occhipinti P, Cennamo V. Endoscopic palliation in patients with incurable malignant colorectal obstruction by means of self-expanding metal stent: analysis of results and predictors of outcomes in a large multicenter series. *Arch Surg* 2011; **146**: 1157-1162 [PMID: 22006874 DOI: 10.1001/archsurg.2011.233]
36 **Kim BC**, Han KS, Hong CW, Sohn DK, Park JW, Park SC, Kim SY, Baek JY, Choi HS, Chang HJ, Kim DY, Oh JH. Clinical outcomes of palliative self-expanding metallic stents in patients with malignant colorectal obstruction. *J Dig Dis* 2012; **13**: 258-266 [PMID: 22500788 DOI: 10.1111/j.1751-2980.2012.00564.x]
37 **Park S**, Cheon JH, Park JJ, Moon CM, Hong SP, Lee SK, Kim TI, Kim WH. Comparison of efficacies between stents for malignant colorectal obstruction: a randomized, prospective study. *Gastrointest Endosc* 2010; **72**: 304-310 [PMID: 20561619 DOI: 10.1016/j.gie.2010.02.046]
38 **Im JP**, Kim SG, Kang HW, Kim JS, Jung HC, Song IS. Clinical outcomes and patency of self-expanding metal stents in patients with malignant colorectal obstruction: a prospective single center study. *Int J Colorectal Dis* 2008; **23**: 789-794 [PMID: 18443807 DOI: 10.1007/s00384-008-0477-1]
39 **Yoon JY**, Jung YS, Hong SP, Kim TI, Kim WH, Cheon JH. Outcomes of secondary stent-in-stent self-expandable metal stent insertion for malignant colorectal obstruction. *Gastrointest Endosc*

2011; **74**: 625-633 [PMID: 21762906]
40 **Yoon JY**, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH. Outcomes of secondary self-expandable metal stents versus surgery after delayed initial palliative stent failure in malignant colorectal obstruction. *Digestion* 2013; **88**: 46-55 [PMID: 23880524 DOI: 10.1159/000351208]
41 **Mauro MA**, Koehler RE, Baron TH. Advances in gastrointestinal intervention: the treatment of gastroduodenal and colorectal obstructions with metallic stents. *Radiology* 2000; **215**: 659-669 [PMID: 10831681 DOI: 10.1148/radiology.215.3.r00jn30659]
42 **Khot UP**, Lang AW, Murali K, Parker MC. Systematic review of the efficacy and safety of colorectal stents. *Br J Surg* 2002; **89**: 1096-1102 [PMID: 12190673 DOI: 10.1046/j.1365-2168.2002.02148.x]
43 **Kim JH**, Song HY, Li YD, Shin JH, Park JH, Yu CS, Kim JC. Dual-design expandable colorectal stent for malignant colorectal obstruction: comparison of flared ends and bent ends. *AJR Am J Roentgenol* 2009; **193**: 248-254 [PMID: 19542421 DOI: 10.2214/AJR.08.2003]
44 **Canena JM**, Liberato M, Marques I, Rodrigues CI, Lagos AC, Patrocínio SD, Tomé MJ, Cpires EN, Romão C, Coutinho AP, Veiga PM, Neves BC, Deus JR, Além HD, Gonçalves JA. Sustained relief of obstructive symptoms for the remaining life of patients following placement of an expandable metal stent for malignant colorectal obstruction. *Rev Esp Enferm Dig* 2012; **104**: 418-425 [PMID: 23039802 DOI: 10.4321/S1130-01082012000800005]
45 **Fernández-Esparrach G**, Bordas JM, Giráldez MD, Ginès A, Pellisé M, Sendino O, Martínez-Pallí G, Castells A, Llach J. Severe complications limit long-term clinical success of self-expanding metal stents in patients with obstructive colorectal cancer. *Am J Gastroenterol* 2010; **105**: 1087-1093 [PMID: 19935785 DOI: 10.1038/ajg.2009.660]
46 **Gianotti L**, Tamini N, Nespoli L, Rota M, Bolzonaro E, Frego R, Redaelli A, Antolini L, Ardito A, Nespoli A, Dinelli M. A prospective evaluation of short-term and long-term results from colonic stenting for palliation or as a bridge to elective operation versus immediate surgery for large-bowel obstruction. *Surg Endosc* 2013; **27**: 832-842 [PMID: 23052501 DOI: 10.1007/s00464-012-2520-0]
47 **Song HY**, Kim JH, Kim KR, Shin JH, Kim HC, Yu CS, Kim JC. Malignant rectal obstruction within 5 cm of the anal verge: is there a role for expandable metallic stent placement? *Gastrointest Endosc* 2008; **68**: 713-720 [PMID: 18561924 DOI: 10.1016/j.gie.2007.12.051]

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MINIREVIEWS

Role of microRNA-7 in digestive system malignancy

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Abstract

There are several malignancies of the digestive system (including gastric, pancreatic and colorectal cancers, and hepatocellular carcinoma), which are the most common types of cancer and a major cause of death worldwide. MicroRNA (miR)-7 is abundant in the pancreas, playing an important role in pancreatic development and endocrine function. Expression of miR-7 is downregulated in digestive system malignancies compared with normal tissue. Although there are contrasting results for miR-7 expression, almost all research reveals that miR-7 is a tumor suppressor, by targeting various genes in specific pathways. Moreover, miR-7 can target different genes simultaneously in different malignancies of the digestive system. By acting on many cytokines, miR-7 is also involved in many gastrointestinal inflammatory diseases as a significant carcinogenic factor. Consequently, miR-7 might be a biomarker or therapeutic target gene in digestive system malignancies.

Key words: MicroRNA-7; Digestive system malignancy; Tumor biomarker; Target gene; Inflammation

Core tip: MicroRNA (miR)-7 targets different genes in various complicated pathways and plays diagnostic, prognostic, anti-metastatic, and therapeutic roles in digestive system malignancies. MiR-7 might be a biomarker or therapeutic target gene in digestive system malignancies, even in the precancerous lesions (inflammatory disease).

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INTRODUCTION

MicroRNAs are small noncoding RNAs consisting of 18-25 nucleotides that post-transcriptionally regulate expression of target genes, and are involved in cell proliferation, epithelial-mesenchymal transition (EMT), apoptosis, migration, invasion and metastasis[1-3]. miRNAs have emerged as potential critical regulators of carcinogenesis and tumor progression[4,5].

The digestive system is composed of many ducts and glands, and because of its complicated physiology and anatomy, numerous diseases may occur, especially malignancies including the third, fourth and eighth most common cancers worldwide: Colorectal cancer (CRC), gastric cancer (GC) and esophageal cancer, respectively[6,7], as wells as the leading cause of cancer-related death: Pancreatic cancer (PC)[8]. According to the 2014 cancer statistics, the combined cancer mortality rates have been continuously declining for the past two decades. However, the incidence of some digestive system malignancies, including cancers of the esophagus, liver, anus and pancreas, is increasing. Moreover, with rising death rates for cancers of the liver, anus and pancreas, and other non-digestive cancers, cancer is still the second leading cause of death following heart disease[8]. Therefore, it is necessary for us to explore novel molecular mechanisms, and screen for the most effective therapeutic methods to avoid the majority of patients succumbing to these digestive malignancies.

MicroRNA (miR)-7 is an evolutionarily conserved miRNA that is involved in the development of the eye and pancreas in Drosophila. Li *et al*[9] reported that miR-7 is repressed by the transcription factor Yan, which is degraded while mediating epidermal growth factor receptor (EGFR) signaling. Also, miR-7 is expressed abundantly in human pancreas and endocrine cells and has a specific role in endocrine cell differentiation and function[10]. It has been demonstrated that miR-7 is a tumor suppressor in breast, lung and ovarian cancers, and glioblastoma, mainly focusing on its relationship with EGFR[11-15]. Accumulating evidence shows that miR-7 can simultaneously target a variety of mRNAs involved in diverse signaling pathways in different tumors. However, no specific review has described the role of miR-7 in digestive tract malignancies. In this review, we focus on current research on miR-7 in order to elucidate its role in digestive system malignancies or their precancerous lesions, with reference to its expression, signaling pathways, and role as a circulatory biomarker.

EXPRESSION OF MIR-7

By comparing the differential expression of miRNAs in pancreatic islets (endocrine) and acinar (exocrine) tissue in rats, using microarray and quantitative polymerase chain reaction (qPCR), Bravo-Egana *et al*[16] revealed that miR-7 was ranked highest among the 17 miRNAs preferentially expressed in islets, suggesting that it acts as an endocrine miRNA. Another two studies reported that miR-7 was expressed at a high level during human pancreatic islet development[10,17]. For malignancy, it has been demonstrated that miR-7 is downregulated in cancer tissue of digestive malignancies such as GC[18-20], CRC[21,22] and hepatocellular carcinoma (HCC)[23] by comparison with normal tissues, suggesting that it acts as a suppressor. A similar conclusion was drawn in a study of hydroxycamptothecin-resistant GC cells[24]. In some inflammatory diseases, such as gastritis and Crohn's disease[25], the level of miR-7 is also lower than that in normal tissue, which suggests that it is an inflammation-related miRNA participating in the process of digestive cancer.

In contrast, using the same method, Suto *et al*[26] discovered that miR-7 level was higher in CRC tissue than in adjacent normal tissue, induced by EGFR mutations. However, it was found that the aforementioned results would be opposite when the EGFR protein expression was positive in CRC. Finally, they concluded that low miR-7 expression resulted in poorer prognosis than high expression. Ahmed *et al*[27] identified the expression of miR-7 in stool samples from 40 cases of colon cancer (TNM stages 1-4), and found that miR-7 was one of the 12 increased miRNAs, which they then recognized as a diagnostic gene. In HCC, Fang *et al*[28] speculated that owing to inactivation of the transcriptional regulators and/or failure to promote miR-7 expression, there is no alteration of its expression between tumor and adjacent normal tissues. However, miR-7 and miR-21 are overexpressed in esophageal squamous cell carcinoma (ESCC) and related to its differentiation[29].

