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World Journal of Gastrointestinal Oncology (*World J Gastrointest Oncol*, *WJGO*, online ISSN 1948-5204, DOI: 10.4251) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJGO*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

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Proteomics approaches for early detection and targeted therapy of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer related mortality worldwide. HCC incidences have increased worldwide though more prevalent in Asia and Africa. Hepatitis B virus and hepatitis C virus infections are mostly responsible of increased number of HCC cases. Biomarkers can help early detection and improve treatment regimen in patients as advanced stage is chemo-refractive with limited treatment options. Potential of proteomics in finding new biomarkers for early detection has been explored more recently. Future developments in this area rely on how efficiently we manage vast amount of data generated by these techniques and speed up the clinical trials to improve patient care.

Key words: Proteomics; Cancer; Biomarker; Hepatocellular carcinoma

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Core tip: Despite ongoing development in treatment for hepatocellular carcinoma (HCC), effective biomarkers for diagnosis and treatment for HCC are not available. Profiling of proteins puts proteomics on the forefront to understand promising new biomarkers and drug targets for HCC. HCC proteome database would be an important step towards identifying tumor associated proteins as potential therapeutic targets in the treatment of HCC.

Khare T, Khare S, Ibdah JA. Proteomics approaches for early detection and targeted therapy of hepatocellular carcinoma. *World J Gastrointest Oncol* 2017; 9(1): 1-3 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v9/i1/1.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v9.i1.1>

Hepatocellular carcinoma (HCC) is most common in

underdeveloped and developing countries^[1,2]. Etiological agents of HCC vary in different part of world. HCC is more common in Asia due to chronic hepatitis B virus (HBV) infections whereas hepatitis C virus (HCV) infection is the major cause of HCC in western countries and Japan. Besides infection, alcoholic fatty liver disease, nonalcoholic fatty liver disease as well as nonalcoholic steatohepatitis also accounts for HCC in developing and developed countries. Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are the risk factors for HCC in China and sub-Saharan Africa. Inherited metabolic diseases, against a background of cirrhosis or without cirrhosis are associated with HCC. Smoking, estrogens, androgens, and thorium oxide (thorotrast) are also associated with HCC. Alcohol is responsible for HCC in both developing and developed countries^[3]. Less common and emerging risk factors also include diabetes and obesity.

Curative surgery is not possible in HCC patients due to late diagnosis and/or advanced underlying liver cirrhosis^[4]. Only limited treatment options such as resection and transplantation, radiofrequency ablation and transarterial chemoembolization with marginal clinical benefits are available for majority of patients. The cytotoxic systemic therapy options usually fail in patients with HCC due to the chemoresistance^[5]. Though most commonly used drug is Doxorubicin^[6], a combination of Sorafenib and Doxorubicin therapy is more beneficial in patients with advanced HCC^[7]. Recent reviews summarized a list of agents for the treatment of HCC^[8,9]. Despite ongoing development in treatment for HCC, effective biomarkers for diagnosis and treatment for HCC are not available.

Alpha-fetoprotein (AFP) is a biomarker, currently used for screening patients at-risk of HCC. AFP is not a good marker as many other liver diseases can also increase blood level of AFP. Furthermore, AFP is not always elevated in early stages of cancer development, when therapy is mostly effective. Other serum markers for HCC have now been identified in addition to AFP. These are, for example, an abnormal prothrombin molecule, des- γ -carboxyprothrombin, cell-surface proteoglycan, glypican-3, glycoprotein, osteopontin, golgi protein 73, microRNA-21, α -1-fucosidase, human telomerase reverse transcriptase, squamous cell carcinoma antigen, and transforming growth factor- β 1. Data suggest that a combination of biomarker may be more effective^[10]. More importantly, nine Food and Drug Administration-approved blood based cancer markers are used to monitor the treatment^[11]. Profiling of proteins puts proteomics on the forefront to understand promising new biomarkers and drug targets for HCC.

HCC proteome database would be an important step towards identifying tumor associated proteins as potential therapeutic targets in the treatment of HCC^[12]. Basic proteomic approaches including 2-dimensional electrophoresis (2DE), reversed-phase high performance liquid chromatography, size-exclusion chromatography, free-flow electrophoresis, capillary electrophoresis, ion-

exchange chromatography along with tools such as MS-based imaging of tissue biopsies, plasmon resonance technique coupled to MS, matrix assisted laser desorption/ionization-time of flight mass spectrometry, surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS), 2DE-LC-MS/MS and laser capture microdissection enable the study of cancer proteomics^[13]. Most significant advancement in 2DE is two-dimensional difference gel electrophoresis (2D-DIGE), with greater sensitivity and dynamic range due to use of fluorescent cyanine dyes (Cye2, Cye3, and Cye5)^[14]. Analysis of HBV related HCC tumor and non-tumor tissues using 2D-DIGE revealed increased expression of heat-shock proteins (hsp70, hsp90) and heterogeneous nuclear ribonucleoproteins (C1 and C2) as tumor biomarkers^[14].

Bioinformatics is essential for proteomic analyses. The Human Proteome Organization (HUPO) has developed the standards for experimental strategies and data exchange^[15]. An open basic XML (extensible markup language) representation of MS data, named mzXML, helped accelerate data management, interpretation and dissemination using different instrumentation platforms^[16]. Commercial tools available to the proteomics community to analyze two-dimensional electrophoresis protein patterns include Delta2D (Decodon), BioNumerics 2D (Applied maths), Melanie (GeneBio), Imagemaster 2D (GE healthcare), Progenesis Samespots (NonLinear Dynamics), PDQuest (BioRad Laboratories), REDFIN (Ludesi), ProteinMineTM (Scimagix), and the Z3:2D-Gel image Analysis System (Compugen Limited).

Global analysis of proteins faces several challenges, for example, tertiary structure of proteins, detection of low abundance proteins and reversible modifications such as glycosylation and phosphorylation when compared to studies of genes and transcripts. Furthermore, RNA splicing can produce splice variants that are homologous but differ in function. The revolution in the field of proteomics can hopefully overcome some of these hurdles. In last forty years, only few new tests have been added in clinics. A four-way collaboration is required between the research laboratory (for developing the fundamental concept), the diagnostic lab or industry (converting the concept into a hands-on reliable tool), the clinical laboratory (assessing the tool in real life practices), and the clinicians (providing clinical specimens) for bringing a biomarker from the research lab successfully into the clinical practice^[17].

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New therapeutic approaches to metastatic gastroenteropancreatic neuroendocrine tumors: A glimpse into the future

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Abstract

Neuroendocrine (NE) gastroenteropancreatic tumors are a heterogeneous group of neoplasias arising from neuroendocrine cells of the embryological gut. Their

incidence have increased significantly over the past 3 decades probably due to the improvements in imaging and diagnosis. The recent advances in molecular biology have translated into an expansion of therapeutic approaches to these patients. Somatostatin analogs, which initially were approved for control of hormonal syndromes, have recently been proven to inhibit tumor growth. Several new drugs such as antiangiogenics and others targeting mammalian target of rapamycin pathways have been approved to treat progressive pancreatic neuroendocrine tumors (NETs) although their role in non-pancreatic is still controversial. The treatment of NETs requires a coordinated multidisciplinary approach. The management of localized NETs primarily involves surgical resection followed by surveillance. However, the treatment of unresectable and/or metastatic disease may involve a combination of surgical resection, systemic therapy, and liver-directed therapies with the goal of alleviating symptoms of peptide release and controlling tumor growth. This article will review the current therapeutic strategies for metastatic gastroenteropancreatic NETs and will take a glimpse into the future approaches.

Key words: Gastroenteropancreatic neuroendocrine tumors; Peptide receptor radionuclide therapy; Somatostatin analogs; Octreotide; Transarterial chemoembolization; Carcinoid syndrome; Setotinin; Chromogranin

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Core tip: The management of localized NETs is straight forward, however, the treatment of advanced tumors involves several disciplines and requires a coordinated multidisciplinary approach. Recent advances in molecular biology have expanded the therapeutic arsenal. Somatostatin analogs, initially approved for control of hormonal syndromes, have recently proven to inhibit tumor growth. Several new drugs, antiangiogenics,

mTOR inhibitors have been tested with promising results and some of them have already been approved. Several trials are still under way but the future should focus on patient selection, predictive markers, and tolerability improvement as critical aspects to continue advancing.

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INTRODUCTION

Neuroendocrine (NE) gastroenteropancreatic tumors are a heterogeneous group of neoplasias arising from neuroendocrine cells of the embryological gut^[1]. Their incidence have increased significantly over the past 3 decades with a crude incidence of 5.25/100000 per year. This is probably due to the improvements in imaging and diagnosis^[1-4].

Usually, the primary lesion is located in the gastric mucosa, the small and large intestine, rectum and pancreas^[2,3]. These tumors can appear at all ages, but the highest incidence is after the fifth decade. The carcinoid of the appendix is an exception as its highest incidence is at around 40 years of age^[1]. Those patients with multiple endocrine neoplasia type 1 or von Hippel-Lindau's disease, may have a clinical onset 15-20 years earlier than patients with sporadic neuroendocrine tumors (NETs)^[5].

The recent advances in molecular biology have translated into an expansion of therapeutic approaches to these patients. Somatostatin analogs, which initially were approved for control of hormonal syndromes, have recently been proven to inhibit tumor growth^[6].

Several new drugs such as antiangiogenics and others targeting mammalian target of rapamycin (mTOR) pathways have been approved to treat progressive pancreatic NETs although their role in non-pancreatic is still controversial^[7].

The treatment of NETs requires a coordinated multi-disciplinary approach. The management of localized NETs primarily involves surgical resection followed by surveillance. However, the treatment of unresectable and/or metastatic disease may involve a combination of surgical resection, systemic therapy, and liver-directed therapies with the goal of alleviating symptoms of peptide release and controlling tumor growth^[7].

Several completed and ongoing studies are evaluating somatostatin analogs (SSAs), vascular endothelial growth factor (VEGF) pathway inhibitors, mTOR inhibitors, cytotoxic chemotherapy, and peptide receptor radionuclide therapy (PRRT)^[7].

This article will review the current therapeutic strategies for metastatic gastroenteropancreatic NETs

and will take a glimpse into the future approaches.

MANAGEMENT OF ADVANCED NETS

NETs present in up to 40% of cases with metastases at diagnosis (mainly in the liver). If metastatic disease is localized or if > 70% of tumor burden can be resected, cytoreductive surgery should be considered. This approach has shown to reduce local symptoms and also systemic endocrine symptoms^[8].

NETs can arise in different organs and from different cell types, and so present a clinical challenge due to their diversity and the variety of symptoms they can cause. Functioning NETs are characterized by the hormones they produce and/or the symptoms they cause; these tumors usually produce clinical symptoms following dissemination to the liver^[8,9].

Carcinoid syndrome

Many functioning NETs release vasoactive peptides and amines (such as serotonin and tachykinins), into the systemic circulation. These can cause a group of symptoms known as "carcinoid syndrome", which appear in 10% of cases of metastatic NETs. This syndrome is characterized by flushing, diarrhea, abdominal pain, telangiectasia and bronchoconstriction^[8,9]. Carcinoid crisis are believed to be caused by a massive release of bioactive products from the tumor and can occur spontaneously or more frequently after stress, chemotherapy, surgery or anesthesia. These episodes are life-threatening^[10]. The clinical picture represents an exacerbation of the usual symptoms of carcinoid syndrome, including severe flushing with/without bronchospasm, tachycardia and hypo/hypertension^[10].

This needs prompt and effective management to prevent any carcinoid heart disease, though 10%-20% of patients suffer from this issue at diagnosis^[11]. This is characterized by fibrous thickening of the endocardium (classically on the right heart)^[12], tricuspid and pulmonary valves^[12].

Other syndromes

Pancreatic NETs can cause several other syndromes, such as Zollinger-Ellison syndrome, which is characterized by peptic ulcers, diarrhea and abdominal pain and caused by gastrinomas. Glucagonomas which produce hyperglycemia, leading to diabetes mellitus and also a chronic necrolytic migratory erythema. Insulinomas cause hypoglycemia and VIPomas a Verner-Morrison syndrome with severe watery diarrhea (10-15 L/d) and flushing^[13].

Nonfunctioning NETs

These are not associated with hormonal syndromes, thus they become more difficult to diagnose and patients present with advanced disease. Anyway, these tumors may secrete bioactive hormones or amines at subclinical levels^[13].

Table 1 Systemic treatment

Ref.	Type of tumor	Treatment	RR %	PFS (mo)	OS (mo)
Moertel <i>et al</i> ^[41]	Pancreatic	STZ	42		16.5
		STZ + 5FU	42		26
Moertel <i>et al</i> ^[39]	Poorly differentiated	CIS + ETO	18		19
Chan <i>et al</i> ^[58]	Carcinoid	TMZ + beva	0		18.8
	Pancreatic		33		41.7
Yao <i>et al</i> ^[72]	Pancreatic	Everolimus		9.7	
		Everolimus + octreotide		16.7	
Yao <i>et al</i> ^[74]	Midgut carcinoid	Everolimus + octreotide		16.4	
		Octreotide		11.3	
Yao <i>et al</i> ^[78]	Pancreatic	Everolimus	34		
		Placebo	9		
Yao <i>et al</i> ^[80]	Lung/GI NETs	Everolimus		11	
		Placebo		3.9	
Ahn <i>et al</i> ^[84]	Carcinoid	Pazopanib	0		
	Pancreatic		21.9		
Kulke <i>et al</i> ^[81]	Pancreatic	Sunitinib		11.4	
		Placebo		5.5	

PFS: Progression-free survival; STZ: Streptozocin; CIS: Cisplatin; ETO: Etoposide; 5FU: 5-fluorouracil; TMZ: Temozolomide; beva: Bevacizumab.

SOMATOSTATINE ANALOGS: PAST, PRESENT AND FUTURE

Most NETs express G-protein-coupled transmembrane somatostatin receptors (SSTRs)^[14]. There are five subtypes of SSTRs, and different NETs have different proportions of receptors expression^[7] (Table 1).

Somatostatin analogs bind to G-protein-linked receptors on the cell surface and inhibits the release of NE hormones. However, somatostatin has a short half-life *in vivo* (< 3 min)^[7] and therefore, synthetic somatostatin analogs have been developed for NET symptom control. These analogues form the first-line medical step for well-differentiated NETs^[3,15,16].

They bind with high affinity to the five SSRT (sstr₁₋₅) on secretory NE cells^[3,16,17], which have different inhibitory effects in the body. Subtypes sstr₂ and sstr₅ are the most important in inhibiting hormonal secretions in functioning NETs, thus dual inhibition of both may have a higher inhibitory benefit^[3,16,17]. These two subtypes may also mediate antiproliferative effects^[7]. Octreotide and lanreotide bind to the SSTR and decreased hormonal secretion, growth and proliferation, increased apoptosis, inhibit protein synthesis and have a direct antiproliferative activity^[17,18].

There is evidence that octreotide controls severe diarrhea and flushing in carcinoid syndrome^[14,19].

It has long been suggested that somatostatin analogs may exert antitumor effects for NETs^[20,21]. Moreover, there may inhibit the release of growth factor and trophic hormones, angiogenesis and modulation of the immune system.

Octreotide is the first somatostatin analogue available commercially, and it is a sstr₂-preferring agonist, although it has also moderate affinity for sstr₃ and sstr₅^[22,23]. It has a much longer half-life than somatostatin (2 h).

Lanreotide was the second analogue available and

has a similar binding profile to octreotide.

Octreotide was introduced in clinical practice in the 1987 as it confirmed ability to palliate carcinoid syndrome, as well as other hormonal syndromes caused by metastatic gastroenteropancreatic NETs. Several clinical trials of SSAs tested their ability to inhibit the release of NE hormones such as serotonin, glucagon, insulin, gastrin and vasoactive intestinal peptide (VIP)^[14].

Survival rate at 5 years of 67% have been reported in patients receiving somatostatin analogues compared with 18% for historical controls^[3].

Several years after the approval of octreotide, evidence of its antineoplastic activity emerged. Although objective radiographic responses (ORR) were rare, many cases of prolonged stable disease (SD) were documented, leading to the hypothesis that SSAs exert an inhibitory effect on tumor growth^[24-27].

Recently, this has been tested in a phase III trial. Initial evidence demonstrating that octreotide can reduce symptoms of carcinoid syndrome and decrease 5-HIAA levels was shown with the subcutaneous formulation^[28].

The first controlled study of octreotide LAR for treating carcinoid syndrome was conducted in 93 patients with NETs over at least 20 wk^[29].

There was a significant decrease in the number of daily stools and incidence of flushing. Treatment success was obtained in 66% of patients receiving octreotide LAR 10-30 mg/mo. It also decreased 5-HIAA levels by 50%^[29].

This study demonstrated that monthly octreotide LAR was at least as effective as subcutaneous octreotide for symptom control. Its efficacy for the symptomatic and biochemical control in NETs have subsequently been demonstrated in other studies^[21,22].

The mechanism by which somatostatin analogues normalize bowel function is not clear, however, it is hypothesised that involves inhibition of gut hormone

secretion, lengthening of intestinal transit time, increased water and electrolyte absorption and reduced splanchnic blood flow^[23-26]. Treatment with octreotide improves survival in patients with carcinoid crisis^[27]. Therefore, its prophylactic use is mandatory to prevent the development of a crisis. It is generally well tolerated, being the most common side effects, abdominal discomfort and bloating, generally mild and resolve spontaneously within the first week^[27].

Gallstones can develop, although only a small proportion of patients develop clinical symptoms. Local pain at the injection site has also been reported^[27].

A second somatostatin analog, lanreotide, was licensed in Europe in 1998 for the treatment of symptoms associated with NETs (particularly carcinoid).

Lanreotide is less widely studied than octreotide for symptomatic and biochemical control and no directly comparative trials have been conducted. The effects of lanreotide on symptom relief are comparable with those of octreotide^[28].

Ruszniewski *et al*^[29] carried out a study with 71 patients who received lanreotide for 6 mo and reported that 65% of the patients documented a 50% or greater reduction in flushing episodes, and 18% had a 50% or greater reduction in diarrhea episodes. The biochemical response rate is similar to octreotide, with higher responses in patients naive to somatostatin analogue therapy^[30].

Somatostatin analogs have got minimal adverse effects and have demonstrated antiproliferative activity *in vitro*^[23].

These have been used for patients with metastatic disease when surgical cure is not possible and have been also indicated for the relief of symptoms in patients with functionally active NETs^[31].

It has been controversial if somatostatin analogs control the growth of well-differentiated metastatic NETs. Uncontrolled studies have shown tumor shrinkage in response to somatostatin analogs^[32] and their combination with interferon alfa^[18].

Later trials were only able to confirm tumor stabilization in up to 50% of patients, but these studies were not placebo controlled^[30-35].

