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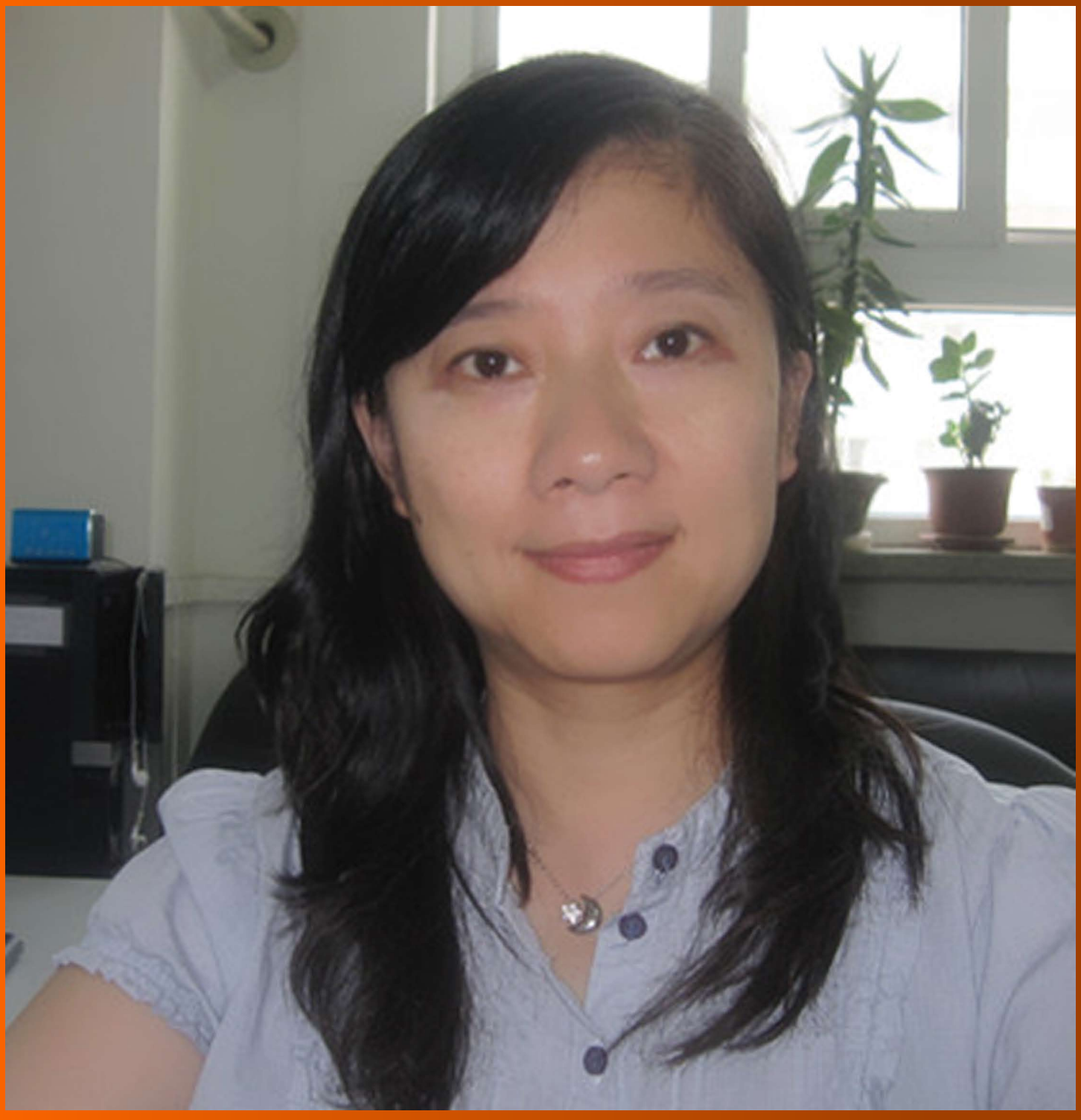

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EDITORIAL

Neoadjuvant treatment for resectable pancreatic adenocarcinoma

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Abstract

Pancreatic adenocarcinoma is the fourth leading cause of cancer mortality in the United States in both men and women, with a 5-year survival rate of less than 5%. Surgical resection remains the only curative treatment, but most patients develop systemic recurrence within 2 years of surgery. Adjuvant treatment with chemotherapy or chemoradiotherapy has been shown to improve overall survival, but the delivery of treatment remains problematic with up to 50% of patients not receiving postoperative treatment. Neoadjuvant therapy can provide benefits of eradication of micrometastasis and improved delivery of intended treatment. We have reviewed the findings from completed neoadjuvant clinical trials, and discussed the ongoing studies. Combinational cytotoxic chemotherapy such as fluorouracil, leucovorin, irinotecan, and oxaliplatin and gemcitabine plus nanoparticle albumin-bound (nab)-paclitaxel, active in the metastatic setting, are being studied in the neoadjuvant setting. In addition, novel targeted agents such as inhibitor of immune checkpoint are incorporated with cytotoxic chemotherapy in early-phase clinical trial. Furthermore we have explored the utility of biomarkers which can personalize treatment and select patients for target-driven therapy to improve treatment outcome. The treatment of resectable pancreatic adenocarcinoma requires multidisciplinary approach and novel strategies including innovative trials to make progress.

Key words: Pancreatic cancer; Resectable pancreatic adenocarcinoma; Neoadjuvant treatment; Biomarkers; Chemotherapy; Surgery

Core tip: The treatment of resectable pancreatic adenocarcinoma requires multidisciplinary approach and novel strategies including innovative trials to make progress. Data from completed neoadjuvant clinical trials are reviewed, and important ongoing studies are presented. Biomarkers for patient selection and personalized medicine are discussed.

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INTRODUCTION

Pancreatic cancer can arise from either the exocrine or endocrine cells. Cancer of the endocrine pancreas, also known as pancreatic neuroendocrine tumor, is uncommon and has a relatively better prognosis than pancreatic adenocarcinoma (cancer of the exocrine pancreas). In the United States, approximately 48960 people are diagnosed with cancer of the exocrine pancreas each year, and an estimated 40560 people will die from their disease; it is the fourth leading cause of cancer mortality in men and women[1]. Globally, it is the seventh leading cause of cancer mortality in men and women, causing more than 300000 deaths annually[2]. Most patients with pancreatic adenocarcinoma will die within two years of diagnosis, and the 5-year survival rate is less than 5%[3]. The lack of a low cost screening test with high sensitivity and specificity contributes to most cases being diagnosed at an advanced stage.

Staging of pancreatic adenocarcinoma is usually done with tri-phasic pancreatic-protocol computed tomography scan of abdomen and pelvis and chest imaging. Based upon imaging, the tumor is classified as resectable, borderline resectable, locally advanced, or metastatic. Approximately 15% to 20% of patients are diagnosed with resectable disease and 45%-55% of patients are diagnosed with metastatic disease[4]. Appropriate staging allows the selection of patients who will have the best chance for curative intent resection (R0). Patients with borderline resectable disease are often given neoadjuvant treatment for tumor down-staging to render resection afterwards. Up to about one-third of patients with borderline-resectable tumors could have resectable disease after neoadjuvant treatment[5]. However, the role of neoadjuvant treatment for resectable pancreatic cancer remains unclear.

Surgical resection remains the only curative treatment for pancreatic cancer. A tumor is considered resectable if there is no arterial tumor contact [celiac axis (CA), superior mesenteric artery (SMA), common hepatic artery] and there is no tumor contact with the superior mesenteric vein (SMV) or portal vein (PV) or ≤ 180-degrees contact without vein contour irregularity on imaging study[6]. A tumor is considered unresectable if there is distant metastasis or unreconstructible SMV/PV due to tumor; if the tumor involves the pancreatic head it is unresectable if there is more than 180-degree encasement of the SMA or CA; a tumor of the pancreatic body or tail is unresectable if there is more than 180-degree encasement of the SMA or CA or tumor contact with the CA and aortic involvement[6].

Surgical management may include pancreaticoduodenectomy or pancreatectomy. It has been shown that pancreatic cancer patients undergoing surgery have better outcomes at high-volume hospitals, and the multidisciplinary approach and experienced surgeon seem to contribute most to the outcome of patients receiving pancreatic surgery[7]. The incomplete resection with positive surgical margins is frequent, reported 40% to 50% in most series[8]. The survival rate for patients with positive surgical margins is similar to that of patients who have locally-advanced disease[9]. The long-term survival after surgery remains low due to high rate of systemic recurrence: About 10% for node-positive disease and about 25% to 30% for node-negative disease[10-13]. Adjuvant treatment has been shown to improve survival as demonstrated in studies such as ESPAC-1, CONKO-001, ESPAC-3, RTOG 9704, and GITSG[14]. However, the delivery of postoperative treatment can be problematic with up to 50% of patients not receiving the intended treatment due to post-operative complications[15,16]. About 15% of patients may develop overt metastatic disease during postoperative recovery period, therefore early initiation of adjuvant chemotherapy within 20 d after surgery has been shown to improve disease-free and overall survival[8,17].

COMPLETED NEOADJUVANT TREATMENT STUDIES FOR RESECTABLE PANCREATIC CANCER

Due to the high rate of micrometastasis with systemic recurrence and difficulty in achieving R0 resection and delivering adjuvant therapy in time, neoadjuvant therapy has been studied to seek improvement in resection and survival. Potential benefits include early treatment of micrometastatic disease, tumor shrinkage for complete resection, and better tumor oxygenation plus drug delivery during chemoradiotherapy. It can also facilitate patient selection for surgery since those patients with disease progression at restaging would likely not benefit from resection.

Currently there is no data that clearly demonstrates improved resectability or survival with neoadjuvant treatment compared with initial surgery followed by adjuvant therapy. The National Comprehensive Cancer Network suggests not administering neoadjuvant therapy outside of a clinical trial. There have been several meta-analyses that have examined the benefit of neoadjuvant therapy. One analysis of 4394 patients in 111 phase Ⅰ/Ⅱ studies with about 1120 patients with resectable disease in 35 studies found no difference in overall survival between neoadjuvant and adjuvant treatment[5]. A meta-analysis of 19 studies in neoadjuvant chemoradiation showed that patients receiving neoadjuvant chemoradiotherapy were less likely to have a positive resection margin but they had an increased risk of perioperative death[18]. Another study of 536 patients in 14 phase Ⅱ studies concluded that patients with locally advanced

Table 1 Selected published neoadjuvant phase Ⅱ trials in resectable pancreatic adenocarcinoma between 2006-2015

Trial/reference published year	Trial phase/patient number	Treatment regimen	Primary endpoint	Result
NCT00335543/2015[63]	Ⅱ, randomized/66 (254 planned)	Upfront surgery *vs* chemoradiation with gemcitabine/cisplatin and radiotherapy of 55.8 Gy	Median survival	Median survival: 14.4 mo *vs* 17.4 mo (P = 0.96). Overall R0: 48% *vs* 52% (P = 0.81)
NCT00536874/2014[64]	Ⅱ/38	Gemcitabine and oxaliplatin	18-mo overall survival	18-mo overall survival: 63%. Median overall survival: 27.2 mo. Resection rate was 71%, and 74% of resection was R0
NCT00490360/2008[20,65]	Ⅱ/28	Gemcitabine and cisplatin	Resectability rate ⩾ 70%	Resection rate was 89%, and 80% of resection was R0. Overall survival was 26.5 mo
Evans *et al*[21]	Ⅱ/86	Chemoradiation with gemcitabine and radiotherapy of 30 Gy (in 10 fractions) for pancreatic head cancer	Clinical outcome	Overall R0: 74%. Median overall survival was 22.7 mo with a 5-yr survival of 27% (36% in R0)
Varadhachary[22]	Ⅱ/90	Gemcitabine and cisplatin followed by chemoradiation with gemcitabine and radiotherapy of 30 Gy for pancreatic head cancer	Clinical outcome	Overall R0 was 58%. Additional chemotherapy did not improve clinical outcome
Palmer *et al*[66]	Ⅱ, randomized/50	Gemcitabine *vs* gemcitabine and cisplatin	Resection rate	Resection rate was 54%: 9 (38%) in the gemcitabine arm and 18 (70%) in the combination arm

pancreatic cancer would benefit from neoadjuvant treatment[19]. Of note, some trials included in these analyses used older generation of chemotherapy and chemoradiation regimens such as 5-fluorouracil, cisplatin and mitomycin-C. Additionally, not only perspective studies, but retrospective cohort studies and case reports were also included in these meta-analyses, which are subjective to confounding and bias errors. Therefore, we have summarized the 6 published phase Ⅱ trials with gemcitabine-based regimen in the last 10 years (Table 1). Most of these trials have shown overall R0 rate around 50%. Two studies, neoadjuvant chemotherapy with gemcitabine and cisplatin reported by Heinrich *et al*[20], and neoadjuvant chemoradiation with gemcitabine reported by Evans *et al*[21], have demonstrated overall R0 rate of more than 70%. However, adding chemotherapy with gemcitabine and cisplatin before gemcitabine-based chemoradiation did not improve clinical outcome including overall R0 and survival rate[22].

Barbour *et al*[23] reported the result of GAP study: Phase Ⅱ study of gemcitabine and nab-paclitaxel for resectable pancreas cancer, a muticenter study conducted in Australia in 2015 ASCO Gastrointestinal Cancers Symposium. Patients in this study received 2 mo of pre-operative chemotherapy with gemcitabine and nab-paclitaxel, then underwent surgical resection. Patients received post-operative treatment based on their resection status (R0 *vs* R1). The primary endpoint was to examine the rate of R0 resection with all margins microscopically clear (minimum distance from tumor to resection margin ⩾ 1.0 mm), with a planned enrollment of 50 patients to aim for R0 rate of 85% or greater. However, this study was stopped after enrolling 42 patients due to a review by Independent Data and Safety Monitoring Committee showing the primary endpoint could not be met.

The ACOSOG Z5041 (NCT00733746) is a phase Ⅱ study in United States investigating overall survival at 2 years in patients receiving perioperative gemcitabine and erlotinib[24]. Erlotinib, an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, has been shown to deliver statistically significant but marginal benefit in overall survival when adding to gemcitabine compared to gemcitabine alone as first-line treatment in patients with advanced pancreatic cancer[25]. The ACOSOG Z5041 met the accrual goal of 123 patients at end of 2013, and the result of the study is highly anticipated. The ACOSOG Z5041 will address the benefit of erlotinib as an adjunct to gemcitabine given perioperatively in resectable setting[26]. Additionally, this study will explore the biomarkers for response to erlotinib, such as E-cadherin whose expression is lost during epithelial-mesenchymal transition (EMT) contributing to cellular insensitivity to EGFR inhibition[27]. The NEOPAC (NCT01521702) is a phase Ⅲ randomized study in Europe comparing adjuvant gemcitabine *vs* neoadjuvant gemcitabine and oxaliplatin plus adjuvant gemcitabine[28]. The primary endpoint is progression-free survival, and the study has been terminated after enrolling about 25% of planned accrual.

ONGOING NEOADJUVANT TREATMENT STUDIES FOR RESECTABLE PANCREATIC CANCER

The landscape of systemic treatment in metastatic pancreatic cancer has changed significantly since 2011. Conroy *et al*[29] have shown FOLFIRINOX (5-fluorouracil plus leucovorin, irinotecan and oxaliplatin) delivered significant improvement of median overall survival when compared to gemcitabine alone in a randomized phase Ⅲ study enrolling 342 patients [11.1 mo *vs* 6.8 mo (P < 0.001)]. Of note, this study excluded patients

Table 2 Selected ongoing neoadjuvant trials for resectable pancreatic cancer

Trial	Trial phase	Treatment regimen	Primary endpoint	Planned accrual (patients)
NCT01900327 (NEOPA)	Ⅲ	Chemoradiation with gemcitabine and radiotherapy of 50.4 Gy *vs* upfront surgery	3 yr survival rate	410
NCT02172976	Ⅱ/Ⅲ	Perioperative FOLFIRINOX *vs* adjuvant gemcitabine	Median overall survival	126
NCT02047513 (NEONAX)	Ⅱ	Perioperative nab-paclitaxel/gemcitabine *vs* adjuvant nab-paclitaxel/gemcitabine	Disease free survival	166
NCT02305186	Ⅰ/Ⅱ	Neoadjuvant pembrolizumab plus chemoradiation with capecitabine and radiotherapy of 50.4 Gy *vs* neoadjuvant chemoradiation	Dose limiting toxicities; # of tumor infiltrating lymphocytes per high power field in resected tissue	56

with suboptimal performance status (ECOG 2 and beyond) or ages older than 76 years old. Von Hoff and colleagues[30] reported increased median overall survival with nab-paclitaxel plus gemcitabine compared to gemcitabine alone in a randomized phase Ⅲ study with 861 patients [8.5 mo *vs* 6.7 mo ($P < 0.001$)]. Therefore both FOLFIRINOX and nab-paclitaxel/gemcitabine have become preferred regimens in advanced pancreatic cancer, and are currently explored in the neoadjuvant setting (Table 2).

The NEPAFOX is a phase Ⅱ/Ⅲ multicenter study (NCT02172976) conducted in Germany with primary endpoint being median overall survival that has started recruiting patients with resectable or borderline resectable pancreatic cancer since November 2014. The phase Ⅱ study will randomize 126 patients to either surgery followed by 6 mo of gemcitabine or perioperative FOLFIRINOX (3 mo before surgery and 3 mo after surgery). After an interims analysis, the trial can be continued as phase Ⅲ to enroll 310 patients[31].

The NEONAX study (AIO-PAK-0313, NCT02047513) is a phase Ⅱ study conducted in Germany that has started recruiting patients with resectable pancreatic cancer since April 2015. This trial will randomize 166 patients to either perioperative treatment with nab-paclitaxel and gemcitabine (2 mo before surgery and 4 mo after surgery) or adjuvant treatment with nab-paclitaxel and gemcitabine. The primary outcome measure is disease-free survival, and aims to improve the disease-free survival rate at 18 mo in at least one arm to ⩾ 55%. This study will conduct biomarker study by collecting tumor tissue for exome sequencing, and circulating tumor DNA for biocorrelate and pharmacogenomic study[32,33].

The NEOPA study (NCT01900327; Neoadjuvant Treatment in Resectable Pancreatic Cancer) is an ongoing phase Ⅲ study in Germany that will randomize 410 patients to neoadjuvant gemcitabine-based chemoradiation *vs* upfront surgery[34]. Both groups will receive post-operative adjuvant treatment with gemcitabine. The primary endpoint is 3-year survival rate. This study is to examine the hypothesis that neoadjuvant gemcitabine-based chemoradiation increases the three-year overall survival by 12% compared to upfront surgery for resectable pancreatic cancer.