From Table 1, we can speculate the reasons for the divergent views about the expression of miR-7 under different conditions, including[30]: (1) heterogeneity of different malignancies/diseases; (2) different study sample sizes; and (3) the standards were not the same (*e.g.,* whether or not to include patients with prior cytotoxic therapy). Based on the published studies, we conclude that miR-7 could be an oncogene or tumor suppressor in the digestive system depending on the specific gene targeted (Table 2).

GC

The pathogenesis of GC has been extensively studied, and there is a consensus that intestinal gastric carcinogenesis is a multistep process starting with chronic gastritis triggered by *Helicobacter pylori*, progressing through atrophy, intestinal metaplasia and dysplasia to carcinoma (Correa model)[31]. Thus, inflammation is a significant event in gastric carcinogenesis, whereas miR-7 is an inflammation-mediated miRNA inversely

Table 1 Expression of microRNA-7 in the digestive system

Cancer	Sample	Sap No.	Method	Exp	Role	Ref.
Colon	Stool	60	qPCR	↑	Diagnostic	[27]
CRC	Tissue	80	qPCR	↓	Diagnostic/therapeutic	[21]
CRC	Tissue	8	RT-PCR	↓	Therapeutic	[22]
CRC	Tissue	105	qRT-PCR	↑	Prognostic	[26]
ESCC	Tissue	34	Microarray/qRT-PCR	↑	Differentiation	[29]
GC	Tissue	40	ISH/IHC	↓	Inhibits metastasis/EMT	[18]
GC	Tissue	23	Microarray qRT-PCR	↓	Inhibits invasion/metastasis	[19]
GC	Tissue	28	RT-PCR	↓	Represses inflammation	[20]
HCC	Tissue	10	Microarray qRT-PCR	-	Therapeutic/diagnostic/prognostic	[28]
HCC	Tissue	12	qRT-PCR	↓	Tumor suppressor	[23]
HCC	Tissue	429	Chip assay	↓	Prognostic	[43]
CD	Tissue	-	RT-PCR	↓	Therapeutic	[25]

↓: MiR-7 is downregulated; ↑: MiR-7 is upregulated; −: There is no alteration for expression of miR-7, or no mention. Sap No.: Sample number; Exp: Expression; ISH/IHC: *In situ* hybridization/immunohistochemistry; CRC: Colorectal cancer; GC: Gastric cancer; HCC: Hepatocellular carcinoma; ESCC: Esophageal squamous cell carcinoma; EMT: Epithelial–mesenchymal transition; qPCR: Quantitative polymerase chain reaction.

Table 2 Function of microRNA-7 by targeting diverse genes

Cells	Function of miR-7	Target	Ref.
CCA	Reduces migration, invasion and metastasis	LAT1	[41]
CRC	Inhibits proliferation, invasion and metastasis, and induces G1 arrest	PAX6	[21]
CRC	Inhibits proliferation and induces apoptosis	XRCC2	[22]
CRC	Suppresses proliferation, induces G1 arrest, and induces apoptosis	YY1	[37]
GC	Suppresses invasion and metastasis	IGF1R	[18]
GC	Inhibits proliferation, invasion and metastasis	EGFR	[19]
HCC	Decreases invasion and migration	PIK3CD/mTOR/p70S6K	[28]
HCC	Suppresses colony formation and induces cell cycle arrest	CUL5	[23]

CCA: Cholangiocarcinoma; CRC: Colorectal cancer; GC: Gastric cancer; HCC: Hepatocellular carcinoma; miR-7: MicroRNA; XRCC2: X-ray repair complementing defective repair in Chinese hamster cells 2; IGF1R: Insulin-like growth factor 1 receptor; EGFR: Epidermal growth factor receptor; mTOR: Mammalian target of rapamycin; CUL5: Cullin 5.

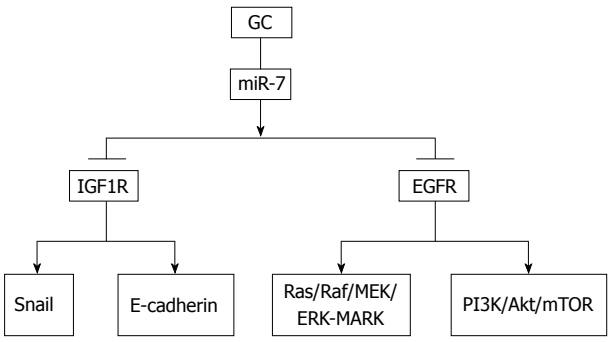

Figure 1 Pathway of microRNA-7 in gastric cancer. It has been revealed that miR-7 targets mainly IGF1R and EGFR. IGF1R: Insulin-like growth factor 1 receptor; EGFR: Epidermal growth factor receptor; GC: Gastric cancer; miR-7: MicroRNA-7; PI3K: Phosphoinositide 3-kinase; mTOR: Mammalian target of rapamycin.

correlated with many proinflammatory cytokines and inflammatory factors such as interleukin-1β and tumor necrosis factor-α. Three genes, *LPHN2*, *BASP1* and *MAFG*, targeted by miR-7 are induced in the cyclo-oxygenase (COX)-2/prostaglandin (PG) E2 pathways, which shows that miR-7 plays a significant part in gastric tumorigenesis with an inflammatory response[20].

MiR-7 suppresses GC cell invasion and metastasis both *in vitro* and *in vivo* by targeting the miR-7/insulin-like growth factor 1 receptor/Snail axis, which shows its EMT function and suggests that it can act as a therapeutic biomarker to prevent GC metastasis[18]. Xie *et al*[19] demonstrated that restoration of miR-7 significantly inhibited tumor cell viability, invasion and migration by suppressing EGFR expression. These results suggest that targeting miR-7 is a potential therapeutic option for GC (Figure 1).

PC

PC is one of the major leading causes of cancer mortality; the 5-year survival rate for pancreatic adenocarcinoma is < 5%, and most patients die within the first 2 years[8]. Therefore, there is an urgent need to explore novel therapeutic methods. In accordance with the expression of miR-7 in the pancreas, miR-7-3, which is one of the three endogenous genes potentially transcribed in the human genome, is upregulated by targeting mitogen-activated protein kinase (MAPK), suggesting that miR-7 is negatively modulated by an EGFR-MAPK feedback loop[32]. In an *in vitro* study, in

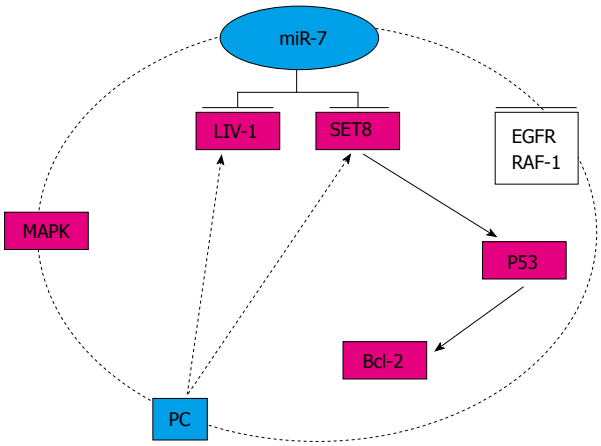

Figure 2 Pathway of microRNA-7 in pancreatic cancer. In PC, there is an EGFR-MAPK-miR-7 negative feedback loop, and LIV-1 and SET8 are two other targets. EGFR: Epidermal growth factor receptor; MAPK: Mitogen-activated protein kinase; PC: Pancreatic cancer; miR-7: MicroRNA-7; SET8: SET domain containing 8.

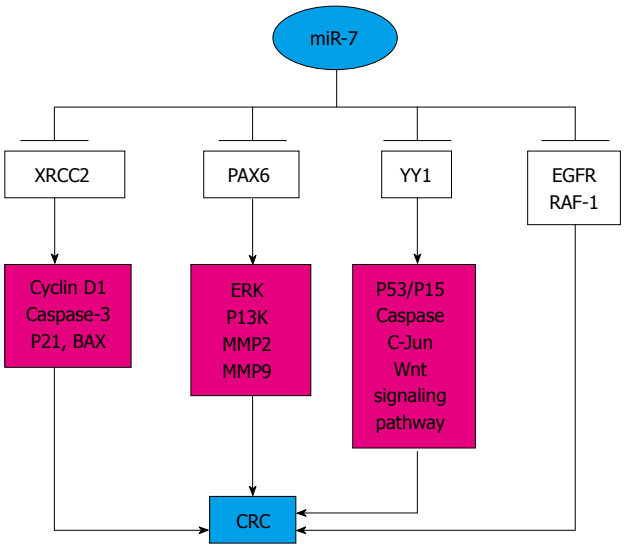

Figure 3 Pathway of microRNA-7 in colorectal cancer. MiR-7 can target various genes involving different pathways and act as a suppressor. CRC: Colorectal cancer; EGFR: Epidermal growth factor receptor; miR-7: MicroRNA-7. XRCC2: X-ray repair complementing defective repair in Chinese hamster cells 2; ERK: Extracellular signal-regulated kinase.

which miR-7 targeted SET domain containing 8 leading to increased p53 expression and decreased Bcl-2 level, curcumin suppressed cell growth, migration and invasion, and induced apoptosis in PC cells, indicating that targeting miR-7 is a useful therapeutic option for PC[33]. Although knockdown of LIV-1 (a zinc transporter) can upregulate expression of miR-7 in PC cells, the exact role of miR-7 in the maintenance of cancer-stem-cell-related phenotypes in PC remains unclear[34]. Future research will focus on identifying the exact pathway of miR-7 in PC, and only in this way, can research proceed from bench to bedside (Figure 2).