In 2009, Panzuto *et al*^[36] carried out a prospective, phase IIIB, double-blind, placebo-controlled trial to check the effect of octreotide LAR in the control of tumor growth in patients with well-differentiated metastatic midgut NETs. Treatment-naïve patients were randomly assigned to either placebo or octreotide LAR 30 mg intramuscularly in monthly intervals until tumor progression or death.

The primary end point was time to tumor progression. Most patients (75%) had evidence of somatostatin receptor expression as evidenced by radiotracer uptake on Octreoscan. Thirty-eight percent had carcinoid syndrome (flushing and/or diarrhea associated with elevation in urine 5-HIAA). Only patients with mild carcinoid syndrome who tolerated flushing without intervention or responded to treatment with loperamide

and/or cholestyramine in cases of diarrhea were included. The trial showed a median time to tumor progression of 14.3 mo in the octreotide LAR compared to 6 mo in the placebo arm (HR = 0.34; 95%CI: 0.20-0.59, $P = 0.000072$). Functionally active and inactive tumors responded similarly. Chromogranin A or age did not make any impact on the result either. At 6 mo, tumor progression was seen in 24% of patients on the octreotide LAR arm vs 66% of patients receiving placebo ($P = 0.0079$)^[36].

Serious adverse events were balanced (11 patients in the octreotide LAR 30 mg arm and 10 patients in the placebo arm).

The most favorable effect was observed in patients with low hepatic tumor load (10%) and resected primary tumor, however both of these subgroups contained the majority of study patients. Even patients with higher hepatic tumor burden (> 10%) experienced a near doubling in time to progression on the octreotide LAR arm^[36].

The small number of deaths in both treatment arms (seven in the octreotide LAR 30 mg arm; nine in the placebo arm) precluded any analysis of differences in survival.

Authors concluded that Octreotide LAR significantly increased the time to tumor progression in patients with functionally active and inactive metastatic midgut NETs.

This PROMID trial unfortunately does not clarify the appropriate timing for the treatment, either at initial diagnosis or at the moment of tumor progression or if these data can be extrapolated to patients with G2 NETs^[36].

No major differences in classical efficacy have been seen between octreotide and lanreotide^[36,37].

One study has evaluated the antiproliferative efficacy of lanreotide in 25 patients. They found partial tumor remission in one patient and stable disease in seven patients, whereas tumor progression occurred in 14.

The CLARINET study is a randomized, double-blind, placebo-controlled study of lanreotide in advanced, well or moderately differentiated, non-functioning, SSTR-positive NETs (Ki-67 < 10%)^[38]. Tumors could be in the pancreas, midgut, or hindgut or unknown origin. Patients were randomized to receive an lanreotide 120 mg or placebo every 28 d for 24 mo. The primary end point was progression-free survival (PFS). Secondary end-points were overall survival (OS), quality of life and safety. Thirty-three percent had liver tumor > 25%. Lanreotide significantly prolonged PFS (median not reached vs median of 18.0 mo, $P < 0.001$). The estimated rates of PFS at 24 mo were 65.1% and 33.0% for the lanreotide and the placebo group respectively.

There were no significant differences in quality of life or OS between-groups. The most common adverse effect was diarrhea (26% vs 9% for lanreotide and placebo respectively)^[36].

The somatostatin analogue should be the first

approach for Grade 1 and 2 gastrointestinal NETs^[39].

Predictive factors for no-response to somatostatin analog are Ki67 > 5%^[39] and distant extra-hepatic metastasis. In these situations chemotherapy should be considered alternatively^[39].

SOM230 (Pasireotide) is a novel multireceptor ligand analogue that has high affinity for four of the five somatostatin receptor SSTR (sstr₁, 2, 3 and sstr₅); it has 40-fold higher affinity and 158-fold higher functional activity for sstr₅ than octreotide^[37,38].

CHEMOTHERAPY: PAST, PRESENT AND FUTURE

Responses to chemotherapeutics are extremely heterogeneous in gastroentero-pancreatic NETs. These responses are influenced by tumor differentiation/grade and primary site. Poorly differentiated gastroenteropancreatic NETs respond typically to platinum-based regimens, and the reported RR > 50%^[40].

Though recent data point to the relevance of proliferative rate (Ki-67) as higher proliferative levels (> 55%) are significantly linked to higher response to platinum/etoposide compared with high-grade tumors with lower rates of proliferative activity^[40].

Pancreatic NETs are sensitive to alkylating agents, including streptozocin, dacarbazine, and temozolomide, as well as fluoropyrimidines. Streptozocin showed response rates of 63% in combination with fluorouracil vs 36% in monotherapy^[41].

When combined with doxorubicin vs streptozocin + 5-FU, the response rates and time to progression benefited the first combination (69% and 20 mo vs 45% and 6.9 mo respectively)^[42]. However, radiographic assessment was not accurate and this fact makes difficult to draw final conclusions about the efficacy of streptozocin.

Unfortunately the use of streptozocin is limited due to its toxicity such as myelosuppression, nausea, and renal insufficiency.

But the role of chemotherapy in NETs has evolved in recent years. It represents a useful option mainly for symptomatic patients, progressive disease, G2 differentiation, and a more aggressive behavior. It also should be considered when the primary objective is tumor load reduction for bulky lesions.

Single agents such as fluorouracil, dacarbazine, doxorubicin and streptozotocin were initially assessed in midgut carcinoid tumors with little benefit^[43]. Therefore these monotherapies could be reserved to pretreated patients or for patients with a poor performance status. In fact midgut NETs are particularly chemoresistant, possibly due to their low proliferative activity as well as their high expression of methyl guanine methyl transferase (MGMT), which is a DNA repair enzyme^[44]. For many years there was no evidence that combination regimens were any more effective. None of the regimens demonstrated a response rate (RR) greater than

15%^[45]. Though recently this has changed. Combination of chemotherapy and IFN- α therapy does not appear to improve on the results of monotherapy^[46,47].

Pancreatic NETs RR of approximately 40% have been reported for streptozotocin in combination with other agents such as 5-fluorouracil, cisplatin or doxorubicin^[22,45]. Temozolomide has also demonstrated promising anti-tumor effects in pancreatic NETs^[48]. Kulke *et al*^[49] carried out a phase II trial of temozolomide and thalidomide in patients with metastatic NETs. This combination was associated with a biochemical (chromogranin A) response of 40%, and radiologic response of 25% (45% among pancreatic NETs, 33% among pheochromocytomas, and 7% among carcinoid tumors). Median duration of response was 13.5 mo, 1-year survival was 79%, and 2-year survival was 61%. The response rate seems to be related to the expression of 06-MGMT. Low expression gives a higher response rate (40%) vs high expression (0%).

Orally administered temozolomide and thalidomide seems to be an active regimen for the treatment of NETs. This regimen appeared more active in pancreatic NETs than in carcinoid tumors^[49].

Saif *et al*^[50] carried out a retrospective study of capecitabine and temozolomide (CAPTEM) in patients with metastatic pancreatic NETs who have failed prior therapies (long-acting release octreotide, chemotherapy and hepatic chemoembolization). Seven patients were treated, and authors reported a total response rate of 43%, and clinical benefit (responders and stable disease) 71%. The median duration of response was 8 mo and the most common toxicities were grade 1-2 neutropenia, fatigue and hand-foot syndrome.

Authors concluded that CAPTEM was well tolerated and further prospective studies are warranted to evaluate this regimen with targeted therapies in pNETs^[50].

Recently Ramirez *et al*^[51] reported the results of another study reviewing the CAPTEM regimen again but in a wide variety of metastatic NETs. Twenty nine patients were included, small bowel (31%), pancreas (52%), lung (10%), and rectum (7%)^[51].

Partial response was documented in 17% and stable disease in 48%. According to Ki-67 values, partial response (PR)/stable disease (SD) were noted in 13/63% if Ki-67 < 2%^[51]. Values 2%-20%, PR/SD 19%/50%. If Ki-67 > 20% PR/SD were 20% each. Authors reported a median PFS of 12 mo. They concluded that this regimen may prolong survival although prospective data are needed. Although adverse reactions were experienced, most patients tolerated this regimen, thus CAPTEM should be considered as a reasonable option for metastatic NET patients^[50].

A phase II study carried out by Claringbold *et al*^[52] assessed the role of the radiopeptide ¹⁷⁷Lu-octreotate and capecitabine as a treatment for progressive disseminated NETs. Thirty-three patients were included to receive four cycles of 7.8 GBq (¹⁷⁷Lu)-octreotate 8-weekly, with 14 d of capecitabine.

Twenty-four percent showed PR, 70% SD. Median PFS and median OS had not been reached at a median follow-up of 16 mo with the survival at 1 and 2 years 91% and 88% respectively. Minimal transient myelosuppression with one grade 3 thrombocytopenia but no neutropenia were seen and nephrotoxicity was absent.

The addition of capecitabine radiosensitizing chemotherapy did not increase the minimal toxicity of ¹⁷⁷Lu-octreotate and led to significant clinical benefit in terms of response and SD in patients with progressive metastatic NETs^[52].

A phase I-II study to assess the safety and efficacy of combining lutetium-177 octreotate with capecitabine/temozolomide in advanced low-grade NETs was published in 2012. Thirty-five patients received fixed activities of 7.8 GBq lutetium-177 octreotate each 8 wk, with capecitabine for 4 cycles^[53].

In phase I, successive cohorts of patients received escalating doses of temozolomide in the last 5 d of each capecitabine cycle^[54].

In phase II, patients were treated with 200 mg/m² temozolomide. Adverse events were mild to moderate. Complete response was achieved in 15%, PR 38%, SD 38%. Median PFS was 31 mo and median OS was not reached with 90% surviving at 24 mo. Response rates were higher in patients with gastropancreatic NETs than in those with bowel primaries. This study showed that lutetium-177 octreotate in combination with capecitabine and temozolomide was well tolerated in patients with advanced low-grade NETs with significant tumor control rates^[55].

Temozolomide, an oral analog of dacarbazine, has activity against NETs when administered alone or in combination with other agents.

A systematic review of temozolomide in advanced NETs has been published by Abdel-Rahman *et al*^[54] in 2015. These authors assessed 16 trials including 348 patients. Median PFS reported ranged from 6 to 31 mo. Disease control rate 65%-100%. They found that most frequent toxicities were leukopenia, lymphopenia and elevated transaminases.

The data suggested that temozolomide-based combinations with some antineoplastic agents (especially capecitabine) could be an effective treatment for advanced low-intermediate grade NETs^[54,55].

NEW AGENTS: PRESENT BUT LOOKING MORE INTO THE FUTURE

NETs are highly vascularised tumours that express high levels of the VEGF ligand together with its receptor VEGFR. These tumors may show 30%-40% RR to combination chemotherapies but the response to single-agents is only 10%^[55].

Bevacizumab

Tyrosine kinase inhibitors targeting the VEGF receptor

and bevacizumab, a monoclonal antibody targeting VEGF, have demonstrated activity in NETs.

Bevacizumab has been shown to induce objective tumour responses and improvement in median time to progression in advanced carcinoid tumours^[56,57].

Several studies have found that temozolomide had significant effect on NETs.

A previous report examining a variety of NETs suggested that the combination of bevacizumab and temozolomide can be safely administered and showed promising activity in patients who had progressed after prior treatments^[57].

A phase II study evaluating the same combination in advanced/metastatic NETs was carried out including 34 patients with carcinoid and pancreatic NETs. All patients received prophylaxis against *Pneumocystis carinii* and varicella zoster. The combination of temozolomide and bevacizumab was associated with grade 3-4 toxicities, including lymphopenia (53%) and thrombocytopenia (18%)^[58].

Although overall radiographic response rate was 15%, response rates were different between pancreatic NETs (33%) and carcinoids (0%). The median PFS was 11 mo (14.3 mo for pancreatic NETs vs 7.3 mo for carcinoid tumors). Median OS was 33.3 mo (41.7 mo for pancreatic NETs vs 18.8 mo for carcinoid tumors). Authors concluded that this combination could be safely administered and seemed to be promising in pancreatic NETs^[59].

Koumarianou *et al*^[60] had carried out a similar study where temozolomide was delivered continuously at 100 mg daily, a so-called metronomic schedule, together with bevacizumab 7.5 mg/kg once every 3 wk and somatostatin long-acting release 30 mg once every 4 wk. The number of patients with carcinoids was small but authors found occasional durable responses. In their comment published in JCO 2013, these authors suggested the the necessity of further studies with larger numbers of patients to be able to identify who those patients are.

This combination seems to be an important approach as it uses treatments with possibly direct antiangiogenic action on the endothelial cells together with an antibody that blocks the action of VEGF produced by the tumor cells. And therefore, this dual antiangiogenic activity may prove to be an efficacious therapy in NETs which are highly vascularized tumors^[11].

Several combinations with bevacizumab have been studied with different results^[61-66].

These approaches are mainly effective in G1 and G2 tumors, with a Ki-67 < 20%. However, it is relevant to identify the patients who will most probably benefit from this approach. Koumarianou *et al*^[60,67] proposed that this combination should be restricted to advanced NET G1/2 tumors, possibly with a Ki-67 < 20%.

mTOR inhibitors

mTOR is a key regulator of protein synthesis in cancer,

cell growth, proliferation, angiogenesis and cell metabolism. Abnormal PI3K-Akt/PKB-mTOR pathway signaling has been implicated in the pathogenesis of pancreatic NETs.

Everolimus, or RAD001 is an oral, once-daily mTOR inhibitor that blocks the mTOR pathway by binding to its intracellular receptor, FKBP-12. It has shown synergistic anti-tumor activity when combined with other anticancer therapies^[68,69].

In a phase III study, patients with low- and intermediate-grade advanced pancreatic NETs were randomized to receive everolimus 10 mg/d or placebo. Median PFS was significantly prolonged in the everolimus arm, 11 mo vs 4.6 mo^[70].

Everolimus may have a similar effect when used in combination with a somatostatin analogue. In the study by Grozinsky-Glasberg *et al*^[71], octreotide and everolimus showed significant anti-proliferative effects and they suggested that everolimus could interact with the same pathway at a site or sites similar to octreotide.

A study to assess the antiproliferative effect of combining everolimus with octreotide in patients with metastatic low to intermediate grade NETs was carried out. It enrolled 60 patients. Authors found promising activity in those receiving everolimus 10 mg daily^[72,73].

A Phase II trial of everolimus with or without octreotide LAR in patients with advanced pancreatic NETs following chemotherapy failure (RADIANT-1) found that in those receiving everolimus monotherapy, median PFS was 9.7 mo. PR 9.6%, 67.8% SD and 13.9% showed progressive disease. In the combination arm, median PFS was 16.7 mo, 4.4% PR, 80% SD, and no patients with progressive disease^[74]. Authors found that an early CgA or NSE response was associated with a longer PFS compared with those without an early response^[74]. Most adverse events were mild to moderate.

A Phase III trial, RADIANT-2, was carried out in advanced (unresectable locally advanced or distant metastatic and disease progression within the past 12 mo) midgut carcinoid tumors with low-grade or intermediate-grade NETs (carcinoid). It compared everolimus 10 mg/d plus octreotide LAR 30 mg every 28 d with placebo and octreotide LAR every 28 d. Four hundred and twenty-nine patients were randomly assigned to study groups. The combination arm showed a median PFS of 16.4 mo vs 11.3 mo for the control arm ($P = 0.026$). This did not meet a prespecified significance level by central review ($P = 0.024$)^[75].

However, by an investigator review the median PFS was 12.0 mo for the combination arm and 8.6 mo for the control arm ($P = 0.018$).

Authors concluded that everolimus plus octreotide LAR, compared with placebo plus octreotide LAR, improved PFS in advanced NETs associated with carcinoid syndrome^[76].

Both everolimus and temozolomide are associated with single-agent activity in patients with pancreatic NETs.

A phase I-II study was performed to evaluate the safety and efficacy of temozolomide in combination with everolimus in advanced pancreatic NET. Patients received temozolomide 150 mg/m² per day on days 1 through 7 and days 15 through 21 in combination with everolimus daily in each 28-d cycle.

In cohort 1, everolimus as administered at 5 mg daily. In cohort 2 it was increased to 10 mg daily. Temozolomide was administered for 6 mo^[77]. Forty-three patients were enrolled. No synergistic toxicities were reported. Forty percent had PR. The median PFS was 15.4 mo. Median OS was not reached. Authors concluded that this regimen could be safely given to advanced pancreatic NETs with significant antitumor activity^[77].

RADIANT-3 trial is another phase III prospective, double-blind, randomized, placebo-controlled study carried out in patients with advanced, low or intermediate grade pancreatic NETs. Patients were randomised to receive everolimus 10 mg daily or placebo. Four hundred and ten patients were included. The median PFS was 11.0 mo with everolimus and 4.6 mo with placebo ($P < 0.001$). Estimates of the proportion of patients who were alive and progression-free at 18 mo were 34% with everolimus as compared with 9% with placebo^[78].

Adverse events were mostly grade 1 or 2, mainly stomatitis rash, diarrhea, fatigue and infections primarily upper respiratory. Grade 3 or 4 included anemia and hyperglycemia.

Everolimus significantly prolonged PFS among patients with progressive advanced pancreatic NETs and was associated with a low rate of severe adverse events.

Mature data showed a median OS of 44.02 mo in the everolimus arm compared with 37.68 mo in the placebo; However, a high crossover of patients from placebo to everolimus (85%) may have contributed to the long median OS in the placebo arm and may have confounded the ability to detect a difference in the overall survival results^[79].

RADIANT-4 is another phase III study assessing the efficacy and safety of everolimus compared with placebo in patients with advanced, progressive, well-differentiated, non-functional NETs of the lung or gastrointestinal tract^[80]. Patients were randomised to receive everolimus 10 mg per day or placebo. Three hundred and two patients were enrolled. Median PFS was 11 mo in the everolimus group and 3.9 mo in the placebo arm. Everolimus was associated with a 52% reduction in the estimated risk of progression. In the first pre-planned interim OS analysis, the results of everolimus showed a reduction in the risk of death, although not statistically significant. The safety findings were consistent with the known side-effect profile of everolimus.

Authors concluded that everolimus is the first targeted agent to show robust anti-tumour activity with acceptable tolerability across a broad range of NETs (pancreas, lung, and gastrointestinal tract)^[80].

Sunitinib

NETs express VEGF and its receptor VEGFR. Sunitinib malate, an oral multi-targeted tyrosine kinase inhibitor targets VEGFR-1, -2, and -3; platelet-derived growth factor receptor; and c-KIT. Sunitinib is currently approved for treatment of pancreatic NETs. Its toxicity profile includes diarrhea, fatigue, cytopenias, nausea, hypertension, and palmar-plantar erythrodysesthesia. The efficacy of sunitinib was assessed in a two-cohort, phase II study of advanced carcinoid and pancreatic NETs. Patients were treated with repeated 6-wk cycles of oral sunitinib (50 mg/d for 4 wk, followed by 2 wk off treatment). The trial showed an overall response rate of 2.4% and 16.7% in patients with carcinoid tumors and pancreatic NETs, respectively. Median time to tumor progression was 7.7 mo in pancreatic NETs and 10.2 mo in carcinoid. The authors concluded that sunitinib has antitumor activity in pancreatic NETs whereas its activity against carcinoid tumors could not be definitively determined^[81].