The Prep-02/JSAP05 study is a prospective randomized phase Ⅱ/Ⅲ trial conducted in Japan since January 2013 (clinical trial information: UMIN000009634)[35]. This study plans to enroll 360 patients with resectable pancreatic cancer, and randomizes them to either surgery followed by adjuvant chemotherapy with S1 for 6 mo or 2 mo of neoadjuvant chemotherapy with gemcitabine and S1 followed by surgery then 6 mo of adjuvant chemotherapy with S1. The primary study endpoint is resection rate for phase Ⅱ and overall survival for phase Ⅲ. This study plans to have 40 patients in each arm of the phase Ⅱ part, and moves on to phase Ⅲ if there are no more than 14 cases of non-resection in each arm of phase Ⅱ study. S-1 is an oral fluorinated pyrimidine, containing tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate at a molar ratio of 1:0.4:1[36]. Tegafur is a pro-drug of 5-fluorouracil, and S1 has been shown to deliver higher 5-fluorouracil levels in the plasma and the tumor tissue. The safety and efficacy of combination chemotherapy with gemcitabine and S1 for resectable pancreatic cancer have been reported in pilot study[37].

The UVA-PC-PD101 study (NCT02305186; Safety and Immunological Effect of Pembrolizumab in Resectable or Borderline Resectable Pancreatic Cancer) is a phase Ⅰb/Ⅱ multicenter study in patients with resectable or borderline resectable pancreatic cancer. This study will randomize 56 subjects in 2:1 to the experimental arm with pembrolizumab given concurrently with chemoradiation or control arm receiving chemoradiation only. Patients in both arms will receive surgery and adjuvant chemotherapy with gemcitabine. The primary outcome measures are to determine the safety of neoadjuvant chemoradiation with capecitabine in combination with pembrolizumab, and to examine and compare the difference in the number of tumor infiltrating lymphocytes (TILs) in resected pancreatic tissue between experimental and control arms[38]. The investigators hypothesize chemoradiation recruits TILs to the microenvironment of pancreatic cancer causing overexpression of programmed death-ligand 1 (PD-L1). PD-L1 binds to PD-1 on T-cells, and suppress cytotoxic T-cells. Pembrolizumab is a monoclonal antibody that targets the PD-1, and release the inhibition on cytotoxic T-cells. Therefore, it is expected that there are more immune effects at tumor tissues in the experimental arm than control arm. It will be interesting to see if this will

translate into improved clinical outcome.

BIOMARKERS IN RESECTABLE PANCREATIC CANCER

At this time there are no validated biomarkers for early pancreatic cancer. Carbohydrate antigen 19-9 (CA-19-9) is currently used as a marker for following patients during treatment for pancreatic cancer but it is non-specific and can be positive in other conditions such as cirrhosis of the liver, pancreatitis, cholangitis, and other GI cancers. Presence of circulating tumor cells in the peripheral blood has been found to be a negative prognostic factor in pancreatic cancer and potentially may have a role in patient selection for neoadjuvant treatment[39].

Whole exome sequencing in pancreatic cancer demonstrated four frequently mutated genes: *KRAS*, *TP53*, *CDKN2a/p16*, and *SMAD4/DPC4*. KRAS was found to be mutated in virtually all pancreatic cancer patients but genetic alterations in the other three genes were found to be associated with malignant behavior and may be a prognostic tool[40]. The inflammatory markers ferritin and C-reactive protein (CRP) have also been studied for prognostic and predictive value in advanced pancreatic cancer. The study demonstrated that patients with elevation in both biomarkers had a notable decrease in overall survival, and can possibly be a clinically useful tool[41].

The expression of E-cadherin, a calcium-dependent adhesion molecule, is frequently suppressed or lost during EMT of solid tumor malignancy including non-small cell lung and pancreatic cancers, which renders invasiveness and drug resistance[42,43]. Several retrospective studies in pancreatic cancer have shown poorer clinical outcome with decreased expression of E-cadherin[44-46]. Furthermore, E-cadherin interacts with EGFR, and down-regulation of E-cadherin contributes to decreased response and survival in patients receiving EGFR inhibitors for non-small cell lung cancer[47,48]. Ko *et al*[49] have recently reported the result of a phase Ⅱ study with combined inhibitors of EGFR, and MEK which is a key downstream effector of EGFR signaling, in advanced pancreatic cancer. They have found patients with tumors exhibiting an epithelial phenotype (demonstrated by high level of E-cadherin expression) were more likely to be sensitive to study treatment. The planned correlative investigation of ACOSOG Z5041 (NCT00733746), a completed perioperative phase Ⅱ study of gemcitabine and erlotinib for resectable pancreatic cancer, will provide further information on the interaction between EMT marker status and overall/progression-free survival after treatment.

Gemcitabine is a prodrug that is taken into cells *via* a nucleoside transporter[50]. The human equilibrative nucleoside transporter 1 (hENT1) has been studied as a predictive marker for treatment response, and there is data that hENT1 expression may correlate with response to gemcitabine[51-53]. These findings were not able to be validated in the metastatic setting in the LEAP trial[54].

The PD-1 is encoded by the *PDCD1* gene. It is primarily expressed by activated T-cells as negative co-stimulatory receptor; binding of PD-1 to its ligands, PD-L1 and PD-L2, downregulates T-cells and the immune system[55,56]. Many tumor cells express PD-L1 and PD-L2 which is a mechanism which allows escape from immune destruction of the tumor cells. Pembrolizumab is an anti-PD-1 antibody that is approved for use in metastatic melanoma and metastatic non-small cell lung cancer and is currently under study for other malignancies, and PD-L1 expression may be a potential marker for efficacy of anti-PD-1 studies for pancreatic cancer.

Elevated CRP levels in the plasma, a well-established marker of inflammation, at diagnosis correlate with higher tumor stage and grading and poorer clinical outcome in pancreatic cancer[57]. Patients with CRP greater than 13 mg/L had improved survival with ruxolitinib and capecitabine compared to capecitabine and placebo in a randomized phase Ⅱ study also known as the RECAP study enrolling 127 patients with metastatic pancreatic cancer (median overall survival of 83 d *vs* 55 d, $P = 0.01$)[58]. The CRP level could be a useful marker for patient stratification in the management of pancreatic cancer, and the JAK inhibitor ruxolitinib may improve clinical outcome in patients with elevated CRP. An ongoing phase Ⅲ study, known as JANUS 2, is examining these promising leads as a second-line setting in patients with advanced pancreatic cancer[59].

There is high prevalence of BRCA1/2 mutations in Ashkenazi Jewish with pancreatic cancer[60]. The *BRCA1* and *BRCA2* gene encodes large proteins that coordinate the homologous recombination repair double strand breaks (DSBs) pathway. Poly ADP-ribose polymerases (PARP) are a family of nuclear enzymes that regulates the repair of DNA single-strand breaks through the base-excision repair (BER) pathway. Since BRCA1/2-mutated tumors cannot utilize homologous recombination to repair DSBs, exposing these cells to PARP inhibitor, which shuts down BER rescue pathway, will lead to accumulation of DNA damage, genomic instability and cell death, also known as synthetic lethality[61]. Investigators from Memorial Sloan-Kettering Cancer Center have reported high response rate with combination of gemcitabine, cisplatin and veliparib, a PARP inhibitor, as first-line treatment in patients with andvanced pancreatic cancer and mutant BRCA[62]. Ongoing phase Ⅱ randomized study comparing gemcitabine and cisplatin with and without veliparib is currently underway (NCT01585805). This study will most likely provide us the information on using BRAC mutation as a biomarker for personalized treatment.

CONCLUSION

The need for more effective treatment regimens for resectable pancreatic cancer is highlighted by the

continued relatively low survival even in patients who receive surgical resection. Several studies utilizing more active chemotherapy regimens are pending results, and additional studies are ongoing. There also remains the need for accurate and cost-effective biomarkers to aid in the management of pancreatic cancer.

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EDITORIAL

Current treatment options for patients with initially unresectable isolated colorectal liver metastases

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Abstract

The development of liver metastases is a common clinical entity in the clinical course of colorectal cancer. For patients with isolated liver involvement, surgical resection is the only treatment that can provide a chance of prolonged survival and cure. However, most of these patients are not initially eligible for the surgery. Selected patients with initially considered to have unresectable disease may become resectable after systemic (chemotherapy ± biological therapy) and loco-regional treatment modalities including hepatic arterial infusion. Patients who have colorectal liver metastases ideally should be referred to a multidisciplinary cancer care team in order to identify the most optimal management approach.

Key words: Colorectal cancer; Conversion therapy; Liver metastases; Targeted therapy; Hepatic arterial infusion

Core tip: A subset of patients presenting with unresectable colorectal liver metastases (CLM) patients may become eligible for resection following systemic (chemotherapy ± biological therapy) and loco-regional treatments, including hepatic arterial infusion. After successful complete (R0) resection of liver lesions, these patients can achieve long-term survival. Therefore, all patients with CLM should be discussed in a multidisciplinary team meeting to identify appropriate treatment options.

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INTRODUCTION

The liver is the one of the most attractive site for colorectal cancer (CRC) metastases. Up to 50% of CRC patients will experience liver metastases at some point during their clinical follow-up, and approximately 20%-30% of these patients will have isolated liver metastases. Complete surgical removal of all liver meta-

stases is the only treatment option providing the best opportunity for long-term survival in these patients[1-4].

However, most patients with isolated colorectal liver metastases (CLM) are not eligible for surgical resection due to the size, location or number of the lesions and anatomical constraints[5]. On the other hand, a subset of patients initially considered to have unresectable CLM may become eligible for resection following effective systemic chemotherapy. When the chemotherapy is used for that purpose, it is called conversion chemotherapy. Patients with resected CLM following conversion chemotherapy can achieve similar survival to those with initially resectable tumors[4]. In a large series reported by Adam *et al*[6], 1104 patients with unresectable CLM received chronomodulated chemotherapy regimens combining 5-fluorouracil (5-FU), leucovorin (LV), and oxaliplatin or irinotecan. After an average of 10 cycles of chemotherapy, tumor response that allowed secondary curative hepatic resection was seen in 138 (12.5%) of these patients. The 5- and 10-year overall survival (OS) rates after resection were 33% and 23%, respectively, which are better than those in patients who did not undergo resection, but less than those observed in patients who underwent initial complete metastasectomy at the same institution during the same period of time (48% and 30%, respectively, $P = 0.01$).

According to the new systematic review that looked at ten observational studies, the use of modern chemotherapy regimens including irinotecan and oxaliplatin with or without biological agents permitted secondary curative (R0) resection of CLM in 436 of 1886 patients (23.1%). The median OS following surgery was 45 (range, 36-60) mo and recurrence-free survival rate was 19%[7].

IS THERE AN OPTIMAL CHEMOTHERAPY REGIMEN FOR CONVERSION THERAPY?

It is clear that the regimens that can produce high response rates with an acceptable toxicity will lead to a higher rate of R0 resections of liver metastases and improved survival rates[8]. Folprecht *et al*[9] examined all published or presented trials as well as retrospective studies that report the rate of objective response and the rate of secondary resection following systemic chemotherapy in patients with initially unresectable CLM. They demonstrated a strong relationship between response rates to the regimen used and the liver resection rates in patients who have isolated liver involvement, and also speculated that highly active schedules can provide response rates as high as 70% and complete surgical resection rates as high as 50% in selected cases.

The standard doublet combinations of fluoropyrimidines plus either oxaliplatin (XELOX, FOLFOX) or irinotecan (FOLFIRI) offer conversion rates of between 9 and 33%, and their use remains a reasonable option for patients with unresectable CLM[8,10-12]. On the other hand, the administration of intensified triplet chemotherapy regimen of 5-FU, oxaliplatin and irinotecan (FOLFOXIRI) is an attractive strategy that may potentially increase response and resectability rates[13]. The Gruppo Oncologico Nord Ovest (GONO) performed a phase Ⅲ clinical study comparing FOLFOXIRI with FOLFIRI in front-line chemotherapy for patients with initially unresectable CRC[14]. In this trial, response rate (60% *vs* 34%, $P < 0.0001$) and R0 secondary resection rate was significantly greater in patients treated with FOLFOXIRI (15% *vs* 6%; $P = 0.033$, among all participants; and 36% *vs* 12%; $P = 0.017$ among patients with liver-only metastases). Furthermore, patients who received FOLFOXIRI had significantly improved progression-free survival (PFS) and OS than those who received FOLFIRI (median PFS, 6.9 mo *vs* 9.8 mo, $P = 0.0006$; median OS, 16.7 mo *vs* 22.6 mo, $P = 0.032$). As expected, FOLFOXIRI was found to be more toxic with regard to peripheral neurotoxicity and neutropenia, but they were manageable.

Masi *et al*[15] recently performed a retrospective analysis of pooled clinical data from 196 patients who received FOLFOXIRI because of initially unresectable metastatic CRC in phase Ⅰ-Ⅲ GONO studies. The primary aim of the investigators was to determine the long-term clinical results of patients undergoing a secondary complete resection and the effects of this regimen on perioperative surgical morbidity and mortality. They demonstrated that administration of this intensified regimen was associated with a high response rate of 70.4%, and a secondary complete (R0) resection was possible in 37 of 196 patients (19%) after a median of 5.5 mo of chemotherapy. In addition, four patients achieved a complete pathologic response. No perioperative mortality was recorded. Although 27% of patients developed perioperative complications, all of them resolved without sequelae. After a median follow-up period of 67 mo, the estimated OS rate at 5 and 8 years were 42% and 33%, respectively for the total patients population. For patients who had liver-only metastatic disease ($n = 25$), however, the median survival was 65 mo, with the estimated 5-year and 8-year survival rate was 43%. In the histopathological examination of chemotherapy-induced hepatic injury, a major cause for concern when treating patients with CLM, neither grade 3 vascular toxicity nor grade 4 steatosis, was detected.

The addition of targeted agents to chemotherapy backbones may further improve resectability rates of CLM[16-18]. According to the results of an initial phase 3 trial, bevacizumab, a monoclonal antibody directed against the vascular endothelial growth factor, only moderately improved resectability rates when added to oxaliplatin-based chemotherapy (8.4% *vs* 6.1%) in an unselected patient population with metastatic CRC[19]. Further data on the effects of bevacizumab on resection rates of CLM came from the Bevacizumab Expanded Access Trial investigating the safety of bevacizumab with fluoropyrimidine-based chemotherapy in the

first- line treatment of 1914 patients with metastatic CRC[20]. In 704 patients with liver-only metastases, 107 patients (15.2%) underwent hepatectomy, which was R0 resection in 85 out of 107 patients (79.4%). The 2-year survival rate was 89% in patients who underwent resection with curative intent and 94% in those who achieved complete R0 resection.

The BOXER (bevacizumab, oxaliplatin, xeloda in unresectable liver metastases) study investigated the efficacy of perioperative chemotherapy with bevacizumab plus capecitabine and oxaliplatin in patients with CLM who were considered ineligible for upfront resection due to following poor-risk features: The presence of more than four metastatic lesions, metastasis diameter > 5 cm, unlikely R0 resection, inadequate viable liver function if undergoing upfront surgical resection, inability to maintain adequate liver vascular perfusion, or the presence of synchronous metastases. After a median number of four cycles (range, 3-9) preoperative chemotherapy, objective tumor response was observed in 78% of patients and 40% of patients were converted from unresectable to resectable disease. Of these patients, 20% achieved an R0 resection[21].

In a phase 2 study, Masi *et al*[22] assessed the feasibility of FOLFOXIRI and bevacizumab combination in 57 patients with metastatic CRC. Among the 30 patients with liver-only metastatic disease, this regimen yielded an 80% objective response rate and 40% of these patients could undergo a curative (R0) resection. No perioperative mortality was recorded. Subsequently, the GONO reported the results of the phase 3 TRIBE (combination chemotherapy and bevacizumab as first-line therapy in treating patients with metastatic CRC) study comparing FOLFOXIRI plus bevacizumab with FOLFIRI plus bevacizumab in metastatic CRC patients[23]. FOLFOXIRI plus bevacizumab provided a significant increase in response rates (65% *vs* 53%) and PFS (median 12.1 mo *vs* 9.7 mo) compared with FOLFIRI plus bevacizumab. However, FOLFOXIRI plus bevacizumab did not improve the secondary curative R0 resection rate in the liver-only patient subgroup (28% *vs* 32%, $P = 0.823$).

In the OLIVIA randomized phase Ⅱ trial, 80 patients with initially unresectable CLM were randomized to receive bevacizumab plus modified FOLFOX6 or bevacizumab plus FOLFOXIRI[24]. The results showed that the combination of bevacizumab plus FOLFOXIRI improved overall resection rate (61% *vs* 49%) and R0 resection rate (49% *vs* 23%), and PFS (18.6 mo *vs* 11.5 mo) compared with bevacizumab plus FOLFOX6.

The results of phase Ⅲ CRYSTAL (cetuximab combined with irinotecan in first-line therapy for metastatic colorectal cancer) trial and phase Ⅱ OPUS (oxaliplatin and cetuximab in first-line treatment of metastatic colorectal cancer) trial have showed that the integration of cetuximab, a chimeric immunoglobulin G1 anti-epidermal growth factor receptor (EGFR) monoclonal antibody, to irinotecan or oxaliplatin-based first-line chemotherapy significantly improved response rates, R0 resection rates, PFS, and OS compared with chemotherapy alone in patients with metastatic CRC whose tumors did not harbor a KRAS mutation[25,26]. In the CRYSTAL trial, combined administration cetuximab and FOLFIRI resulted in an increase in the resection rate from 4.5% to 9.8% in the subgroup of patients with disease confined to the liver at presentation[25]. Similarly, in the OPUS study, the R0 resection rate for hepatic metastases doubled from 2.4% to 4.7% when cetuximab was added to FOLFOX4 regimen[26].

The CELIM randomized phase Ⅱ study was designed to assess the effect of cetuximab combined with chemotherapy (FOLFOX6 or FOLFIRI) on tumor response and secondary resectability of CLM[27]. A retrospective analysis of the study revealed that 70% of patients with KRAS wild-type disease achieved either a complete or partial or complete response after chemotherapy-biologic therapy, and the resectability rates increased from 32% (at baseline) to 60% (after treatment).

In the study by Ye *et al*[28], patients with KRAS wild-type unresectable colorectal liver-limited metastases were randomly assigned to receive chemotherapy (FOLFIRI or FOLFOX6) plus cetuximab or chemotherapy alone. Patients who received cetuximab plus chemotherapy had improved objective response rates (57.1% *vs* 29.4%; $P < 0.01$), and the R0 hepatic resection rates (25.7% *vs* 7.4%, $P < 0.01$) compared to patients who received chemotherapy alone.

Preliminary reports have suggested that response rates can be increased further by combining cetuximab with FOLFOXIRI regimen. The POCHER study investigated secondary liver resection rates following neoadjuvant treatment with cetuximab plus chronomodulated FOLFOXIRI in patients who were considered unsuitable for resection of their CLM at presentation[29]. After a median of six cycles of chemotherapy, a partial response was obtained in 79% of patients and R0 liver resection was possible in 60% of patients.

The MetaPan study evaluated the activity of adding panitumumab, a fully human monoclonal anti-EGFR antibody, to the capecitabine plus oxaliplatin (XELOX) combination as perioperative conversion treatment in CRC patients with unresectable liver-only metastases[30]. After conversion therapy, the overall response rate in the unselected patient population was 54%. However, in 35 patients with KRAS wild-type, response rate reached to 65%, which allowed for liver resection in 15 of these patients.