CRC

CRC is related to the mutation of genes such as P53, APC, SMAD4, PIK3CA, KRAS, ARID1A, SOX9 and FAM123B. Some minimal tailoring of therapy (selecting a chemotherapeutic agent based on toxicity, or not using anti-EGFR in those with KRAS-mutated tumors) can be offered to patients, however, the dream of truly individualized therapy remains elusive[35,36]. Based on the present studies, miR-7 can target specific genes to modulate the correlated pathways, and its decreased expression continuously participates in the process of CRC. MiR-7 is a tumor suppressor, which is mediated through the YY1-P53-Wnt signaling pathway, and plays pivotal roles in many cellular processes, such as development, differentiation, proliferation and apoptosis[37]. By targeting EGFR and *v-raf-1* murine leukemia viral oncogene homolog 1 (RAF-1), a low level of miR-7 suggests poor prognosis for CRC, and miR-7 precursor, alone or in combination with a monoclonal antibody, could be a novel therapy against CRC[26]. XRCC2 (X-ray repair complementing defective repair in Chinese hamster cells 2) participates in homologous recombination, and its relationship with miR-7 has been studied. *In vitro*, overexpression of miR-7 suppressed proliferation and induced apoptosis of CRC cells by directly targeting XRCC2 through decreasing cyclin D1 and increasing p21, caspase-3 and BAX expression[22]. In addition, the expression of paired box (PAX) 6 is inversely correlated with that of miR-7, and simultaneous activation of the extracellular signal-regulated kinase and phosphoinositide 3-kinase (PI3K) signaling pathways and regulation of the levels of matrix metalloproteinase (MMP) 2 and MMP9 could modulate the expression of PAX6 and miR-7 in opposing ways, which suggests that miR-7 is a promising therapeutic target for CRC[21]. Thus, further mechanisms mediated by miR-7 should be explored, which could be a promising approach for individually tailored therapy of CRC (Figure 3).

HCC

HCC, the third most common cause of cancer mortality worldwide, which develops from activation of cellular oncogenic pathways and abrogation of tumor suppressor pathways including the p53/p21^{WAF1} pathway, the p16^{INK4a}/CDK4/RB1/E2F pathway, the Wnt/β-catenin signaling pathway, transforming growth factor-α, c-myc, transcription factor NF-κB, insulin/IGF-I, and receptor tyrosine kinases and their downstream activators[38,39]. Several studies have shown that miR-7 participates in several pathways by targeting different genes in HCC. MiR-7 regulates the PI3K/Akt/mammalian target of rapamycin *in vitro* and *in vivo*, which functions downstream of EGFR, suggesting that miR-7 is a potential target for treating or diagnosing/prognosing HCC[28]. Likewise, ectopic expression of cullin 5, a novel target gene of miR-7, inhibits HCC cell proliferation, arrests cell cycle progression, and suppresses colony formation, although the exact pathway remains unclear[23]. Moreover,

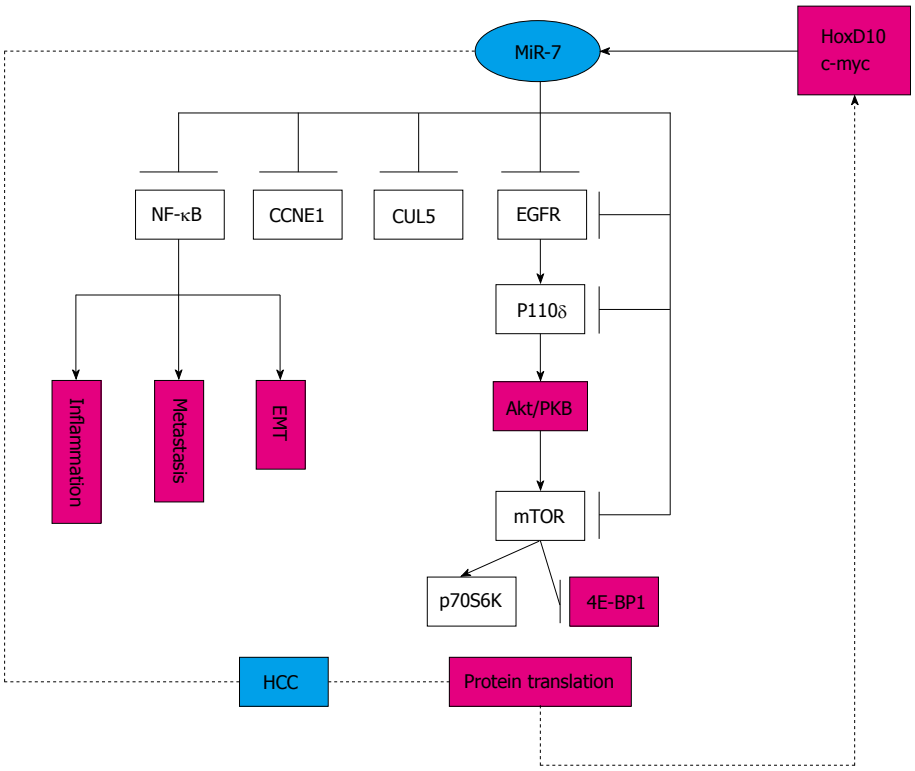

Figure 4 Pathway of miR-7 in hepatocellular carcinoma. MiR-7 can target various genes in the specific signaling pathway. HCC: Hepatocellular carcinoma; miR-7: MicroRNA-7; NF-κB: Nuclear factor κB; CCNE1: Cyclin E1; CUL5: Cullin 5; EGFR: Epidermal growth factor receptor; mTOR: Mammalian target of rapamycin; EMT: Epithelial–mesenchymal transition.

a member of the highly conserved cyclin family, CCNE1 (cyclin E1), is inversely correlated with miR-7 expression in HCC cell lines and clinical samples, indicating that it is a downstream mediator for miR-7, and miR-7 might be a candidate for the treatment of HCC[40]. Most studies in this field have been *in vitro* experiments, except one[28]. The detailed mechanisms remain to be elucidated, thus, the exact role of miR-7 in HCC needs further research (Figure 4).

OTHER DIGESTIVE MALIGNANCIES

MiR-7 also plays a role in other digestive tract malignancies such as cholangiocarcinoma and ESCC[29,41]. However, the expression and exact role of miR-7 in these two malignancies need to be verified.

INFLAMMATORY DISEASE

Inflammation makes a significant contribution to carcinogenesis and progression of malignancies[42]. In some conditions, inflammation such as chronic atrophic gastritis is defined as a precancerous lesion of GC. MiR-7 also participates in some inflammatory diseases, in addition to malignancies. The role of miR-7 in the progression from chronic inflammation to GC has been studied more thoroughly compared with other inflammatory diseases. Using established mouse models, Kong *et al*[20] have demonstrated that downregulation of miR-7 induced by PGE2 associated with inflammation, and activation of EGFR are critical steps in gastric carcinogenesis. Although the COX-2/mPGES-1/PGE2/EP2 pathway has been identified in gastric tumorigenesis, whether there is a similar mechanism mediated by miR-7 has not been established in other malignancies. It has been revealed that the expression of miR-7 is decreased in actively inflamed colonic tissues form patients with Crohn's disease, which is regulated by hCD98[25]. Similarly, chronic hepatitis has an important influence on HCC development, and hepatocyte nuclear factor 4α and NF-κB form a feedback circuit, for which miR-7 and miR-124 could be the targets[43]. These findings suggest that miR-7 is involved in many inflammatory diseases by activating many inflammatory/proinflammatory cytokines, and it will be intriguing to demonstrate the role of miR-7 in the regulation of alimentary inflammatory responses and carcinogenesis.

CIRCULATORY BIOMARKER

The diagnostic and prognostic biomarkers for some digestive malignancies including GC and CRC are still limited. Common circulatory markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 have inadequate sensitivity, therefore, exploring specific biomarkers is a significant breakthrough.

MiRNAs are stable in serum, plasma and body fluids (*e.g.*, stools and gastric juice), and their expression differs between tumor and non-tumor tissue. Some miRNAs, like miR-21, have been subjected to meta-

analysis and concluded to be diagnostic biomarkers for GC[44]. Wang *et al*[45] designed their study with three phases. In the discovery phase, they detected 723 miRNAs in 80 serum samples using microarrays; in the training phase they experimented on another 112 plasma samples using qPCR; and finally, they confirmed the results with 49 samples using a logistic model, and screened miR-7 as one of a panel that yielded high diagnostic accuracy to diagnose CRC. Compared with CEA, miR-7 has a higher receiver operating characteristic curve, sensitivity and specificity (0.897, 82% and 89%, respectively). Similarly, by analyzing the serum from 12 acute pancreatitis patients and three healthy controls, Liu *et al*[46] identified miR-7 as one of the three diagnostic and prognostic biomarkers. Although several systematic reviews[44,47-49] have investigated biomarkers for GC, none has shown that miR-7 could be a biomarker of GC.

CONCLUSION

Several studies have identified possible mechanisms mediated by miR-7 in specific malignancies of the digestive system, including some inflammatory diseases. No study has investigated miR-7 comprehensively, which may explain why different studies have discovered different targets for miR-7, or it may be because miRNA can form one-to-one, one-to-multiple or multiple-to-one relationships with its target genes[50]. Disruption of homeostasis in the digestive system is due to many pathways acting together in a complicated manner, which contributes to the progression from inflammatory diseases to malignancy. Furthermore, the genetic abnormalities in tumors are highly heterogeneous, and no two tumors are exactly alike, which raises a serious challenge. Consequently, more research should be conducted to verify whether miR-7 could be a biomarker or therapeutic target gene for digestive system malignancies.