A phase III randomized, double-blind trial in low and intermediate grade pancreatic NETs with sunitinib 37.5 mg/d orally or placebo showed. PFS of 11.4 mo vs 5.5 mo in the sunitinib and placebo arms respectively was statistically significant^[82].

A phase II study testing the efficacy and safety of everolimus and octreotide LAR or everolimus, bevacizumab, and octreotide LAR in advanced pancreatic NET with evidence of progression was presented at ASCO annual conference 2015. PFS was 16.7 mo on everolimus + bevacizumab + octreotide LAR vs 14 mo for everolimus + octreotide LAR. Response rate 31% in triplet arm vs 12% in doublet. Toxicity was significantly higher on the triplet with 81% grade 3-4 adverse events vs 49% on the doublet. Investigators commented on promising results but future trials to learn about patients selection are warranted^[82].

Pazopanib

Pazopanib is an orally bioavailable, multitargeted kinase inhibitor that inhibits VEGF receptors 1, 2, and 3. It has been evaluated in a nonrandomized phase II study of 37 patients with gastroenteropancreatic NETs. The overall response rate was 24% with a median PFS of 9.1 mo^[83].

The PAZONET study is another phase II which showed clinical activity of pazopanib in patients with advanced NETs regardless of previous treatments. Authors suggested that circulating tumor cell counts and soluble *VEGFR2* and *VEGFR3* gene polymorphisms could be potential biomarkers for selecting patients for pazopanib^[84].

Another phase II study in metastatic or locally advanced grade 1-2 carcinoid tumours or pancreatic NETs, using pazopanib 800 mg orally once per day and octreotide showed a response of 21.9% in pancreatic NETs whereas no responses in carcinoid tumours^[85].

Based on all these results, a randomized phase III trial

of pazopanib vs placebo for advanced carcinoid tumors is ongoing and other phase III trials are warranted^[86].

There are few effective therapies for pancreatic NETs. Recent placebo-controlled phase III trials of everolimus and sunitinib have reported improved PFS. Preclinical studies have suggested enhanced antitumor effects with combined mTOR and VEGF pathway-targeted therapy. A phase II trial was carried out with a combination of temsirolimus 25 mg intravenously (iv) once per week and bevacizumab 10 mg/kg iv once every 2 wk in well or moderately differentiated pancreatic NETs and progressive disease^[87]. Fifty-eight patients were enrolled, response rate was 41%, PFS at 6 mo was 79% with median PFS 13.2 mo. Median OS 34 mo. The investigators concluded that this combination had shown significant activity with acceptable toxicity in pancreatic NETs with progressive disease^[87].

Interferon

Interferon therapy is generally recommended as a second-line in patients with functioning NETs and low proliferation^[28-30]. The benefits of interferons on symptom control is similar to that of somatostatin analogues but they may have higher antiproliferative activity^[30]. Unfortunately their safety profile is not as favourable, with fever, fatigue, anorexia and weight loss among others^[29,30].

IFN- α has shown in a pooled analysis of trials in patients with NETs a 40% of biochemical responses (similar to octreotide and lanreotide) with 10% of objective tumor responses^[9,29,30,87,88].

Bondanelli *et al*^[89] have suggested that a combination of IFN- α with somatostatin analogues might have a synergistic effect. A phase II prospective trial randomized 44 patients to receive bevacizumab or pegylated IFN- α for 18 wk, followed by both agents in combination. At the end of the single-agent administration period, the rate of PFS was 95% in the bevacizumab arm vs 68% in the IFN- α arm. This study demonstrated activity with bevacizumab in patients with carcinoid tumor^[56].

A phase III trial comparing bevacizumab vs IFN- α showed that a combination with bevacizumab obtained longer time to failure compared to IFN- α arm. Responses were also higher with bevacizumab. However, it did not meet its primary endpoint of improvement in PFS.

Participants had advanced NETs with poor prognosis, as defined by one or more of the following criteria: Progressive disease, G2 with 6+ lesions, colorectal or gastric primaries.

Toxicity was higher on the interferon arm with 26% grade 3-4 fatigue. Based on these results, the investigators concluded that neither bevacizumab nor IFN α -2b arm should be used as standard treatment^[90].

LIVER-DIRECTED THERAPIES

NETs present in up to 40% of cases with metastases at

diagnosis (liver mainly). Although radical surgery would be the treatment of choice, however, it is generally not possible. Liver resection is generally advocated in those cases with limited hepatic disease in which more than 90% of tumors can be successfully resected or ablated^[91].

Patients with liver metastases may experience symptoms such as pain, anorexia, and weight loss related to tumor burden. Additional symptoms include flushing and diarrhea caused by secretion of hormones directly into the systemic circulation. Medical treatments and locoregional therapies are palliative approaches in symptomatic patients or in cases of progressive disease. As the liver metastases from NET are hypervascular, endovascular treatments are interesting^[92,93] too as a cytoreductive technique.

Overall hepatic-directed therapies include liver resection or ablation, hepatic artery embolization (transarterial embolization, transarterial chemoembolization and radioembolization) and liver transplantation. These therapies are generally reserved for patients whose tumors are predominantly confined to the liver.

Ablation

Ablation techniques are generally reserved for unresectable metastases smaller than 5 to 7 cm in diameter. Several ablation techniques have been described. Those include cryoablation, alcohol ablation and radiofrequency ablation (RFA).

There are no randomized studies comparing surgical to nonsurgical treatments and though long survivals have been observed in surgically treated patients, these could be due to the fact those patients have got favourable prognosis as low tumour burden.

RFA has been used with good results and minimal morbidity for the treatment of patients with NET hepatic metastases^[94]. One disadvantage with this therapy has been the relatively small volume of tissue that can be coagulated and clinical trials with RFA have shown that complete responses are more likely to occur with tumours ≤ 4 cm^[95].

With the use of simultaneous multiple fiber laser induced thermotherapy or next generation bipolar RFA, some authors have reported ablation of tumours as large as 7 cm in diameter^[95,96].

Moreover, up to 7 lesions at one time may be ablated using specialized techniques to increase lesion size^[96,97].

The morbidity associated is 5%-10% and mortality rate is about 0.5%^[98,99].

Berber *et al*^[98] reported a total and significant symptom relief in 95% and 80% respectively in 34 patients with NET liver metastases. The median duration of the benefit was 10 mo. These benefits were seen even in patients with extrahepatic disease.

These techniques are suitable for repeated treatments in patients with local recurrence or new metastases.

Microwave ablation

Microwave ablation (MWA) is more appropriate than RFA to treat tumours next to major hepatic vasculature. In those areas the adjacent blood flow theoretically predisposes RFA to a heat sink effect^[100].

Although clinical experience with MWA has mostly involved hepatocellular carcinoma, though NETs have also been included in some series.

Martin *et al*^[101] reported NET patients undergoing MWA with a 90% success rate for complete ablation with no recurrences at the ablation sites. Most of these patients had MWA performed under ultrasound guidance during open surgery (concomitant hepatectomy and/or extrahepatic metastasectomy). Median overall survival reported was 41 mo. But these results need further studies to be confirmed as these authors only included 11 patients.

There is a lack of data comparing MWA (especially percutaneous) to RFA but geographic patterns of preference have been described. Whereas RFA is widely adopted in the United States, MWA is in Europe and Asia^[101].

Hepatic artery embolization

This technique is performed in patients with diffuse, unresectable liver metastases. The rationale for embolization is related to liver blood supply. Liver metastases get the majority of their blood supply from the hepatic artery, while the normal liver parenchyma gets blood supply primarily from the portal vein. In patients with bilobar hepatic metastases, staged lobar embolizations are typically performed at 4- to 6-wk intervals^[102-104]. Several techniques have been included such as TAE, TACE and drug eluting beads (DEB)-TACE.

TACE and DEB-TACE

TACE has been used several decades. It combines the benefits of embolization and locoregional chemotherapy and provides with a high rate of tumour and symptomatic response^[103].

TACE follows the same principles as TAE, but the intra-arterial administration of a chemotherapeutic agent is added at the time of embolization. With this technique intratumoral concentrations of the drug are over 20 times higher than those obtained by systemic administration of the same drug. Moreover, with this technique, it exists the further potential clinical benefit of tumour ischaemia as a result of embolization.

This technique is indicated in nonsurgical candidates with progressive or refractory disease despite medical treatment (SSAs) and no contraindication to TACE. The best results are obtained if liver involvement is $< 60\%$ and good ECOG (0-1).

Conventional TACE uses a mixture of doxorubicin, lipiodol and embolic agent. The symptoms response has been as high as 73% to 100%, objective response 55% to 80% and time to progression from 8 to 42 mo^[103,104].

Toxicity profile shows grade 2 alopecia, 2-3 nausea and vomiting, postembolization syndrome, acute meta-

bolic syndrome or infection^[91,104]. Some of these toxicities blamed the unfavorable pharmacokinetic profile of doxorubicin binding to lipiodol^[105].

DEB-TACE more recently has improved the pharmacokinetics of the delivered drug. However, a higher incidence of bile duct injury has made its indication controversial in NET metastasis in some institutions.

DEB is a new product which has been shown to achieve higher intratumoral drug concentration and less concentration in the bloodstream TACE in animal studies^[106].

De Baere *et al*^[107] carried out a study of 20 patients and showed 80% objective tumor response and disease control, with time to progression of 15 mo. Drug toxicity was very low with grade 2 alopecia around 1% and only a few cases of mild nausea and vomiting. They reported high rate of liver infarction and bile duct injuries although most cases were asymptomatic^[107].

In normal liver parenchyma, intrahepatic bile ducts do not have a dual blood supply and are fed only from the hepatic arterial branches that form a vascular plexus (peribiliary capillary plexus) around the bile ducts. Therefore, ischemia of the intrahepatic bile ducts can easily occur after TACE^[108].

Some authors have suggested that the incidence of DEB-TACE-related bile duct injury is the result of inadvertent retention at the capillary peribiliary network of DEB loaded with doxorubicin. This may occur as a consequence of over-embolization related to a very aggressive TACE resulting in a high DEB dose and/or complete vessel occlusion.

Experienced operators are aware that the technique is different from conventional TACE notably the embolization. In 35 consecutive patients with liver NET treated in a single institution with DEB-TACE, two different embolization endpoints were compared (complete vs limited embolization). The results showed lower rate of adverse events (14% vs 57%, $P < 0.05$) using the latter. No statistically significant difference in response comparing the two endpoints^[109].

It seems not to be definitively clear which technique should be used, although DEB-TACE has an excellent pharmacokinetic profile which results only in minimal drug toxicity, but it has shown to increase the risk of biliary tree injury, albeit asymptomatic in most cases.

Both techniques, conventional TACE and DEB-TACE offer a high objective response rate and disease control with satisfactory duration of the response. Some authors have suggested that there seems not to be rational in performing conventional TACE or DEB-TACE, because of doxorubicin has no proven effect in NETs. Moreover, the highest benefit from these techniques seems to be due to the embolization rather than the drug effect^[110].

The ablation techniques include cryoablation, alcohol ablation and radiofrequency ablation. These methods are reserved for unresectable oligometastases smaller than 5-7 cm. There are no randomized trials

comparing surgical vs nonsurgical approaches in the management of gastroenteropancreatic NETs with liver metastases^[96,111].

TAE

In targeted embolization of the hepatic artery (TAE) several occlusive materials have been used such lipiodol, gel foam particles, polyvinyl alcohol foam or bland microspheres. It produces tumoral ischemic necrosis while the surrounding liver is perfused by the portal vein. If bilobar metastases, staged lobar embolizations may be needed.

Contraindications to TAE include > 75% replacement of liver parenchyma by tumour, predominant extrahepatic tumour burden, indolent tumours, and hepatic dysfunction.

In cases of revascularization, TAE or TACE can often be repeated. Postembolization syndrome can occur after this technique. It consists of self-limiting pain, fever and nausea/vomiting. This syndrome occurred in most patients, with an 11% major complication rate. There are no completed randomized trials comparing TAE with TACE and the superiority of one technique to another has never been shown^[111].

Selective internal radiotherapy

A novel approach to liver metastases from gastroenteropancreatic NETs involves embolization of ⁹⁰Y embedded either in a resin microsphere (SIR-Sphere) or a glass microsphere (TheraSphere)^[112].

This technique also known as selective internal radiotherapy (SIRT) will produce tumor necrosis through direct delivery of radiation. Response rates in metastatic GEP-NETs have been encouraging. A retrospective multicenter study of 148 patients treated with SIR-Spheres showed overall response rate of 63%^[113].

SIRT has never been compared prospectively to other embolic therapies and long-term toxicities such as radiation fibrosis represent potential risks. Its cost is substantially higher than more traditional embolization therapies, therefore its widespread adoption should await prospective randomized trials.

HIGH-INTENSITY FOCUSED ULTRASOUND

High-intensity focused ultrasound (HIFU) has been recently introduced for the treatment of pancreatic cancer^[114]. HIFU is a non-invasive technique for the treatment of several primary tumors and metastases. Wu *et al*^[115] have reported large areas of coagulation necrosis with this technique in hepatocellular carcinoma. Zhang *et al*^[116] have documented complete tumour necrosis even in lesions adjacent to major hepatic blood vessels.

HIFU achieves ablation by focused ultrasound energy

from an external source that is targeted within the body and induces thermally necrosis. The acoustic intensity is high only within the focal region and therefore it minimizes the risk of injury to the surrounding tissues.

This technique can reach tumours in unfavourable locations for a needle placement and it has proved to offer better disease control and quality of life.

HIFU appears to be an alternative for pancreatic NETs when no indication for a different minimally invasive approach exists. It may be easily repeated and provides good local tumour control^[116].

Moreover, it could be used as a cytoreductive therapy aiming at improving the palliation of patients with locally advanced pancreatic malignancies. However, more studies are needed to evaluate its real impact on survival or quality of life.

Currently and until solid data become available in NETs, HIFU should be reserve for patients whose symptoms cannot be controlled by medical therapy and they are not candidates for surgery or a different minimally invasive therapy^[116].

THE FUTURE

Mutations in the PI3-kinase (PI3K) pathway occur in 16% of patients with pancreatic NETs. Therefore, these tumors are a potential setting for PI3K/AKT/mTOR pharmacological interventions^[117].

Everolimus, a mTOR inhibitor, is used to treat patients with advanced pancreatic NETs. However, resistance to mTOR targeted therapy is emerging partially due to the loss of mTOR-dependent feedback inhibition of AKT. In contrast, the response to PI3K inhibitors in pancreatic NETs is unknown^[118].

Soler *et al*^[118] carried out a study to assess the frequency of PI3K pathway activation in human pancreatic NETs and in RIP1-Tag2 mice, which is a preclinical tumor model of pancreatic NETs. They investigated the therapeutic efficacy of inhibiting PI3K in RIP1-Tag2 mice using a combination of pan (GDC-0941) and p110 α selective (GDC-0326) inhibitors and isoform specific PI3K kinase-dead mutant mice. They found that treatment of these mice with GDC-0941 reduced tumor growth without impact on vascular area and the selective inactivation of the p110 α PI3K isoform reduced tumor growth as well as vascular area.

The authors concluded that p110 α could have a role in pancreatic NETs and unravel a new function of this kinase in cancer biology through its role in promoting metastasis^[118].

Andersson *et al*^[119] carried out a study to define the transcriptome of small intestinal NETs to identify clinically relevant subgroups of tumors, prognostic markers and novel targets for treatment.

Genome-wide expression profiling was conducted on biopsies from 33 patients with well-differentiated metastatic NETs of the distal ileum. They identified three groups: The largest, characterized by longer patient survival and higher expression of NE markers,

including SSTR2. Then, tumors with higher grade (G2/3) or gain of chromosome 14 which were associated with shorter survival and increased expression of cell cycle-promoting genes^[118].

The prostaglandin E receptor 2 is the most significantly activated regulator in tumors of higher grade, whereas Forkhead box M1 was the most significantly activated regulator in tumors with gain of chromosome 14^[118].

Evaluation of candidate drug targets on NET cells (GOT1) showed significant inhibition of tumor cell growth after treatment with tyrosine kinase inhibitors or inhibitors of HDAC, HSP90 and AKT^[118].

Authors found specific gene expression patterns associated with tumor grade and chromosomal alterations^[118]. The results of several practice-changing phase III clinical trials have been presented at The North American Neuroendocrine Tumor Society symposium 2015: The TELESTAR randomized phase III trial of telotristat vs placebo in patients with carcinoid syndrome^[119,120].

Telotristat etiprate is an oral inhibitor of tryptophan hydroxylase (This enzyme triggers the excess serotonin production within metastatic NET cells that leads to carcinoid syndrome). It decreased significantly the mean of daily bowel movements by 35% among patient who received 500 mg of the drug three times a day and 29% among those who received 250 mg three times a day, compared with 17% for those who received placebo^[119,120].

Urinary 5-HIAA levels were significantly reduced as well, for patients receiving the active drug, suggesting effective inhibition of serotonin production.

Telotristat etiprate has received Fast Track and Orphan Drug designation from the United States Food and Drug Administration. Whereas current treatments for carcinoid syndrome reduce the release of serotonin outside tumor cells, telotristat etiprate works to reduce serotonin production within the tumor cells^[119,120].

Currently, there are limited therapeutic options for patients with advanced midgut neuroendocrine tumors progressing on first-line somatostatin analog therapy.

NETTER-1 randomized phase III trial of radiolabelled somatostatin analog 177-Lutetium-dotatate vs high dose octreotide (LAR) 60 mg in patients with progressive midgut NETs. Results showed that the median PFS, the trial's primary endpoint, improved by nearly 80%. The median PFS with high-dose octreotide was 8.4 mo and was not yet reached in the ¹⁷⁷Lu-Dotatate arm at a median follow-up of 18 mo but update data indicate that it will probably be in excess of three years. Although the OS data were not mature enough for a definitive analysis, the number of deaths was 13 in the Lutathera group and 22 in the Octreotide LAR 60 mg group at interim analysis which suggests an improvement in OS. The overall response rate was 18% vs 3%. Safety data confirmed favorable results of the preceding phase I/II studies^[121,122].

Serious adverse events related to treatment were 9%

for Lu-Dotatate and 1% for octreotide. Withdrawals due to adverse events were 5% for Lu-Dotatate and did not occur in patients treated with octreotide. Lu-Dotatate is the most advanced candidate in development of PRRTs, which target tumors with radiolabelled somatostatin analog peptides. In April 2015, the FDA granted a fast track designation to Lu-Dotatate for the treatment of inoperable progressive midgut NETs^[122,123].