Petrelli *et al*[31] have performed a literature-based meta-analysis to determine the effects of cetuximab and panitumumab on objective response rate, the conversion rate, and survival outcome in patients with KRAS wild-type unresectable colorectal liver-limited metastases. They found that compared to chemotherapy alone, the addition of anti-EGFR agents significantly increased the response rate of liver metastases from 43% to 72% ($P = 0.0001$), and the curative (R0) resection rate of liver metastases from 11% to 18% ($P = 0.04$). Although anti-EGFR agents significantly reduced the risk of progression

by 32% (P = 0.002), they did not show any significant favorable effect on OS (P = 0.42).

Neither study found a significant increase in surgical complications with the use of biological agents in this setting[16-18]. There are currently no specific recommendations regarding the use of anti-EGFR antibodies in the preoperative period[16,17]. However, bevacizumab should be stopped at least 6-8 wk before surgery to reduce the risk of specific postoperative complications, including wound healing or bleeding problems[18,32].

HEPATIC ARTERIAL INFUSION

Since CLMs predominantly receive their blood supply from the hepatic artery, infusion of chemotherapeutic agents that have a high hepatic extraction rate [such as 5-fluorouridine (FUDR)] *via* the hepatic artery results higher drug concentrations in liver metastases compared with tumor-free liver parenchyma, which is supplied mainly by portals vein[33]. This can be achieved thorough a biocompatible pump that is implanted under the subcutaneous tissue of the abdomen and attached to a catheter placed in the hepatic artery, which delivers the chemotherapeutic drugs at a slow fixed rate.

Currently hepatic arterial infusion (HAI) chemotherapy is primarily recommended for the treatment of patients with unresectable liver confined metastatic CRC who had disease progression after first-line systemic chemotherapy[34]. However, available data suggest that HAI in combination with systemic chemotherapy or chemo-biologic therapy offer a chance for curative rescue resection to a substantial proportion of patients presenting with liver-limited metastatic CRC[34,35]. With this approach, down-staging to resectability occurs in 25%-50% of patients; the percentage can reach up to 57% in chemotherapy-naïve patients[10,11,35,36]. Importantly, the long-term overall survival can be obtained[10,11,35-37]. In the series reported by Goere *et al*[37], 87 patients with isolated unresectable CLM were treated with HAI of oxaliplatin with systemic 5-FU and LV. Seventy-nine percent of patients had previously received systemic chemotherapy. After the treatment, a curative resection was possible in 26% of patients, and the 5-year survival for these patients was 56% *vs* 0% for non-resected patients.

Ammori *et al*[38] reported the largest institutional series of 373 patients with unresectable CLM who were treated with HAI FUDR and systemic chemotherapy. Two hundred and ninety-six patients (79%) had been treated with systemic chemotherapy before HAI, and 43 of these patients received multiple lines of chemotherapy. Sixty patients (16%) had also extrahepatic disease at the time of HAI pump placement. Despite these unfavorable features, 25% of patients responded sufficiently to treatment and subsequently underwent complete resection and/or radiofrequency/microwave ablation. The median and the estimated 5-year survival for this conversion group were 59 mo and 47%, respectively, which were comparable to reports in the literature of patients initially presenting with resectable disease.

HAI can be associated with technical and liver-related complications. Technical complications including arterial thrombosis, catheter occlusion or dislodgement, extrahepatic perfusion, pump pocket infections or hematoma, have been reported up to 22% of patients[39]. However, most of these complications are manageable and overall rate of pump failure is around 9% at 1 year[39]. The most limiting hepatic toxicity related to HAI is biliary sclerosis, which has been reported in 4.6% of patients receiving HAI FUDR for unresectable CLM, and it can often be effectively managed, if detected early[40].

CONCLUSION

Surgical resection is currently the only curative approach for patients with isolated CRC liver metastases. Conversion chemotherapy may offer a chance for secondary resection in about one-third of these patients. Although, the optimal regimen for this is still unclear, a doublet combination of 5-FU plus either oxaliplatin or irinotecan remain the standard first-line option. The FOLFOXIRI triplet is a very attractive treatment option especially for patients who can tolerate this regimen. Despite lacking specifically designed randomized trials, available data suggest that the integration of targeted biological agents into chemotherapy may further improve tumor response and resectability. HAI should be considered in patients with extensive liver tumor burden and chemotherapy-refractory disease.

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REVIEW

Advanced oropharyngeal squamous cell carcinoma: Pathogenesis, treatment, and novel therapeutic approaches

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Abstract

Oropharyngeal cancer accounts for approximately 2.8% of newly cancer cases. Although classically a tobacco related disease, most cases today are related to infection with human papilloma virus (HPV) and present with locally advanced tumors. HPV related tumors have been recognized as a molecularly distinct entity with higher response rates to therapy, lower rates of relapse, and improved overall survival. Treatment of oropharyngeal cancer entails a multi-disciplinary approach with concomitant chemoradiation. The role of induction chemotherapy in locally advanced tumors continues to be controversial however large studies have demonstrated no difference in survival or time to treatment failure. Surgical approaches may be employed with low volume oropharyngeal cancers and with development new endoscopic tools, more tumors are able to be resected *via* an endoscopic approach. Given advances in the understanding of HPV related oropharyngeal cancer, ongoing research is looking at ways to minimize toxicities *via* de-intensification of therapy. Unfortunately, some patients develop recurrent or metastatic disease. Novel therapeutics are currently being investigated for this patient population including immunotherapeutics. This review discusses the current understanding of the pathogenesis of oropharyngeal cancer and treatment. We also discuss emerging areas of research as it pertains to de-intensification as well novel therapeutics for the management of metastatic disease.

Key words: Oropharyngeal cancer; Human papilloma virus; Transoral robotic surgery; Immunotherapy; Metastatic head and neck squamous cell carcinoma

Core tip: Treatment of oropharyngeal cancer entails a

multi-disciplinary approach with concomitant chemoradiation. Given advances in the understanding of human papilloma virus related oropharyngeal cancer, ongoing research is looking at ways to minimize toxicities *via* de-intensification of therapy. Unfortunately, some patients develop recurrent or metastatic disease. This review discusses the current understanding of the pathogenesis of oropharyngeal cancer and treatment. We also discuss emerging areas of research as it pertains to de-intensification as well novel therapeutics for the management of metastatic disease.

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INTRODUCTION

Oropharyngeal cancer accounts for approximately 2.8% of newly diagnosed cancer cases and, in 2015, will result in 8650 estimated deaths[1]. Today, most cases are related to human papilloma virus (HPV) infections and many are curable with definitive combinations of surgery and radiation or chemoradiotherapy. Hence, HPV is a prognostic biomarker, but not yet predictive. As the field of clinical research continues to advance, methods for de-intensifying treatment for such patients are becoming more important. Here, we will review the epidemiology of oropharyngeal cancer as well as treatment strategies and areas of developing research for those afflicted with this disease.

EPIDEMIOLOGY, PATHOGENESIS, AND RISK STRATIFICATION

Classically, use of tobacco products has been the leading factor for development of oropharyngeal cancer, although this has been shifting with changes in societal trends in tobacco usage[2-4]. This increased risk pertains to use of cigarettes, cigars, and pipes and increases with the number of years an individual has smoked[5]. Smoking cessation resulted in a normalization of risk in casual smokers after approximately 15 years[6,7]. Additionally, tobacco usage during definitive therapy for head and neck cancer is associated with an increased rate of disease progression and death, particularly in those whose cancers are not related to HPV or are p16 negative[8]. Similarly, alcohol intake increases the risk of head and neck cancers in a dose dependent manner[7,9-11].

HPV, most notably genotype 16, has been identified as an increasing causative factor for oropharyngeal cancer and is chiefly seen in patients with minimal tobacco and alcohol use. This is especially important since the pathogenesis, presentation, and prognosis differ in HPV(+) *vs* HPV(-) oropharyngeal carcinomas. The molecular carcinogenesis of HPV associated oropharyngeal cancer has been explored in detail and is separate from that seen in HPV(-) cancer and relates to loss of cell cycle checkpoints[12,13]. In a subset of patients with chronic HPV infections, the viral oncoproteins E6 and E7 bind p53 and pRb/p21, respectively. The resultant effect is that E6 binding causes p53 degradation whereas E7 binding to pRb and p21 leads to an activation of transcription factors. These transcription factors cause malignant cells to progress into the G1 cell cycle phase which is unopposed due to the loss of p53. The latency from time of primary infection to development of malignancy is approximately 15-20 years. Over the last 20 years there has been an steady rise in the number of newly diagnosed HPV(+) oropharyngeal cancers, increasing from 16.3% to 71.7%, accompanied by a corresponding 50% decline in the incidence HPV(-) oropharyngeal carcinomas[3,14-16].

Clinically, HPV+ cancers are more likely to present in younger patients and involve the base of the tongue or tonsils[3,17,18]. Additionally, patients with HPV+ oropharyngeal cancers are much more likely to respond to therapy, have lower rates of disease relapse, and enjoy improved overall survivals. Furthermore, such tumors are less likely to develop second malignancies compared to matched HPV(-) patients[3,14-16,19]. Based on these studies, a model for risk stratification has been generated based on HPV status, smoking history, tumor stage, and nodal involvement. A classification of low, intermediate, or high risk disease has been generated, predicting 3 year overall survivals of 93%, 70.8%, and 46.2%, respectively[15]. Interestingly, a single center study analyzing survival and TNM staging in oropharyngeal cancers found that survival based on TNM status did not correlate with survival in those patients with HPV(+) disease, but it did correlate with survival in those with HPV(-) disease. A retrospective, multivariate analysis of the HPV+ patients, however, was able to generate an accurate prognostic model by including tumor stage, smoking status, and age by recursive partitioning analysis (RPA). Thus, the authors propose an RPA-based staging system in HPV-related oropharynx cancers, whereby stage Ⅰ cancers would be classified by T1-3, N0-N2b tumors, stage Ⅱ by T1-3, N2c, and stage III by T4 or N3 disease[20].

TREATMENT STRATEGIES

Surgical approaches

Surgical approaches are currently one of the primary modalities in the treatment of low volume oropharyngeal cancers. Early stage squamous cell carcinomas of the oropharynx can be managed with either surgery or radiation therapy. Given the significant acute and long term side effects of radiation therapy, minimally invasive surgical approaches [including transoral robotic surgery (TORS) and transoral laser microsurgery (TLM)] have

been increasingly employed for the management of early stage tumors. This increased utilization has been further driven by development of new endoscopic tools including the da Vinci Robot, enabling better visualization and surgical manipulation in the oropharynx. These technologies have allowed tumors only previously resectable *via* external and highly morbid approaches (mandibular split and pharyngotomy approaches) to now be treatable *via* the transoral route with significantly less morbidity. One report of TLM demonstrated the promise of this modality in patients with early stage oropharyngeal cancer (T1-4a, N0). In this study, sixty-nine patients in two centers underwent TLM and neck dissection, of which no patients were treated with adjuvant radiation. Excellent patient outcomes were reported, including a five year overall survival of 86%. Similarly, locoregional recurrences were quite low, with a 90% locoregional control rate in patients with T1 disease, and a 94% control rate in patients with T2 disease[21].

Although treatments with TORS and TLM are increasingly becoming employed in early stage oropharyngeal carcinomas, the bulk of the evidence supporting their use stems from the surgical management of patients with locally advanced (stage Ⅲ/Ⅳ) disease. The utilization of TORS was first reported in 2005[22], and since then has been described in numerous publications as an effective treatment for oropharyngeal cancers[23-25]. In one large case series of patients with locally advanced oropharyngeal cancers (T2-4a, N0-2c), treatment with TORS and selective neck dissections resulted in excellent outcomes, notably with a 98% 1-year disease specific survival. Regarding the need for further multimodality therapy, only 39% required radiation and 39% received chemoradiation. Based on these results, the use of TORS accompanied by selective neck dissection may be a method to de-intensify therapy, sparing patients from the toxic effects of adjuvant chemotherapy, and in some select cases, adjuvant radiation as well[26]. Further matched retrospective patient studies, directly comparing TORS to chemoradiation, have demonstrated that patients treated with TORS have less acute toxicities and a higher rate of recovery to baseline swallowing function at 12 mo[27]. Although these studies support the use of transoral surgery in select patient populations for both early and locally advanced, low volume oropharyngeal cancers, further multi-center, randomized studies comparing transoral surgery-based approaches to definitive chemoradiotherapy are needed in order to establish the role of primary surgery in standard of care practice.

Chemoradiotherapy

The management of locoregionally advanced oropharyngeal cancer (stage Ⅲ-ⅣB) is complex and emphasizes the need for a multidisciplinary approach as treatment for each patient is individualized based on the clinical setting. Currently, the treatment of locally advanced disease focuses around definitive chemoradiotherapy.

Organ preservation with chemoradiation has been studied exhaustively over the last 20 years. The relative benefit of concomitant chemotherapy and radiation has been established through numerous trials; however, the MACH-NC meta-analysis, which combined 93 randomized trials and more than 17000 patients, offers the most comprehensive perspective to date. In this study, concomitant chemotherapy and radiation was found to offer a significant improvement in 5-year overall survival compared to radiation therapy alone (33.7% *vs* 27.2%, absolute difference of 6.5% ± 1%). In an exploratory multivariate analysis, the observed effect of chemotherapy on improved survival decreased as a function of age; in the group of patients 70 and older, no improvement in survival was observed[28]. A similar analysis, presented at the 2015 American Society of Clinical Oncology (ASCO) annual meeting in Chicago, also noted lower survival rates in patients 70 years or older collectively from three previously published Radiation Thoracic Oncology Group (RTOG) studies[29]. A subsequent analysis, based on tumor site, also noted improvement of the 5-year overall survival rate in patients with oropharyngeal cancers, whereby the absolute benefit in 5-year overall survival was 8.1%[30].

A number of chemotherapeutic agents have been utilized as radiation sensitizers during concomitant therapy. However, the most commonly used regimens include high-dose cisplatin (100 mg/m^2 every 21 d for two or three doses), weekly cisplatin (30-40 mg/m^2), weekly carboplatin (AUC = 2) plus paclitaxel (45 mg/m^2), and weekly cetuximab. Landmark studies defining non-surgical approaches established high-dose bolus cisplatin as the original, standard concomitant agent[31-33]. Given the proven efficacy of bolus cisplatin, several phase Ⅱ studies and retrospective case series have sought to establish if weekly cisplatin is an effective and well-tolerated alternative[34,35]. Sharma *et al*[34] demonstrated that the addition of weekly cisplatin (40 mg/m^2) to radiotherapy improved overall survival when compared to radiation alone, though 40% of patients experienced Grade 3 or 4 toxicities in the concomitant arm as compared to 20% treated with radiation alone. Similarly, 29% of patients receiving cisplatin required treatment interruptions, compared to 9% in the radiation alone arm[34]. One meta-analysis found that increased cumulative cisplatin dose, regardless of schedule (bolus *vs* weekly), was associated with improvement in survival[36]. To date, there still are still no prospective, randomized published trials comparing weekly cisplatin and radiation with bolus cisplatin and radiation. Several retrospective reviews presented as abstracts suggest that survival may not be compromised with weekly platinum *vs* high-dose platinum-radiation regimens. Furthermore, patients with low risk disease (*i.e.*, p16+, low tumor volume, < 10 pack smoking histories) will inherently enjoy longer survival times regardless of the chemoradiotherapy regimen administered. Patients with poor prognosis tumors (T4, N2c, N3 tumors, > 10 pack year smoking histories), on the other hand, may benefit from high-dose cisplatin combined with radiation[37].

Given the persistent toxicities with weekly cisplatin and issues with renal failure, carboplatin has been explored alone or in combination with 5-fluorouracil or paclitaxel for use with radiation therapy[38,39]. In a pilot study of 60 patients, the combination of carboplatin and paclitaxel given concomitantly with radiation was well tolerated. Eighty-two percent of patients achieved a complete response and the 2 year overall survival rate was 62%. Fifty nine of the patients completed treatment, with the most common grade 3 toxicities being mucositis, dysphagia, leukopenia, and skin desquamation[38]. In another multicenter phase Ⅲ study, weekly carboplatin and 5-flurouracil given with radiation was compared to radiation alone in patients with locally advanced oropharyngeal carcinomas. Although this study demonstrated increased rates of grade 3 or 4 toxicities in patients receiving chemoradiation *vs* radiation alone (71% *vs* 29%), the three year overall survival rates favoring the chemoradiotherapy arm were impressive (51% *vs* 31%)[39].

Randomized, prospective studies comparing weekly platinum regimens to high-dose cisplatin with radiation have yet to be conducted. Investigators at the University of Michigan compared their institutional studies, utilizing weekly carboplatin and paclitaxel with intense modulated radiation therapy (IMRT) and bolus cisplatin with IMRT, in stage Ⅲ/Ⅳ oropharyngeal cancer patients *via* a matched, paired, retrospective analysis. This evaluation demonstrated that patients treated with high dose cisplatin had higher numbers of grade 3 or 4 toxicities (54% *vs* 40%). After accounting for HPV status, there was no significant difference noted in overall or progression-free survival between the two treatment arms[40].

The anti-EGFR monoclonal IgG1 antibody Cetuximab has been established as an effective agent for use with radiation therapy. In a large Phase Ⅲ trial, the median overall survival and 5-year overall survivals were both significantly improved with the addition of Cetuximab to radiation therapy over radiotherapy alone (49 mo *vs* 29.3 mo and 45.6% *vs* 36.4%, respectively). Of note, on exploratory multivariate analysis it was noted that the greatest benefit was seen in patients with oropharyngeal cancers but a benefit was not seen in those > 65 years old. In addition, it was noted that the development of a prominent acneiform rash (grade 2 or greater) was associated with a significantly improved overall survival[41,42]. Analysis of the effect of cetuximab on overall survival based on pre-treatment characteristics demonstrated that the addition was most beneficial in non-elderly men with oropharyngeal tumors, grade 1-3 tumors, node positive (N1-3), with good performance status[42]. A biomarker analysis evaluating outcomes related to HPV status was recently conducted on this study, and the results were presented at the 2014 ASCO annual meeting in Chicago. This investigation demonstrated improvement in OS with the addition of cetuximab to radiation in both HPV+ *vs* HPV- tumors, though a greater degree of improvement was seen in those tumors which were p16+. This study was exploratory in nature and not powered to make definitive conclusions; however, it does confirm that HPV is a prognostic biomarker, not yet predictive[43].

Given the improvement in clinical outcomes seen with cetuximab, several large trials have sought to answer whether the addition of anti-EGFR monoclonal antibodies (cetuximab or panitumumab) to conventional platinum based chemoradiation results in clinical improvement. Each of these studies has failed to demonstrate improvement in clinical outcomes with the addition of EGFR inhibition[44,45]. One of these studies did demonstrate that although EGFR expression did not distinguish outcome in patients treated with cetuximab, patients with p16 positive oropharyngeal carcinomas had a better 3 year progression free survival (72.8% *vs* 49.2%) and overall survival (85% *vs* 60.1%)[44]. Unplanned post-hoc analysis of RTOG 0522 (reviewing the role of cisplatin based chemoradiotherapy plus cetuximab) demonstrated that patients with high baseline metabolic tumor volumes on PET/CT had an inferior response to chemoradiotherapy in terms of progression-free survival and locoregional control. Interestingly, this remained an independent prognostic factor on multivariate analysis even after factoring for T stage[46].