REFERENCES

1 **Calin GA**, Croce CM, MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945]
2 **Croce CM**. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet* 2009; **10**: 704-714 [PMID: 19763153 DOI: 10.1038/nrg2634]
3 **Bushati N**, Cohen SM. microRNA functions. *Annu Rev Cell Dev Biol* 2007; **23**: 175-205 [PMID: 17506695]
4 **Garzon R**, Marcucci G, Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. *Nat Rev Drug Discov* 2010; **9**: 775-789 [PMID: 20885409 DOI: 10.1038/nrd3179]
5 **Ruan K**, Fang X, Ouyang G. MicroRNAs: novel regulators in the hallmarks of human cancer. *Cancer Lett* 2009; **285**: 116-126 [PMID: 19464788 DOI: 10.1016/j.canlet.2009.04.031]
6 **Cunningham D**, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N. Colorectal cancer. *Lancet* 2010; **375**: 1030-1047 [PMID: 20304247]
7 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
8 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786]
9 **Li X**, Carthew RW. A microRNA mediates EGF receptor signaling and promotes photoreceptor differentiation in the Drosophila eye. *Cell* 2005; **123**: 1267-1277 [PMID: 16377567]
10 **Correa-Medina M**, Bravo-Egana V, Rosero S, Ricordi C, Edlund H, Diez J, Pastori RL. MicroRNA miR-7 is preferentially expressed in endocrine cells of the developing and adult human pancreas. *Gene Expr Patterns* 2009; **9**: 193-199 [PMID: 19135553 DOI: 10.1016/j.gep.2008.12.003]
11 **Masuda M**, Miki Y, Hata S, Takagi K, Sakurai M, Ono K, Suzuki K, Yang Y, Abe E, Hirakawa H, Ishida T, Suzuki T, Ohuchi N, Sasano H. An induction of microRNA, miR-7 through estrogen treatment in breast carcinoma. *J Transl Med* 2012; **10** Suppl 1: S2 [PMID: 23227519]
12 **Li J**, Zheng Y, Sun G, Xiong S. Restoration of miR-7 expression suppresses the growth of Lewis lung cancer cells by modulating epidermal growth factor receptor signaling. *Oncol Rep* 2014; **32**: 2511-2516 [PMID: 25334070 DOI: 10.3892/or.2014.3519]
13 **Zhou X**, Hu Y, Dai L, Wang Y, Zhou J, Wang W, Di W, Qiu L. MicroRNA-7 inhibits tumor metastasis and reverses epithelial-mesenchymal transition through AKT/ERK1/2 inactivation by targeting EGFR in epithelial ovarian cancer. *PLoS One* 2014; **9**: e96718 [PMID: 24816687 DOI: 10.1371/journal.pone.0096718]
14 **Liu Z**, Jiang Z, Huang J, Huang S, Li Y, Yu S, Yu S, Liu X. miR-7 inhibits glioblastoma growth by simultaneously interfering with the PI3K/ATK and Raf/MEK/ERK pathways. *Int J Oncol* 2014; **44**: 1571-1580 [PMID: 24603851 DOI: 10.3892/ijo.2014.2322]
15 **Wang B**, Sun F, Dong N, Sun Z, Diao Y, Zheng C, Sun J, Yang Y, Jiang D. MicroRNA-7 directly targets insulin-like growth factor 1 receptor to inhibit cellular growth and glucose metabolism in gliomas. *Diagn Pathol* 2014; **9**: 211 [PMID: 25394492]
16 **Bravo-Egana V**, Rosero S, Molano RD, Pileggi A, Ricordi C, Domínguez-Bendala J, Pastori RL. Quantitative differential expression analysis reveals miR-7 as major islet microRNA. *Biochem Biophys Res Commun* 2008; **366**: 922-926 [PMID: 18086561]
17 **Joglekar MV**, Joglekar VM, Hardikar AA. Expression of islet-specific microRNAs during human pancreatic development. *Gene Expr Patterns* 2009; **9**: 109-113 [PMID: 18977315]
18 **Zhao X**, Dou W, He L, Liang S, Tie J, Liu C, Li T, Lu Y, Mo P, Shi Y, Wu K, Nie Y, Fan D. MicroRNA-7 functions as an anti-metastatic microRNA in gastric cancer by targeting insulin-like growth factor-1 receptor. *Oncogene* 2013; **32**: 1363-1372 [PMID: 22614005 DOI: 10.1038/onc.2012.156]
19 **Xie J**, Chen M, Zhou J, Mo MS, Zhu LH, Liu YP, Gui QJ, Zhang L, Li GQ. miR-7 inhibits the invasion and metastasis of gastric cancer cells by suppressing epidermal growth factor receptor expression. *Oncol Rep* 2014; **31**: 1715-1722 [PMID: 24573489 DOI: 10.3892/or.2014.3052]
20 **Kong D**, Piao YS, Yamashita S, Oshima H, Oguma K, Fushida S, Fujimura T, Minamoto T, Seno H, Yamada Y, Satou K, Ushijima T, Ishikawa TO, Oshima M. Inflammation-induced repression of tumor suppressor miR-7 in gastric tumor cells. *Oncogene* 2012; **31**: 3949-3960 [PMID: 22139078 DOI: 10.1038/onc.2011.558]
21 **Li Y**, Li Y, Liu Y, Xie P, Li F, Li G. PAX6, a novel target of microRNA-7, promotes cellular proliferation and invasion in human colorectal cancer cells. *Dig Dis Sci* 2014; **59**: 598-606 [PMID: 24185687 DOI: 10.1007/s10620-013-2929-x]
22 **Xu K**, Chen Z, Qin C, Song X. miR-7 inhibits colorectal cancer cell proliferation and induces apoptosis by targeting XRCC2. *Onco Targets Ther* 2014; **7**: 325-332 [PMID: 24570594 DOI: 10.2147/OTT.S59364]
23 **Ma C**, Qi Y, Shao L, Liu M, Li X, Tang H. Downregulation of miR-7 upregulates Cullin 5 (CUL5) to facilitate G1/S transition in human hepatocellular carcinoma cells. *IUBMB Life* 2013; **65**: 1026-1034 [PMID: 24339204 DOI: 10.1002/iub.1231]
24 **Wu XM**, Shao XQ, Meng XX, Zhang XN, Zhu L, Liu SX, Lin J, Xiao HS. Genome-wide analysis of microRNA and mRNA expression signatures in hydroxycamptothecin-resistant gastric cancer cells. *Acta Pharmacol Sin* 2011; **32**: 259-269 [PMID: 21293479 DOI: 10.1038/aps.2010.204]

25 **Nguyen HT**, Dalmasso G, Yan Y, Laroui H, Dahan S, Mayer L, Sitaraman SV, Merlin D. MicroRNA-7 modulates CD98 expression during intestinal epithelial cell differentiation. *J Biol Chem* 2010; **285**: 1479-1489 [PMID: 19892711 DOI: 10.1074/jbc.M109.057141]

26 **Suto T**, Yokobori T, Yajima R, Morita H, Fujii T, Yamaguchi S, Altan B, Tsutsumi S, Asao T, Kuwano H. MicroRNA-7 expression in colorectal cancer is associated with poor prognosis and regulates cetuximab sensitivity via EGFR regulation. *Carcinogenesis* 2015; **36**: 338-345 [PMID: 25503932 DOI: 10.1093/carcin/bgu242]

27 **Ahmed FE**, Ahmed NC, Vos PW, Bonnerup C, Atkins JN, Casey M, Nuovo GJ, Naziri W, Wiley JE, Mota H, Allison RR. Diagnostic microRNA markers to screen for sporadic human colon cancer in stool: I. Proof of principle. *Cancer Genomics Proteomics* 2013; **10**: 93-113 [PMID: 23741026]

28 **Fang Y**, Xue JL, Shen Q, Chen J, Tian L. MicroRNA-7 inhibits tumor growth and metastasis by targeting the phosphoinositide 3-kinase/Akt pathway in hepatocellular carcinoma. *Hepatology* 2012; **55**: 1852-1862 [PMID: 22234835 DOI: 10.1002/hep.25576]

29 **Fu HL**, Wu de P, Wang XF, Wang JG, Jiao F, Song LL, Xie H, Wen XY, Shan HS, Du YX, Zhao YP. Altered miRNA expression is associated with differentiation, invasion, and metastasis of esophageal squamous cell carcinoma (ESCC) in patients from Huaian, China. *Cell Biochem Biophys* 2013; **67**: 657-668 [PMID: 23516093 DOI: 10.1007/s12013-013-9554-3]

30 **Gao M**, Yin H, Fei ZW. Clinical application of microRNA in gastric cancer in Eastern Asian area. *World J Gastroenterol* 2013; **19**: 2019-2027 [PMID: 23599620 DOI: 10.3748/wjg.v19.i13.2019]

31 **Yakirevich E**, Resnick MB. Pathology of gastric cancer and its precursor lesions. *Gastroenterol Clin North Am* 2013; **42**: 261-284 [PMID: 23639640 DOI: 10.1016/j.gtc.2013.01.004]

32 **Ikeda Y**, Tanji E, Makino N, Kawata S, Furukawa T. MicroRNAs associated with mitogen-activated protein kinase in human pancreatic cancer. *Mol Cancer Res* 2012; **10**: 259-269 [PMID: 22188669 DOI: 10.1158/1541-7786.MCR-11-0035]

33 **Ma J**, Fang B, Zeng F, Pang H, Zhang J, Shi Y, Wu X, Cheng L, Ma C, Xia J, Wang Z. Curcumin inhibits cell growth and invasion through up-regulation of miR-7 in pancreatic cancer cells. *Toxicol Lett* 2014; **231**: 82-91 [PMID: 25256401 DOI: 10.1016/j.toxlet.2014.09.014]

34 **Unno J**, Masamune A, Hamada S, Shimosegawa T. The zinc transporter LIV-1 is a novel regulator of stemness in pancreatic cancer cells. *Scand J Gastroenterol* 2014; **49**: 215-221 [PMID: 24294832 DOI: 10.3109/00365521.2013.865075]

35 **Kaemmerer E**, Klaus C, Jeon MK, Gassler N. Molecular classification of colorectal carcinomas: the genotype-to-phenotype relation. *World J Gastroenterol* 2013; **19**: 8163-8167 [PMID: 24363505 DOI: 10.3748/wjg.v19.i45.8163]

36 Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

37 **Zhang N**, Li X, Wu CW, Dong Y, Cai M, Mok MT, Wang H, Chen J, Ng SS, Chen M, Sung JJ, Yu J. microRNA-7 is a novel inhibitor of YY1 contributing to colorectal tumorigenesis. *Oncogene* 2013; **32**: 5078-5088 [PMID: 23208495 DOI: 10.1038/onc.2012.526]