Radiolabeled SSA therapy (also called peptide receptor radiotherapy or PRRT) has shown to be an effective treatment for gastroenteropancreatic NETs, as it allows targeted delivery of radionuclides to SSTR-expressing tumor cells. Selection criteria for PRRT include evidence of strong radiotracer uptake on somatostatin-receptor scintigraphy, ideally higher than in normal liver tissue.

The agents ⁹⁰Y-DOTATOC and ¹⁷⁷Lu-Dotatate are the latest generation of PRRT. ⁹⁰Y is a high-energy β -particle emitter. Strosberg *et al*^[123], Kwekkeboom *et al*^[124] and Valkema *et al*^[125] reported ORR > 25%. A later large multicenter trial of 90 patients with metastatic carcinoids showed a RR of 4%, and 70% of SD^[126].

¹⁷⁷Lu emits both β and γ rays. A large nonrandomized trial including 310 patients, has reported a 30% RR with gastroenteropancreatic NETs receiving ¹⁷⁷Lu-octreotate.

Responses were particularly high in patients with pancreatic NETs^[127]. PRRT toxicities include myelosuppression and renal insufficiency, with the latter generally ameliorated by concurrent amino acid infusion.

CONCLUSION

NE gastroenteropancreatic tumors are a heterogeneous group of neoplasias arising from NE cells of the embryological gut^[1] whose incidence have increased due probably to the improvements in diagnosis^[1-4]. Many recent advances in molecular biology have expanded the therapeutic arsenal and we have shifted from somatostatin analogs^[6] only, to a new scenario where antiangiogenics and mTOR inhibitors among others have started to take over^[7]. The treatment of NETs continues being a challenge and requires a coordinated multidisciplinary approach. Although the management of localized NETs involves surgical resection followed by surveillance, the treatment of unresectable and/or metastatic disease may involve several disciplines (surgical resection, systemic therapy, liver-directed therapies)^[7].

Several completed and ongoing studies are evaluating somatostatin analogs, VEGF pathway and mTOR inhibitors, cytotoxic chemotherapy, PRRT^[7], new liver-directed therapies, *etc.* but future trials should focus on patient selection, predictive markers, and tolerability improvement as these aspects are critical to continue advancing.

The circulating tumour cells (CTCs), which are detectable in the blood of 50% of patients with functioning midgut NETs, are usually related to poor prognosis. The CALM-NET, a phase IV, multicentre, open label, single

group exploratory study to assess the clinical value of enumeration of CTCs to predict clinical symptomatic response and PFS in patients receiving lanreotide to treat the symptoms of functioning midgut NETs is under way. The results of this trial could be valuable as if positive, CTCs could be used as predictive markers to help make therapeutic decisions.

Pancreatic NETs are heterogenous neoplasms still with limited therapeutic options but everolimus has recently been approved for the treatment of progressive, well-differentiated, non-functional, unresectable, locally advanced or metastatic NETs of gastrointestinal or lung origin. This is the first approved treatment for these rare cancers whose prognosis is poor and their options limited.

Alkylating cytotoxic agents, such as streptozocin and temozolomide, play an important role in the treatment of pancreatic NETs, although RR varies widely. Future studies of cytotoxics in gastroenteropancreatic NETs should stratify patients based on primary site and tumor grade. Over the next years, randomized clinical trials are expected to provide more data about role of radiolabeled somatostatin analogs. Predictive biomarkers that would allow for individualized selection of treatments are needed.

New findings have shed light on the biological processes of pancreatic NETs and have identified a tumorigenic cell population that suggest these cells can hide from immune surveillance.

These discoveries will hopefully open the door to new potential therapeutic targets^[128] which can lead to personalised treatments and optimize the results in this heterogeneous group of tumors.

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Novel therapeutic approaches targeting L-type amino acid transporters for cancer treatment

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Abstract

L-type amino acid transporters (LATs) mainly assist the

uptake of neutral amino acids into cells. Four LATs (LAT1, LAT2, LAT3 and LAT4) have so far been identified. LAT1 (SLC7A5) has been attracting much attention in the field of cancer research since it is commonly up-regulated in various cancers. Basic research has made it increasingly clear that LAT1 plays a predominant role in malignancy. The functional significance of LAT1 in cancer and the potential therapeutic application of the features of LAT1 to cancer management are described in this review.

Key words: LAT1; Amino acid transporter; Molecular target drug; Amino acid starvation response; Signal transduction

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Core tip: The discovery of molecules preferentially expressed in cancer cells is extremely valuable for the development of molecular target drugs in cancer therapy. Amino acid transporters have been receiving a great amount of attention as a candidate of such molecular targets. This review summarizes new initiatives for clinical applications of the basic research relative to L-type amino acid transporters, which are commonly expressed in cancers.

Hayashi K, Anzai N. Novel therapeutic approaches targeting L-type amino acid transporters for cancer treatment. *World J Gastrointest Oncol* 2017; 9(1): 21-29 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v9/i1/21.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v9.i1.21>

INTRODUCTION

Cancers consume a huge amount of materials for biochemical reactions, and a continuous supply of sufficient nutrients is essential for their survival. Hydrophilic nutrients are delivered into cells by transporters.

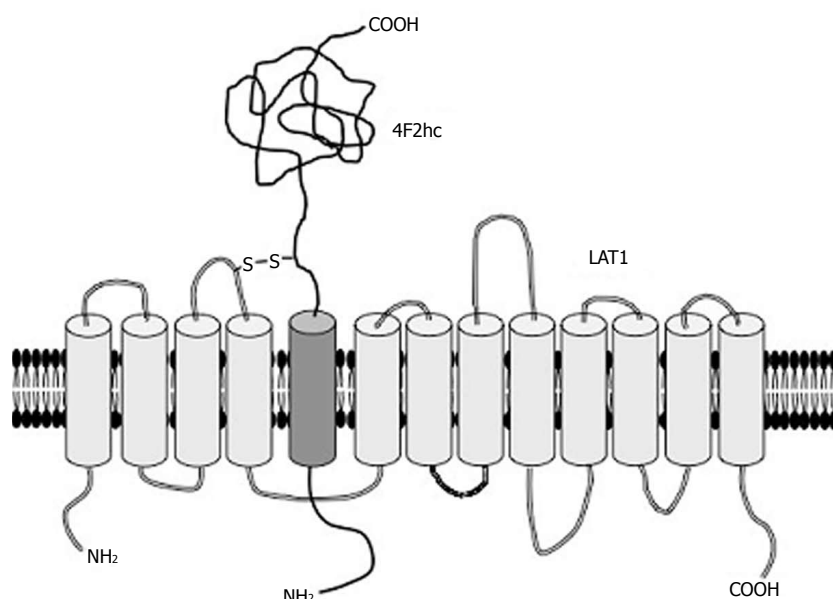


Figure 1 Structure of LAT1. LAT1 is composed of 12 transmembrane helices that are predicted to form a cylindrical conformation penetrating the cellular membrane. LAT1 associates with 4F2hc for stable localization at the cellular membrane. LAT2 is similar in structure to LAT1, whereas LAT3 and LAT4 function independently of 4F2hc.

Recent studies have revealed several transporters preferentially expressed in cancers. Inhibition of cancer specific-nutrient transporters would be a good strategy for cancer management with minimal side effects. Indeed, a therapeutic approach using transporter inhibitors for cancer prevention has been proven to be efficacious in cell lines and animal experiments and is now under evaluation in a clinical trial.

L-TYPE AMINO ACID TRANSPORTERS

Many cells take advantage of transporters to incorporate what is necessary at the time of need. Transporters fall into two broad categories based on ATP dependency for their transport form^[1]. ATP-dependent transporters, known as ATP-binding cassette, hydrolyze ATP to obtain the energy for translocation of their substrates across the membrane (active transport). Transporters with no ATPase, called solute carriers (SLCs), facilitate diffusive transport. Each SLC transporter is named in combination with the family numeral based on the sequence similarity and individual number with letter A between them (e.g., SLC3A2), with a few exceptions. Most of the amino acid transporters were formerly categorized into several groups ("System") on the basis of their substrates and sodium dependency (e.g., System L, which incorporates neutral amino acids without sodium), but they are currently classified into SLCs according to their protein homology.

L-type amino acid transporters (LATs) are categorized as system L transporters. LATs mainly deliver neutral amino acids into cells in a sodium-independent manner. So far, four LATs have been identified.

LAT1 (SLC7A5) was identified as the first LAT by

two groups in 1998^[2,3]. The major substrate of LAT1 is large neutral amino acids as typified by leucine. The expression of LAT1 in normal adults is detected in proliferative zones of gastrointestinal mucosa, testicular sertoli cells, ovarian follicular cells, pancreatic islet cells, and some endothelial cells that serve as a barrier between tissues (blood-brain, blood-retinal and blood-follicle barrier)^[4]. Recent studies revealed a crucial role of LAT1 in activated T cells^[5,6]. As described below, LAT1 expression is commonly up-regulated in various cancers.

LAT2 (SLC7A8) was subsequently isolated on the basis of sequence similarity to LAT1^[7-9]. LAT2 has broader specificity of its substrates including polar uncharged and small neutral amino acids than that of LAT1^[8]. LAT2 is ubiquitously expressed in normal body^[4], though LAT2 knockout mice show a mild phenotype and almost no visible symptoms except aminoaciduria^[10]. Both LAT1 and LAT2 are composed of 12 transmembrane domains that form the pathway of their substrates^[11] (Figure 1). They associate with the heavy glycoprotein subunit 4F2hc (SLC3A2) by sulfur bond^[11]. Although 4F2hc does not seem to have a function to directly transfer the substrates, it makes the localization of its partner LATs more stable at the plasma membrane^[12].

LAT3 (SLC43A1) was isolated by expression cloning from hepatocarcinoma cells^[13]. Sequence analysis revealed that LAT3 was identical to POV1, which was originally identified as a cancer-up-regulated gene^[14,15]. The substrate selectivity of LAT3 was similar to that of LAT1. LAT3 mRNA is expressed in the liver, skeletal muscle, and pancreas^[16]. The physiological role of LAT3 in normal individuals of mammals remains unknown,

but it was shown that LAT3 functions for podocyte development in zebrafish^[17].

LAT3 appears to behave as a critical transporter in several cancers. LAT3 is up-regulated in response to androgen and knockdown of LAT3 expression by RNA interference (RNAi) significantly inhibits the leucine uptake and cell proliferation in human prostate cancer cell lines *in vitro*^[18]. Furthermore, high expression of LAT3 is detected in prostate cancer patients, and stably knockdown of LAT3 by RNAi in human prostate cancer cell lines results in decrease of their growth and metastatic potential with alteration of cell cycle gene expression after xenografts into mice^[19].

LAT4 (SLC43A2) was identified by searching for sequence homology to LAT3^[20]. LAT4 is expressed in the basolateral membrane of the small intestine, kidney proximal tubule and thick ascending limb epithelial cells. LAT4 knockout mice are smaller than their controls and die within 9 d, presumably because of defective amino acid absorption^[21]. Unlike LAT1 and LAT2, LAT3 as well as LAT4 functions independently of heavy chain.

LAT1

LAT1 is the most extensively studied transporter among LATs. The interest in LAT1 is because of its extremely high expression in diverse human cancers. LAT1 was originally cloned from mRNA of C6 glioma cells^[2]. Subsequent studies have shown that LAT1 is highly expressed in many cancer cell lines. Histological analysis with qualitatively enhanced antibodies confirmed potent expression of LAT1 in human cancers in a broad range of tissues. The number of cancer types that were reported to express a high level of LAT1 is well above twenty (Table 1). LAT1 is thus a commonly up-regulated amino acid transporter in multiple human cancers. Furthermore, LAT1 expression level appears to be associated with prognosis of cancer patients. For example, elevated expression of LAT1 correlates with an adverse prognosis in prostate^[22], gastric^[23], and pancreatic cancers^[24], suggesting that higher-grade tumors are more dependent on LAT1. Not only the expression of LAT1 but also the functional significance of LAT1 in cancers has been verified by use of its inhibitors, by knockdown with RNAi and by gene disruption. 2-Aminobicyclo (2,2,1) heptane-2-carboxylic acid (BCH) is an inhibitor of system L transporters. BCH inhibits leucine uptake and strongly suppresses the proliferation of many cancer cells (Table 1). Genetic manipulation confirmed the functional significance of LAT1 in cancer cells. Knockdown of LAT1 with RNAi^[25-29] as well as genetic disruption of *LAT1* by zinc fingers nucleases-mediated gene knockout^[12] in cancer cells reduces leucine uptake and cell proliferation, indicating that LAT1 is a predominant transporter that is essential for growth of cancers. The reason that so many cancers use LAT1 despite the presence of many other amino acid transporters might be that LAT1 has a prominent capability for substrate transport. Indeed,

the affinity of LAT1 for leucine is much higher than that of LAT2^[30], although LAT2 is ubiquitously expressed in the normal body^[4]. Cancers may therefore be more dependent on LAT1 for rapid uptake of sufficient amino acids, whereas normal cells need less amino acid delivery that can be supported by LAT2.

The definite effect of LAT1 on the growth of various cancer cell lines prompted researchers to apply the LAT1 inhibitor in a clinical setting. However, the concentration of BCH required for suppression of cancer growth is extremely high (usually around 10 mmol/L). Moreover, the unselective effect of BCH that inhibits all LATs is another problem, since LATs other than LAT1 are considered to have functions in the normal body. It has been necessary to develop drugs that act on just LAT1 but not other transporters at a low concentration. In 2010, Endo and colleagues designed a new compound named JPH203 ((S)-2-amino-3-(4-((5-amino-2-phenylbenzo[d]oxazol-7-yl) methoxy)-3,5-dichlorophenyl) propanoic acid)^[31]. JPH203 has structural analogy to tyrosine, but it inhibits only LAT1 without affecting any other LATs. JPH203 displayed potent suppressive effects on the growth of cancers *in vitro*^[12,32,33]. Moreover, this compound has the ability to powerfully inhibit the proliferation of tumor cell lines of the colon and leukemia injected into nude mice^[31,33]. Following improvements in its specificity and pharmacological effect, JPH203 is under evaluation in a phase I clinical trial of cancer patients.

CLINICAL APPLICATION OF LAT1

Positron emission tomography

By exploiting the characteristics of LAT1 expression, an approach for the diagnosis of cancers through radiolabeled substrates of LAT1 has been attempted. [¹⁸F] or [¹¹C]-labeled compound administered into the body can be visualized by positron emission tomography (PET)^[34]. Cancers incorporating an isotopically labeled probe can be located by tracing the body with PET. In the past, 2-¹⁸F-fluoro-2-deoxy-d-glucose ([¹⁸F]FDG) was one of the most commonly used probe for diagnosis of cancer with PET. This strategy exploits the characteristic of cancers consuming a huge amount of glucose compared to that consumed by normal cells. Although [¹⁸F]FDG has been of assistance in the clinical diagnosis of many cancers, it sometimes showed false positive results, especially in brain, because even normal brain cells take up a relatively large amount of glucose. To overcome this problem, amino acids have attracted attention as alternative probes to glucose. Representative amino acids or their analogs developed as probes of PET are L-3-[¹⁸F]-fluoro- α -methyl tyrosine ([¹⁸F]FAMT), 6-¹⁸F-fluoro-L-3,4-dihydroxy-phenylalanine (¹⁸F-DOPA), L-[¹¹C-methyl] methionine ([¹¹C]MET) and O-(2-[¹⁸F]fluoroethyl)-L-tyrosine ([¹⁸F]FET). If the compounds are delivered into cells specifically through LAT1, those cells are likely to be cancers. Indeed,

Table 1 Summary of studies for expression and functions of LAT1 in cancers

Cancer	Expression (method of detection)	Inhibition of amino acid uptake by	Growth inhibition by	Ref.
Biliary tract	Immunohistochemistry	BCH	BCH	[71]
Bladder	Northern blot (cell line)	BCH		[72]
Bone	Immunohistochemistry			[73]
Brain	Immunohistochemistry, RT-PCR (cell line), Western blot (tissue, cell line)	BCH	BCH	[74,75]
Breast	Immunohistochemistry, RT-PCR (cell line)	BCH	RNAi, BCH	[29,76-78]
Colon	Western blot (cell line)	Knockout (cell line)	Knockout (cell line) JPH203	[12]
Esophagus	Immunohistochemistry			[79,80]
Hepatocyte	Immunohistochemistry			[81]
Gastrointestine	Immunohistochemistry, Western blot (cell line)		RNAi	[23,45]
Laryngeal	Immunohistochemistry			[82]
Leukemia	RT-PCR (cell line)		BCH, JPH203	[33]
Lung	Immunohistochemistry			[41,83-85]
Melanoma	Immunohistochemistry, Microarray (tissue), Western blot (cell line)	BCH		[86,87]
Myeloma	RT-PCR (cell line)	RNAi		[88]
Neuroendocrine	Immunohistochemistry, RT-PCR (tissue), Western blot (tissue)			[89]
Ovarian	Immunohistochemistry, RT-PCR (cell line), Western blot (tissue, cell line)	BCH	BCH	[47,65,90]
Oral	RT-PCR (cell line)	RNAi	RNAi	[25]
Pancreas	Immunohistochemistry Western blot (cell line)	RNAi	RNAi	[24,27,91]
Pleura	Immunohistochemistry			[92]
Prostate	Immunohistochemistry, Western blot (cell line)	RNAi, BCH	RNAi, BCH	[18,19,22,28]
Tongue	Immunohistochemistry			[93]
Thymus	Immunohistochemistry, Western blot (cell line)	JPH203	JPH203	[94,95]
Urinary tract	Immunohistochemistry			[96]

RT-PCR: Reverse transcription polymerase chain reaction; BCH: 2-aminobicyclo (2,2,1) heptane-2-carboxylic acid.

[¹⁸F]FAMT images accord well with LAT1 distribution^[35]. Moreover, FAMT is incorporated by LAT1 but not by other amino acid transporters^[35]. Although there is still room for improvement in its specificity, this method is powerful tool for diagnosis of cancers including microcarcinoma.

Boron neutron capture therapy

LAT1 is an attractive molecular target for boron neutron capture therapy (BNCT). BNCT is an anticancer therapy that utilizes high linear energy transfer alpha particles. Particle radiation is produced by fission reaction when irradiated thermal neutrons collide with boron incorporated by a malignant tumor. The traveling distance of particle radiation is limited (5-9 μ m), and it therefore disrupts only cancer cells incorporating boron without damage to other cells around target cells^[36,37]. A key component of BNCT success is accumulation of boron specifically in cancer cells. This difficult task could be achieved by the synthesis of a boron compound that is selectively delivered by LAT1. Indeed, p-boronophenylalanine (BPA), a boron compound commonly used in BNCT, is incorporated by LAT1^[38-40], suggesting that LAT1 is an optimal mediator for delivery of boron in BNCT. However, since we cannot still completely rule out the possibility of BPA uptake by other transporters, it is necessary to develop compounds that exhibit strict selectivity to LAT1. BNCT has accomplished certain clinical outcomes so far, but the problem in the

past was that it required a large-scale nuclear reactor to generate neutrons. However, a compact accelerator has been developed as an alternative to a nuclear reactor and it can be installed in a hospital, making BNCT easier to perform. Such technology will expand the applications of BNCT in the future.