Based on the evidence of efficacy with the use of Cetuximab as a radio-sensitizing agent, the question has arisen regarding the comparative efficacy *vs* a platinum based regimen. A published single center retrospective study was recently published describing the outcomes of patients with locally advanced head and neck squamous cell carcinoma treated with concurrent chemoradiation stratified by chemotherapeutic agent. It was noted that patients treated with platinum based chemotherapy had significantly superior relapse free and overall survival compared to those treated with cetuximab monotherapy or in combination with chemotherapy[47]. One meta-analysis including 15 trials and 1808 patients which was presented in a preliminary form demonstrates that studies to date support a greater improvement in both locoregional recurrence and overall survival with the use of cisplatin. However, this study had significant heterogeneity and did not account for p16 status[48]. Other studies comparing panitumumab and radiation with cisplatin and radiation have also failed to demonstrate the improvements of this fully human monoclonal antibody against EGFR to the standard of care[49,50]. Ongoing studies are still seeking to answer this question in select populations, including RTOG 1016.

The role of induction chemotherapy in oropharyngeal cancer has been debated extensively and there continues to be some controversy regarding its role. In general, the use of induction chemotherapy has been intended to decrease the rate of distant metastases, to cause rapid cytoreduction, to offer high doses of chemotherapy to tumor prior to disruption of vasculature by radiation, and to decrease tissue volume requiring

exposure to radiation[51]. Three large, randomized phase Ⅲ studies have been performed to date evaluating the role of induction *vs* concurrent chemoradiation, all of which demonstrated no difference in survival or time to treatment failure[52-54]. In the recently published DeCIDE trial, evaluating induction chemotherapy primarily in oropharyngeal cancer, enrollment was difficult and the study was closed after enrollment of 285 of the planned 400 patients. Although overall survival was no different between the arms at three years, one should note that (albeit not statistically significant) the difference in the rate of distant failure was 10% in the induction chemotherapy group *vs* 19% in the concurrent chemoradiation group. HPV status was available for only 49 patients and on subgroup analysis is was noted that there was no statistically significant difference in overall survival between HPV(+) and HPV(-) patients[54]. Early results of a phase Ⅲ trial from Italy, comparing induction chemotherapy followed by definitive chemoradiotherapy *vs* concomitant chemoradiation with cetuximab *vs* cisplatin and 5-flourouracil (5-FU) *via* 2 × 2 factorial design, were presented at the 2014 ASCO annual meeting in Chicago. This trial had a primary endpoint of 3 year overall survival between the induction *vs* no induction groups. Preliminary results demonstrated a statistically significant improvement with induction chemotherapy in both median progression-free (29.7 mo *vs* 18.5 mo, $P = 0.12$) and overall survival (57.6 mo *vs* 45.7 mo, $P = 0.03$). On unplanned subgroup analysis, these improvements were not seen in patients with oropharyngeal cancers. Additionally, when compared with similar previously published trials as historical controls[41,44,54], both progression-free survival and overall survival appear to be lower across the board, for which the etiology is unclear. Reporting of HPV status amongst the treatment groups is pending and will be important in fully interpreting the results of this study[55].

Investigators at the University of Michigan have studied the use of induction chemotherapy as a means of chemoselection, whereby patients with oropharyngeal cancers who had a response to one cycle of induction chemotherapy were treated with definitive chemoradiation, whereas those patients without evidence of response proceeded to salvage surgery. In this study, induction therapy failed to successfully select patients for surgical salvage, but a subgroup analysis demonstrated that higher HPV titers were associated with a significant reduction in tumor burden following the administration of a single cycle of chemotherapy, demonstrating the robust response of p16 positive oropharyngeal tumors to cytotoxic agents[56]. In the companion paper published with this article, correlative analysis noted that EGFR expression was inversely associated with response to chemoselection as well as patient outcomes including disease specific survival and overall survival. Moreover, when biomarkers were combined low EGFR and high p16 expression were associated with a good response to chemoselection however the combination of high EGFR expression, low p53 expression, and high Bcl-xL expression was associate with a poor response to chemoselection and overall survival[57].

DE-INTENSIFICATION OF THERAPY

Although chemoradiotherapy has improved survival outcomes in patients with loco-regionally advanced oropharyngeal cancers, this has come at the expense of both acute and late treatment related toxicities. These toxicities substantially impair patients' quality of life, potentially for the remainder of their lives, and include long-term swallowing dysfunction as a result of radiation. HPV+ oropharyngeal cancer is now being increasingly recognized as a biologically distinct malignancy with a distinct disease course and response to therapy. Moreover, HPV+ tumors have higher response rates to multimodality therapies, lower rates of disease relapse, and improved overall survivals compared with HPV- tumors. In an attempt to mitigate acute and late toxicities, an area of research looking to define patients with low risk oropharyngeal cancer who may be candidates for de-intensification of therapy is actively underway. Proposed methods of de-intensification include decreasing doses of radiation (so called de-escalation) or switching from cisplatin based radio-sensitization to targeted therapy with cetuximab.

To date, few published trials provide insight into this matter, and hopefully with the maturity of several ongoing prospective trials, there will be a body of literature as to guide the field. One retrospective study sought to define the pattern of recurrence in HPV + low risk patients (< 10 pack-year smoking and T1-T3 disease) based on treatment with radiation alone *vs* concomitant chemoradiation. It was shown that low risk patients, those with N0-N2a nodal involvement, had no difference in disease control rates with the introduction of chemo-sensitization as compared to those receiving only radiotherapy[58]. Given the retrospective nature of this study and the fact that the majority of patients not receiving chemotherapy were those with advanced age or restricting medical co-morbidities, it is difficult to draw definitive conclusions. However, this research certainly supports the consideration for de-escalation of therapy in a subset of low risk patients. Currently, RTOG 1333 is assessing such an approach with the primary endpoint of 2 year progression free survival. In this study low, risk patients (HPV+ with a ⩽ 10 pack-year smoking history) with oropharyngeal cancer are being randomized to either radiation (60 Gy, 2.0 Gy/fraction in 6 wk) with concurrent weekly cisplatin (40 mg/m^2 × 6 doses) or radiation alone (60 Gy of radiation, 2.0 Gy/fraction over 5 wk)[59]. As a chief aim of de-escalation is improving treatment related toxicities, one of the main secondary endpoints being followed in this trial includes quality life, most notably swallowing function. ECOG 3311 is an ongoing risk stratified randomized phase Ⅱ

study evaluating an approach of TORS followed by a risk adapted approach in patients with HPV(+) stage Ⅲ/Ⅳ oropharyngeal carcinoma. In this study, based on post-operative findings low risk patients will be observed, intermediate risk patients will be treated with radiation alone, and high risk patients will be treated with chemoradiation.

ECOG 1308 is a prospective, phase Ⅱ study that also examined the role of de-escalation. In this trial, patients were treated with 3 cycles of induction chemotherapy, and if they were found to have a complete response, they were treated with weekly cetuximab and low dose intensity IMRT (54 Gy/27 fractions). If, on the other hand, patients had less than complete response, they received weekly cetuximab with full dose IMRT (68.3 Gy/33 fractions). Preliminary analyses demonstrated that patients with complete responses, treated with low dose IMRT, had an improved 2 years progression free and overall survival compared to those patients in the standard-dose IMRT arm. Additional insights from the analysis of the patient cohort receiving low dose radiotherapy demonstrate that progression-free survival and overall survival were better in patients with a ⩽ 10 pack-year smoking histories and low volume (< T4, T1-N2b) disease. This favorable risk cohort had a significantly improved 2 year progression-free survival compared to other enrolled patients (96% *vs* 64%)[60]. Although this data yields valuable insights into the potential for reducing intensity of treatment for a select population of oropharyngeal cancer patients, a larger, multi-center phase Ⅲ is needed study to verify the results of this de-escalation trial, comparing this concept to standard cisplatin and radiotherapy.

Finally, RTOG 1016 is an ongoing non-inferiority phase Ⅲ trial that is seeking to identify the role of substituting Cetuximab for high dose bolus Cisplatin (100 mg/m^2 q 21 d × 2 doses) in combination with accelerated IMRT. This protocol exclusively enrolled 1000 patients with p16+ locoregionally advanced oropharyngeal cancer (clinical stage T1-2 N2a-N3 or T3-4 any N) with any smoking status. In addition to defining whether the substitution of cisplatin is non-inferior to standard therapy, this study will assess the effect of tobacco exposure and molecular profiles on patient outcomes. This study is now closed to accrual and the results are eagerly awaited.

LOCALLY RECURRENT AND METASTATIC DISEASE

Despite increased understanding of oropharyngeal cancer and advances in treatment of both early stage and loco-regionally advanced disease, a number of patients still develop locally recurrent and metastatic disease. Evidence now supports that HPV(+) oropharyngeal cancer patients who develop progression have a better median overall survival than those cancers which are HPV(-) (2.6 years *vs* 0.8 years). Fakhry *et al*[61] noted a worse survival upon progression in patients with distant metastases or those who initially presented with T4 lesions. Patterns of recurrence are also related to HPV status in oropharyngeal cancers. HPV(+) status markedly reduces the risk for loco-regional recurrence (HR = 0.09, P = 0.03)[62] and in one study was associated with a longer time to distant failure (16.4 mo *vs* 7.2 mo)[63].

The goal of therapy in patients with locally recurrent or metastatic oropharyngeal cancer who are treated with chemotherapy is palliative. As prognosis is poor and effective treatment options are limited, enrollment onto clinical trials offers the best possible care, especially for those who have failed a front-line platinum containing regimens. If trial involvement is not possible, numerous treatment modalities with standard agents may be considered.

Surgical salvage should be entertained in select situations as a treatment for locally recurrent or metastatic oropharyngeal cancer. Recent studies have demonstrated that surgery is an effective treatment option, often improving survival. One large study of 181 patients demonstrated that even when factoring in T/N stage, progression type (distant *vs* locoregional), smoking history, and p16 status to a multivariate analysis, salvage surgery still remained a significant predictor of overall survival (HR = 0.56, P = 0.02)[61]. Another similar retrospective study attempted to gain similar insight; however, this evaluation also considered whether salvage treatment with nonsurgical methods or with surgical methods offered superior overall survival. The investigators found that surgical salvage offered an improvement in overall survival compared to those treated with salvage radiation or chemotherapy. Similar to previous studies, this finding remained significant even on multivariate analysis when p16 status, T/N stage, smoking history, site of disease recurrence, and number of sites with disease recurrence were factored in[64].

If surgical salvage is not an option, there are numerous classes of cytotoxic chemotherapy drugs including platinum agents, taxanes, methotrexate, 5-FU as well as the anti-EGFR targeted therapy, cetuximab, which have proven efficacy in metastatic head and neck cancer. Response rates to chemotherapy range between 10%-30% with single agent regimens and 20%-40% for multi-drug regimens[65-67]. It is important to appreciate that although conventional cytotoxic agents may be combined as doublet therapies (traditionally platinum based), these combinations increase response rates but not overall survival, and they have notable increases in toxicities[66]. There have been no studies showing superiority of one cytotoxic regimen over the other, median overall survivals ranging from 6.6-8.7 mo[65-68]. Incorporation of cetuximab into a 5-FU and platinum containing regimens is associated with an increased objective response rate (36% *vs* 20%), progression free survival (5.6 mo *vs* 3.3 mo), and overall survival (10.1 mo

vs 7.4 mo) relative to platinum-5 FU doublet therapy in patients with metastatic head and neck cancer[69]. Although underpowered to draw conclusions, a post-hoc analysis of p16+ oropharyngeal cancers seemed to have a greater degree of benefit with the incorporation of cetuximab compared to those that were p16-[70].

FUTURE DIRECTIONS

There are currently numerous ongoing trials involving the treatment of oropharyngeal cancer. Among the current research avenues are novel predictive factors for recurrence and the development of immunotherapeutics. Although the prognosis of HPV+ advanced oropharyngeal cancer is impressive with 3 year survival rates of 62%-83%[71,72], there is an increasing rate of distant treatment failure, not accounting for 45% of long term deaths in the population[15,73]. Numerous prognostic factors have been explored as methods to better tailor therapy for those at increased risk, including micro-RNA, advanced T and N classification, and smoking status[58,74,75]. One novel finding, identified as prognostic as well as predictive, is the presence of matted nodes on pre-treatment imaging (CT or PET/CT). Matted nodes are defined as the presence of three lymph nodes abutting one another with loss of the intervening fat plane which is thought to represent radiologic evidence of extracapsular spread. Matted nodes have been identified in 20% of patients presenting with advanced oropharyngeal cancer. In one analysis, patients presenting with matted nodes had a three year disease specific survival of 58% *vs* 97% in those without. This bore out as a predictive marker on a further analysis and on a multivariate analysis whereby the presence of matted nodes remained an independent predictor of poor prognosis even when controlling for age, tumor classification, HPV status, and smoking status[76,77].

There has also been interest in searching for novel biomarkers as to guide patients at risk for reoccurrence. Retrospective analysis of patients with locally advanced HPV+ oropharyngeal cancer has demonstrated that patients who recurred were noted to have a significantly lower rate of E7 antibody clearance[78]. Prospective analyses are needed to determine the utility of E6 and E7 antibody clearance perhaps in combination with plasma HPV DNA levels. Two abstracts presented at the 2015 ASCO annual meeting may also aid in identifying patients at high risk for recurrence. In one study, loss of function tumor suppressor gene mutations appears to decrease the efficacy of treatments for locally advanced squamous cell carcinomas of the head and neck. Activating driver gene mutations, on the other hand, may define poor risk patients, in particular those with HPV(+) oropharyngeal carcinomas[79]. A second study evaluated the implication of persistent HPV-16 DNA detection in oral rinses in patients with p16 positive oropharyngeal carcinomas, treated for locally advanced disease. Data from this evaluation suggests that persistent oral HPV DNA in post-treatment rinses is strongly associated with poorer outcomes[80]. These findings may help to tailor intensification of therapy in high risk populations as to improve patient outcomes.

Immunotherapy [namely Programmed Death-1 (PD-1) inhibition] is currently one of the most exciting and rapidly changing areas of oncology with impressive response rates and improvements in overall survival seen in melanoma and lung cancer[81-83]. PD-1 targeting in head and neck cancer has been of interest as these malignancies [especially HPV(+) tumors] are thought to be quite antigenic[84]. In addition, pathologic samples in both HPV(+) and negative tumors have demonstrated a high frequency of PD-1 and PD-L1 expression, suggestive of a potential role for checkpoint inhibitors[85,86]. Preliminary results of the KEYNOTE-012 study, a phase 1b multisite study evaluating the activity of Pembrolizumab in patients with recurrent or metastatic HNSCC regardless of PD-L1 or HPV status, were reported at the ASCO Annual Meeting in 2015. An overall response rate of 24.8% and stable disease rate of 24.8% was reported with activity observed in both HPV(+) and HPV(-) patients. Although follow up was limited as only preliminary results were available, it was intriguing that the median duration of response was not reached[87]. An accompanying study analyzed this population as to try and identify predictors of response as both HPV and PD-L1 status have been non-discriminatory. It was demonstrated that an inflamed-phenotype gene expression, chiefly interferon gamma, was able to predict 6 mo progression free survival with a 95% negative predictive value and 40% positive predictive value[88]. Similar findings have been reported in melanoma where inflamed-phenotype gene expression signatures appear to predict benefit from pembrolizumab[89]. There are multiple ongoing phase Ⅱ/Ⅲ clinical trials investigating the role for Pembolizumab and Nivolumab in the setting of metastatic disease for head and neck cancer, which include the evaluation of markers to potentially identify responders[87]. Results of these studies will offer new insights and may drastically alter the treatment of metastatic oropharyngeal cancer.

CONCLUSION

The management of oropharyngeal cancer is complex and depends on a multidisciplinary team including otolaryngologists, medical oncologists, and radiation oncologists. Although great strides have been made in the last 20 years in approaches to organ preservation and risk stratification, improvements are needed in delineating the role of treatment de-intensification and development of novel therapeutics for the treatment of metastatic disease. We eagerly await final publications of the data from the recent ASCO annual meetings to further validate the use of several novel agents and treatment approaches.

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REVIEW

Current therapeutic strategies for advanced pancreatic cancer: A review for clinicians

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Abstract

Pancreatic cancer (PC) would become the second leading cause of cancer death in the near future, despite representing only 3% of new cancer diagnosis. Survival improvement will come from a better knowledge of risk factors, earlier diagnosis, better integration of locoregional and systemic therapies, as well as the development of more efficacious drugs rising from a deeper understanding of disease biology. For patients with unresectable, non-metastatic disease, combined strategies encompassing primary chemotherapy and radiation seems to be promising. In fit patients, new polychemotherapy regimens can lead to better outcomes in terms of slight but significant survival improvement associated with a positive impact on quality of life. The upfront use of these regimes can also increase the rate of radical resections in borderline resectable and locally advanced PC. Second line treatments showed to positively affect both overall survival and quality of life in fit patients affected by metastatic disease. At present, oxaliplatin-based regimens are the most extensively studied. Nonetheless, other promising drugs are currently under evaluation. Presently, in addition to surgery and conventional radiation therapy, new locoregional treatment techniques are emerging as alternative options in the multimodal approach to patients or diseases not suitable for radical surgery. As of today, in contrast with other types of cancer, targeted therapies failed to show relevant activity either alone or in combination with chemotherapy and, thus, current clinical practice does not include them. Up to now, despite the fact of extremely promising results in different tumors, also immunotherapy is not in the actual therapeutic armamentarium for PC. In the present paper, we provide a comprehensive review of the current state of the art of clinical practice and research in PC aiming to offer a guide for clinicians on the most relevant topics in the management of this disease.

Key words: Pancreatic cancer; Chemotherapy; Radiofrequency; Stereotactic radiotherapy; Irreversible electroporation

Core tip: This review focuses on the current clinical practice in the treatment of pancreatic cancer (PC), and outlines research topics. PC is still a highly lethal disease, for a usual presentation stage not manageable with curative surgery. Up to now, new targeted therapies have not shown any positive impact on its dismal prognosis. Only slight improvements ensued from the availability of more active polychemotherapy regimens. From the point of view of a multimodal approach, in addition to surgery, new locoregional techniques are nowadays available, suitable for combination with systemic treatments, to increase disease control and survival.

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INTRODUCTION

In contrast to the general trend of increase in cancer survival, advances have been slow for pancreatic cancer (PC). Therefore PC is actually the fourth cause of cancer death, and it is expected it will be the second cause of cancer death by 2030 in Western countries. The American Cancer Society estimated that there will be 48960 new cases of PC in the United States in 2015, with 40560 deaths[1,2]. Despite surgery, locoregional therapy, chemotherapy and molecular therapies, the overall median survival is less than 1 year from diagnosis, highlighting the need for better therapeutic options. In fact, PC is frequently undiagnosed until the sudden appearance of prominent clinical symptoms and signs for advanced disease. Only in 10%-20% of cases the disease is resectable or borderline resectable, therefore suitable to surgery associated with neoadjuvant or adjuvant treatment, with curative purposes. In the last years different ablative techniques such as irreversible electroporation (IRE), radiofrequency ablation (RFA) and stereotactic body radiation therapy (SBRT) caught the attention of the scientific community. Such techniques may be an alternative to surgery in patients with a locally advanced disease, poor response to systemic therapy, and with a locoregional rather than metastatic growth pattern.