38 **Kudo M**. Signaling pathway/molecular targets and new targeted agents under development in hepatocellular carcinoma. *World J Gastroenterol* 2012; **18**: 6005-6017 [PMID: 23155330 DOI: 10.3748/wjg.v18.i42.6005]

39 **Alves RC**, Alves D, Guz B, Matos C, Viana M, Harriz M, Terrabuio D, Kondo M, Gampel O, Polletti P. Advanced hepatocellular carcinoma. Review of targeted molecular drugs. *Ann Hepatol* 2011; **10**: 21-27 [PMID: 21301005]

40 **Zhang X**, Hu S, Zhang X, Wang L, Zhang X, Yan B, Zhao J, Yang A, Zhang R. MicroRNA-7 arrests cell cycle in G1 phase by directly targeting CCNE1 in human hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2014; **443**: 1078-1084 [PMID: 24370822 DOI: 10.1016/j.bbrc.2013.12.095]

41 **Janpipatkul K**, Suksen K, Borwornpinyo S, Jearawiriyapaisarn N, Hongeng S, Piyachaturawat P, Chairoungdua A. Downregulation of LAT1 expression suppresses cholangiocarcinoma cell invasion and migration. *Cell Signal* 2014; **26**: 1668-1679 [PMID: 24726839 DOI: 10.1016/j.cellsig.2014.04.002]

42 **Diakos CI**, Charles KA, McMillan DC, Clarke SJ. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol* 2014; **15**: e493-e503 [PMID: 25281468 DOI: 10.1016/S1470-2045(14)70263-3]

43 **Ning BF**, Ding J, Liu J, Yin C, Xu WP, Cong WM, Zhang Q, Chen F, Han T, Deng X, Wang PQ, Jiang CF, Zhang JP, Zhang X, Wang HY, Xie WF. Hepatocyte nuclear factor 4α-nuclear factor-κB feedback circuit modulates liver cancer progression. *Hepatology* 2014; **60**: 1607-1619 [PMID: 24752868 DOI: 10.1002/hep.27177]

44 **Zeng Z**, Wang J, Zhao L, Hu P, Zhang H, Tang X, He D, Tang S, Zeng Z. Potential role of microRNA-21 in the diagnosis of gastric cancer: a meta-analysis. *PLoS One* 2013; **8**: e73278 [PMID: 24023850 DOI: 10.1371/journal.pone.0073278]

45 **Wang S**, Xiang J, Li Z, Lu S, Hu J, Gao X, Yu L, Wang L, Wang J, Wu Y, Chen Z, Zhu H. A plasma microRNA panel for early detection of colorectal cancer. *Int J Cancer* 2015; **136**: 152-161 [PMID: 23456911 DOI: 10.1002/ijc.28136]

46 **Liu P**, Xia L, Zhang WL, Ke HJ, Su T, Deng LB, Chen YX, Lv NH. Identification of serum microRNAs as diagnostic and prognostic biomarkers for acute pancreatitis. *Pancreatology* 2014; **14**: 159-166 [PMID: 24854610 DOI: 10.1016/j.pan.2014.03.019]

47 **Shrestha S**, Hsu SD, Huang WY, Huang HY, Chen W, Weng SL, Huang HD. A systematic review of microRNA expression profiling studies in human gastric cancer. *Cancer Med* 2014; **3**: 878-888 [PMID: 24902858 DOI: 10.1002/cam4.246]

48 **Wang JL**, Hu Y, Kong X, Wang ZH, Chen HY, Xu J, Fang JY. Candidate microRNA biomarkers in human gastric cancer: a systematic review and validation study. *PLoS One* 2013; **8**: e73683 [PMID: 24040025 DOI: 10.1371/journal.pone.0073683]

49 **Fu X**, Han Y, Wu Y, Zhu X, Lu X, Mao F, Wang X, He X, Zhao Y, Zhao Y. Prognostic role of microRNA-21 in various carcinomas: a systematic review and meta-analysis. *Eur J Clin Invest* 2011; **41**: 1245-1253 [PMID: 21521185 DOI: 10.1111/j.1365-2362.2011.02535.x]

50 **Hashimoto Y**, Akiyama Y, Yuasa Y. Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. *PLoS One* 2013; **8**: e62589 [PMID: 23667495 DOI: 10.1371/journal.pone.0062589]

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ORIGINAL ARTICLE

Retrospective Study

Impact of *RAS* and *BRAF* mutations on carcinoembryonic antigen production and pattern of colorectal metastases

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Abstract

AIM: To investigate the impact of *RAS* and *BRAF* mutations on the pattern of metastatic disease and carcinoembryonic antigen (CEA) production.

METHODS: In this retrospective study, we investigated the impact of *RAS* and *BRAF* mutational status on pattern of metastatic disease and CEA production. Only patients presenting with a newly diagnosed metastatic colorectal cancer (CRC) were included. Patients' characteristics, primary tumor location, site of metastatic disease and CEA at presentation were compared between those with and without *RAS* and *BRAF* mutations.

RESULTS: Among 174 patients, mutations in *KRAS*, *NRAS* and *BRAF* were detected in 47%, 3% and 6% respectively. *RAS* mutations (*KRAS* and *NRAS*) were more likely to be found in African American patients (87% *vs* 13%; *P* value = 0.0158). *RAS* mutations were associated with a higher likelihood of a normal CEA (< 5 ng/mL) at presentation. *BRAF* mutations were more likely to occur in females. We were not able to confirm

any association between mutational status and site of metastatic disease at initial diagnosis.

CONCLUSION: No association was found between *RAS* and *BRAF* mutations and sites of metastatic disease at the time of initial diagnosis in our cohort. Patients with *RAS* mutations were more likely to present with CEA levels < 5 ng/mL. These findings may have clinical implications on surveillance strategies for *RAS* mutant patients with earlier stages of CRC.

Key words: *RAS*; *BRAF*; Carcinoembryonic antigen; Pattern of metastatic disease; Surveillance

Core tip: We investigated the impact of *RAS* and *BRAF* mutations on pattern of colorectal cancer (CRC) metastases and carcinoembryonic antigen (CEA) production. Patients with *RAS* mutations were more likely to present with CEA levels < 5 ng/mL. No association was found between *RAS* and *BRAF* mutations and sites of metastatic disease at the time of initial diagnosis in our cohort. Our study is the first study to link low CEA production with a *RAS* mutant status at the time of initial presentation of metastatic CRC. These findings may have clinical implications on surveillance strategies for *RAS* mutant patients with earlier stages of CRC.

Cho M, Akiba C, Lau C, Smith D, Telatar M, Afkhami M, Sentovich S, Melstrom K, Fakih M. Impact of *RAS* and *BRAF* mutations on carcinoembryonic antigen production and pattern of colorectal metastases. *World J Gastrointest Oncol* 2016; 8(1): 128-135 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i1/128.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i1.128

INTRODUCTION

Colorectal cancer (CRC) continues to be the second leading cause of cancer-related death in the United States. It is projected that 136830 individuals will be diagnosed with CRC in 2014 in the United States, 50310 of whom will succumb to this disease[1]. While significant progress has been made in the treatment of metastatic CRC (mCRC) over the last two decades, cure amongst these patients remains rare and is only achievable in approximately 20% of patients who are amenable to metastases resection[2,3].

It is estimated that 20% of patients with CRC present with metastatic disease while another 30% develop metastatic disease after an initial presentation with local or regional disease[2,4]. Patients with limited oligometastatic disease are the ones who benefit the most from aggressive surgical strategies[5]. Therefore, early identification of metastatic disease remains key in improving the outcome of patients with metastatic disease. Indeed, intensive surveillance strategies in patients with earlier stages of CRC have been associated with an increased rate of metastectomies in several prospective and retrospective clinical trials[6]. However, these surveillance strategies are not standardized amongst different medical societies and do not take into account the molecular heterogeneity of CRC[7]. It has been recently shown that certain oncogenic alterations have significant impact on disease biology, response to treatment, and overall outcome. For example, *BRAF* mutations, present in 5%-10% of CRCs, are associated with worse prognosis, a worse overall survival after disease recurrence, and a tendency to metastasize to the peritoneum and distant lymph nodes[8,9]. The impact of *KRAS* and *NRAS* mutations, which occur in approximately 50% of CRCs, on the pattern of metastatic disease at initial presentation has been more controversial[10-13].

To better understand the impact of the commonly tested *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations on metastatic disease pattern and on surveillance strategies, we conducted a single institute retrospective study that investigates the impact of *RAS* and *BRAF* mutations on the pattern of metastatic disease and carcinoembryonic antigen (CEA) production.

MATERIALS AND METHODS

Study population

We retrospectively reviewed all cases with metastatic colon cancer patients who presented to City of Hope Comprehensive Cancer Center from 2007 to 2014. Inclusion on study required all the following criteria: (1) confirmed CRC by pathology; (2) availability of imaging studies confirming metastatic disease at the time of presentation; (3) availability of *KRAS* or *BRAF* testing by PCR or by ONCO44 or ONCO48 next generation sequencing; and (4) available CEA level at the time of presentation of metastatic CRC.

Patients' characteristics including age, gender, race, location of the primary tumor, CEA, and sites of metastatic disease at the time of presentation were reviewed and collected from corresponding electronic medical records. Primary tumor location was categorized as right or transverse colon, left colon, and rectum. Metastatic sites were categorized into 3 groups: (1) lung; (2) liver; and (3) mesenteric or distal lymph nodes or peritoneum. The study was approved by the local institutional review board.

RAS and BRAF analysis

To allow for a more powerful sample size, we included *RAS* and *BRAF* analysis performed by either a CLIA certified next generation sequencing or a CLIA certified PCR assay.