LAT1 AND METASTASIS

It has been suggested that LAT1 is involved in cancer metastasis. A number of studies have shown a correlation of increase in LAT1 expression with metastasis of multiple cancers. Lymph node metastasis-positive squamous cell carcinomas express LAT1 whereas there is no positive signal of LAT1 in metastasis-negative cells^[41]. LAT1 mRNA level was significantly higher in renal cell carcinoma with metastasis^[42]. A group of cells with high LAT1 expression showed a larger size of the metastatic lesion of gastric carcinoma^[43]. LAT1 expression in neuroendocrine tumors was significantly associated with lymph node metastasis^[44]. The potency of the functional significance of LAT1 in metastasis has been shown. Knockdown of LAT1 by RNAi inhibited the migration and invasion of gastric cancer^[45] and a cholangiocarcinoma cell line^[46]. BCH inhibited the proliferation and migration of a human epithelial ovarian cancer cell line^[47]. On the basis of these findings, inhibition of LAT1 will be good strategy to prevent metastasis of cancer. However, it remains to

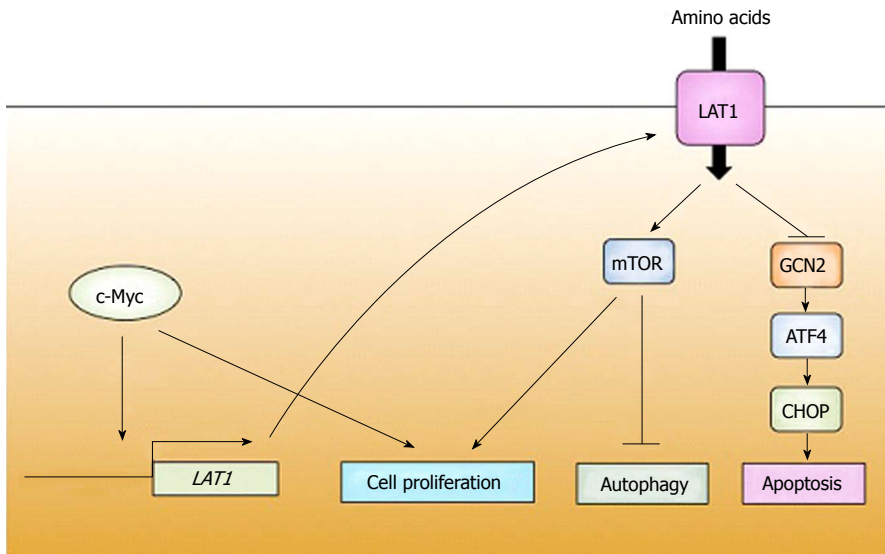


Figure 2 Schematic model of acquisition and monitoring of amino acids in cancer. c-Myc promotes expression of LAT1, which supplies amino acids necessary for growth of cancers. The availability of amino acids is constantly monitored by factors such as mTOR and GCN2. Once amino acid deficiency is detected, cancers suppress their proliferation and, as occasion demands, induce apoptosis. mTOR: Mechanistic target of rapamycin; GCN2: General control non-derepressible 2; ATF4: Activating transcription factor 4; CHOP: C/EBP homologous protein.

be determined whether the metastasis defect is derived from amino acid starvation or from other factors such as an aberrance of adhesion molecules. It would thus be valuable to investigate the relevance of LAT1 and integrin in metastasis, since they form a complex^[48].

MECHANISM OF LAT1 EXPRESSION

Although it remains unknown how LAT1 expression is facilitated in cancers, some possible molecular mechanisms have been proposed. c-Myc, a proto-oncogenic transcription factor, has been demonstrated to be an upstream of LAT1. The expression of c-Myc in normal adults is generally low^[49], but overexpression of c-Myc triggered by some cues such as gene amplification, gene translocation or other gene mutations^[50] is responsible for malignant transformation. Numerous human cancer tissues strongly express c-Myc. Target genes of c-Myc include many factors involved in progression of the cell cycle^[51]. On the other hand, the consensus binding sequence of c-Myc is also located at the *LAT1* promoter^[27]. Moreover, knockdown of c-Myc leads to reduction of LAT1 expression in cancer cell lines^[27]. These results suggest that up-regulation of LAT1 is mediated, at least in part, by c-Myc (Figure 2). Of note is that c-Myc also enhances the metabolic reprogram in cancers by promoting the expression of enzymes of glycolysis and glucose transporter^[52,53]. This is an ingenious strategy of cancers since they can coordinate multiple events required for cell growth by just one factor.

Some other factors appear to regulate LAT1 expression. Hypoxia-inducible factor (Hif) is a critical regulator in response to hypoxia. Hif2 α , an isoform of the Hif family, binds to the *LAT1* promoter and enhances LAT1 expression in renal carcinoma cell

lines^[54]. Artificial manipulation to elevate Hif2 α activity induces LAT1 expression in lung and liver tissues, in which LAT1 expression is usually low^[54]. Aryl hydrocarbon receptor (AHR) is a transcription factor that is activated by interaction with its ligands such as dioxin, and it promotes tumorigenesis^[55]. AHR binds to its consensus binding sequence in *LAT1* and drives LAT1 expression in breast cancer cell lines^[56], suggesting that LAT1 contributes to tumorigenesis induced by an environmental carcinogen. As described previously, T cell activation induces LAT1 expression^[5,6]. Nuclear factor kappa B, AP-1 and nuclear factor of activated T-cells are critical transcription factors that are activated by T cell stimulation and enhance immunological reactions. The expression of LAT1 is prevented by inhibitors of these transcription factors^[5,6]. This means that LAT1 expression is induced by the common regulators that also boost immunological reaction in T cells.

DOWNSTREAM OF LAT1

Ensuring a sufficient supply of nutrients is an issue of vital importance for cancers. The majority of cancers are thought to constantly monitor the availability of amino acids. Starvation of amino acids rapidly induces a stress response that puts a brake on cellular biochemical reactions to avoid wasting energy and materials. The most extensively studied system for monitoring the amino acid availability is mechanistic target of rapamycin (mTOR)^[57], a serine-threonine kinase. Plenty of amino acids maintains mTOR kinase activity, resulting in progression of the cell cycle, protein synthesis, or inhibition of autophagy induction (Figure 2). Some mTOR regulators such as SLC38A9^[58-60], Cellular arginine sensor for mTORC1 (CASTOR1)^[61] and Sestrin2^[62] have

been demonstrated to associate with amino acids to dictate mTOR activity. Dissociation of those interactions caused by amino acid deficiency inactivates mTOR and inverses the reaction of its downstream, resulting in a halt of cancer growth. Growing evidence suggests that LAT1 disruption leads to the inhibition of mTOR. LAT1 inhibition decreases mTOR activity in many cancer cell lines^[28,33,63-65]. These findings suggest that the arrest of cell growth of cancers by a defect of LAT1 is derived from inactivation of mTOR (Figure 2). mTOR inhibitors are being used in practical trials for therapeutic management of several cancers^[66]. Application of JPH203 together with an mTOR inhibitor probably creates a synergistic effect and might be useful for maximizing the benefit of treatment with a low-dose drug, which would help to minimize adverse effects.

General control non-derepressible 2 (GCN2) is another factor for detection of amino acid starvation^[67]. GCN2 is a serine-threonine kinase that is activated by amino acid deficiency. Uncharged tRNAs caused by a decline of amino acid concentration activates GCN2, which eventually induces activity of activating transcription factor 4 (ATF4). ATF4 regulates the expression of genes responsible for coping with amino acid deficiency^[68]. Several studies have shown that dysfunction of LAT1 initiates the GCN2 signal. JPH203 promotes the expression of C/EBP homologous protein [CHOP, also known as DNA damage inducible transcript 3 (DDIT3)], which is up-regulated by ATF4^[68] and probably takes part in apoptosis in leukemia^[33]. Gene disruption of LAT1 in cancer cell lines activates the GCN2-ATF4 cascade^[12]. Activation of ATF4 by LAT1 defect was also shown in cells other than cancer. JPH203 triggers the expression of CHOP^[5,69] and homeobox B9^[70], a novel target of ATF4, in human T cells to repress cytokine production. These findings suggest that GCN2-ATF4 is another critical system for detecting amino acid deficiency evoked by LAT1 inhibition (Figure 2).

CONCLUSION

After the importance of LAT1 in cancer cells had been established, basic studies on LAT1 have progressed with remarkable speed. Better still, research achievements are potentially capable of technical developments for the use of LAT1 as a molecular target in clinical practice. However, although JPH203 is more effective and specific than BCH, it still requires a high concentration for sufficient suppression of the growth of cancers, and wariness of adverse effect persists. Nevertheless, such concerns might be overcome, at least for the time being, by virtue of the proper combinational use of multiple drugs with different action points in cellular metabolism (*e.g.*, mTOR inhibitor). However, further improvements in selectivity of the inhibitor, boron donor of BNCT and PET probe to LAT1 will raise the quality of cancer treatment. Besides, although not to the extent to LAT1, there are several cancers that rely on LAT3

for their growth and development of a LAT3-specific inhibitors is also encouraged. Advances in technologies are expected to resolve such issues.

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Pancreatic resection in very elderly patients: A critical analysis of existing evidence

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Abstract

The aging of the population results in a rise of number of elderly patients (aged 80 years and older)

with pancreatic or periampullary cancer, and more pancreatectomies could eventually be performed in such complex patients. However, early and long-term results after pancreatic resection in octogenarians are still controversial, and may trouble the surgeon when approaching this type of population. Evaluation of reported experiences shows that for almost all Authors, pancreatectomy can be performed safely in elderly population, although overall morbidity and mortality rates were 34.9% and 13.2% respectively, with a mean length of hospital stay of 18 d. These features appear higher in older patients compared to the younger counterpart. Less than 50% of patients underwent adjuvant therapy after operation. Long-term survival is reported not significantly different in aged 80 years and older patients, with a median overall survival time of 17.6 mo. The quality of life after pancreatic resection is only sporadically evaluated but, when considered, it highlights the need of health facility service after operation for these "frail" patients. Prospective studies on the quality of life of pancreatectomized octogenarians are welcome. Proper selection of patients, geriatric assessment with multidisciplinary approach, centralization of pancreatic surgery in high-volume centres and rehabilitation programs after surgery appear to be crucial points in order to improve surgical treatments of pancreatic tumors in very elderly patients.

Key words: Elderly; Octogenarian; Pancreatectomy; Pancreatic neoplasms; Survival

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Core tip: Although not statistically significant, pancreatic resection in very older patients carried a greater risk of complications, mortality and nursing facility after discharge than in younger patients. Thus, pancreatectomy in 80 years and older patients, should be performed after careful consideration of potential benefit, surgical risk, and patient's preferences.

Sperti C, Moletta L, Pozza G. Pancreatic resection in very elderly patients: A critical analysis of existing evidence. *World J Gastrointest Oncol* 2017; 9(1): 30-36 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v9/i1/30.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v9.i1.30>

INTRODUCTION

The number of elderly in Western countries is rapidly increasing and it constitutes the fastest-growing age group of the population^[1]. In the United States, the proportion of people 65 years of age or older will reach 18.2% by 2025^[2], and the oldest elderly (individuals 85 years old or older) will account for 5% of the overall population^[3]. The number of octogenarian patients referred to surgeons is going to gradually increase as well. This is particularly true for gastrointestinal cancers, which are characterized by the greatest incidence in the elderly, pancreatic cancer included. In past years, the high mortality and morbidity rates associated with pancreatic resections made this kind of surgery a rare indication for elderly people, considering also the limited survival time associated with pancreatic cancer. Recent data have clearly shown that pancreatic surgery is safe and feasible in high-volume centres, with reported mortality rates less than 2% and acceptable morbidity rates^[4,5]. As postoperative outcome after pancreatic resections improved, many authors began to report pancreatectomies also in elderly patients. However, there are limited data on outcomes in octogenarians patients after pancreatic surgery. So, some crucial points may arise when treating very elderly patients with pancreatic tumors: (1) Is pancreatic resection safe and feasible in octogenarians? (2) Is surgical risk justified by long-term outcome after resection of malignancy? (3) Is quality of life preserved after major pancreatic resection?

The aim of this study was to analyse the existing literature and the available data on early postoperative outcomes and long-term results after pancreatic resection in patients 80 years and older.

EVIDENCE ACQUISITION

The published Literature was systematically searched using PubMed and free text search engines up to December 2015. The following search terms were used: Pancreaticoduodenectomy, pancreatectomy, duodenal neoplasm/surgery, pancreatic neoplasm/surgery, pancreatic neoplasm/surgery, 80 years of age and over, elderly and octogenarian. The "related articles" function was used to broaden the search and all abstracts, studies, and citations retrieved were reviewed. The preliminary literature search showed 113 studies matching the initial criteria. After screening, 16 studies evaluating octogenarians patients and their outcome

Table 1 Type of periampullary neoplasms

Ref.	n	Age (mean)	Benign disease	Malignant disease	Pancreatic adenocarcinoma
Chen <i>et al</i> ^[6]	16	82.3	1	15	5
Makary <i>et al</i> ^[7]	207	82	30	177	96
Finlayson <i>et al</i> ^[8]	2915	NR	0	2915	NR
Riall <i>et al</i> ^[9]	214	NR	50	164	NR
Hardacre <i>et al</i> ^[3]	32	82	2	30	25
Tani <i>et al</i> ^[2]	25	82.3	3	22	10
Lee <i>et al</i> ^[10]	74	82.6	16	58	45
Khan <i>et al</i> ^[11]	53	NR	0	53	53
Stauffer <i>et al</i> ^[12]	32	82.1	11	21	18
Hatzaras <i>et al</i> ^[13]	27	83.4	0	27	24
Melis <i>et al</i> ^[14]	25	83	0	25	25
Oguro <i>et al</i> ^[15]	22	81.5	0	22	8
Turrini <i>et al</i> ^[16]	64	83	0	64	64
Belyaev <i>et al</i> ^[17]	38	82	NR	NR	NR
Beltrame <i>et al</i> ^[18]	23	82.6	1	22	20
Kinoshita <i>et al</i> ^[19]	26	82	0	26	26
Total	3793	82.2	114	3641	419

NR: Not reported.

after pancreatic resections were selected^[2,3,6-19]. Information about 3793 aged 80-years or older patients who underwent pancreatic resections, were collected (Table 1). There were 13 single institution's series, 2 nation or regional inpatient samples, and 1 multicentric report. In the population selected, there were 1710 male patients (45.1%) and the mean age was 82.2 years. Information about preoperative comorbidities were available for 489 patients. The most frequent reported comorbidities were cardiovascular disease (53.8%, $n = 263$ patients), in particular hypertension was reported for 168 patients, and coronary disease for 95 patients. Other frequent major comorbidities were diabetes mellitus ($n = 94$, 19.2%), pulmonary disease ($n = 30$, 6.1%) and chronic renal failure ($n = 10$, 2.0%). Elderly patients are often reported to have two or more concomitant major comorbidities. Finlayson *et al*^[8] and Khan *et al*^[11] reported a percentage of respectively 67.6% and 51% patients with 2 or more concomitant diseases. Six studies^[3,11,14-16,18] reported data on the American Society of Anesthesiologists (ASA) score, with ASA grades 3 or 4 more frequently observed (60.3% of patients) (Table 2).

SURGERY

Thirteen studies^[2,3,6,7,10-16,18,19] reported the type of pancreatic neoplasm treated ($n = 626$ patients). In particular, four reports evaluated only outcome after resections for pancreatic adenocarcinoma, whereas other two authors considered also patients with other primary malignancies. Finally, the remaining 7 studies addressed also resections for benign pancreatic conditions. Malignant indications for surgery accounted for 89.8% of cases ($n = 562$), with pancreatic adenocarcinoma being the most frequent primary

Table 2 Type of surgical resections and American Society of Anesthesiologists score

Ref.	Type of surgical procedure			Vascular resections % (n)	ASA SCORE	
	PD	DP	TP		1-2	3-4
Chen <i>et al</i> ^[6]	16	0	0	NR	NR	NR
Makary <i>et al</i> ^[7]	197	0	10	2.4 (5)	NR	NR
Finlayson <i>et al</i> ^[8]	NR	NR	NR	NR	NR	NR
Riall <i>et al</i> ^[9]	155	48	NR	NR	NR	NR
Hardacre <i>et al</i> ^[3]	26	5	1	12.5 (4)	8	24
Tani <i>et al</i> ^[2]	25	0	0	4 (1)	NR	NR
Lee <i>et al</i> ^[10]	74	0	0	14.9 (11)	NR	NR
Khan <i>et al</i> ^[11]	18	10	4	6.25 (2)	7	46
Stauffer <i>et al</i> ^[12]	20	5	0	NR	NR	NR
Hatzaras <i>et al</i> ^[13]	53	0	0	NR	NR	NR
Melis <i>et al</i> ^[14]	25	0	0	4 (1)	7	17
Oguro <i>et al</i> ^[15]	22	0	0	23 (5)	21	1
Turrini <i>et al</i> ^[16]	56	8	0	11 (7)	37	23
Belyaev <i>et al</i> ^[17]	27	3	8	NR	NR	NR
Beltrame <i>et al</i> ^[18]	21	2	0	8.7 (2)	5	18
Kinoshita <i>et al</i> ^[19]	16	9	1	39 (10)	NR	NR
Total	751	90	24		85	129

PD: Pancreatoduodenectomy; DP: Distal pancreatectomy; TP: Total pancreatectomy; NR: Not reported; ASA: American Society of Anesthesiologists.

tumor (74.6% of malignant cases, $n = 419$), followed by periampullary carcinoma (11.6%, $n = 65$) and cholangiocarcinoma (6.6%, $n = 37$). Other malignant tumor types reported were neuroendocrine tumors, intraductal papillary mucinous neoplasms (IPMNs) with invasive carcinoma, and pancreatic secondary tumors. Among benign neoplasms ($n = 64$, 10.2%), the most frequent indications were benign or borderline IPMNs ($n = 25$, 39.1%), cystic neoplasms (20.3%, $n = 13$) and neuroendocrine tumors (9.4%, $n = 6$). A total of 3793 resections were performed, with data on 751 pancreatoduodenectomy, 90 distal pancreatectomy and 24 total pancreatectomy (TP). A vascular resection was reported in 48 cases (Table 2).