This review aims to explore the major questions still open regarding the management of the disease. We identified studies and systematic reviews by searching PubMed, ClinicalTrials.gov, and the Cochrane database from database inception to April 2015.

ARE THERE PROVEN RISK FACTORS IN PC? CAN WE PREVENT IT?

Current knowledge and unmet needs

Facing such a dismal prognosis cancer, a frequent question from patients is "Why? Why to me". PC has a multifactor etiology, whose better knowledge could be helpful to identify groups of people worthy of surveillance trials. A study on 117 meta-analytical and pooled reports estimated risk factors and the fraction of PCs attributable to them[3]. There is a moderately sized association between a family history of PC in first degree relatives, with multivariate-adjusted odds ratios (OR) of 1.8[4], justifying 5%-10% of cases. There is a significant association between PC risk and AB0 phenotypes (OR = 1.4 in non 0 blood type), and up to 19.5% of all cases of PC in populations with European ancestry could be attributable to a non-0 blood group[5]. Moreover, a multistage genome-wide association study[6] identified multiple susceptibility alleles to be further evaluated. A study of Maisonneuve and Lowenfels[7] however suggests that nearly two thirds of PC are due to potentially avoidable causes. The strongest associations are with tobacco smoking, that is the greatest behavioral risk factor for PC, and *Helicobacter pylori* infection, with estimated population attributable fractions of 11%-32% and 4%-25% respectively. Besides carcinogens, smoking also generates agents perpetuating inflammatory response, and heightens the risk of chronic pancreatitis. A higher risk of PC indeed is associated with chronic pancreatitis. In this perspective, Helicobacter pylori infection could have a role in pancreas carcinogenesis, through the induction of autoimmune pancreatitis[8]. Heavy alcohol intake, defined as a daily consumption of over 30 g, has a strong association with PC, with an attributable increased risk of 20%-30%. All or many of these risk factors could concur through complex interactions involving different pathways[9]. Diabetes, obesity and reduced adiponectin level are all related to insulin resistance, and probably share common pathways, which can be responsible for attributable fraction up to 16%-19%, with the opposite postulated protective effect of higher physical activity[10].

The strongest evidence for a protective effect is for atopic allergy, especially hay fever or allergy to animals, that could reduce PC risk up to 20%-30%[11]. A number of other postulated risk factors or protective factors like meat and fruit intake, or vitamin D circulating levels have a lower level of evidence and deserve further studies.

Cystic lesions occasionally detected with non-invasive abdominal imaging, prescribed for unrelated indications, deserve a separate discussion. Prevalence range of incidental pancreatic cysts in the adult population is from 2.2%-5.9% (depending on imaging technique)[12]. Their correct management is crucial for preventing and early treating of the disease. Especially intraductal papillary mucinous neoplasms can have a progression model similar to that of colonic polyps, with the risk of transforming into invasive cancer, more likely in cases with involvement of main pancreatic duct or with multiple lesions. But only a few of them actually progress to malignancy. Their optimal management is still controversial, based more on experts' opinions than on evidence from randomised studies. This uncertainty

about the prediction of future behavior is due to a lack of accurate diagnostic tools and prognostic factors. It exposes patients to a risk of overtreatment with unnecessary high-risk surgery, undertreatment or expensive long term imaging follow-up[13].

IS INTEGRATED APPROACH (SURGERY, RADIOTHERAPY AND CHEMOTHERAPY) THE GOLD STANDARD IN LOCALLY ADVANCED PC? WHAT IS TODAY THE ROLE OF SURGERY?

Current knowledge

Nearly 30%-40% patients at diagnosis have a borderline-resectable (BRPC) or locally advanced PC (LAPC)[14]. But despite the absence of distant metastasis, the overall survival (OS) of these patients is absolutely poor[15,16], and only radical surgery can give a chance for a cure[17]. Selected patients can have an improved outcome with a multimodal approach, combining chemotherapy with radiation therapy or surgery. The selection of a population of patients suitable to multimodal approach, however, needs an accurate identification of LAPC and BRPC. LAPC refers to cases with an extended involvement of adjacent structures[18]; whereas BRPC comprises a subset of patients eligible to an upfront resection, but with a high risk of residual microscopic disease (R1, according to the International Union Against Cancer Classification) caused by an involvement of nearby structures, such as superior mesenteric artery or celiac artery, not allowing a removal of the tumour without an arterial resection, thus greatly increasing the risk of R1 or R2 surgery. R0 resection only can cure PC. Unfortunately, there is a wide heterogeneity in the literature regarding the definition of resectability criteria. Moreover, BRPC patients are an ill-represented population in the majority of chemotherapy clinical trials.

In this context, upfront resection has been rated as a 2B recommendation in the National Comprehensive Cancer Network Guideline[18-21].

Although lacking high level evidence, there is a general consensus for neo-adjuvant chemotherapy, estimated able to convert to R0 resection 33% of LAPC/BRPC patients[22,23].

This therapeutic strategy has been historically based on fluoropyrimidines, 5-fluorouracil (5-FU) or capecitabine, combined with radiation and recently on gemcitabine induction followed by concomitant chemo-radiation with either gemcitabine or fluoropyrimidines[24,25]. At present, there are no data about the better neo-adjuvant chemotherapy regimen. But based on the observed results in the metastatic settings, FOLFIRINOX or gemcitabine plus nab - paclitaxel with or without subsequent chemoradiation might represent promising options. However, especially FOLFIRINOX suits only to fit patients, for high rate of G3-G4 toxicities[26-29]. The results of ongoing Alliance A021101 pilot trial (NCT01821612) could help clarify the role of a multimodal strategy of neoadjuvant FOLFIRINOX, followed by chemoradiation [50.4 Gy external beam radiation therapy (EBRT) with concomitant capecitabine], definitive surgery and postoperative adjuvant gemcitabine in BRPC patients.

For LAPC affected patients as well, a combined approach in LAPC could allow radical resection also in cases not eligible to upfront surgery. In several studies[30,31], and a meta-analysis by Gillen *et al*[23], gemcitabine-based combination regimens allowed a higher resection rate than single agent chemotherapy (33% *vs* 27%). In this meta-analysis OS was almost doubled in patients who finally underwent surgical resection of their tumour (20.5 mo *vs* 10.2 mo). Moreover, three meta-analyses have suggested a survival advantage in patients treated with gemcitabine-based chemo-radiation (CRT)[32-34].

Nevertheless, the role of chemoradiation in LAPC is still unclear, for conflicting results of clinical trials. Indeed, two studies reported improved OS with CRT (Gastrointestinal Tumor Study Group 9283[35] and ECOG 4201[36]) and Huguet *et al*[37] reviewed two perspective trials finding a survival advantage in patients treated with chemotherapy and chemoradiation *vs* patients treated with chemotherapy alone. Other interesting results were recently reported by Sherman *et al*[38] using docetaxel and capecitabine followed by gemcitabine and capecitabine combined with radiation therapy and surgery[38]. In this phase 2 trial, 20 out of 45 treated patients (44%) had R0 resection.

On the opposite, Chauffert *et al*[39] reported no advantage in OS and more toxicity with the addition of CRT to chemotherapy, and preliminary results of the international phase 3 GERCOR LAP-07 study demonstrated improved local control with the addition of chemoradiation to chemotherapy, but no difference in OS[40].

Waiting for definitive evidence about the usefulness of CRT, at present, the most widespread approach in fit patients is to start with induction chemotherapy followed by chemoradiation in absence of disease progression at the time of first radiological evaluation. This approach has two advantages: It avoids unnecessary radiotherapy in the nearly 30% of patients who undergo widespread disease progression during initial treatment, and it permits to test patient's tolerance to chemotherapy alone, before adding the relevant toxicities of a radiation concomitant to chemotherapy. Radiotherapy[41].

Standard dose radiation therapy is usually 50.4 Gy in 1.8-Gy fractions, although some trials reported the use of a 30-36 Gy in 3-Gy fractions schedule[42]. Better outcomes could come from the use of newer radiotherapy techniques like intensity-modulated radiation therapy (IMRT) and SBRT, suited to deliver higher biological dose[42]. Indeed, in a phase 2 multi-institutional trial, SBRT was feasible without unexpected toxicities and obtained a 1-year local progression-free survival (PFS) of 78%[43].

Unmet needs

An agreement about an unambiguous, rigorous definition of the BRPC could help to reach a homogeneous approach to borderline resectable disease, thus allowing comparison among different trials results. Despite multimodal treatments, not all BRPC and LAPC will become resectable up to R0, missing their chance for a cure. Deeper exploration of combination regimens is necessary to improve this outcome, especially through the identification of prognostic factors and biomarkers to predict the response or the resistance to the different treatments. At present, little evidence is available. As an example, SMAD4-deleted tumours are associated with widespread disease, whereas SMAD4-proficient tumours are associated with a more locally aggressive disease[44]. Nevertheless, the impact of SMAD4 on treatment outcome is far to be defined. In any case, a multidisciplinary management in high-expertise centers can increase the chance of cure for all patients with PC, and even more for those with BRPC and LAPC.

WHAT IS THE BEST FIRST LINE CHEMOTHERAPY IN INOPERABLE PC?

Current knowledge

Despite recent advances in our understanding of the molecular biology of PC, there has been limited progress in therapeutic options for metastatic disease, and traditional chemotherapy outcomes, even though improved, are still disappointing. The overall median survival from diagnosis is still less than 1 year, underscoring the need for the development of newer therapeutic options. The goals of chemotherapy are: The improvement of survival, the control of symptoms and the need to ensure a good quality of life for the patient. In the past, several studies have demonstrated the superiority of chemotherapy compared to best supportive care alone (BSC) and fluorouracil (5-FU), in different doses, schedules, and combination regimens, has been considered the cornerstone in the palliative treatment of metastatic PC[45]. Since 1997, gemcitabine monotherapy has represented the standard of care for patients with metastatic PC, when Burris *et al*[46] demonstrated that it was superior to 5-FU in terms of clinical benefit/efficacy, outcome measures and safety profile in patients with a baseline Karnofsky performance status (PS) $\geqslant$ 50. Gemcitabine subsequently represented a backbone in chemotherapy, in clinical trials investigating more intensive combination regimens. Due to its good tolerability and demonstrated efficacy, from 1997 to 2010 several studies had combined it with many other active cytotoxic agents, including fluorouracil[39], capecitabine[47], cisplatin[48], epirubicin[49], docetaxel[50-52], oxaliplatin[31], irinotecan[53,54], and pemetrexed[55]; but up to now, no conclusive results about an effective impact on survival. In contrast to other tumour types, with the exception of the negligible benefit showed by erlotinib[56], tested targeted therapies as cetuximab[57], bevacizumab[58], axitinib[59], tipirarnib[60], oftrametinib[61], trastuzumab[62], have largely failed to show any significant benefit when added to standard chemotherapy in metastatic PC.

In 2011 a combination regimen of leucovorin, fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX) obtained a meaningful survival benefit over single agent gemcitabine. FOLFIRINOX, providing a significant survival improvement of 4.3 mo in comparison to gemcitabine alone[63]. The median OS, PFS, and objective response rate (ORR) were significantly higher with FOLFIRINOX compared with gemcitabine alone (median OS, 11.1 mo *vs* 6.8 mo; PFS, 6.4 mo *vs* 3.3 mo; ORR, 32% *vs* 9%). FOLFIRINOX, however, showed an unfavourable toxicity profile compared to gemcitabine alone, including grade 3/4 neutropenia (46% *vs* 21%), febrile neutropenia (5.4% *vs* 1.2%), thrombocytopenia (9.1% *vs* 3.6%), sensory neuropathy (9% *vs* 0%), vomiting (15% *vs* 8%), fatigue (23% *vs* 18%), and diarrhea (13% *vs* 2%). Only well-selected patients with metastatic PC can therefore bear such a treatment without heavy side effects.

In 2013, nab-paclitaxel in combination with gemcitabine showed an improved median survival of almost two months (1.8), compared to gemcitabine alone[64]. It also increased OS at 1 and 2 years, with a tolerable toxicity profile. Grade 3/4 adverse events occurred, as expected, more often with the combination therapy and included neutropenia (38% *vs* 27%), febrile neutropenia (3% *vs* 1%), fatigue (17% *vs* 7%), diarrhea (6% *vs* 1%), and neuropathy (17% *vs* 1%). In September 2013, nab-paclitaxel in combination with gemcitabine was approved by the FDA for first-line treatment of metastatic PC of the pancreas.

Unmet needs and proposals

Clinical trials results suggest that combination chemotherapy with regimens FOLFIRINOX or gemcitabine plus nab-paclitaxel are an acceptable option for patients with good PS, good pain management, and adequate nutritional intake. It is still not clear which is the best: FOLFIRINOX or gemcitabine and nab-paclitaxel? The median OS obtained in the two different trials was 11.1 mo with FOLFIRINOX and 8.5 mo with gemcitabine plus nab-paclitaxel. A direct comparison of the results of the two trials, conducted on different populations, is impossible.

In our opinion, both FOLFIRINOX and gemcitabine plus nab-paclitaxel are reasonable choices for first-line therapy in patients with Eastern Cooperative Oncology Group (ECOG) PS 0 or 1. For a better tolerability, the combination of gemcitabine and nab-paclitaxel could be an option also for patients with a slight worse PS, who cannot tolerate a FOLFIRINOX regimen, or in patients who have received FOLFIRINOX as neoadjuvant treatment. However, in common clinical practice only a small number of patients with metastatic PC presents with good PS. For the other patients gemcitabine

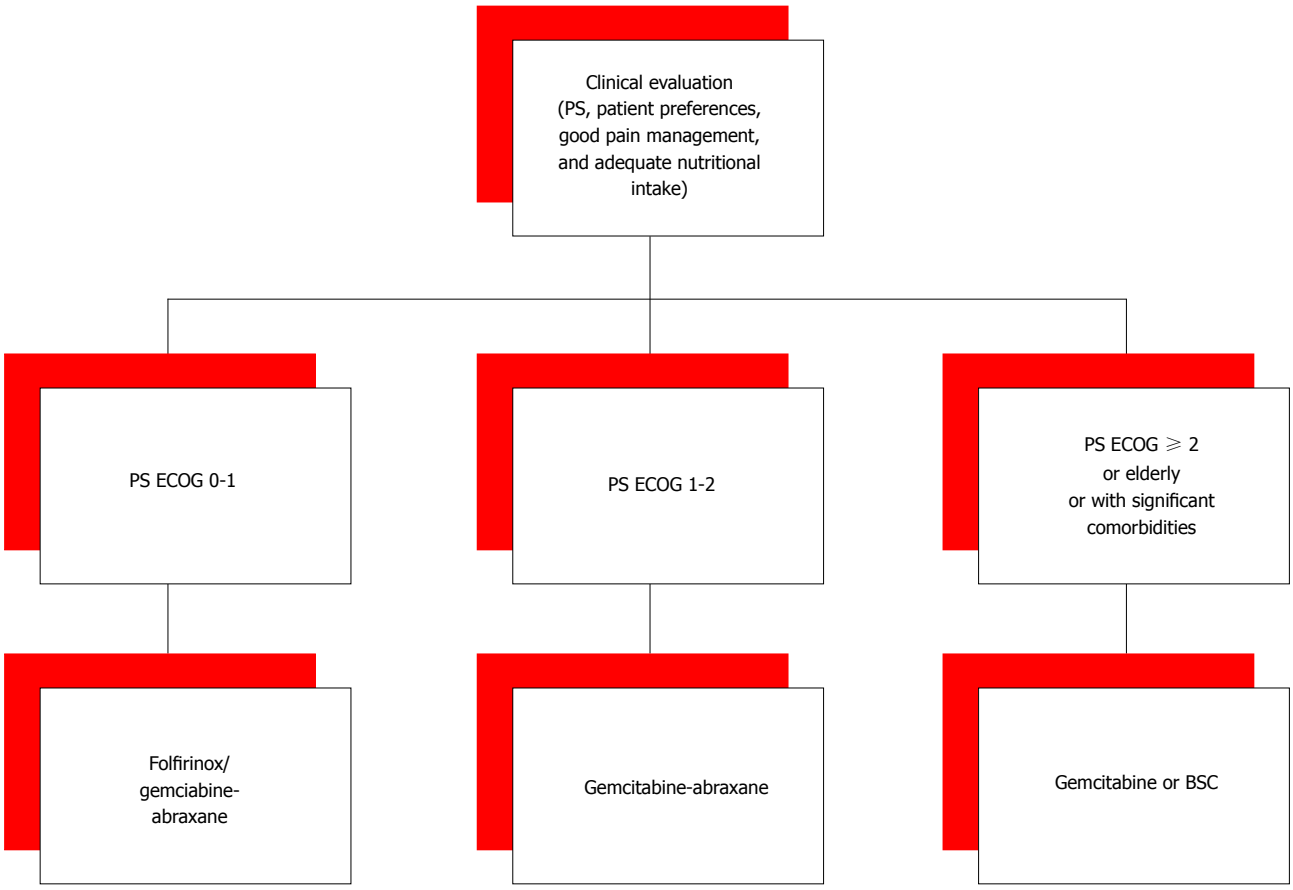

Figure 1 Proposal for the choice of the first line. PS ECOG: The eastern cooperative oncology group score of performance status; BSC: Best supportive care.

monotherapy is still the only therapeutic option (Figure 1).

Additional therapeutic advances are expected from studies evaluating strategies for depletion stromal, inhibition pathways of cancer (*i.e.,* Hedgehog, RAS-RAF-MAPK and PI3K-AKT), new chemotherapeutic drugs (*i.e.,* MM-398 irinotecan encapsulated into liposomal-based nano particles)[65,66], or the new era of immunotherapy. The identification of biomarkers continues to be clinically challenging but essential in order to tailor therapy to specific patients' subgroups in which the maximal antitumour effect from novel agents can be obtained.

WHAT IS THE ROLE OF THE LINES OF CHEMOTHERAPY SUBSEQUENT TO THE FIRST?