Onco 44: Genomic DNA is extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue with minimum 30% tumor cellularity. A targeted DNA library is generated using the Ion AmpliSeq™ Cancer

Table 1 *RAS* and *BRAF* status and patient demographics

		All		*BRAF* MT		*BRAF* WT		*P*	*RAS* MT		*RAS* WT		*P*
		n	%	*n*	%	*n*	%		*n*	%	*n*	%	
Age	< 60	87	50	5	6	82	94	1.00	44	51	43	49	1.00
	⩾ 60	87	50	6	7	81	93		43	49	44	51	
Gender	Male	103	59	3	3	100	97	0.052	49	48	54	52	0.54
	Female	71	41	8	11	63	89		38	54	33	46	
Race	White	122	70	7	6	115	94	0.53	57	47	65	53	0.015
	Asian, PI	41	24	4	10	37	90		23	56	18	44	
	Black	8	5	0	--	8	100		7	87	1	13	
	Unknown	3	2	0	--	3	100		0	--	3	100	

n: Number of patients; PI: Pacific Islander; MT: Mutant; WT: Wild type.

Hotspot Panel Kit, and sequenced by semiconductor-based next-generation sequencing technology on an Ion Torrent PGM. The Onco 44 pannel is designed to target 713 mutations in 44 key cancer genes that include *KRAS, NRAS,* and *BRAF.* Tested *KRAS* mutations include codon 12, 13, 61 and 146. Tested NRAS mutations include codon 12, 13, 61.

Onco 48: The Onco48 Panel is designed to target 2800 mutations in 48 key cancer genes. The difference between Onco 44 and 48 is the additional sequencing of 4 target genes: EZH2, GNA11, GNAQ, and IDH2. In addition, the Onco48 panel identifies the rare *KRAS* codon 117 and *NRAS* codon 146 mutations.

***KRAS*-PCR:** DNA was extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue with minimum 30% tumor cellularity. A PCR based fragment analysis with 5% sensitivity using "Shift Termination" technology was used to detect mutations in the *KRAS* gene. This assay is CLIA approved and detects 6 mutations on codon 12 and 1 mutation on codon 13 of exon 2.

***BRAF*:** Genomic DNA is extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue with minimum 30% tumor cellularity. A real-time PCR assay with 1% sensitivity was performed to detect the c.1799 T > A (V600E) mutation in the *BRAF* gene.

CEA assay

CEA was tested *via* Siemens Advia Centaur chemiluminescent immunoassay and normal range is 0.5 ng/mL to 4.5 ng/mL.

Statistical analysis

We tested for differences in proportions between rate of mutations *vs* clinical and demographic factors with Fisher's Exact Tests. We also tested for differences in proportions between rate of mutations *vs* site of metastatic disease, location of primary disease, and CEA (cut point of 5 ng/mL) with Fisher's Exact Tests. For testing the association between metastases site and CEA as a continuous variable, we transformed CEA using the natural logarithm and used it as the independent variable in a logistic regression. The dependent variable in the logistic regression was presence or absence of a given metastases location.

KRAS and *NRAS* mutations were categorized under *RAS* mutations, irrespective of the testing methodology. Comparative analysis was performed on 4 distinct subgroups: *RAS* mutant, *RAS* wild type, *BRAF* mutant, and *BRAF* wild type populations.

RESULTS

The study population consisted of 174 patients who presented with metastatic colon cancer patients and documented *RAS* and *BRAF* mutational analysis. Genomic evaluation for *KRAS, NRAS*, and *BRAF* was performed by next generation sequencing using ONCO44 or ONCO48 in 122 patients. 52 patients were evaluated for *KRAS* (no *NRAS* evaluation) and *BRAF* mutation by PCR. Eighty-seven (50%) of patients had an identifiable *RAS* mutations (47% *KRAS* and 3% *NRAS*). Only 11 patients (6%) had *BRAF* mutation (Table 1).

RAS and BRAF mutations and patients' demographics

The median age of the study population was 60 years (range 23 to 87 years). There was no difference in *RAS* or *BRAF* mutation status by age, or gender. However, females had a trend towards a higher incidence of *BRAF* mutation. No distinct variations were noted in *KRAS* or *BRAF* mutations by race, with the exception of an increased rate of *RAS* mutations among African Americans. However, African American representation on this study was low (5%), limiting the interpretation of this finding (Table 1).

RAS and BRAF status, primary tumor location and pattern of metastases

There was no difference in primary tumor sites by *KRAS* or *BRAF* status, with the exception of a lower likelihood of *BRAF* mutations among rectal cancers. In addition, no difference in tumor spread pattern at the time of metastatic disease presentation was noted among the 4 molecular subgroups (Table 2).

Table 2 *RAS* and *BRAF* status and primary tumor location and pattern of metastasis

		All		*BRAF* MT		*BRAF* WT		*P*	*RAS* MT		*RAS* WT		*P*
		n	%	*n*	%	*n*	%		*n*	%	*n*	%	
Primary lesion	Rectal	43	25	0	--	43	100	0.022	23	53	20	47	0.23
	Left colon	79	45	5	6	74	94		34	43	45	57	
	Right colon	52	30	6	12	46	88		30	58	22	42	
Site of metastasis	Lung	72	42	3	4	69	96	0.36	41	57	31	42	0.17
	Liver	98	56	5	5	93	95	0.54	46	47	52	53	0.44
	Peritoneal	49	28	5	10	44	90	0.3	23	47	26	53	0.74

n: Number of patients; MT: Mutant; WT: Wild type.

Table 3 *RAS* and *BRAF* status and carcinoembryonic antigen levels

	All		*BRAF* MT		*BRAF* WT		*P*	*RAS* MT		*RAS* WT		*P*
	n	%	*n*	%	*n*	%		*n*	%	*n*	%	
< 5 ng/mL	60	34	4	7	56	93	1.00	37	62	23	38	0.037
⩾ 5 ng/mL	114	66	7	6	107	94		64	56	50	44	

n: Number of patients; MT: Mutant; WT: Wild type.

RAS and BRAF status and CEA production

Thirty-four percent of the total cohort were non-CEA producers (CEA < 5 ng/mL). Patients with liver metastases were more likely to produce CEA (OR = 0.639; $P < 0.0001$) while patients with peritoneal/mesenteric metastases were less likely to produce CEA (OR = 1.315; $P = 0.0010$). Patients with *RAS* mutation were more likely to be low-CEA producers at the time of metastatic disease presentation (Table 3). There was no significant association between *BRAF* mutation status and CEA production.

DISCUSSION

In this study we sought to explore correlations between *RAS* and *BRAF* mutational status, patient demographics, metastatic disease pattern, and CEA production. No distinct demographic characteristics were associated with *RAS* or *BRAF* status, with the exception of *BRAF* mutations which were less likely to occur with a rectal primary. Although not statistically significant, females were more likely to harbor a *BRAF* mutation. These findings are consistent with prior reports[9,12,14,15]. We were not able to confirm an association between *BRAF* mutations and age or a right colon primary, contrary to previous reports[9,12,14,15]. This discordance is likely related to our more limited sample size, especially that the percentages of *RAS*-mutant and *RAS*-wild type patients with right colonic primaries were in line with the above referenced studies. We also investigated the impact of race on *RAS* and *BRAF* mutational status. The only positive association was for *RAS* mutation and African American race. Several studies have previously evaluated the impact of race on *RAS* and *BRAF* mutational status[16,17]. The N0147 adjuvant clinical trial in patients with stage Ⅲ colon cancer reported an increased likelihood of *BRAF* mutation amongst White and an increased *KRAS* mutation frequency in African Americans[18]. In addition, N0147 reported a lower frequency of *KRAS* mutations in Asians, a finding not supported by our study.

Contrary to the current literature, we did not find an association between *BRAF* mutation and peritoneal mestastases at the time of presentation, likely due to our small *BRAF* mutant sample size. Several studies have reported an increased likelihood of peritoneal dissemination in *BRAF* mutant mCRC patients[8,9,19]. Yaeger *et al*[9] reported that patients with *BRAF* mutations were more likely to present with peritoneal metastases at initial diagnosis and less likely to have liver-limited metastases. Moreover, the 2-year cumulative incidence of peritoneal metastases was higher with *BRAF* mutated tumors[9]. Tran *et al*[8] reported a higher rate of peritoneal and distant lymph node metastases and a lower rate of lung metastases in *BRAF* mutated tumors. Similarly, Russo *et al*[19] reported a higher likelihood of *BRAF* mutations in patients with distant lymph node metastases at the site of first recurrence. Finally, Kawazoe *et al*[12] retrospectively studied the clinical-pathological features of *BRAF* mutations in Japanese patients with metastatic CRC and found that peritoneal metastases are more frequently observed in *BRAF* mutated patients. Since the presence of peritoneal metastases has been identified as a poor prognostic factor, a higher incidence of peritoneal metastases in *BRAF* tumors may partly explain the poor prognosis associated with this subgroup[12,20,21]. These studies are summarized in Table 4.

Our study did not confirm an association between *RAS* mutations and lung metastases at initial mCRC presentation. There is discordance among studies on the impact of *RAS* mutational status on lung metastases at the time of initial mCRC presentation. However,

Table 4 *BRAF* status and pattern of colon cancer metastases

Ref.	*n* (%*BRAF*)	End point	%*BRAF* MT *vs* %*BRAF* WT	*P*
Tran *et al*[8]	524 (11%)	Rate of peritoneal metastases	46% *vs* 24%	0.001
		Rate of distant lymph node metastases	53% *vs* 38%	0.008
		Rate of lung metastases	35% *vs* 49%	0.049
Yaeger *et al*[9]	515 (18%)	Peritoneal involvement at presentation	26% *vs* 14%	< 0.01
Kawazoe *et al*[12]	264 (5%)	Peritoneal metastasis	50% *vs* 18%	0.009

n: Total number of patients; %*BRAF*: %patients with *BRAF* mutation; MT: Mutant; WT: Wild type.