EARLY OUTCOME

Overall morbidity and mortality rates were 34.9% and 13.2% respectively, with a mean length of hospital stay of 18 d (Table 3). Detailed information on specific type of postsurgical complications were available for 569 patients. Most frequent complications were pancreatic fistula (12.1%, $n = 69$), delayed gastric emptying (10.9%, $n = 62$) and cardiopulmonary complications (9.3%, $n = 53$). Reoperations rate was 7.5% (Table 4). Four studies^[2,8,9,11] focused on hospital discharge and the need for skilled nurse facilities after surgical resection. Finlayson *et al*^[8], Riall *et al*^[9] and Khan *et al*^[11] observed a percentage of respectively 63.3%, 61.8% and 79% of resected patients who were discharged directly home, with or without home health care support. The other patients were discharged to health care facilities (nursing home, skilled care or other intermediate care facilities)

Table 3 Perioperative outcomes after pancreatic resection

Ref.	Mortality % (n)	Morbidity % (n)	Mean length of stay (d)
Chen <i>et al</i> ^[6]	13.0 (2)	51 (8)	25.0
Makary <i>et al</i> ^[7]	4.0 (8)	53 (109)	11.0
Finlayson <i>et al</i> ^[8]	15.5 (452)	NR	20.4
Riall <i>et al</i> ^[9]	11.4 (24)	NR	15.0
Hardacre <i>et al</i> ^[3]	0	66 (21)	11.0
Tani <i>et al</i> ^[2]	NR	44 (11)	25.0
Lee <i>et al</i> ^[10]	5.4 (4)	47 (35)	10.5
Khan <i>et al</i> ^[11]	2.0 (1)	51 (27)	13.5
Stauffer <i>et al</i> ^[12]	0	50 (16)	13.3
Hatzaras <i>et al</i> ^[13]	3.7 (1)	52 (14)	12.0
Melis <i>et al</i> ^[14]	4.0 (1)	68 (17)	20.0
Oguro <i>et al</i> ^[15]	4.5 (1)	27 (6)	31.5
Turrini <i>et al</i> ^[16]	4.7 (3)	56 (36)	24.9
Belyaev <i>et al</i> ^[17]	11.4 (4)	NR	15.0
Beltrame <i>et al</i> ^[18]	0 (0)	43 (10)	13.5
Kinoshita <i>et al</i> ^[19]	0 (0)	8 (2) ¹	25.8
Total	13.2 (501)	34.9 (306) ²	18.0

NR: Not reported. ¹Clavien-Dindo classification \geq III; ²Riall *et al*^[9] excluded, because only severe complications were reported.

or required other inpatient acute care hospitals.

ADJUVANT THERAPY

Only few studies reported data on adjuvant therapy after pancreatic resections for cancer (Table 5). Kinoshita *et al*^[19] reported 13 out of 26 resected patients (50%) who received adjuvant treatment. Six out of 13 patients completed the planned adjuvant chemotherapy, which was discontinued in the other patients because of poor general conditions, chemotherapy-related adverse-events or postoperative recurrence. Beltrame *et al*^[18] reported 30% of resected patients who received adjuvant treatment, while in the study by Turrini *et al*^[16] the patients' rate receiving postsurgical treatment was as low as 23.4%. Finally, Hardacre *et al*^[3] reported 10/25 patients resected for adenocarcinoma who were administered adjuvant chemotherapy. Specific survival outcome for patients receiving adjuvant treatment were not reported.

SURVIVAL

Median overall survival was 17.6 mo. One-year and 5-year survival rates were not always reported and varies among different series; at that point it is important to keep in mind that different reports consider different surgical indications. One-year survival rates range from 50% to 75.7%, while 5-year survival rates range from 0% to 46% (Table 5). When considering only those studies focusing on pancreatic adenocarcinoma, median overall survival is 15.4 mo. Melis *et al*^[14] reported a 1-year and 5-year survival rate of 68.2% and 4.5% respectively. Turrini *et al*^[16] reported a 1-year survival rate of 75.7% while 5-year survival rate was 0%.

Table 4 Postoperative complications and reoperation rates

Ref.	Pancreatic Fistula	Delayed gastric emptying	Postpan- createctomy haemorrhage	Reoperation rate % (n)
Chen <i>et al</i> ^[6]	2	3	3	NR
Makary <i>et al</i> ^[7]	21	32	0	5.6 (11)
Finlayson <i>et al</i> ^[8]	NR	NR	NR	NR
Riall <i>et al</i> ^[9]	NR	NR	NR	NR
Hardacre <i>et al</i> ^[3]	3	4	5	22.0 (7)
Tani <i>et al</i> ^[2]	1	6	0	NR
Lee <i>et al</i> ^[10]	3	NR	NR	5.4 (4)
Khan <i>et al</i> ^[11]	6	9	5	1.9 (1)
Stauffer <i>et al</i> ^[12]	NR	NR	NR	6.2 (2)
Hatzaras <i>et al</i> ^[13]	3	0	NR	4.0 (1)
Melis <i>et al</i> ^[14]	NR	NR	NR	NR
Oguro <i>et al</i> ^[15]	11	5	4	4.5 (1)
Turrini <i>et al</i> ^[16]	10	NR	10	10.9 (7)
Belyaev <i>et al</i> ^[17]	NR	NR	NR	13.1 (5)
Beltrame <i>et al</i> ^[18]	4	0	1	13.0 (3)
Kinoshita <i>et al</i> ^[19]	5	3	1	NR
Total	69	62	29	7.5 (43)

NR: Not reported.

PROGNOSTIC FACTORS

Six authors^[9,10,13,15,16,19] examined clinical variables and risk factors that could be associated with poorer survival in octogenarian patients. Hatzaras *et al*^[13] reported lymphovascular invasion as the only predictor of survival. Oguro *et al*^[15] found that pancreatic cancer was an independent poor prognostic factor in the multivariate analysis with a hazard ratio of 4.398. Turrini *et al*^[16] identified 4 independent prognostic indicators of overall survival: Venous invasion, arterial invasion, positive lymph nodes and adjuvant treatment. In their multivariate analysis, Kinoshita *et al*^[19] indicated that distant metastasis and the conclusion of the planned adjuvant therapy were independent prognostic factors of surgical resection. Lee *et al*^[10] reported female gender, non-caucasian race and positive lymph nodes as factors associated with shorter survival time in the multivariate model. In none of the aforementioned studies, age 80 or more resulted to be a significant predictor of long-term survival. On the contrary, Riall *et al*^[9] in a population-based study used logistic regression models to assess the independent effect of age group on mortality. When compared with patients < 69 years of age, age group was an independent predictor of mortality after pancreatic resection.

QUALITY OF LIFE AFTER RESECTION

Although quality of life (QoL) is an important aspect of surgical result, this point is not evaluated in most of the studies. Gerstenhaber *et al*^[20] firstly assessed QoL after pancreaticoduodenectomy in 70 elderly patients (aged 70 years and older). Fatigue was the most common symptom for the first 6 mo after surgery, but QoL quickly improved to normal scores. However, it has been reported that patients 80 years or older required

Table 5 Long-term results

Ref.	Adjuvant therapy % (NR)	Median overall survival (mo)	1-yr survival rate (%)	5-yr survival rate (%)
Chen <i>et al</i> ^[6]	NR	17.6	NR	NR
Makary <i>et al</i> ^[7]	NR	19	59.1	24.4
Finlayson <i>et al</i> ^[8]	NR	NR	NR	11.3
Riall <i>et al</i> ^[9]	NR	NR	NR	NR
Hardacre <i>et al</i> ^[3]	31.2 (10)	14.4	57.0	24.0
Tani <i>et al</i> ^[2]	NR	NR	NR	NR
Lee <i>et al</i> ^[10]	NR	11.6	NR	NR
Khan <i>et al</i> ^[11]	22	13.5	NR	NR
Stauffer <i>et al</i> ^[12]	NR	NR	67.0	42.0
Hatzaras <i>et al</i> ^[13]	NR	33.3	NR	33.1
Melis <i>et al</i> ^[14]	NR	17.3	68.2	4.5
Oguro <i>et al</i> ^[15]	0 (0)	13.0	NR	46.0
Turrini <i>et al</i> ^[16]	23.4 (15)	30.0	75.7	0
Belyaev <i>et al</i> ^[17]	NR	NR	NR	NR
Beltrame <i>et al</i> ^[18]	30.0 (7)	19.0	NR	NR
Kinoshita <i>et al</i> ^[19]	50.0 (13)	12.4	50.0	NR

NR: Not reported.

discharge to a nursing facility more frequently when compared to younger patients^[21]. This is obviously due to the need of rehabilitation program both in the physical activity and digestive function.

DISCUSSION

The higher incidence of morbidity, risk of mortality and of a prolonged recovery in an extended care facility following hospital discharge, made in past years pancreatic surgery a rare indication for older patients. The improved outcomes after pancreatic resections performed in high-volume centres have allowed to broaden the selection criteria for surgery and to include more elderly patients. The first study considering octogenarian patients and pancreatic surgery was published by Sohn *et al*^[22]. Authors compared postoperative outcome of octogenarian patients undergoing pancreaticoduodenectomy with patients younger than 80 years, and reported similar morbidity and mortality rates in the two different age groups. This observation was then confirmed in other subsequent studies, showing similar results in postoperative outcome in elderly patients^[2,7,11]. On the contrary, two large population-based studies^[8,9] showed high mortality rates after pancreatic surgery in octogenarians with a high rate of discharge to health facilities. Sukhramwala *et al*^[23] performed a systematic review and meta-analysis comparing the results of four studies^[7,11,13,22] and showed that patients 80-years or older had a higher incidence of postoperative mortality, morbidity and pneumonia in comparison to younger patients. A recent meta-analysis by Casadei *et al*^[24], showed a higher postoperative mortality rate in patients 80 years or older when compared to younger patients. These conflicting results may have different possible explanations. First of all, it

has to be considered that the presence of an increased prevalence of preoperative comorbidities, represents a potential selection bias in those studies comparing outcome of elderly to younger patients. Therefore, preoperative studies play a major role in recognizing high-risk patients and in selecting the most appropriate treatment. The identification of modifiable preoperative risk factors for morbidity and mortality would improve the surgical outcome of patients^[25]. Several scoring systems are available in the clinical practice to assess the surgical risk of old patients: Adult Comorbidity Evaluation-27 (ACE-27)^[26], Charlson Comorbidity index^[27] and G8 geriatric screening tool^[28]. These tools allow a risk stratification in order to evaluate the impact of age in the surgical management of elderly patients. Old patients require a multidisciplinary evaluation (geriatric assessment) in order to identify those individuals who are at high-risk of complication^[29]. Another reason of difference in postoperative outcome may be the hospital load for pancreatic resections. In fact, the importance of hospital volume for improving outcome after pancreatic surgery has already been demonstrated^[30] and better prognosis after centralization of pancreatic cancer resection is reported^[31]. Riall *et al*^[9] reported a mortality rate following surgery in octogenarians nearly doubled at low-volume facilities compared to high-volume centres. Management of elderly patients requires a multidisciplinary evaluation prior to surgery in order to have a precise risk stratification and a selection of patients undergoing surgery. Moreover, postoperative care requires a specialized staff (surgeons, anaesthesiologists, interventional radiologists, endoscopists, etc.) and specialized facilities commonly available in high-volume centres. Finally, only few reports^[3,16,18,19] in the literature reported the patients' rate undergoing adjuvant treatment after surgery and their specific outcome. It is increasingly recognized that elderly patients are underrepresented in cancer trials and that elderly patients are less likely to receive adjuvant chemotherapy^[32,33]. Reluctance to administer postoperative treatment is often based on the presence of comorbidities in elderly patients and by the perception that there is an increased risk of non-cancer-related cause of death, limiting the overall benefit of adjuvant treatment. Nagrial *et al*^[33] showed that this is not the case, being cancer the predominant cause of death in older patients. Given the role of adjuvant therapies in prolonging overall survival and delaying time to recurrence in resectable pancreatic cancer^[34,35], the advancing age alone should not preclude the use of adjuvant treatment. Although these limitations, most Authors reported that overall survival after resection for pancreatic cancer in octogenarians is not statistically different from younger patients^[18,21,36,37].

CONCLUSION

Although several authors say that major pancreatic

surgery is safe and feasible in very old patients, the risk of postoperative complications and troubled outcome objectively exist, and may explain the reluctance to perform such complex operation in older patients^[38]. To overcome any prejudice, a careful patient selection is fundamental to avoid or reduce postoperative mortality and morbidity. It seems reasonable to consider elderly patients with 2 or 3 ASA score, with a low rate of comorbidity and good performance status, as good candidate for surgical resection. Even if caution is recommended when treating elderly patients, the morbidity and mortality rates of octogenarians appear within the acceptable range for pancreatic surgery when performed at experienced centres. Geriatric assessment and centralization of pancreatic cancer are key to treatment decisions for patients 80 years and older. Survival after pancreatic resection appears similar in old and young patients, but we are lacking sufficient information about the quality of life of elderly pancreatectomized patients. Additionally, prospective studies are needed in order to determine the quality of life and long-term outcome in this subset of patients, because these features have to be considered in planning of the surgical treatment of octogenarians.

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Epithelial-mesenchymal transition as a therapeutic target for overcoming chemoresistance in pancreatic cancer

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Abstract

Pancreatic cancer has one of the worst prognoses among all cancers due to the late manifestation of identifiable symptoms and high resistance to chemo- and radiation therapies. In recent years, a cancer development phase termed epithelial-mesenchymal transition (EMT) has gained increasing research focus. The process is implicated in tumour metastasis, and emerging evidence suggests EMT also contributes or induces chemoresistance in several cancers. Nevertheless, the applicability of therapeutic targeting of EMT faces many challenges. In this mini-review, we summarise the evidence supporting the role of EMT in pancreatic cancer progression, focusing particularly on its association with chemoresistance.

Key words: Epithelial-mesenchymal transition; Drug resistance; Pancreatic cancer; Chemotherapy

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Core tip: This mini-review examines the role of epithelial-mesenchymal transition in the development of drug resistant pancreatic cancer and a possible use of this process as a drug target.

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INTRODUCTION

Epithelial-mesenchymal transition (EMT) is a stage of phenotypic alteration in cancer cells instigated by several paracrine and autocrine stimuli, leading to a morphological transformation of epithelial-like cancer cells to an elongated mesenchymal phenotype. The phenotypic change is thought to derive from a shift in the balance between epithelial (*e.g.*, E-cadherin and Claudin-1) and mesenchymal (*e.g.*, N-cadherin, Snail, Zeb-1 and Twist-1) factors. Once described as a key step for successful metastasis in some types of cancers^[1], the role of EMT in chemotherapy resistance has attracted much interest recently. Indeed, EMT has been shown to contribute to drug resistance in pancreatic cancer. For instance, in a recent study, the patterns of sensitivity and resistance to three conventional chemotherapeutic agents with divergent mechanisms of action were investigated in several pancreatic cancer cell lines^[2]. Interestingly, gene expression profiling revealed that the sensitive and resistant cells formed two distinct groups with resistant cells showing several features consistent with EMT. Additionally, an inverse correlation between E-cadherin and its transcriptional suppressor, Zeb-1, was observed in the gene expression. Moreover, silencing of Zeb-1 restored drug sensitivity in pancreatic cancer cells. The implication of this study is that effectors of EMT, in particular Zeb-1, are potential molecules to target to overcome drug resistance. Recently, a study casts doubt on the role of EMT in metastasis, while a separate study strongly corroborates the key role of EMT in drug resistance. Indeed, both Fischer *et al.*^[3] and Zheng *et al.*^[4] demonstrated that not only was EMT able to aid chemotherapy resistance in pancreatic and lung cancer cells but that EMT was not needed for metastasis, as inhibition of EMT did not prevent metastasis of pancreatic and lung cancer cells.

EMT SIGNALLING PATHWAYS IN PANCREATIC CANCER

Several signalling pathways can regulate EMT during tumorigenesis^[5-7]. Major pathways which can induce EMT include those activated by growth factors such as transforming growth factor- β (TGF β), epidermal growth factor and hepatocyte growth factor or the Wnt/beta-catenin and Notch pathway. Notch-2 activation was identified in gemcitabine-resistant (GR) pancreatic cancer cells that have acquired EMT. By using pancreatic cancer cells that have developed gemcitabine resistance through exposure to increasing concentrations of gemcitabine, Wang *et al.*^[8] were the first to delineate the EMT profile of GR cells. GR but not gemcitabine sensitive (GS) cells had an elongated and irregular shape, increased expression of Zeb-1, vimentin, fibronectin and alpha-SMA, as well as translocation of E-cadherin from the plasma membrane to the nucleus. In concordance with previous reports

regarding the role of Notch signalling in EMT, Wang *et al.*^[8] further characterised the Notch activation status in GR cells. Notch-2 and its ligands, Jagged-1 and Notch-4, were upregulated in GR cells. The Notch signalling also upregulates NF- κ B, a critical mediator of the EMT process. Indeed, NF- κ B was upregulated in GR cells as well as its downstream target, matrix metalloproteinase 9 (MMP-9). The activity of MMP-9 is governed by urinary plasminogen activator (uPA), also upregulated in GR cells, and both proteins are known for their role in cancer invasion and metastasis. Using Notch-2 and Jagged-1 siRNA, the interrelation between Notch, EMT and NF- κ B were further delineated. Notch-2 and Jagged-1 knock-down in GR cells led to mesenchymal-epithelial transition (MET) morphological changes which include upregulation of E-cadherin, and reduction of vimentin, slug, snail, Zeb-1, and NF- κ B (p65 subunit). As expected, Notch-2 and Jagged-1 downregulation by siRNA also led to a reduction in GR cells invasion and migration^[8]. The study characterised for the first time the Notch-driven EMT pathway in GR pancreatic cancer cells, however, the authors did not address whether silencing or inhibition of Notch could reverse GR.

EMT AND GEMCITABINE RESISTANCE

The development of gemcitabine resistance and its association with EMT phenotype has drawn attention to a possible role of gemcitabine in inducing EMT. Güngör *et al.*^[9] examined the role of Midkine (MK) in orchestrating the interplay between Notch, EMT and gemcitabine resistance. MK is a heparin-binding growth factor overexpressed in some types of cancers^[10,11]. High MK mRNA expression was detected in pancreatic cancer cell lines that developed gemcitabine resistance following repeated exposure to gemcitabine, and in PDAC tumour samples isolated from patients who underwent total pancreaticoduodenectomy. Gemcitabine treatment in GR cells led to a dose-dependent increase in MK mRNA expression and protein secretion, an effect not observed in GS cells. Knockdown of MK with siRNA restored gemcitabine sensitivity in GR cells, while the addition of recombinant human MK (rh-MK) to MK-knockdown or GS cells, restored or induced resistance, respectively. GR cells overexpressing MK displayed EMT characteristics while MK-depleted GR cells displayed MET characteristics as evidenced by downregulation of vimentin and NF- κ B, and upregulation of E-cadherin. As expected, there was a reduction in migration and invasion in MK-depleted cells compared to control. As Notch-2 and EMT are associated with gemcitabine resistance, Güngör *et al.*^[9] further examined the impact of MK on Notch-2 activation. Treatment of GR cells with rh-MK resulted in enhanced cleavage of Notch^{ICD} and expression of Hes-1 (Notch-2 target). Expectedly, silencing of Notch-2 improved gemcitabine efficacy in GR cells. Güngör *et al.*^[9] were the first to pinpoint the role of MK in gemcitabine resistance and its impact on

Notch-2 activation and EMT phenotype.