Current knowledge

Outside the context of clinical trials[67], median OS in patients with metastatic PC is 2.8-5.7 mo. However, despite the aggressiveness of this disease, in recent years the better results obtained with first-line chemotherapy have allowed a wider use of second-line treatments. In a retrospective study, the French and British oncologists analysed data of 400 patients treated for metastatic PC between 2009 and 2012. They collected patients' information about sex, age, PS, comorbidities, cancer-directed treatment, supportive care, adverse events and complications. The most common used first-line chemotherapy regimens were gemcitabine alone (46%), FOLFIRINOX (20.1%), gemcitabine/capecitabine (10.8%), and gemcitabine/oxaliplatin (9.5%). Approximately 40% of patients received second-line systemic therapy, whereas less than 20% received third-line systemic therapy[68]. About 45% of patients in phase Ⅱ-Ⅲ trial PRODIGE 4-ACCORD 11 received second-line therapy. FOLFIRINOX, despite significantly higher chemotherapy-related adverse events, allowed a better Quality of Life (QoL) than gemcitabine[69]. Since the QoL of patients with metastatic PC is more influenced by disease symptoms than by chemotherapy-related toxicity, the second-line chemotherapy could be a good option for selected patients. In a phase Ⅱ study, oxaliplatin-based regimen showed some activity in metastatic PC patients after failure of first-line chemotherapy with gemcitabine[70]. The CONKO-01 randomised phase Ⅲ multicenter study compared OFF (oxaliplatin, folinic acid and 5-FU 24 h) to BSC in patients with PC progressing while on gemcitabine therapy. Stratification included duration of first-line therapy, PS, and tumour stage. Trial terminated prematurely, after the accrual of 46 patients instead of 165 planned, probably for patients and physicians unwillingness to a randomisation in a BSC arm. Median second-line survival was 4.82 mo with OFF treatment,

compared to 2.30 mo with BSC. Median OS for the sequence GEM-OFF and for GEM-BSC was 9.09 and 7.9 mo, respectively. The OFF regimen was well tolerated with 13% of grade Ⅱ/Ⅲ gastrointestinal toxicities. This randomised trial has supported the hypothesis of the benefit of second-line chemotherapy in comparison to BSC alone, for patients with PC[71]. A further phase Ⅲ trial, CONKO-003, evaluated the effect on survival of oxaliplatin added to 5-FU, on second-line therapy. This trial randomised 168 patients with disease progression during first-line gemcitabine therapy, to folinic acid and 5-FU or oxaliplatin and 5-FU (OFF). In the OFF arm, the median OS and TTP were significantly extended in comparison to the 5-FU arm. The toxicities were similar between the two groups except for neurotoxicity, in 38.2% of OFF group patients[72]. However, oxaliplatin-based regimens for second-line chemotherapy have not given only positive outcomes. The PANCREOX trial randomised 108 patients after first-line gemcitabine, to mFOLFOX6 *vs* infusional 5-FU and folinic acid (5-FU/LV). The study showed no difference in median PFS (3.1 mo *vs* 2.9 mo, $P = 0.99$). Moreover mFOLFOX6 arm had a shorter OS and a higher patients number in mFOLFOX6 group withdrew for adverse events, thus the conclusion could be that this regimen is too toxic for this patients[73]. Irinotecan, alone or in combination with other drugs, could be another promising option for second-line therapy, in patients with metastatic PC after failure of gemcitabine. Also FOLFIRI has been proved, by some phase Ⅱ trials, to be a safe and potentially active regimen in this setting[74,75]. But a more interesting aspect is the availability of a new irinotecan formulation, encapsulated into liposome-based nanoparticles, potentially increasing drug stability and sustaining drug release in the tumour area. The NAPOLI-1 trial, a multicentre, open- label, three-arm, randomised phase Ⅲ trial, randomised 417 patients affected with metastatic PC, after prior gemcitabine-based therapy, to nano-liposomal irinotecan (MM-398) alone, or combined with 5-FU/LV, in comparison to 5-FU/LV. The combination of MM-398 + 5-FU/LV significantly improved OS, PFS, TTF, and ORR in comparison to 5-FU/LV. Median OS was 6.1 and 4.2 mo respectively. And median PFS 3.1 and 1.5 mo. MM-398 alone did not demonstrate any statistical improvement in efficacy. Many phase Ⅱ trials have investigated other therapeutic options as taxanes[76,77], capecitabine[78], S1[79], FOLFIRI and FOLFOX[80], FOLFIRINOX[81,82], nab-paclitaxel[83] for the treatment of chemorefractory patients, but more confirmation studies are needed.

Unmet needs and proposals

Given the evidence of some benefit from second-line therapy, questions still remain about which optimal drugs and regimens and for which patients. Moreover, available second-lines therapies further questions concern the optimal treatment sequences. For patients who received FOLFIRINOX in the first-line setting, the second-line option is often a gemcitabine-based therapy. The association nab-paclitaxel with gemcitabine proved to be effective in the front-line setting, but lack efficacy data in second-line setting. While, for the patients who received nab-paclitaxel and gemcitabine in the first-line setting, an oxaliplatin-based treatment may be considered in the second-line (Figure 2). Choosing second-lines options very aggressive behaviour of PC and the relatively rapid QoL deterioration have not to be forgotten. The choice of second-line treatment should always be done with close attention to PS, patient's age, the presence of comorbidities, and patient preferences.

ARE LOCOREGIONAL TREATMENTS ACHIEVABLE ALTERNATIVES TO SURGERY? ARE THEY USEFUL IN LAPC OR METASTATIC PC?

Current knowledge

Roughly 40% of PC diagnosis are of LAPC, because non-metastatic but unresectable disease, not suited to surgery with radical intent. So far, in this setting, sole palliative chemotherapy can only give slight survival improvement. But there are further options of several innovative local ablative therapies, including RFA, IRE, SBRT, high-intensity focused ultrasound (HIFU), microwave ablation (MWA), photodynamic therapy (PDT), and cryoablation (Table 1). Ablative therapies based on thermal tumour damage include RFA, HIFU, cryoablation and MWA while IRE, PDT and SBRT are non-thermal ablative methods. Actually, despite their proven safety, feasibility and reproducibility, novel ablative methods in LAPC or metastatic PC have still to demonstrate a benefit on survival in large prospective randomised studies[84].

Stereotactic body radiotherapy: In the last few years, SBRT gained increasing interest for its better and longer lasting outcomes, as well less toxicity than conventional EBRT. The SBRT can selectively deliver a higher dose of radiation to a target lesion, in single or multiple sessions. When using SBRT it is of paramount importance the precise delineation of the therapeutic target and the correct evaluation of possible target motion, in particular for pancreas, in a site affected from breathing movements. For this reason, the treatment planning uses four-dimensional diagnostic imaging. SBRT may be delivered using non-isocentric technique, IMRT, or volumetric-modulated arc therapy[85]. Despite the above mentioned characteristics which seem to improve some of the major limits of EBRT, the role of SBRT in LAPC and BRPC is not clearly defined yet, though some interesting preliminary evidence of its activity has been recently reported. As an example, in a single centre institution experience, the authors reported a median OS of 18.4 mo and median PFS of 9.8 mo in 88 patients affected by LAPC and BRPC treated with SBRT (2-30 Gy in five fractions on the planning target volume) with an acceptable toxicity profile (3.4% of

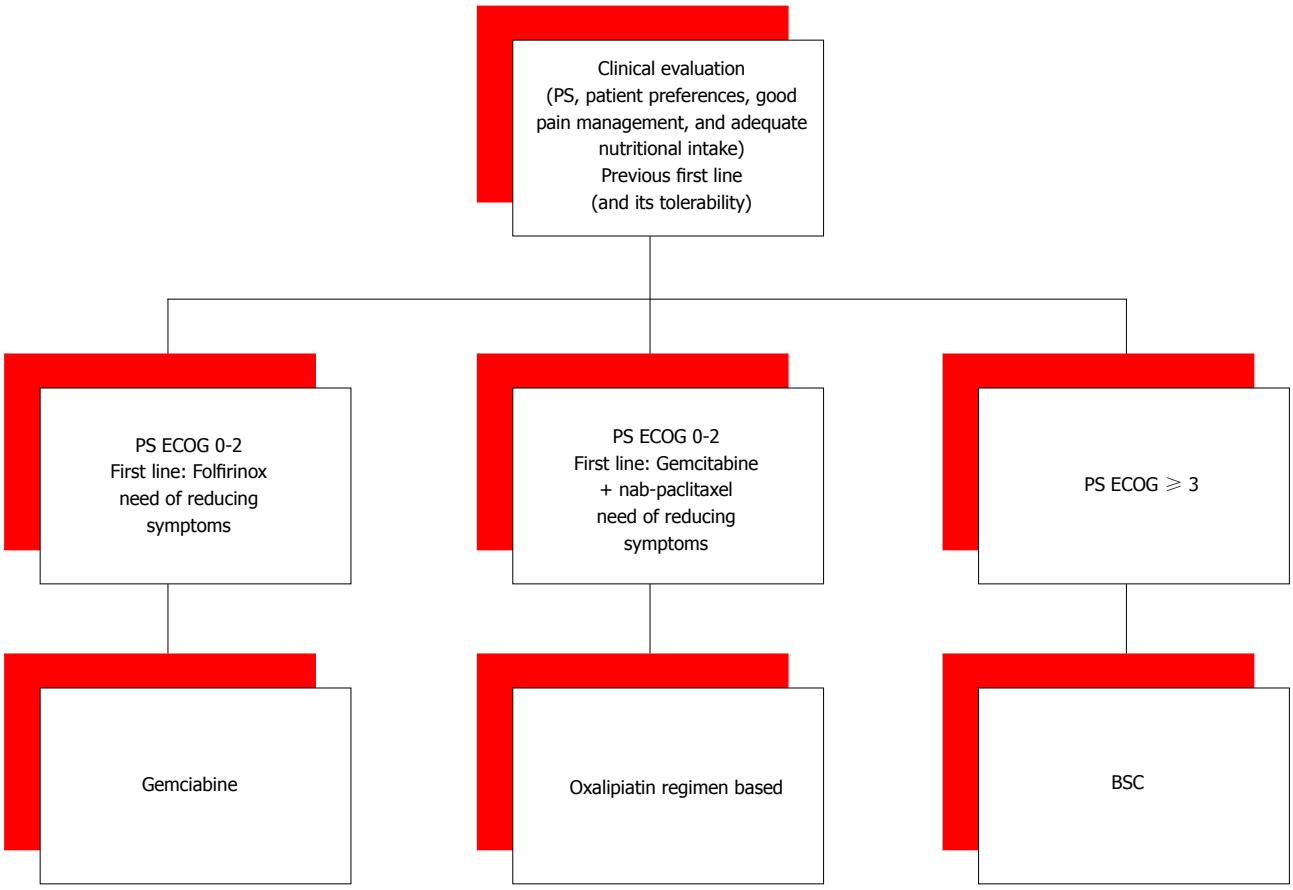

Figure 2 Proposal for the choice of the second line. PS ECOG: The eastern cooperative oncology group score of performance status; BSC: Best supportive care.

Table 1 Ongoing clinical trials about locoregional treatments in locally advanced pancreatic cancer

Combination	Inclusion criteria	Start	Clinical trial identifier[1]	Expected end of accrual
FOLFIRINOX + SBRT	T ⩽ 7 cm, non-metastatic	November 14	NCT02292745	November 20
RFA	Unresectable, also metastatic	June 14	NCT02166190	June 16
Cryoablation	Borderline resectable/locally advanced	November 14	NCT02336672	November 16
Radioembolization	unresectable/failure of celiac alcholization	Not available	NCT01786850	Not available
Irreversible electroporation (PAN.FIRE)	T < 5 cm, non-metastatic	September 13	NCT01939665	June 16

RFA: Radiofrequency ablation; SBRT: Stereotactic body radiation therapy; [1]Data Available from: URL: http// www.clinicaltrials.gov (last access 2015 May 24).

> G3 gastrointestinal toxicity)[86]. Furthermore, SBRT led to improved pain control in five out of six studies in which this outcome has been evaluated[87]. Moreover, as previously mentioned, SBRT can be delivered in association with chemotherapy with interesting preliminary evidence of activity (*e.g.*, gemcitabine). Some trials are currently ongoing trying to better clarify the role of SBRT in PC and its activity in association with more recent combination regimens (*e.g.*, SBRT with FOLFIRINOX, NCT 02292745).

RFA is the commonest thermal ablative technique used to treat tumours. It causes both direct thermal destructive effect and stimulation of antitumour immunity, through the expression of heat shock protein. RFA appears to be an attractive treatment for LAPC. According to the experience of Spiliotis *et al*[88] it should not be offered as an option for resectable PC, but it has shown to improve survival in 25 consecutive patients with inoperable LAPC who underwent palliative therapy with or without RFA. Median OS was 13 mo in patients receiving palliative therapy alone, compared to 33 mo in those who received RFA too (P = 0.0048). Moreover, RFA could be an option for patients with liver only metastasis in locally controlled PC. In a retrospective review by Park *et al*[89], RFA of liver metastasis was performed on 34 patients with PC, after the pancreatectomy or at the same time of the pancreatectomy. Median OS after liver metastasis treatment was 14 mo. In multivariate analysis, a single < 2 cm diameter liver metastasis and good or moderate differentiation were independent predictors for longer patient survival (P = 0.27, P = 0.16)[90].

Pancreatic Cryo Ablation (PCA) is a technique that uses single (or multiple) argon based probe in order to freeze the tumour. In most cases two cycles of freezing are used. It is currently used in several centers in the Far East for unresectable and often metastatic pancreatic ductal adenocarcinoma. Reported complications include acute pancreatitis, bleeding, leakage of bile, and delayed gastric emptying. No randomised trials have evaluated the efficacy of cryoablation, but Niu *et al*[91] retrospectively assessed the effect of comprehensive cryosurgery (ablation of intrapancreatic and extrapancreatic tumours) plus immunotherapy in 106 metastatic PC patients (cryoimmunotherapy: 31 patients, cryotherapy: 36 patients, immunotherapy: 17 patients and chemotherapy: 22 patients). Median OS was higher in the cryoimmunotherapy (13 mo) and cryotherapy groups (7 mo) than in the chemotherapy group (3.5 mo; both $P < 0.001$) and was higher in the cryoimmunotherapy group than in the cryotherapy ($P < 0.05$) and immunotherapy groups (5 mo; $P < 0.001$). In both the cryoimmunotherapy and cryotherapy groups, median OS was higher after multiple cryoablations than after a single cryoablation ($P = 0.0048$ and 0.041, respectively). A single institution retrospective review suggested effectiveness of PCA in palliation of cancer pain, on 62 patients, in combination with celiac plexus block. Some slight adverse effects (*e.g.*, increased serum amylase, abdominal distension and nausea, abdominal bleeding) had disappeared by 3 wk, spontaneously or after symptomatic treatment. A significant difference was found between pretreatment and post-treatment pain frequency ($P = 0.0019$), regardless of the presence of advanced ($P = 0.0096$) or metastatic ($P = 0.0072$) cancer, and pain control was reported to last for more than 8 wk, without severe side effects[92].

Radio Embolization (RE) is a form of brachytherapy, which involves the direct intra-arterial delivery of radioactive isotopes close to or into a tumour. RE with intravascular yttrium-90 microspheres has been shown to be a safe and efficacious treatment of unresectable primary and metastatic hepatic tumours. RE is well tolerated with minimal toxicity. Patients may experience a short lasting post embolization syndrome, characterized by fatigue, nausea, abdominal pain, and/or a transient rise in liver function tests. RE for the treatment of liver metastasis from PC is investigational[93].

MWA is an emerging modality, performed either under percutaneous ultrasound guidance or through a laparotomy. Although operative temperatures may be higher with MW than with RFA, heat sink effects are less prominent, with less procedure related pain. Multiple probes can be used at the same time, reducing operative time. In a retrospective series[94], 10 patients with unresectable LAPC were treated with MW and palliative bypass surgery. In 5 of them MW was administered percutaneously, while in the other 5 it was delivered during laparotomy. One late major complication occurred, without any visceral injury being detected. No patient underwent further surgery. All patients had an improvement in QOL. In conclusion, MW ablation is a feasible approach in the palliative treatment of PC, but further studies are necessary.

Trans Artherial Chemo Embolization it is an interventional radiology procedure, of intra-arterial catheter-based chemotherapy. The selective administration of small drug-coated particles allow high doses directly to the tumour bed while sparing the surrounding liver tissue. For reported very limited experience, regarding liver metastasis from PC, its use is purely investigational[95].

PDT is a minimally invasive and safe method of treating cancer using an intravenous adinistered photosensitizer, activated by a specific wavelength of light, to kill tumour cells. The activated photosensitizer, produces singlet oxygen from molecular oxygen, which in turn causes tumour necrosis. There is also indirect cell death caused by induced hypoxia through tumour vasculature damage, without significant damage to connective tissues. The VERTPAC-01 trial investigated the safety and efficacy of PDT in 15 patients with LAPC using Verteroporphin. In 11 of 13 assessable patients, tumour size was stable at 1 mo, and in 6 of them stability was maintained at 3 mo. The technique proved to be feasible and safe and the authors concluded that it warrants further studies and may have a role in the multimodal treatment of PC[96].

HIFU The intention of a HIFU treatment is to deliver ultrasound energy to a well-defined targeted volume at depth, and to induce complete coagulation necrosis of the tumour. It can be administered with continuous or pulsed modality. HIFU doesn't need the placement of a needle and it is characterized by a low rate of adverse events[87]. In a recent trial of HIFU, administered in addition chemotherapy or chemoradiotherapy to 30 patients with stage Ⅲ/Ⅳ PC, the rate of symptom relief effect was 66.7% and the disease control-rate was 86% (mainly stable disease). The procedure was well tolerated, with moderate adverse events occurred in 10% of cases, mainly pseudocyst formation and mild pancreatitis[97,98].

IRE is a nonthermal ablative technique that uses ultrashort pulsed but very strong electrical fields. Formation of nanopores and micropores in the lipid bilayer of cell membranes induces cancer cells apoptosis[99,100]. No results of randomised trial are currently available. In the largest prospective series in LAPC, 54 patients have undergone an open approach IRE for unresectable cancer. The outcomes were compared to those obtained in 85 matched stage Ⅲ patients, treated with chemotherapy and radiation therapy alone. The IRE procedure was given in addition to standard treatment: Chemotherapy or chemoradiation therapy in forty-nine (90%) patients and chemotherapy or chemoradiation after IRE in forty patients (73%). The 90 d mortality was 2%. IRE was associated with an increase in local progression-free survival (14 mo *vs* 6 mo; $P = 0.01$),

distant progression-free survival (15 *vs* mo 9 mo; P = 0.02), and OS (20 mo *vs* 13 mo; P = 0.03)[101]. In a percutaneous approach IRE study, in 14 patients IRE was performed. All patients had received chemotherapy or radiation previously. Two patients underwent surgical resection with margin-negative and both had long disease-free survival (11 and 14 mo). There were no procedure-related deaths[100]. IRE appears to be feasible and safe, but it doesn't improve OS compared with standard treatments, because of rapid progression of distant metastasis. IRE could be used as an additional treatment when surgical resection is possible but with hight risk of margin-positive (R1).

Regional intra-arterial chemotherapy: Intra-arterial chemotherapy aims both to increase drug concentrations in tumours tissues and to maintain low systemic drug levels. A recent meta-analysis and systematic review of randomised controlled trials included 155 patients receiving Regional Intra-Arterial Chemotherapy (RIAC) and 143 patients receiving systemic chemotherapy[102]. The RIAC efficacy seems to be evidenced by response rates of 58.06% with RIAC *vs* 29.37% with systemic treatment. Also, clinical benefit seems to be in favor of the RIAC (78.06% *vs* 29.37% respectively). The median survival time with RIAC (5-21 mo) was longer than for systemic chemotherapy (2.7-14 mo). Side effects were fewer in patients treated with RIAC (49.03%) than in those treated with systemic chemotherapy (71.33%), but the only statistically significant difference was for hematological side effects (60.87% *vs* 85.71% respectively). Despite these results, RIAC is not commonly used in clinical practice because it is invasive and requests hospitalization, with consequent risks of complications. A possible application of this technique, to further explore, could be in the neoadjuvant setting, in order to increase the resection rates and then probably OS with local advanced PC[103].