Table 5 *RAS* status and pattern of colon cancer metastases

Ref.	*n* (%MT)	Results	*P*
Cejas *et al*[29]	110 (34% *KRAS* MT)	Frequency of *KRAS* mutation in primary tumor of patients with lung *vs* liver metastases 59% *vs* 32%	0.054
Tie *et al*[10]	Cohort A 161 (48.4% *KRAS* MT)	Mutation frequencies in lung in *KRAS* MT *vs* WT 62% *vs* 38%	0.003
		Mutation frequencies in brain in *KRAS* MT *vs* WT 56.5% *vs* 43.5%	0.003
	Cohort C 859 (33.8% *KRAS* MT)	Relapse in lung in *KRAS* MT HR 2.1, 95%CI: 1.2-3.5	0.007
Kim *et al*[23]	143 (43.4% *KRAS* MT)	Lung as initial metastatic site in *KRAS* MT *vs* WT 45.3% *vs* 22.1%	0.003
		Liver as initial metastatic site in *KRAS* MT *vs* WT 37.3% *vs* 70.6%	< 0.001
		Distant lymph node as initial metastatic site in *KRAS* MT *vs* WT 6.7% *vs* 19.1%	0.025
Vauthey *et al*[25]	193 (18% All *RAS* MT)	3-yr lung RFS rate in patients undergoing curative resection of liver metastases in *RAS* MT *vs* WT 34.6% *vs* 59.3%	< 0.001
Yaeger *et al*[11]	918 (48% All *RAS* MT)	Lung as site of first metastasis in *RAS* MT *vs* WT 22% *vs*%	< 0.01
		Cumulative incidence of lung as subsequent metastasis at 2 yr after diagnosis in RAS MT *vs* WT 32.5% *vs* 19%	< 0.001
Kemeny *et al*[24]	169 (30% *KRAS* MT)	3-yr cumulative recurrence rate to lung after hepatic resection and HAI in *KRAS* MT *vs* WT 58% *vs* 32%	< 0.01
		3-yr cumulative recurrence rate to brain after hepatic resection and HAI in *KRAS* MT *vs* WT 14.5% *vs* 2%	0.05
		3-yr cumulative recurrence rate to bone after hepatic resection and HAI in *KRAS* MT *vs* WT 13.4% *vs* 2%	< 0.01
Pereira *et al*[13]	494 (41% *KRAS* MT)	Time to lung metastasis (median months) in *KRAS* MT *vs* WT 15.2 *vs* 22.4 (HR 1.4)	0.002

n: Total number of patients; %MT: %patients with mutation; MT: Mutant; WT: Wild type; RFS: Recurrence free survival; HAI: Hepatic arterial infusion.

clinical studies have consistently shown an association between *KRAS* mutation and lifetime likelihood of lung metastases in patients with mCRC, but not at initial presentation (Table 5). In our previous study, conducted on a different patient data set, Sharma *et al*[22] reported no predictive role for *KRAS* mutations on the site(s) of metastatic disease at the time of presentation. Pereira *et al*[13] retrospectively evaluated patients with mCRC who were tested for *KRAS* mutation at MD Anderson Cancer Center. They did not report an increase rate of lung metastases in *KRAS* mutated patients at the time of diagnosis of mCRC. However, *KRAS* mutation was found to have a shorter time to lung metastases and a two-fold greater odd of developing lifetime lung metastases in a cohort of a liver-limited CRC. However, several other studies reported that *KRAS* mutant patients were more likely to present with lung metastases than *KRAS* wild type patients. Kim *et al*[23] reported on the initial metastatic disease patterns in South Korean patients with mCRC. Lung metastases were more frequent

as the initial metastasic site in *KRAS* mutant patients while liver and distant lymph node metastases were less likely[23]. Yaeger *et al*[11] reported on the impact of *KRAS* mutations on the pattern of metastatic spread in CRC. In this retrospective study, *KRAS* mutant patients had a higher incidence of lung metastases at initial presentation compared to *KRAS* wild type patients. In addition, *KRAS* mutated patients had higher cumulative incidence of lung, bone and brain metastases at two years from initial mCRC presentation. Fewer patients had liver-limited disease at the initial presentation in *KRAS* mutated patients than *KRAS* wild type patients[11]. *KRAS* mutations have also been associated with a higher risk of lung relapse while *NRAS* mutations were associated with increased local recurrence after curative resection of primary CRC or after curative intent hepatectomy[10,24,25]. Review of patients with stage Ⅱ and Ⅲ primary CRC who participated in VICTOR clinical trial showed an association between *KRAS* mutations and an increased relapse rate in the lung. Relapse in the liver was similar between *KRAS* mutant and wild type patients[10]. Kemeny *et al*[24] reported on the pattern of metastatic disease recurrence in patients who underwent hepatic resection and adjuvant HAI plus systemic chemotherapy. The three-year cumulative incidence of lung metastases was higher in the *KRAS* mutant patients. The cumulative incidence of bone and brain metastases was also increased in the *KRAS* mutant patients. Similarly, Vauthey *et al*[25] reported that patients with *KRAS* mutant tumors who underwent curative intent liver resection at MD Anderson cancer center had a lower three-year lung RFS in comparison to patients with *KRAS* wild type tumors. Based on the above studies (summarized in Table 5), *KRAS* mutant mCRC patients have an increased lifetime risk of developing lung metastases. However, the impact of *KRAS* mutational status on the incidence of lung metastases at the initial time of diagnosis of metastatic disease remains controversial. Whether the lack of association between lung metastases at presentation and *KRAS* mutations is related to a limited sample size on those studies *vs* being the result of tumor biology remains unclear.

We have studied the impact of *RAS* and *BRAF* mutational status on CEA levels at the time of initial diagnosis of metastatic disease. We did not find any difference in CEA levels between *BRAF* mutant and *BRAF* wild type mCRC at initial presentation. In contrast, *RAS* mutant mCRC patients were more likely to be non-CEA producers (62% *RAS*-MT *vs* 38% *RAS*-WT) (Table 3). Our findings are in contrast to a study by Selcukbiricik *et al*[26] which reported a higher percentage of patients with CEA > 5 ng/mL among the *KRAS* mutant cohort. Selcurkbiricik study was limited by stage heterogeneity (stages Ⅰ-Ⅳ) and did not include an analysis of the impact of *RAS* mutation within the stage Ⅳ disease cohort. Our study also showed an association between CEA levels and site of metastatic disease. CEA was more likely to be elevated in patients with liver metastases and lower in patients with peritoneal or mesenteric recurrence, which is consistent with prior reports[27].

Our study has several limitations. This is a single institution study with a relatively small size. Modest associations between *RAS* and *BRAF* status and other clinical variables may have therefore been missed due to the lack of adequate power. In addition, the diagnosis of metastatic disease on this study could have been made during surveillance for disease recurrence or during the work-up of symptomatic disease. Therefore, the conclusions derived from this study may not be clearly generalizable to the surveillance population or to the population presenting with symptomatic stage Ⅳ disease. Other limitations include the inclusion of patients with *KRAS* PCR mutation assay (no ONCO48 analysis). This implies that some patients may have been assigned to the *RAS* wild type subgroup without ruling out the possibility of *NRAS* or non-exon 2 *KRAS* mutations. The likelihood of this event impacting our overall results is low as only 52 patients (30%) of our study population was analyzed by *KRAS*-PCR only. Given that less than 10% of the general population carries a non-exon 2 *KRAS* mutation or *NRAS* mutations, we expect that less than 10 patients may have been inappropriately labeled.

In summary, our study is the first study to link low CEA production with a *RAS* mutant status at the time of initial presentation of metastatic CRC. If validated in larger studies, especially in surveillance settings, our findings would have major clinical significance. It has been recently confirmed that *RAS* mutations increase the risk of systemic disease recurrence after a curative resection in patients with stage Ⅲ colon cancer[28]. Reliable screening strategies are especially important in this high risk population in order to diagnosis early recurrence and increase the likelihood of curative-intent metastectomies. If CEA is confirmed as a less reliable screening strategy, intense radiographic screening will be especially important as a complement to CEA screening in this population.

COMMENTS

Background

It is estimated that 20% of patients with colorectal cancer (CRC) present with metastatic disease while another 30% develop metastatic disease after an initial presentation with local or regional disease. Patients with limited oligometastatic disease are the ones who benefit the most from aggressive surgical strategies. Therefore, early identification of metastatic disease remains key in improving outcome. It has been recently shown that certain oncogenic alterations have significant impact on disease biology, response to treatment, and overall outcome. To better understand the impact of the commonly presented *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations on metastatic disease pattern and on surveillance strategies, the authors conducted a single institute retrospective study that investigates the impact of *RAS* and *BRAF* mutations on the pattern of metastatic disease and carcinoembryonic antigen (CEA) production.

Research frontiers

BRAF mutations, present in 5%-10% of CRCs, are associated with worse prognosis, a worse overall survival after disease recurrence, and a tendency to metastasize to the peritoneum and distant lymph nodes. The impact of *KRAS* and *NRAS* mutations, which occur in approximately 50% of CRCs, on the

pattern of metastatic disease at initial presentation has been more controversial. No studies have reported on the impact of either *RAS* or *BRAF* mutations on CEA production.

Innovations and breakthroughs

The authors did not find any difference in CEA levels between *BRAF* mutant and *BRAF* wild type mCRC at initial presentation. In contrast, *RAS* mutant mCRC patients were more likely to be non-CEA producers (62% *RAS*-MT *vs* 38% *RAS*-WT).

Applications

The study is the first study to link low CEA production with a *RAS* mutant status at the time of initial presentation of metastatic CRC. If validated in larger studies, especially in surveillance settings, the authors' findings may indicate that CEA surveillance is less reliable in curatively resected *RAS* mutant CRC patients. Alternative surveillance strategies may be required in this patients population.

Terminology

The ONCO 44/48 is the next-generation sequencing technology at the City of Hope which is designed to target 713 mutations in 44 and 48 key cancer genes.

Peer-review

It is a well-written paper.