The role of Notch-2 activation in EMT, metastasis and chemotherapy resistance has attracted attention to target Notch-2 in pancreatic cancer. Palagani *et al.*^[12] showed the effect of the γ -secretase inhibitor (GSI IX) in preventing Notch-2 activation, EMT, and cancer cell proliferation and migration *in vitro* and pancreatic tumour-initiating CD44⁺/EpCAM⁺ xenograft growth and metastasis *in vivo*. Future studies are needed to examine the effect of GSI IX in reducing gemcitabine resistance in pancreatic cancer.

A few microRNAs are implicated in EMT and chemoresistance. Li *et al.*^[13] were the first to introduce a novel way of reducing gemcitabine resistance in pancreatic cancer (PaCa) through modulation of microRNAs. GR PaCa cells showed downregulation of miR-200 in addition to the typical EMT signature discussed earlier. Upregulation of miR-200 either through the reintroduction of miR-200 or the treatment with the natural compound, isoflavone, resulted in MET as demonstrated by decreased levels of mesenchymal markers (Zeb1, Vimentin and Slug) and induction of epithelial-associated morphological changes, and thus reducing gemcitabine resistance^[13]. Using a similar methodology, Ma *et al.*^[14] recently showed a positive influence of miR-233 in EMT, invasion, migration and gemcitabine resistance in PaCa cells.

The conversion in cancer cells from epithelial to mesenchymal phenotype and its requirement for cancer invasion and migration were clearly demonstrated through *in vitro* studies. However, the consequence of EMT on cancer metastasis had not been investigated *in vivo* until recently. Using the well-established mouse model that mirrors human pancreatic cancer (KPC mice), Zheng *et al.*^[4], produced KPC mice absent in Snail or Twist protein. Although accumulating evidence suggests the requirement of EMT process for cancer migration, the authors showed the ability of pancreatic cancer to metastasize despite deleted EMT-inducing factors, Snail and Twist. Deletion of either one of these proteins did not affect local invasion, metastasis or overall survival compared to control KPC mice. It also resulted in a reduction in expression of other mesenchymal markers (e.g., Slug, Zeb1 and α -SMA) while enhancing the expression of the epithelial factor, E-cadherin. Although the apoptosis of cancer cells was not affected by deletion of Snail or Twist, the proliferation rate of cancer cells significantly increased, while blood dissemination remained unchanged compared to control KPC mice. Examination of EMT profile of the metastatic pancreatic cancer cells at secondary sites (liver, lung and spleen) showed positive for E-cadherin, and negative for Snail or Twist. Moreover, the ability of cancer cells, isolated from either control KPC mice or KPC with deleted Snail or Twist, to form tumour spheres were comparable. In the study, EMT program did not appear essential for primary cancer growth, local invasion, blood dissemination and metastasis. Interestingly, gemcitabine sensitivity was improved in KPC mice with deleted Snail or Twist

compared to KPC control mice which could be explained by a significant upregulation of equilibrative nucleoside transporter 1 and concentrating nucleoside transporter 3 (receptors that mediate uptake of nucleosides) in cancer cells lacking Snail or Twist.

GENERAL CONSIDERATIONS ON PHARMACOLOGICAL APPROACHES TO TARGET EMT

Several strategies have been proposed for the design of EMT-based therapies as recently and extensively described and reviewed by Davis *et al.*^[1]. While major challenges and questions remain regarding the possibility of targeting EMT to counteract metastasis specifically, stronger evidence is accumulating on the use of anti-EMT agents in cancer chemoresistance settings. However, targeting a single receptor, enzyme or transporter that is associated with EMT faces many limitations since several redundant pathways are involved in this process. Strategies focused on targeting microRNAs regulating EMT such as miR-200, or transcription factors might represent a more effective approach since they influence the process more broadly. In addition, key components of the tumour microenvironment are also attractive targets for therapeutic intervention. Indeed, recent evidence has revealed that local tumour microenvironment represents a main driving force for EMT, chemotherapy resistance and cancer progression. Inflammatory cells such as neutrophils and macrophages are contained in the tumour microenvironment, which offers multidirectional interactions leading in some cases to increased chemotherapy resistance and metastasis^[15-18]. Neutrophils have been shown to induce EMT in pancreatic cancer while macrophages induce gemcitabine resistance *via* promoting cytidine deaminase mediated drug inactivation^[15,19,20]. Similarly, platelets were recently shown to be capable of inducing EMT in cancer^[21,22]. Pancreatic cancer is associated with a high risk of venous thromboembolism (VTE) caused by tumour-derived or tumour-elicited tissue factor which can indirectly induce platelets aggregation^[23,24]. Whether targeting platelets can offer an indirect way to reduce EMT and chemoresistance as well as the risk of VTE in pancreatic cancer is yet to be demonstrated.

Therefore, a systematic testing of different methods for targeting EMT in combination with existing chemotherapeutic agents is required for each model of therapy relapse. The excitement elicited by the new reinforcement of the link between EMT and chemoresistance will surely result in a surge of studies in this field, and consequently, further in-depth investigations are warranted, especially in pancreatic cancer.

CONCLUSION

In summary, resistance to treatments such as Gemcitabine in pancreatic cancer can be mediated by several

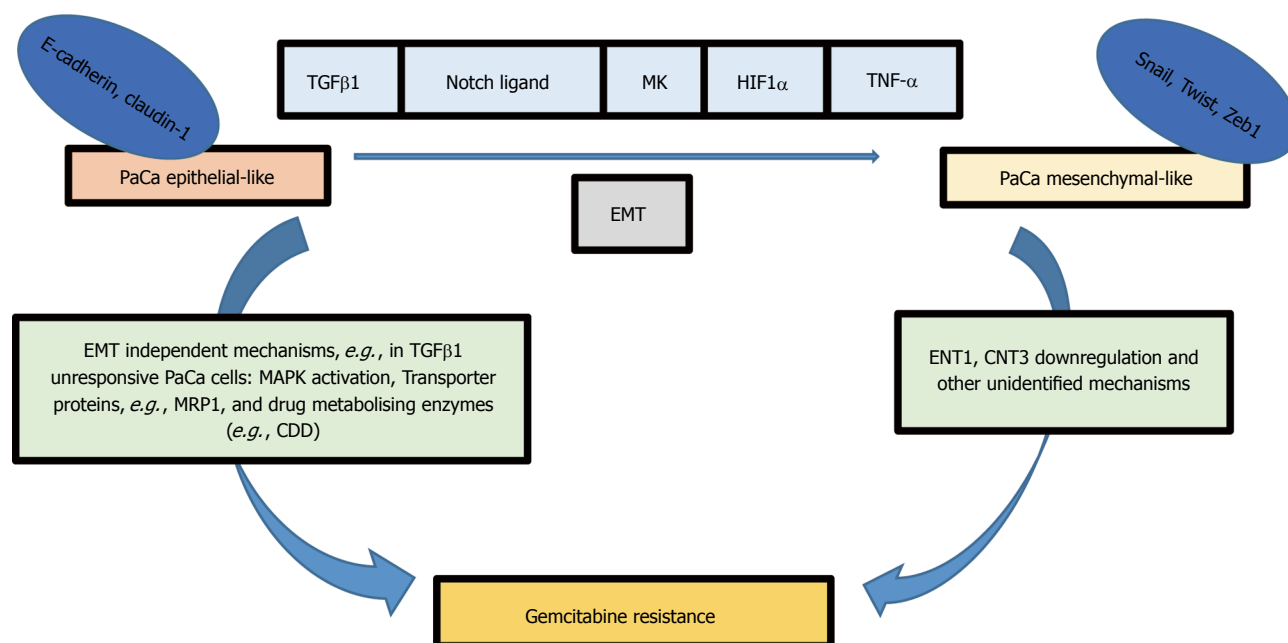


Figure 1 Several signalling pathways can induce epithelial-mesenchymal transition in pancreatic cancer cells for example Notch, transforming growth factor- β , Midkine, hypoxia-inducible factor-1 α and tumor necrosis factor- α . EMT phenotype is associated with gemcitabine resistance; however, the signalling pathway relating EMT factors (e.g., Snail, Twist and Zeb1) to gemcitabine resistance is not clearly identified, although ENT1 and CNT3 upregulation was observed in KPC mice models with deleted Snail or Twist. EMT independent pathways can also lead to gemcitabine resistance for example MAPK activation, transporter proteins, and gemcitabine metabolising enzymes (e.g., CDD). MRP1: Multidrug resistance protein1; CDD: Cytidine deaminase; TGF β : Transforming growth factor- β ; MK: Midkine; HIF1 α : Hypoxia-inducible factor-1 α ; TNF- α : Tumor necrosis factor- α ; EMT: Epithelial-mesenchymal transition; ENT1: Equilibrative nucleoside transporter 1; CNT3: Concentrative nucleotide transporter.

EMT-dependent or independent pathways (Figure 1)^[15,25-29], making the EMT process an attractive target for reducing chemotherapy resistance in pancreatic cancer. EMT can be regulated by blocking extracellular signalling molecules such as TGF β 1 (a cytokine mediator of EMT in many types of cancer) and EMT signal transduction pathways^[1]. Loss of E-cadherin-mediated cell adhesion is a hallmark of EMT and subsequent invasion and metastasis. Since Snail and Zeb family of transcriptional factors mediate E-cadherin translocation, loss of function or downregulation, they can potentially be targeted to avert EMT at its initial steps. Several studies have reported disruption in TGF β signalling in pancreatic cancer^[30-32]. Therefore, the mesenchymal transcriptional factors may be better druggable targets compared to TGF- β receptors to reduce EMT-derived chemoresistance in pancreatic cancer. Despite a significant number of emerging studies examining the role of EMT in cancer, the interplay between different signalling pathways that drive EMT is more complex than we initially thought. The fact that the phenotypic alteration is transient and triggered by several dynamics encountered by tumour cells during their development or metastasis emphasises the challenge of utilising EMT as a lone druggable target. The diversity of EMT-inducing transcriptional factors may enable cancer cells to adapt and survive a single targeted molecular therapy. The association between EMT and chemotherapy resistance is well established in the literature, but it is not well understood how EMT can affect cancer cell survival pathways, drug transporters and drug metabolising enzymes. Delineation of these

interactions may uncover novel approaches to inhibit chemoresistance in pancreatic cancer. Nevertheless, at present, there may be potential benefits in tempering cancer EMT in a combined immunotherapy and molecular-targeted drug strategies to treat pancreatic cancer.

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Retrospective Study

Pre-treatment platelet counts as a prognostic and predictive factor in stage II and III rectal adenocarcinoma

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Abstract

AIM

To investigate if pre-treatment platelet counts could provide prognostic information in patients with rectal adenocarcinoma that received neo-adjuvant treatment.

METHODS

Platelet number on diagnosis of stage II and III rectal cancer was evaluated in 51 patients receiving neo-adjuvant treatment and for whom there were complete follow-up data on progression and survival, as well as pathologic outcome at the time of surgery. Pathologic responses on the surgical specimen of patients with lower platelet counts ($150-300 \times 10^9/L$) were compared with these of patients with higher platelet counts ($> 300 \times 10^9/L$) by the χ^2 test. Overall and progression free survival Kaplan-Meier curves of the two groups were constructed and compared with the Log-Rank test.

RESULTS

A significant difference was present between the two groups in regards to pathologic response with patients with lower platelet counts being more likely to exhibit a

good or complete response to neo-adjuvant treatment than patients with higher platelet counts ($P = 0.015$). Among other factors evaluated, there was also a significant difference between the carcinoembryonic antigen (CEA) at presentation of patients that exhibited a good or complete response and those that had no response or a minimal to moderate response. Patients with a good or complete response were more likely to present with a CEA of less than 5 $\mu\text{g/L}$ ($P = 0.00066$). There was no significant difference in overall and progression free survival between the two platelet count groups (Log-Rank tests $P = 0.42$ and $P = 0.35$, respectively).

CONCLUSION

In this retrospective analysis of stage II and III rectal cancer patients, platelet counts at the time of diagnosis had prognostic value for neo-adjuvant treatment pathologic response. Pre-treatment CEA also held prognostic value in regards to treatment effect.

Key words: Rectal cancer; Platelets; Prognosis; Treatment response; Neo-adjuvant; Chemoradiation

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Core tip: Platelet counts may provide prognostic and treatment efficacy predictive information in various cancers. In this study, platelet number on diagnosis of stage II and III rectal cancer was evaluated in 51 patients before start of neo-adjuvant treatment. A significant difference was present between the two groups, of higher and lower platelets, regarding pathologic response to neo-adjuvant treatment. There was no significant difference in overall and progression free survival between the two platelet count groups. Pre-treatment carcinoembryonic antigen also held prognostic value in regards to treatment effect.

Steele M, Voutsadakis IA. Pre-treatment platelet counts as a prognostic and predictive factor in stage II and III rectal adenocarcinoma. *World J Gastrointest Oncol* 2017; 9(1): 42-49 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v9/i1/42.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v9.i1.42>

INTRODUCTION

Platelets play a crucial role in maintaining hemostasis and vascular integrity. They are produced from bone marrow precursor cells, megakaryocytes, as fragments breaking off of the megakaryocytes cytoplasm^[1]. Abnormalities in platelet number, whether an increase or decrease in their circulating number, are associated with many pathologic conditions^[2]. Cancer is a pathology that is often associated with thrombocytosis as the cytokines that stimulate thrombopoiesis are often elevated in

cancer^[3]. In addition, thrombocytosis has been found to be an adverse prognostic factor in many common cancers^[4].

Colorectal cancer is a common malignancy and one of the leading causes of cancer deaths in both men and women^[5]. Localized stage rectal cancer is typically treated with neoadjuvant chemoradiotherapy in addition to adjuvant chemotherapy^[6]. Pathologic stage is the main prognostic factor and treatment modality determinant. Other prognostic factors include positive surgical margins, pre-treatment elevation of carcinoembryonic antigen (CEA), and high tumour grade^[7]. Prognostic markers of positive pathologic response to neo-adjuvant therapy are also important because such response may be associated with survival outcomes in rectal cancer^[8]. Moreover, being able to predict which patients would benefit from neo-adjuvant chemoradiation could be important for modification of the treatment plan and sparing of patients predicted not to respond to this therapy and its adverse effects. Thus, additional biomarkers are needed to further promote prognostication of rectal adenocarcinomas. In a previous study in patients with colorectal adenocarcinomas of various stages, pre-treatment thrombocytosis was an independent prognostic factor for overall survival (OS) and progression-free survival (PFS)^[9]. Nevertheless, another study in colorectal cancer patients did not observe a difference in survival between patients with thrombocytosis and normal platelet counts^[10]. In the current study, we investigated if pre-treatment thrombocytosis provides prognostic information specifically in patients with stage II and III rectal adenocarcinomas that received neo-adjuvant treatment. We also investigated the effect of thrombocytosis on pathologic outcome at the time of surgery.

MATERIALS AND METHODS

The case records of 130 patients treated for localized rectal cancer at the Algoma District Cancer Clinic between January 2008 and January 2015 were retrospectively reviewed. Patients were included if they had stage II or III disease, had received neo-adjuvant treatment, and had complete follow-up. Follow-up was considered complete if a patient was followed until his or her death, or was seen within the last six months from data collection. Fifty one patients fulfilled the inclusion criteria and were included in the study. Demographic data, as well as histologic characteristics of tumors, stage, tumor marker CEA, and pathologic response were extracted from the medical records. Platelet number at diagnosis of the 51 patients was also evaluated.

Pathologic response at the time of surgery was categorized in a five tier scale ranging from no response (no evidence of treatment effect on tumor), minimal response (some morphologic effect of treatment evident but no significant regression of tumor areas), moderate

Table 1 Baseline characteristics of all patients in the series and comparison of the groups with lower ($\leq 300 \times 10^9/L$) and higher ($> 300 \times 10^9/L$) platelet counts *n* (%)

	Total (%) (<i>n</i> = 51)	≤ 300 (<i>n</i> = 26)	> 300 (<i>n</i> = 25)	χ^2
Age (yr)				
> 60	21 (41.2)	10 (38.5)	11 (44.0)	$P = 0.69$
≤ 60	30 (58.8)	16 (61.5)	14 (56.0)	
Clinical stage				
II	25 (49.0)	15 (57.7)	10 (40.0)	$P = 0.21$
III	26 (51.0)	11 (42.3)	15 (60.0)	
CEA (<i>n</i> = 50)				
> 5	25 (50.0)	11 (44.0)	14 (56.0)	$P = 0.4$
< 5	25 (50.0)	14 (56.0)	11 (44.0)	
Symptoms				
Obstruction/pain	13 (25.5)	6 (23.1)	7 (28.0)	$P = 0.69$
Bleeding/ asymptomatic	38 (74.5)	20 (76.9)	18 (72.0)	
Type of surgery				
Anterior resection	27 (52.9)	13 (50.0)	14 (56.0)	Anterior resection <i>vs</i> APR/ exenteration $P = 0.77$
APR	19 (37.3)	11 (42.3)	8 (32.0)	
Pelvic/exenteration	2 (3.9)	0	2 (8.0)	$P = 0.77$
None	3 (5.9)	2 (7.7)	1 (4.0)	
Pathologic response				
No response	15 (29.4)	8 (30.8)	7 (28.0)	No/ minimal/ Moderate resp <i>vs</i> Good/ complete $P = 0.015$
Minimal	7 (13.7)	4 (15.3)	3 (12.0)	
Moderate	15 (29.4)	3 (11.6)	12 (48.0)	
Good	5 (9.8)	5 (19.2)	0	
Complete	9 (17.6)	6 (23.1)	3 (12.0)	
Lymph nodes at surgery				
Negative	31 (60.8)	15 (57.7)	16 (64.0)	$P = 0.61$
Positive	16 (31.4)	9 (34.6)	7 (28.0)	
No surgery	3 (5.9)	2 (7.7)	1 (4.0)	

Pre-operative CEA not available in one patient. Lymph node evaluation was not available in the pathology report in one patient. APR: Abdomino-perineal resection; CEA: Carcinoembryonic antigen.

response (evident effect of treatment but significant tumor aggregates remaining), good response (only occasional scattered tumor cell aggregates remaining) and complete response (no evidence of tumor in the primary site or the lymph nodes).