Unmet needs and proposals

Locoregional therapies alternative to surgery and radiation, for unresectable PC, are attractive and a number of studies demonstrated that they are feasible and reproducible. All of them should be considered as having a complementary role in the multimodal management care model. In metastatic setting, few data are available, most of them concerning RFA, these could be a safe and feasible strategy for extending survival in selected patients. Albeit several studies have anyway shown improved outcomes (changes in stage, diagnosis, or treatment plan), long-term survival data are lacking. Large prospective randomised studies are mandatory to assess the efficacy of these techniques and define their role/position in future treatment algorithms for the management of LAPC. Their main interest is in the context of a multidisciplinary-team patient evaluation, that is the best option to help patients cope with this challenging cancer[104-106].

IS MOLECULAR BIOLOGY THE NEW ROUTE IN DIAGNOSIS AND THERAPY?

Current knowledge

PC has a mean of 50 to 60 somatic mutations in protein-coding genes and at least 4 to 6 of them are driver mutation-driven in proto-oncogenes or tumour suppressor genes[106]. In addition, these somatic mutations are distributed in several key molecular pathways, probably ten or more[107], thus facilitating the acquisition of both intrinsic and secondary resistance to chemotherapy and targeted agents.

The commonest genome aberrations of PC are[108]: (1) the chromosomal rearrangements, widespread among the cancer genome and very common; (2) the KRAS oncogene mutated in nearly 90% of PC; (3) the tumour-suppressor genes TP53, SMAD4 e CDKN2A inactivated in more than 50% of cases.

Some key features of PC have been recently elucidated by the results of whole genome analyses of 100 cases of PC[109]. In particular, according to structural variations profiles and implicated molecular mechanisms underlying, PC can be classified into 4 subtypes defined as: (1) "stable", 20% of cases, with low (< 50) structural variation events and frequent aneuploidy, suggesting defects in cell cycle/mitosis mechanisms; (2) "locally rearranged", 30% of all samples, exhibiting a significant focal event in 1 or 2 chromosomes. In nearly one-third of cases it was present a gain of known oncogenes, mainly KRAS, SOX9 and GATA6, but also therapeutic target genes as ERBB2, MET, CDK6, PIK3CA, but with a low individual prevalence; (3) "scattered", 36% of samples, exhibiting a moderate range of non-random chromosomal damage and less than 200 structural variation events; (4) "unstable", 14% of cases, with a large (> 200) number of structural variation events suggesting defects in DNA maintenance including both mutations in BRCA pathway and mutations in other pathways involved in genomic instability, with a possible association with sensitivity to platinum agents and PARP inhibitors.

Moreover, the techniques of circulating cell-free tumour DNA (cfct-DNA) or circulating tumour cells, even if there are still very limited data in PC, seem a very interesting and promising way to study dynamically the global amount of cancer mutation. The cfct-DNA can be detected in respectively > 75% and 48% of patients with advanced or localized PC[110].

Unfortunately, up to now no single targeted agent, in preliminary clinical and preclinical data, has demonstrated to have a relevant impact on the natural history of metastatic PC. Strategies employed in these trials have involved mainly the inhibition of EGFR-MEK pathway and farnesyl-transferase. Targeted agents have been studied in PC mainly in combination with standard chemotherapy, in most cases gemcitabine. The association of chemotherapy with targeted agents blocking a single pathway in a molecularly unsele-

Table 2 Available clinical results about multitarget inhibition in pancreatic adenocarcinoma

Combination	Molecular targets	Frequence of mutation[1]	Setting/combination	Results
Everolimus + Erlotinib (Javle 2010)	mTOR + EGFR	+, +	Phase Ⅱ, 16 patients, chemo-refractory	No responses
Bevacizumab + Erlotinib (Van Cutsem 2009)	VEGF + EGFR	+, +	Phase Ⅲ, 301 patients, plus GEM + ERLO	No increase in OS respect GEM+ ERLO
Cixutumumab + Erlotinib (Philip 2014)	IGF-1R + EGFR	+, +	Phase Ⅰb/Ⅱ, 126 patients, plus GEM	No increase in PFS and OS respect GEM + ERLO
Sunitinb (Bergmann 2015)	VEGFR + PDGFR	+, +	Phase Ⅱ, 106 patients, 1st line, plus GEM	No increase in TTP and OS respect GEM

GEM: Gemcitabine; ERLO: Erlotinib; Nab-P: Nab-paclitaxel; OS: Overall survival; PFS: Progression-free survival; mTOR: Mammalian target of rapamycin; EGFR: Epidermal growth factor receptor; IGF-1R: Insulin-like growth factor 1 receptor; VEGFR: Vascular endothelial growth factor receptor; PDGFR: Platelet-derived growth factor receptor; TTP: Time to progression. [1]To obtain this parameter, a mean between the frequency of somatic mutations in the target was calculated from the paper by Biankin *et al*[108] and Waddel *et al*[109], Figure 1. Three parameter were possible: +++ ⩾ 75%, ++ > 50%, + ⩽ 50%.

Table 3 Ongoing clinical trials about multitarget inhibition in pancreatic adenocarcinoma

Combination	Target	Frequence of mutation[1]	Setting	Clinical trial identifier[2]	Expected end of accrual
Dovitinib	FGRFR + PDGFR + VEGFR	+, +, +	Phase Ⅱ, + GEM and CAPE	NCT01497392	Sep-16
Trastuzumab + Erlotinib	EGFR2 + EGFR	+, +	Phase Ⅱ, + GEM	NCT01204372	Apr-15
MEK162 + Ganitumab	MEK1 + IGF-1R	+, +	Phase Ⅱ, multi-disease, chemorefractory	NCT01562899	Apr-15

GEM: Gemcitabine; MEK 1: Mitogen-activated extracellular signal regulated kinase 1; CAPE: Capecitabine. [1]To obtain this parameter, a mean between the frequency of somatic mutations in the target was calculated from the paper by Biankin *et al*[108] and Waddel *et al*[109], Figure 1. Three parameter were possible: +++ ⩾ 75%, ++ > 50%, + ⩽ 50%; [2]Data Available from: URL: http// www.clinicaltrials.gov (last access 2015 May 24).

Table 4 Available and ongoing clinical results about drugs targeting mainly stroma in pancreatic adenocarcinoma

Combination	Target (s)	Setting	Clinical trial identifier[1]	Expected end of accrual
Demcizumab (Gracian 2014)	Cancer stem cells by DLL4 inhibition	Phase Ⅰb, plus GEM +/- Nab-P	NCT01189929	Concluded. presented at ASCO 2014: Increase in ORR, cardiovascular toxicity
Ruxolitinib (Hurwitz 2014)	Inflammation by JAK/STAT inhibition	Phase Ⅱ, 2nd line, plus CAPE	NCT01423604	Concluded. presented at ASCO 2014: Benefit in patients with elevated CRP
PEGPH20 (Hingorani 2013)S	HA by Pegylated-hyaluronidase	Phase Ⅱ, 1st line, plus GEM	NCT01453153	Concluded. presented at ASCO 2013: ORR 33%, especially in patients with high HA expression
″	″	Phase Ⅱ, plus GEM + Nab-P	NCT01839487	July 16

CAPE: Capecitabine; CRP: C-reactive protein; GEM: Gemcitabine; HA: Hyaluronic acid; ORR: Objective response rate; DLL4: Delta like ligand 4; ASCO: American society of clinical oncology; JAK/STAT: Janus kinase/signal transducer and activator of transcription; Nab-P: Nab-paclitaxel. [1]Data Available from: URL: http// www.clinicaltrials.gov (last access 2015 May 24).

cted PC population has not led to relevant increase of treatment outcomes as, for example, in the case of erlotinib, tipifarnib, anti MEK-drugs like selumetinib and trametinib, trastuzumab and bevacizumab. Strategies involving a multiple blockade seem more promising due to the complexity of PC genome: Available clinical data and ongoing trials in this setting are described in Tables 2 and 3[111-115]. Data about multiple pathway inhibition strategies are available only in preclinical models[116].

A very peculiar feature of PC is its ability to promote the growth of a complex peritumoural stroma, with desmoplasia and altered vascularization. This surrounding environment greatly hinders antitumour drugs to reach active concentration into the tumour[117]. As far as inhibition of stroma is concerned, some recent preclinical data showed a possible benefit from hyaluronidase, an enzyme able to dissolve extracellular matrix[118], which is being tested in association with chemotherapy. Moreover, in a preclinical model, the concurrent administration of gemcitabine plus saridegib, a multiple Hedgehog signalling pathway inhibitor, increased vascular density and intratumoural concentration of gemcitabine. Clinical data about these approaches are resumed in Table 4[119-122]. Results from phase Ⅱ and phase Ⅲ trials exploring other treatment targets, as Hedgehog pathway, angiogenesis and immune regulation are expected in the next years[111]. Both the genomic instability and the complex tumour-stroma interactions promote the development of a relevant spatial and temporal molecular heterogeneity[123].

PC stem cells (PCSCs) are believed to promote tumour growth and progression through a number of mechanisms, including differentiation into bulk tumour cells, metastasis, alteration of adjacent stromal cells, and

evasion of conventional therapies. Possible strategies to target PCSCs involve inhibiting specific proteins and pathways, such as c-Met, Alk-4, Notch pathway and gamma-secretase. These approaches are in a preclinical stage of development[124] (Table 3).

Regarding epigenetic modifications, key tumour suppressors genes, with a well-established role in PC, may be altered through hypermethylation. And permissive histone modifications may be the cause of oncogenes upregulation. Moreover, factors involved in tumour invasiveness can be aberrantly expressed through deregulated microRNA. In this perspective, a potential therapeutical target in order to modify epigenetics is the enzyme enhancer of zeste homolog 2 (codified by the gene EZH2) which, when overexpressed, contributes to PC growth. Only preclinical data are available[125].

Unmet needs and proposals

Although presenting a molecular landscape shared with other neoplasms (*e.g.,* colorectal and breast cancer) PC has a worse prognosis. As described above, the very complex genomic landscape with the simultaneous activation of multiple relevant pathways and the complexity of cancer microenvironment could be key factors in determining the disappointing results of targeted agents in PC. From a clinical point of view, due to the increasing availability of targeted agents, a deeper understanding of PC's biology is desirable and remains the mainstay of clinical research in PC.

The recent availability of next-generation sequencing techniques and the creation of joined multicenter working groups has greatly increased the knowledge of the mutational landscape of PC and raised the possibility to perform a personalized medicine even in such as "distressing" setting[126].

Starting from the current knowledges, possible research strategies to improve the results of targeted agents could be the simultaneous inhibition of multiple pathways, the combination of stroma targeting agents with other possibly effective drugs (*e.g.,* chemotherapy), targeting PCSCs, targeting epigenetic alterations.

Moreover, the heterogeneity of the disease has to be taken in account. A better knowledge of pathways and targets and of distinct genetic features can help in defining prognostic and predictive factors to select or stratify patients accrued in clinical trials. As an example, a significant proportion of subtype 2/locally arranged PC harbor mutations in "druggable" genes (as ERBB2 and MET) and many subtype 4/unstable PCs have defects in DNA repair mechanisms suggesting the hypothesis this group could have a particular sensitivity to platinum agents and PARP inhibitors, to prospectively test in further trials.

CONCLUSION

Despite new biomolecular knowledge and the efforts to define new therapeutic approaches in PC in all the setting of care, there are still many unresolved issues. In fact, starting from the definition of resectable disease to the evaluation of the best locoregional treatment in LAPC, everything today is constantly evolving in clinical practice and there is still no uniformity of view from center to center. Moreover in the era of cancer treatment based on specific molecular alterations and of immunotherapy rather than chemotherapy, PC seems to go against the grain. Disappointing results of targeted therapy studies have not allowed us to add new weapons to systemic treatments, and immunotherapy is still object of clinical trials. Furthermore, the high biological aggressiveness of PC and the incomplete knowledge of the biology of this disease have hampered the development of new more efficacious strategies of target selection and drug development. Hence, PC is still an undefeated enemy, with high and early mortality, high genetic complexity and lack of prognostic and predictive factors that can drive the clinical decision. Efforts to define and validate prognostic and predictive factors as well as the genetic and molecular basis that can help the oncologist in everyday clinical practice must be carried over. A multidisciplinary team is crucial in order to rapidly and effectively translate clinical and preclinical findings into valuable and applicable data for the clinical setting.

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REVIEW

Current status of ultrasound-guided surgery in the treatment of breast cancer

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Abstract

The primary goal of breast-conserving surgery (BCS) is to obtain tumour-free resection margins. Margins positive or focally positive for tumour cells are associated with a high risk of local recurrence, and in the case of tumour-positive margins, re-excision or even mastectomy are sometimes needed to achieve definite clear margins. Unfortunately, tumour-involved margins and re-excisions after lumpectomy are still reported in up to 40% of patients and additionally, unnecessary large excision volumes are described. A secondary goal of BCS is the cosmetic outcome and one of the main determinants of worse cosmetic outcome is a large excision volume. Up to 30% of unsatisfied cosmetic outcome is reported. Therefore, the search for better surgical techniques to improve margin status, excision volume and consequently, cosmetic outcome has continued. Nowadays, the most commonly used localization methods for BCS of non-palpable breast cancers are wire-guided localization (WGL) and radio-guided localization (RGL). WGL and RGL are invasive procedures that need to be performed pre-operatively with technical and scheduling difficulties. For palpable breast cancer, tumour excision is usually guided by tactile skills of the surgeon performing "blind" surgery. One of the surgical techniques pursuing the aims of radicality and small excision volumes includes intra-operative ultrasound (IOUS). The best evidence available demonstrates benefits of IOUS with a significantly high proportion of negative margins compared with other localization techniques in palpable and non-palpable breast cancer. Additionally, IOUS is non-invasive, easy to learn and can centralize the tumour in the excised specimen with low amount of healthy breast tissue

being excised. This could lead to better cosmetic results of BCS. Despite the advantages of IOUS, only a small amount of surgeons are performing this technique. This review aims to highlight the position of ultrasound-guided surgery for malignant breast tumours in the search for better oncological and cosmetic outcomes.

Key words: Breast neoplasms; Segmental; Surgery; Ultrasonography; Mastectomy; Cosmetics; Margins; Volume status; Wire localization; Radioguided surgery

Core tip: Despite improved survival and local recurrence rates of breast cancer patients in the past years, there is still much to be gained in surgical treatment. Unacceptable rates of involved margins are described, with up to 25% of the patients undergoing re-excision after breast conserving surgery. The most frequently used excision methods are wire-guided and radioguided localization for non-palpable tumours and palpation-guided localization for palpable tumours. Although ultrasound-guided surgery is a simple and non-invasive technique, it is not frequently used. This review highlights the position of ultrasound-guided surgery for breast cancer in the search for better oncological and cosmetic outcomes.

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INTRODUCTION

Breast conserving therapy

Breast cancer is the most commonly diagnosed cancer worldwide, including low and middle-income countries and incidence is rising with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). In the western world, approximately 1 in 8 women (13%) will develop breast cancer over the course of their lifetime[1].

Since disease-free and overall survival rates after breast conserving therapy (BCT) are known to be comparable with patients treated by mastectomy, BCT is established as the standard of care in women with early stage breast cancer[2-4]. BCT refers to a combination of breast conserving surgery (BCS) followed by whole breast irradiation to eradicate any microscopic residual disease. The widespread use of screening and the development of more effective treatment methods have been associated with improvement in terms of overall survival and recurrence rate, with 5 year survival rates for early stage (Ⅰ and Ⅱ) of more than 92%[5-8].

Primary goal: The primary goal of BCS is to remove the tumour with clear margins. Margins positive or focally positive for tumour cells are associated with a high risk of local recurrence and, in the case of tumour positive margins, re-excision or even mastectomy must be performed[9,10].

Incidences for tumour-involved margins in BCS have been reported up to 40%[11-15]. However, direct comparison of studies is difficult due to the use of varying definitions for positive margins, for instance a "close margin" is used for either a positive and negative margin. In the United Kingdom previous guidelines recommended a margin > 2 mm, however current guidelines do not encompass a clear definition on margin status and they recommend breast units to have local guidelines regarding acceptable margin width[16]. Danish National Guidelines recommend tumour-free margins $\geqslant$ 2 mm[17]. Other European countries such as Germany and France have BCS guidelines on margin status that indicate that patients with margins $\leqslant$ 1 mm should undergo additional surgery[18,19]. In the Netherlands and the United States guidelines for BCS are stating all specimens without tumour-cells at the inked margins are tumour free margins, and these specimens do not necessitate additional local treatment such as surgery or radiotherapy[10,20].

In the recent St. Gallen International Breast Cancer Conference 2015, the majority of the panelists agreed that the minimal acceptable surgical margin was "no ink on invasive tumor" in women undergoing BCS for invasive breast cancer and proceeding to standard radiation and adjuvant systemic therapy[21]. However, two recent surveys in the United States have reported that, against the national breast cancer guidelines, 85% of breast surgeons do not accept a tumour-free margin less than 1 mm[22,23].

In the United States, a striking number of approximately one-fourth patients who undergo initial BCS for breast cancer will have a subsequent operative intervention[24]. Evidently there remains an international controversy regarding the definition of tumour margins. It is, however, important to note that a tumour-free resection margin of > 1 mm is unrelated to local recurrence or overall survival, and the range of local recurrence rates is 2%-5%[5,25].

Secondary goals: Together with increasing breast cancer incidence, the improved outcome has resulted in a growing population of breast cancer survivors and there has been considerable interest in secondary goals such as cosmetic outcome and quality of life of (QOL)[26-33]. The achievement of tumour-free margins during BCS is of great importance for local recurrence but also for the cosmetic outcome. Positive resection margins result in additional treatment, such as higher radiotherapy dose, re-excisions and even mastectomy, these additional therapies will ensure oncological safety but negatively influencing the cosmetic outcome[30,31]. Besides young age, central inner quadrant localization, axillary dissection, re-excision and complications, larger

excision volumes and secondary radiotherapy (administration of boost and whole breast irradiation dose) are the two key determinants of cosmetic outcome[29,32,33].

Poor cosmetic outcomes are observed in up to 30% of patients after BCS[32-34]. In a large survey among 963 women treated with BCS for breast cancer, cosmetic results were scored as 3.4 on a 5-point scale with from 1 (very dissatisfied) to 5 (very satisfied)[35].

The importance of achieving optimal oncological control may lead to an unnecessarily large resection of breast tissue. Literature shows that cosmetic failure rates are significantly higher when a lump exceeds 50-100 cm^3[29,32,36-37]. However, these studies dated from the 90's and recent data on volume are scare. When the surgical accuracy of BCS is improved by a higher rate of margin clearance and smaller excision volume, this will improve not only oncological outcome, but also improves patient satisfaction and cosmetic outcome.

In many cases, an unnecessarily large volume of healthy breast tissue is excised along with the tumour, while clear margins are not assured[13,38,39]. High excision volumes are rarely related to the size of the tumour. Therefore, as a tool to define the amount of tumour and the excess healthy breast tissue in a surgical specimen, the calculated resection ratio (CRR) was introduced, indicating excess healthy tissue resection[13]. The CRR represents a comparison of the total resection volume to the optimal resection volume. This means that in an ideal situation, the specimen volume is equal or smaller than the optimal resection volume and the CRR $\leqslant$ 1. For example, in a retrospective study, 10.7% of 726 patients with T1-T2 tumours still had positive or focally positive margins when the CRR was > 4.0, meaning that the tumour is often located eccentrically in the surgical specimen[13].