REFERENCES

1 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]

2 **Fong Y**, Blumgart LH. Hepatic colorectal metastasis: current status of surgical therapy. *Oncology* (Williston Park) 1998; **12**: 1489-1498; discussion 1489-1500; 1503 [PMID: 9798202]

3 **Park JH**, Kim TY, Lee KH, Han SW, Oh DY, Im SA, Kang GH, Chie EK, Ha SW, Jeong SY, Park KJ, Park JG, Kim TY. The beneficial effect of palliative resection in metastatic colorectal cancer. *Br J Cancer* 2013; **108**: 1425-1431 [PMID: 23481187 DOI: 10.1038/bjc.2013.94]

4 **Fong Y**, Cohen AM, Fortner JG, Enker WE, Turnbull AD, Coit DG, Marrero AM, Prasad M, Blumgart LH, Brennan MF. Liver resection for colorectal metastases. *J Clin Oncol* 1997; **15**: 938-946 [PMID: 9060531]

5 **Van Cutsem E**, Nordlinger B, Adam R, Köhne CH, Pozzo C, Poston G, Ychou M, Rougier P. Towards a pan-European consensus on the treatment of patients with colorectal liver metastases. *Eur J Cancer* 2006; **42**: 2212-2221 [PMID: 16904315]

6 **Pita-Fernández S**, Alhayek-Aí M, González-Martín C, López-Calviño B, Seoane-Pillado T, Pértega-Díaz S. Intensive follow-up strategies improve outcomes in nonmetastatic colorectal cancer patients after curative surgery: a systematic review and meta-analysis. *Ann Oncol* 2015; **26**: 644-656 [PMID: 25411419]

7 **Charnsangavej C**, Clary B, Fong Y, Grothey A, Pawlik TM, Choti MA. Selection of patients for resection of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1261-1268 [PMID: 16947009 DOI: 10.1245/s10434-006-9023-y]

8 **Tran B**, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, Agarwal A, Maru DM, Sieber O, Desai J. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer* 2011; **117**: 4623-4632 [PMID: 21456008 DOI: 10.1002/cncr.26086]

9 **Yaeger R**, Cercek A, Chou JF, Sylvester BE, Kemeny NE, Hechtman JF, Ladanyi M, Rosen N, Weiser MR, Capanu M, Solit DB, D'Angelica MI, Vakiani E, Saltz LB. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. *Cancer* 2014; **120**: 2316-2324 [PMID: 24737664 DOI: 10.1002/cncr.28729]

10 **Tie J**, Lipton L, Desai J, Gibbs P, Jorissen RN, Christie M, Drummond KJ, Thomson BN, Usatoff V, Evans PM, Pick AW, Knight S, Carne PW, Berry R, Polglase A, McMurrick P, Zhao Q, Busam D, Strausberg RL, Domingo E, Tomlinson IP, Midgley R, Kerr D, Sieber OM. KRAS mutation is associated with lung metastasis in patients with curatively resected colorectal cancer. *Clin Cancer Res* 2011; **17**: 1122-1130 [PMID: 21239505]

11 **Yaeger R**, Cowell E, Chou JF, Gewirtz AN, Borsu L, Vakiani E, Solit DB, Rosen N, Capanu M, Ladanyi M, Kemeny N. RAS mutations affect pattern of metastatic spread and increase propensity for brain metastasis in colorectal cancer. *Cancer* 2015; **121**: 1195-1203 [PMID: 25491172 DOI: 10.1002/cncr.29196]

12 **Kawazoe A**, Shitara K, Fukuoka S, Kuboki Y, Bando H, Okamoto W, Kojima T, Fuse N, Yamanaka T, Doi T, Ohtsu A, Yoshino T. A retrospective observational study of clinicopathological features of KRAS, NRAS, BRAF and PIK3CA mutations in Japanese patients with metastatic colorectal cancer. *BMC Cancer* 2015; **15**: 258 [PMID: 25886136 DOI: 10.1186/s12885-015-1276-z]

13 **Pereira AA**, Rego JF, Morris V, Overman MJ, Eng C, Garrett CR, Boutin AT, Ferrarotto R, Lee M, Jiang ZQ, Hoff PM, Vauthey JN, Vilar E, Maru D, Kopetz S. Association between KRAS mutation and lung metastasis in advanced colorectal cancer. *Br J Cancer* 2015; **112**: 424-428 [PMID: 25535726 DOI: 10.1038/bjc.2014.619]

14 **Kalady MF**, Dejulius KL, Sanchez JA, Jarrar A, Liu X, Manilich E, Skacel M, Church JM. BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. *Dis Colon Rectum* 2012; **55**: 128-133 [PMID: 22228154 DOI: 10.1097/DCR.0b013e31823c08b3]

15 **Roth AD**, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010; **28**: 466-474 [PMID: 20008640 DOI: 10.1200/JCO.2009.23.3452]

16 **Gonsalves WI**, Mahoney MR, Sargent DJ, Nelson GD, Alberts SR, Sinicrope FA, Goldberg RM, Limburg PJ, Thibodeau SN, Grothey A, Hubbard JM, Chan E, Nair S, Berenberg JL, McWilliams RR. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. *J Natl Cancer Inst* 2014; **106**: pii: dju106 [PMID: 24925349 DOI: 10.1093/jnci/dju106]

17 **Sylvester BE**, Huo D, Khramtsov A, Zhang J, Smalling RV, Olugbile S, Polite BN, Olopade OI. Molecular analysis of colorectal tumors within a diverse patient cohort at a single institution. *Clin Cancer Res* 2012; **18**: 350-359 [PMID: 22114137 DOI: 10.1158/1078-0432.CCR-11-1397]

18 **Yoon HH**, Shi Q, Alberts SR, Goldberg RM, Thibodeau SN, Sargent DJ, Sinicrope FA, Alliance for Clinical Trials in Oncology. Racial differences in KRAS/BRAF mutation rates and survival in colon cancer (NCCTG N0147 [Alliance]). *J Clin Oncol* 2014; **32**: 5s, (suppl; abstr 3536)

19 **Russo AL**, Borger DR, Szymonifka J, Ryan DP, Wo JY, Blaszkowsky LS, Kwak EL, Allen JN, Wadlow RC, Zhu AX, Murphy JE, Faris JE, Dias-Santagata D, Haigis KM, Ellisen LW, Iafrate AJ, Hong TS. Mutational analysis and clinical correlation of metastatic colorectal cancer. *Cancer* 2014; **120**: 1482-1490 [PMID: 24500602 DOI: 10.1002/cncr.28599]

20 **Assersohn L**, Norman A, Cunningham D, Benepal T, Ross PJ, Oates J. Influence of metastatic site as an additional predictor for response and outcome in advanced colorectal carcinoma. *Br J Cancer* 1999; **79**: 1800-1805 [PMID: 10206296 DOI: 10.1038/sj.bjc.6990287]

21 **Catalano V**, Loupakis F, Graziano F, Torresi U, Bisonni R, Mari D, Fornaro L, Baldelli AM, Giordani P, Rossi D, Alessandroni P, Giustini L, Silva RR, Falcone A, D'Emidio S, Fedeli SL. Mucinous histology predicts for poor response rate and overall survival of patients with colorectal cancer and treated with first-line oxaliplatin- and/or irinotecan-based chemotherapy. *Br J Cancer* 2009; **100**: 881-887 [PMID: 19259089 DOI: 10.1038/sj.bjc.6604955]

22 **Sharma N**, Saifo M, Tamaskar IR, Bhuvaneswari R, Mashtare T, Fakih M. KRAS status and clinical outcome in metastatic colorectal cancer patients treated with first-line FOLFOX chemotherapy. *J Gastrointest Oncol* 2010; **1**: 90-96 [PMID: 22811812]

23 **Kim MJ**, Lee HS, Kim JH, Kim YJ, Kwon JH, Lee JO, Bang SM, Park KU, Kim DW, Kang SB, Kim JS, Lee JS, Lee KW. Different metastatic pattern according to the KRAS mutational status and site-specific discordance of KRAS status in patients with colorectal cancer. *BMC Cancer* 2012; **12**: 347 [PMID: 22876814 DOI: 10.1186/1471-2407-12-347]

24 **Kemeny NE**, Chou JF, Capanu M, Gewirtz AN, Cercek A, Kingham TP, Jarnagin WR, Fong YC, DeMatteo RP, Allen PJ, Shia J, Ang C, Vakiani E, D'Angelica MI. KRAS mutation influences recurrence patterns in patients undergoing hepatic resection of colorectal metastases. *Cancer* 2014; **120**: 3965-3971 [PMID: 25155157 DOI: 10.1002/cncr.28954]

25 **Vauthey JN**, Zimmitti G, Kopetz SE, Shindoh J, Chen SS, Andreou A, Curley SA, Aloia TA, Maru DM. RAS mutation status predicts survival and patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. *Ann Surg* 2013; **258**: 619-626; discussion 626-627 [PMID: 24018645 DOI: 10.1097/SLA.0b013e3182a5025a]

26 **Selcukbiricik F**, Bilici A, Tural D, Erdamar S, Soyluk O, Buyukunal E, Demirelli F, Serdengecti S. Are high initial CEA and CA 19-9 levels associated with the presence of K-ras mutation in patients with metastatic colorectal cancer? *Tumour Biol* 2013; **34**: 2233-2239 [PMID: 23625655]

27 **Fakih MG**, Padmanabhan A. CEA monitoring in colorectal cancer. What you should know. *Oncology* (Williston Park) 2006; **20**: 579-587; discussion 588, 594, 596 passim [PMID: 16773844]

28 **Blons H**, Emile JF, Le Malicot K, Julié C, Zaanan A, Tabernero J, Mini E, Folprecht G, Van Laethem JL, Thaler J, Bridgewater J, Nørgård-Petersen L, Van Cutsem E, Lepage C, Zawadi MA, Salazar R, Laurent-Puig P, Taieb J. Prognostic value of KRAS mutations in stage III colon cancer: post hoc analysis of the PETACC8 phase III trial dataset. *Ann Oncol* 2014; **25**: 2378-2385 [PMID: 25294886]

29 **Cejas P**, López-Gómez M, Aguayo C, Madero R, de Castro Carpeño J, Belda-Iniesta C, Barriuso J, Moreno García V, Larrauri J, López R, Casado E, Gonzalez-Barón M, Feliu J. KRAS mutations in primary colorectal cancer tumors and related metastases: a potential role in prediction of lung metastasis. *PLoS One* 2009; **4**: e8199 [PMID: 20020061 DOI: 10.1371/journal.pone.0008199]

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