OS was defined as the interval from the date of diagnosis to patient death or censored at the date of last contact. PFS was defined as the interval from the date of diagnosis until date of disease progression or censored at the date of last contact without evidence of recurrence. For the purpose of this study, patients with platelet counts of $150\text{--}300 \times 10^9/L$ were included in the lower platelet count group. Patients with counts $> 300 \times 10^9/L$ were included in the higher platelet count group. This value divided patients in two groups with almost equal numbers. Survival plots of the patients with lower and higher platelet counts were constructed using the Kaplan-Meier method and were compared using the Log-Rank test^[11]. The χ^2 test was used to evaluate differences in clinical and biologic characteristics of the two groups^[12]. The Student's *t*

test was used for comparison of means. All *P* values were considered to be significant at a level of $P < 0.05$. Statistical calculations were performed with online tools available from the Technical University of Denmark (<http://www.iscc-serv2.imm.dtu.dk/>) and a noncommercial site (<http://www.statpages.org/>). The study was approved by the Institutional Review Board of our institution. Due to the retrospective nature of the study, no patient consent was required or obtained.

RESULTS

The median age of the patients was 58-year-old. From the 51 patients, 26 patients (51%) were included in the lower platelet ($\leq 300 \times 10^9/L$) group and had mean platelet counts of $232.5 \times 10^9/L$ (range, 167–297) at diagnosis of their disease (Table 1). Twenty-five patients (49%) were in the higher platelet ($> 300 \times 10^9/L$) group and had mean platelet counts of $347 \times 10^9/L$ (range, 303–693). The median age of the patients with lower platelet counts was 59-year-old (range, 32–79) and those with higher counts was 58-year-old (range, 24–74). In the lower platelet group 38.5% of patients were older than 60-year-old while in the higher platelet group 44% were older than 60-year-old (χ^2 test $P = 0.69$). Forty-four patients in the series received neoadjuvant chemoradiation with continuous infusion of 5-FU or capecitabine as the chemotherapy part. Five additional patients (four in the higher platelet group and one patient in the lower platelet group) received 1–2 cycles of neo-adjuvant mFOLFOX before chemoradiation. Two patients (both in the higher platelet group) received neo-adjuvant radiation alone. No differences in the two groups were noted in the clinical stage at presentation, in the tumor marker CEA or patients' symptoms of presentation (Table 1). The type of surgery performed after neo-adjuvant therapy (whether an abdominal resection or abdomino-perineal resection (APR)/pelvic exenteration with permanent colostomy) was also not statistically different in the two groups (Table 1). All patients but two had negative pathologic surgical margins at surgery. Both patients with positive pathologic margins (one in the lower and one in the higher platelet group) underwent an APR, had minimal pathologic responses and had a recurrence 12 and 20 mo postoperatively respectively. All patients but three had post-operative 5-fluoropyrimidine-based chemotherapy. Three patients who had complete pathologic response (two in the lower platelet group and one patient in the higher platelet group) elected not to undergo surgery and were placed in close surveillance.

Overall about one third of patients in the series were lymph node positive on pathologic examination at the time of surgery and the percentage did not differ significantly between the two platelet groups ($P = 0.61$) (Table 1). A complete pathologic response (defined as no pathologic evidence of tumor in either primary site or lymph nodes examined) was obtained after neo-adjuvant treatment in 9 patients (17.6%)

Table 2 Comparison of characteristics of patients according to their pathologic response at surgery (*n* = 48) or at post-neoadjuvant treatment endoscopy (*n* = 3) *n* (%)

	No response/ minimal/ moderate	Good/ complete	χ^2 <i>P</i> value
Age (yr)			
> 60	13 (35.1)	8 (57.1)	0.15
≤ 60	24 (64.9)	6 (42.9)	
Clinical stage			
II	16 (43.2)	9 (64.3)	0.18
III	21 (56.8)	5 (35.7)	
CEA (<i>n</i> = 50)			
> 5	24 (64.9)	1 (7.7)	0.0004
< 5	13 (35.1)	12 (92.3)	
Symptoms			
Obstruction/pain	12 (32.4)	1 (7.1)	0.06
Bleeding/asymptomatic	25 (67.6)	13 (92.9)	
Platelets			
≤ 300	15 (40.5)	11 (78.6)	0.015
> 300	22 (59.5)	3 (21.4)	

Pre-operative CEA not available in one patient. CEA: Carcinoembryonic antigen.

in the series and an additional 5 patients (9.8%) had good pathologic responses. No response, minimal or moderate response were observed in 15 (29.4%), 7 (13.7%), and 15 (29.4%) patients respectively. Overall pathologic response differed between the groups. Eleven patients (42.3%) in the lower platelet group had a good or complete pathologic response while only three patients in the higher platelet group (12%) had such a response (*P* = 0.015). The mean platelet count at diagnosis of good and complete responders was 249.9 (SD = 69.6) while mean platelet count of no/minimal/moderate responders group was 327.0 (SD = 85.6) (*t* test *P* = 0.004). Among the 25 patients in the elevated platelet group, 16 patients had converted to a platelet count below $300 \times 10^9/L$ after the neo-adjuvant treatment, in their pre-operative evaluation, while the remaining nine patients remained with a platelet count above $300 \times 10^9/L$. All three pathologic responders were among the 16 converted patients.

In the analysis for possible associated factors with a good or complete pathologic response a normal range (< 5 µg/L) CEA level at baseline (*P* = 0.0004) and lower platelet counts (*P* = 0.015) were associated with favorable pathologic response (Table 2). Patients that were asymptomatic at presentation (evaluated with a colonoscopy for anemia) or presented with bleeding had a trend towards a better pathologic response than patients presenting with obstruction or pain (*P* = 0.06). Age and clinical stage at presentation was not statistically associated with the degree of pathologic response. Logistic regression analysis with pathologic response as the outcome variable and platelet counts, CEA and symptoms at presentation as the predictor variables confirmed that lower platelet counts (*P* = 0.03, odds ratio 0.15, 95%CI: 0.02-0.85) and a normal CEA

Table 3 Logistic regression analysis of pathologic response (complete or good *vs* moderate or minimal or no response) as the outcome variable and platelet counts (≤ *vs* > $300 \times 10^9/L$), carcinoembryonic antigen (≤ 5 µg/L *vs* > 5 µg/L) and symptoms (obstruction or pain *vs* bleeding or asymptomatic) at presentation as the predictor variables

Variable	OR	95%CI	<i>P</i> value
Platelet count	0.15	0.02-0.85	0.03
CEA	0.04	0.004-0.41	0.006
Symptoms at presentation	4.7	0.39-55.8	0.20

CEA: Carcinoembryonic antigen.

(*P* = 0.006, OR = 0.04, 95%CI: 0.004-0.41) but not symptoms at presentation (*P* = 0.2, OR = 4.7, 95%CI: 0.39-55.89) were significantly associated with a good or complete pathologic response (Table 3).

The median overall survival of patients that had died in the whole cohort (11 of 51 patients) was 21 mo (range 5-68 mo). In the group of patients with lower platelet counts, 4 of the 26 patients (15.4%) had died with a median OS of 20 mo (range 5-24 mo). In the group of patients with higher platelet counts, 7 of the 25 patients (28%) had died with a median OS of 23 mo (range 7-68 mo). Kaplan-Meier survival analysis showed that there was not a statistically significant difference in the OS and PFS between patients in the two platelet groups (Log-Rank test *P* = 0.42 and 0.35 respectively) (Figure 1A and B). PFS of patients with a good or complete pathologic response was significantly better than that of patients with a lesser pathologic response (Log-Rank test *P* = 0.01) (Figure 2A). OS curve of pathologic responders clearly separated from lesser responders after 2 years but the difference did not reach statistical significance (Log-Rank test *P* = 0.15) (Figure 2B).

DISCUSSION

Thrombocytosis is associated with several underlying pathologies among which cancer is included^[2]. About two out of five patients (40%) with thrombocytosis had an occult cancer in one series^[11]. Thrombocytosis or a higher platelet count defined with various cut-offs has been confirmed to be an adverse prognostic factor in several types of cancer including lung, breast, gynecologic and genitourinary^[12-16]. It has also been studied and suggested to have prognostic relevance in virtually every type of gastrointestinal carcinoma including gastric, colon and rectal^[4].

In a series of Asian patients with colorectal carcinoma, thrombocytosis, defined as platelets counts greater than $300 \times 10^9/L$, similarly to our study, was a significant independent prognostic marker for survival^[17]. This was confirmed in another large Japanese series and a smaller study of European patients^[18,19]. An American study of 1513 patients with localized colorectal cancer that had undergone surgery also evaluated pre-operative thrombocytosis (defined in this study as more

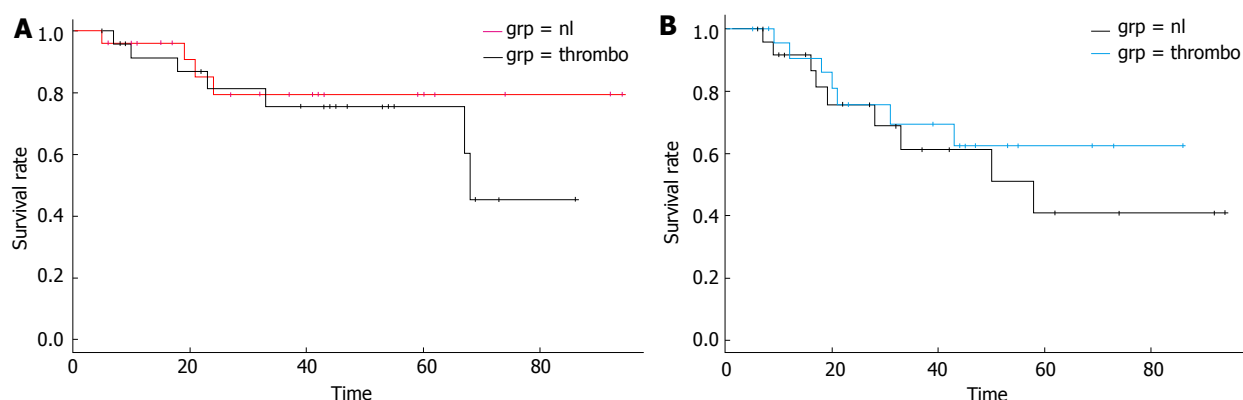


Figure 1 Kaplan-Meier overall survival (A) and progression free survival (B) curves in months from the diagnosis of rectal adenocarcinoma of patients with lower platelet counts ($150-300 \times 10^9/L$, labeled: nl) vs patients with higher platelet counts ($> 300 \times 10^9/L$, labeled: thrombo). Log-Rank tests $P = 0.47$ (A), $P = 0.35$ (B).

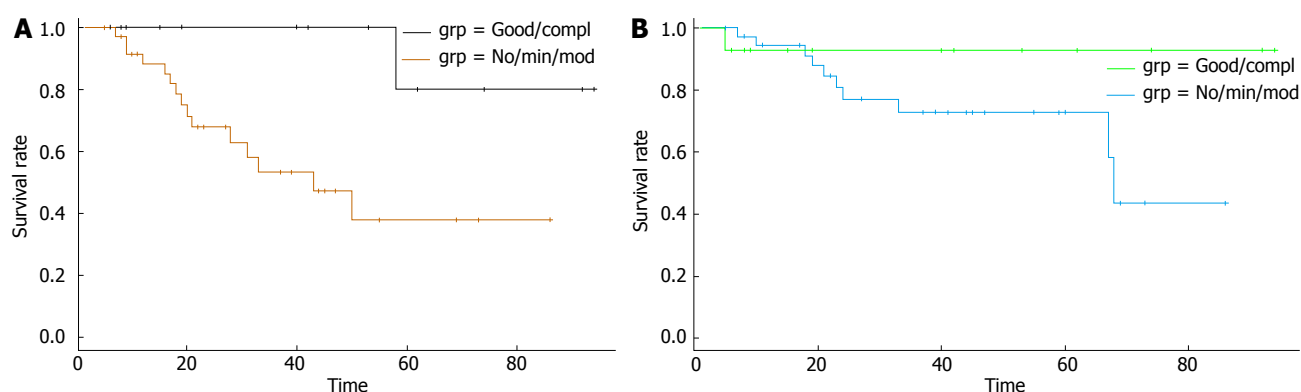


Figure 2 Kaplan-Meier progression-free survival (A) and overall survival (B) curves in months from the diagnosis of rectal adenocarcinoma of patients with a good or complete pathologic response (labeled: Good/compl) vs patients with no response or a minimal or moderate response (labeled: No/min/mod). Log-Rank tests $P = 0.01$ (A), $P = 0.15$ (B).

than $400 \times 10^9/L$) as a prognostic factor for various survival outcomes^[20]. Patients with thrombocytosis had a significantly worse overall survival than patients with normal platelets. Distant metastatic recurrence, but not overall recurrence rate or loco-regional recurrence rate, was also worse in patients with thrombocytosis. In contrast, another study of 630 patients showed no correlation of survival with thrombocytosis defined as platelets above $450 \times 10^9/L$ ^[10]. Nevertheless this study included patients across stages which may have confounded results.

Two studies from the Far East have specifically examined the prognostic role of platelets in rectal cancer patients^[9,21]. Both reports used a similar cut-off for thrombocytosis of 365 to $370 \times 10^9/L$ and showed a similar percentage of thrombocytosis of about 20%. The authorship of the two publications is overlapping and is not clear if the patients of the smaller study^[21] is a subset of the patients included in the larger study^[9]. Both a lower platelet count, below the cut-off, and a normal CEA were associated with pathologic response^[9]. These were the only predictors of such response in logistic regression analysis.

In order to further clarify the prognostic value of

platelets in rectal cancer in a predominantly white population, in this retrospective study we investigated the association between pre-treatment thrombocytosis and the effectiveness of neoadjuvant treatment in patients with stage II and III rectal adenocarcinoma. We also explored the relationship between thrombocytosis and both overall and progression free survival. In the current cohort of 51 patients we found no significant differences between the two groups with lower and higher platelet levels regarding the age of patients, pre-treatment tumor CEA, symptoms at presentation, clinical staging at presentation, and the presence of metastatic lymph nodes at the time of surgery. No difference was also observed in the type of surgery in the two groups. In contrast, a significant difference was present regarding the response to neoadjuvant treatment as patients with lower platelet counts were more likely to have a good or complete pathologic response to pre-operative treatment than patients with higher platelet counts ($P = 0.015$). There was no statistical difference in the OS or PFS of the two platelet groups (Log-Rank test $P = 0.47$ and 0.35 respectively).

The CEA tumor antigen at presentation was also a prognostic marker for pathologic response ($P =$

0.0004). Patients with a CEA of less than 5 were more likely to have a good or complete response to neoadjuvant treatment than those that presented with a CEA greater than 5. A higher pre-treatment CEA has been associated with advanced locoregional disease and therefore may be linked to poorer local control^[22]. Our data confirm that CEA is prognostic for PFS and predictive for pathologic response to neoadjuvant therapy, as suggested previously^[22,23].

The pathogenesis of thrombocytosis in cancer involves production of interleukin-6 (IL-6) at least in some malignancies. In ovarian carcinoma, for example, thrombocytosis was significantly correlated with plasma levels of IL-6^[14]. This was investigated in mice bearing ovarian cancer xenografts of human origin, where human IL-6 was found to stimulate hepatocytes *via* the IL-6 receptor, producing thrombopoietin. Authors proposed that ovarian cancer cells produce IL-6, which functions by stimulating mice liver to produce thrombopoietin, which in turn positively regulates megakaryocyte progenitors in the bone marrow^[14]. In renal carcinoma, most examined cases were positive for IL-6 by immunohistochemistry^[24]. Serum levels of IL-6 were also elevated in prostate and breast cancer patients^[25,26]. Thus cancer cell-derived IL-6 is a trigger of tumor-induced thrombocytosis across various cancers.

The pathophysiology of platelets' contribution to carcinogenesis involves a protective effect on circulating tumor cells from the attack of the immune system^[27]. In addition platelets contribute to the attachment of tumor cells to endothelial cells at sites of metastases. Aggregates of platelets and tumor cells embolize in the microcirculation and facilitate the process of extravasation of tumor cells in metastatic sites. Platelets promote carcinogenesis by their normal function of promoting vascular integrity^[28]. They protect the integrity of newly formed tumor vasculature which is prone to hemorrhage and prevent bleeding in tumor beds^[29]. Platelets contain several types of active macromolecules and cytokines in their granules. These include vascular endothelial growth factor (VEGF), EGF, platelet-derived growth factor, hepatocyte growth factor, transforming growth factor β (TGF β), IL-1 β , IL-8, CXC motif containing ligand 12 and Sphingosine-1-phosphate^[30,31]. These factors have the potential to contribute to metastatic tumor establishment and progression. For example, platelet-derived TGF β promotes epithelial to mesenchymal transition program in tumor cells through Smad and NF- κ B signaling^[32]. This program provides epithelial cells with a mesenchymal phenotype that promotes mobility and metastases while protecting them from apoptosis due to lack of adhesion (anoikis)^[33]. It has also been found that platelets from cancer patients have a higher VEGF level than platelets from patients without cancer^[34]. Interestingly, serum VEGF is not consistently elevated in cancer, with the exception of renal carcinoma, if methods are adequate in order to prevent platelet activation during venipuncture^[34]. As a result, platelet levels could provide a better reflection of VEGF concentrations in the primary and

metastases sites micro-environment where they are activated and participate to tumor angiogenesis.

In conclusion, this retrospective analysis of patients with stage II and stage III localized rectal cancer patients shows that higher platelets counts (defined in the current study as platelets more than $300 \times 10^9/L$) at the time of disease diagnosis has prognostic value regarding treatment effect outcome. Additionally, it was shown that pretreatment tumor CEA also has prognostic value regarding treatment effect outcome. Further study is needed in more extensive series to confirm these results, clarify survival prognostication value and to test whether thrombocytosis may be used as a predictive marker for specific therapies. It would be of particular interest to evaluate the role of thrombocytosis as a predictive element of anti-VEGF treatments. In this regard a study of metastatic renal cell cancer patients has shown that those with thrombocytosis have an increased probability for refractoriness to anti-VEGF treatments than patients with normal platelets counts^[35]. Moreover, given the presumed role of platelets as protectors of circulating tumor cells from immune attack, an investigation into thrombocytosis as a predictive marker of response to the newly introduced immune checkpoint inhibitors may be worth pursuing.

COMMENTS

Background

Platelet counts are easily measurable laboratory values that are usually measured in all patients with a newly diagnosed cancer as part of a general evaluation.

Research frontiers

This paper proposes the evaluation of this easily available laboratory evaluation as part of the prognostic armamentarium in better defining the therapeutic prospects of rectal cancer patients.

Innovations and breakthrough

This is one of the first studies to specifically evaluate platelets as prognostic factors in neo-adjuvant treatment of newly diagnosed rectal cancer patients to be treated with neo-adjuvant therapy.

Applications

Platelet counts measurement could be used in the clinic to predict the effectiveness of neo-adjuvant cancer treatment.

Terminology

Platelet counts are part of the Complete Blood Count standard laboratory evaluation. Rectal cancers are adenocarcinomas of the terminal part of the colon below the peritoneal fold.

Peer-review

The manuscript is concise, clear, and comprehensive. The purpose, results, and conclusion are clearly stated. The manuscript provides new information and it induce new research.

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