Despite the ongoing development of techniques for diagnosing and treating breast cancer, the current techniques BCS in many cases do not meet primary and secondary goals and there is still much to be gained. This review aims to highlight the position of ultrasound-guided surgery for breast tumours in the search for better oncological and cosmetic outcomes.

Non-palpable breast cancer

Due to the development of imaging techniques and screening programs, the incidence of non-palpable breast cancer has increased with up one third of the diagnosed breast cancer being non-palpable. In this group, DCIS represents a challenging problem for breast-conserving surgery (BCS) given that it is typically non-palpable and non-contiguous. Different management procedures are used to remove the non-palpable tumour with optimal resection margins: Wire guided localization (WGL), radio guided localization (RGL) or intra-operative ultrasound-guided surgery (IOUS)[33-52]. Despite improved techniques intra-operatively, unfortunately no assessment can ensure clear lumpectomy margins during surgery.

Wire guided surgery: WGL has been the most commonly used technique for non-palpable breast cancer in the past years. Pre-operatively, a thin, hooked wire is placed into a non-palpable lesion under mammographic, sonographic, or CT guidance. When the lesion is visible on ultrasound, this is the easiest approach because the wire can directly be placed under ultrasound guidance. The mammographic approach is based on measurements of distances between the lesion and the nipple (or other reference points) performed on the two projections of the mammogram. Subsequent mammograms are then obtained to reposition the wire more accurately, and a confirmatory mammogram is finally obtained.

After WGL, positive margins are described in 10%-43% of patients with up to 40% re-excisions after initial surgery[38,42-44]. The reported CRR after WGL is 2.8-4.3[13,39]. Volumes and cosmetic outcome must be compared to other techniques in the same study groups and therefore are mentioned in the next paragraph.

Even though WGL has proven to be a useful localization tool, it is associated with several shortcomings. The wire tip gives no indication of the extent of the tumour and the amount of tissue to be excised is estimated by the surgeon intraoperatively. This could explain the high amount of incomplete tumour resections.

Additionally, the wire may migrate, become displaced or transected[45]. Also the extra pre-operative procedure is demanding for the patient pre-operatively with pain and discomfort caused by the wire.

Radio-guided surgery: Due to the technical and scheduling difficulties of WGL, radio-guided surgery (RGS) was developed, in the form of radio-guided occult lesion localization (ROLL) and radio-guided seed localization (RSL). ROLL uses the radiotracer which is injected intra-tumourally for the sentinel lymph node procures to guide surgical excision of the primary tumour. The gamma-detecting probe guides the localization of the lesion throughout the surgical procedure. In RSL, a radio-opaque titanium seed containing Iodine-125 is placed into the tumor under stereotactic or ultrasound guidance. The seed can be placed days to weeks preoperatively. Again, a handheld gamma probe is used to guide surgical resection of the tumor during surgery.

RGS has been prospectively compared to WGL. Overall tumour free margin rates of RGS range from 73% to 96% with a weighted average of 90%[42,47,48]. The range of re-excisions reported is 4.6%-27%[38,45].

Sajid *et al*[42] showed in a meta-analysis the risk of having positive resection margins following WGL was higher than ROLL. (OR = 0.47; 95%CI: 0.22-0.99; z = 1.99; P < 0.05). A systematic review by Lovrics *et al*[40] demonstrates that WGL produces higher positive margins rates and more re-operations. However, Postma in their randomized controlled trial, showed WGL is comparable to ROLL in terms of complete tumour excision and re-excision rates and ROLL was leading to larger excision volumes (71 cm^3 *vs* 64 cm^3). The average excision volume of five studies including 1077 patients with DCIS or invasive breast cancer was 86 cm^3[47].

Further evaluation about the effect of the specimen volume on margins status demonstrated no correlation and was difficult to interpret because of varying patient populations and the fact that excision volumes were missing in most studies.

One retrospective study mentioned CRR with a significant, and clinically relevant difference for ROLL (CRR = 3.8) compared to WGL (CRR = 2.8, $P = 0.043$)[15]. However, in both techniques CRR is more than one, meaning a large amount of healthy tissue is resected. Cosmetic outcomes assessed were similar after WGL and RGS in a prospective, randomized trial. Most patients rated their overall cosmesis as "excellent" or "good" (76% WGL, 80% RSL). This comparable outcome may reflect the similar reoperation rates and volumes of excision between groups[34]. An advantage of RGS compared to WGL is the fact that it can be done weeks pre-operatively. However, it still requires radioactive material being transported and attendance to the radiology department prior to surgery. Therefore, it does not overcome the scheduling conflicts between radiology or nuclear department and the surgery department. Additionally, with RGS, the borders of the tumour are not visible during surgery, there is only a diffusion zone guided by the gamma probe. The risk of seed migration and failure of seed placement ranged from 0%-0.6% and 0%-7.2% respectively[45]. Unfortunately RGS remains having an invasive component with discomfort for the patient.

Intra operative ultrasound: Since high-frequency real-time ultrasonography was introduced in the 1970s, technological advances have improved sensitivity and reduced the size of ultrasound scanners, making them practical and able to be used close to the bed-side or in the operating theatre. In the 1980s Schwartz *et al*[48] were the first to describe IOUS as an effective and accurate technique for localizing non-palpable breast masses, facilitating excision and diagnosis with a minimum of patient inconvenience and discomfort as well as utilizing hospital resources efficiently. The ideal localization procedure would be non-stressful for the patient and would allow accurate targeting and removal of the lesion central in the specimen, while removing as little tissue as possible with tumour-free margins. In the current series, IOUS met these objectives better than did WGL. The rate of successful intra-operative localization in, ultrasound visible, non-palpable tumours varies between 95%-100%[49-53]. In 2002, a randomized clinical trial from Rahusen *et al*[53] involving 49 patients with non-palpable breast cancer, demonstrated IOUS to be superior to WGL concerning margin clearance. In 2013, a systematic review and meta-analysis of patients with non-palpable breast cancer treated with IOUS *vs* WGL was performed. One RCT[50] and nine cohort studies with control WGL groups were identified, containing 739 patients[15,39,52-58]. The rate of involved surgical margins for IOUS varies between 0%-19%. In the effects model there was a statistically significant difference between IOUS and WGL in terms of tumour-free margins favoring IOUS. (OR = 0.52; 95%CI: 0.38-0.71)[49]. Another meta-analysis of Pan *et al*[59] also demonstrated a statistically significant increase in the incidence of pathologically negative margins with the use of IOUS, for both non-palpable and palpable breast cancers. A limitation of a meta-analysis by Ahmed *et al*[49] is the heterogeneity of *in situ* cancer (DCIS) amongst the small cohorts studies. The trend is towards higher percentages of *in situ* cancer in the WGL groups within the meta-analysis, but this trend does not reach a statistically significant value ($P = 0.65$). Because patients were not randomized to either cohort, more difficult cases with extensive DCIS may have been selectively approached with bracketed needle localization, whereas more "straightforward" cases of limited disease may have been chosen for ultrasound-guided excision.

Excision volumes for non-palpable breast cancer after IOUS and WGL are mostly mentioned in (retrospective) cohort-controlled studies. Different outcome of resection volume between groups were seen ranging from no difference to smaller volume in IOUS[39,58]. However, selection bias has occurred in the localization technique to assess large and clearly visible tumours without microcalcification; on average there was a larger tumour size in the IOUS group. This is indicating that, with IOUS more optimal resection volumes are obtained. The excess breast tissue resection therefore must be determined using the CRR. For example, Barentsz *et al*[39] showed total resection volumes was similar in both groups. (56.6 cm^3 *vs* 62.8 cm^3, $P = 0.66$) Because of the larger tumour size in the IOUS group, the CRR was smaller. (3.3 *vs* 4.3) in the IOUS group ($P = 0.018$). No study specifically measured cosmetic outcome after IOUS in non-palpable breast cancer compared with other techniques. However, as the volume of resection decreases and better margins are achieved, we expect cosmesis to be positively affected, as is patient satisfaction[38].

IOUS is accurate, simple and it carries a minimal risk of procedure-related complications[50,51,60]. It overcomes the issues associated with WGL an RGS because it does not require preoperative localization at the radiology or nuclear department and being a less invasive procedure. IOUS can also be used *ex vivo* to verify the presence of a tumour in a resected specimen. Downsides of IOUS include the need for or visibility of the breast lesion on ultrasound. IOUS is not very accurate for lesions presenting as clustered microcalcifications. Patients with DCIS and multifocal invasive cancer will be at increased risk for a positive margin and at increased risk for re-excision or mastectomy, also after IOUS. However, to overcome this problem, a marker which is visible on ultrasound could be placed intra-tumourally. Additionally, the availability of a surgeon with ultrasound experience or a preoperative assisting radiologist is mandatory in performing IOUS.

Palpable breast cancer

In palpable tumours, surgeons are performing blind surgery trusting on pre-operative imaging and their tactile skills, which can be problematic, especially in dense breasts[61]. A high incidence of positive margins after palpation guided surgery (PGS) up to 40% is described[13,61-63]. Moreover, it has been shown that many surgeons tend to overexcise volumes of healthy tissue in an effort to obtain adequate margins. Median excision volume of PGS is over two times too large[13].

Only a few reports have been published of the use of ultrasound-guided surgery in palpable breast cancer. In 2001, Moore and colleagues were prompted to prospectively evaluate IOUS in women with palpable breast cancer because of poor results obtained with PGS. They compared 27 patients undergoing IOUS with 24 undergoing PGS and their findings were striking. Only 3% positive tumour margins were noted in the ultrasound-guided surgery group compared with 29% in the palpation-guided surgery group (P < 005)[61]. After this, other retrospective studies showed IOUS of palpable breast cancers to be associated with markedly reduced rates of involved margins and re-excisions[59,61-64]. The COBALT-trial was the first multicenter randomized controlled trial for palpable cancer comparing IOUS with the PGS, in order to improve both oncological and cosmetic outcomes. The primary results of this trial showed a dramatic difference in margin involvement with 3% of tumour-involved margins for the invasive component in the IOUS-group compared to 17% in the PGS-group, and thus a significant decrease in additional treatment required in the IOUS group [2% re-excision and 9% boost in IOUS (total, 11%) *vs* 7% mastectomy, 4% re-excision and 16% boost in the PGS group (total, 27%)][65]. Moore *et al*[61] found that the volume of the lumpectomy specimen was smaller in the IOUS group (104 cm^3) *vs* their palpation-guided group (114 cm^3). In the COBALT study, IOUS results in significantly reduced excision volumes and CRR compared with PGS[65]. A CRR greater than 2.0 was seen in only three (5%) women in the ultrasound-guided surgery group *vs* 20 (29%) patients in the palpation-guided surgery arm (P < 0.0001). Minor lesions of additional ductal carcinoma *in situ* (DCIS) were found inside or within several millimeters of the invasive tumour by the pathologist in 73 (55%) of the 132 palpable tumours in the COBALT trial. Despite the fact that United States cannot always detect DCIS, the rate of tumour-free margins was high, even in cases with additional *in situ* carcinoma (11% in the IOUS group compared with 28% in the PGS group). It could be explained by the increased accuracy with IOUS in the localization of the central point of the tumour, which allowed complete resection of the additional DCIS. Earlier studies have mentioned IOUS to improve the cosmetic results and patient satisfaction in palpable breast cancer[61,63]. The COBALT-study clearly showed IOUS resulting in better cosmetic outcome than PGS; 21% of the overall responses were excellent and 6% were poor with IOUS, while 14% and 13% of responses were excellent and poor, respectively, with PGS. Consistently, IOUS had smaller odds of having worse cosmetic outcome than PGS (OR = 0.51, P = 0.045)[66].

Learning curve

Hands-on ultrasound education for surgeons and the ongoing improvements in imaging technology have made surgeon-performed breast ultrasound an effective method of identifying palpable breast lesions. With proper teaching, adequate practice, and close supervision leading to progressive independence, breast surgeons can acquire comprehensive skills that will enable them to successfully incorporate breast ultrasound and ultrasound-guided breast procedures into their clinical practice[50,51,67-72]. A weekly half-day training minimally invasive breast biopsy for breast fellows with hands-on, "live-patient" breast ultrasound training, showed proficiency in performing breast ultrasound by the 12th week[69]. In the study by Krekel *et al*[70] surgeons underwent an ultrasound-training program performing ten cases, under the strict supervision of a breast radiologist. The learning curve for surgeons to develop the adequate skills was short, after the first two supervised cases, resections reached optimal volumes. After eight procedures, surgeons acquire the expertise to perform IOUS.

Despite the good results of IOUS, the utilization of this technique amongst breast surgeons remains consistently low, with surveys of American and Australasian breast surgeons suggesting figures between 2.8%-17%, respectively[73,74]. The main reasons for the low amount of American surgeons performing IOUS were related to their radiology department with almost half stating that radiologists had prohibited them from scanning, the remainder being due to a combination of a lack of time, hospital restrictions, lack of confidence and reimbursement as well as medico-legal liability[71]. The lack of performing IOUS by breast surgeons gains more relevance with the increasing evidence of improving outcome of BCS in palpable and non-palpable breast cancer.

Cost-effectivity

With experienced surgeons, excision time is similar between IOUS and other guidance techniques, although there is extra time for the pre- and post-surgical use of the United States system which will account for 5-10 min[53,57].

In the ROLL-study, QOL effects between ROLL and WGL were similar (difference 0.00 QALYs 95%CI: -0.04-0.05). Total costs were also similar for ROLL and WGL[73]. In palpable breast cancer, a cost-benefit analysis applied to IOUS *vs* other localization techniques for invasive breast tumours evaluating costs in terms of reoperation and complication-related costs as well as the procedural costs themselves has been performed by Haloua *et al*[74] Although the cost of IOUS is more expensive, the overall cost of performing an IOUS procedure *vs* palpation only was €154 cheaper per patient

due to a reduced rate of tumour involved margins and thereby the avoidance of cost of additional treatments. Above 30 patients, use of the USS system leads to cost savings.

Future directions

Because most of the current operative techniques for BCS in palpable and non-palpable breast cancer result in a high rate of positive margins, re-excisions and resection volumes with impact on cosmetic results, surgeons have been proactive in searching for better surgical techniques of BCS in two ways.

Firstly, surgeons have continually used ways to decrease the amount of positive margins and rate of re-excisions such as cavity shaves and touch-prep or intra-operative frozen section assessment of the margins. There is still much debate about the usefulness of these methods and their influence on re-excisions and volume resected[75-80]. These methods are potentially useful as additional methods to decrease the overall volume of excised tissue and re-excisions. However, it is preferable to perform a small lumpectomy with adequate CRR and the tumour centrally in the specimen in the first place.

Another available method of intraoperative margin evaluation is the MarginProbe (Dune Medical Devices, PA, United States). This device allows for *ex vivo* evaluation of the resected specimen and is especially useful in detecting DCIS. Adjunctive use of the Margin Probe device during BCS improved surgeons' ability to identify and resect positive lumpectomy margins in the absence of intraoperative pathology assessment, reducing the number of patients requiring re-excision[81-82]. Due to the *ex vivo* use of the margin probe, we think this method could be used alongside IOUS but should not be seen as a replacement method.

Additionally, the search for improving ultrasound-guided surgery is ongoing. Technical aspects are improved, such as the development of a portable three-dimensional ultrasound systems[83]. Also, IOUS is combined with other techniques such as needle localization or intraoperative margin assessment[75,77,84]. Combined techniques are especially useful in those tumours who are non-palpable and not visible on ultrasound. Ivanovic *et al*[75] recently showed a technique of excising palpable and non-palpable breast cancer by intraoperative ultrasound with an especially constructed marking needle, being placed while the patient is anesthetized. Preliminary results showed this technique to be feasible with good oncological safety with only one patient with a positive resection margin (3%).

Secondly, the volume of normal breast tissue excised at the time of BCS must be minimized by centralizing the tumour in the surgical specimen and, in cases where a larger excision volume is necessary for oncological reasons, volume displacement and replacement techniques are utilized. This last mentioned approach is referred to as oncoplastic breast surgery (OPBS) and combines oncological resection with plastic surgery techniques in a single procedure. The term may refer to simple volume-displacement techniques or to more complex techniques of volume replacement by using local or regional flaps. The proposed benefit of OPBS is the ability to achieve wide surgical margins, with a higher chance of obtaining tumour-free resection margins than with standard BCS. A recent study showed 11.9% positive margins and a 91% breast conservation rate[85]. Additionally, it is surprising to notice in a systematic review on OPBS that tumour-free margins ranged in the included studies from 78% to 93% with OPBS, resulting in a conversion to mastectomy in 3% to 16% of all OPBS cases. However, most studies showed significant weaknesses including lack of robust design and important methodological shortcomings, negatively influencing generalizability[86]. Therefore there is a need for well-designed comparative studies to create high quality guidelines, ensuring uniform indications for OPBS in breast cancer patients.

Considering the outcomes of studies performing IOUS, the results of OPBS may also be improved with the use of ultrasound-guidance. In patients with large tumours, a high amount of volume must be resected, even with IOUS. In these cases, by performing IOUS a safe margin with minimal volume of healthy breast tissue will be excised while achieving a good cosmetic outcome. We do think that if the tumour volume to mammary volume ratio is low, IOUS could be sufficient to achieve both tumour-free surgical margins and a good cosmetic result without concomitant reconstruction techniques.

As mentioned earlier, IOUS is not applicable in every patient. However, when applicable, IOUS is an accurate, non-invasive and technically feasible method. Although we embrace new studies to improving surgical outcome and reducing the need for re-excision in BCS, it does not seems necessary to develop new and expensive techniques for patients already suitable for IOUS. Despite advances and usability of ultrasound, the main problem remains that the use of ultrasound by breast surgeons is consistently low, as is the presence of a radiologist in the operating theatre. Surgeons who wish to provide optimal, state-of the art care for patients with breast cancer should embrace IOUS. Adequate ultrasound training should be included in the surgical curriculum for breast surgical trainees.

CONCLUSION

The best evidence available demonstrates the benefits of IOUS in BCS with a high proportion of negative margins and optimum resection volumes compared with other localization techniques in palpable and non-palpable breast cancer visible on ultrasound. With intra-operative United States guidance, surgeons can more accurately delineate the tumour by direct feedback and thereby achieving a centrally localized tumour in the specimen. Next to this, IOUS is shown to be a method which can be learned easily by surgeons and being cost-effectiveness because of less additional therapy

applied as a result of negative margins. The oncological, cosmetic and logistical advantages of IOUS for patients with breast cancer have to be recognized by every surgeon performing BCS.

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REVIEW

Historical review of the causes of cancer

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Abstract

In the early 1900s, numerous seminal publications reported that high rates of cancer occurred in certain occupations. During this period, work with infectious agents produced only meager results which seemed irrelevant to humans. Then in the 1980s ground breaking evidence began to emerge that a variety of viruses also cause cancer in humans. There is now sufficient evidence of carcinogenicity in humans for human T-cell lymphotrophic virus, human immunodeficiency virus, hepatitis B virus, hepatitis C virus, human papillomavirus, Epstein-Barr virus, and human herpes virus 8 according to the International Agency for Research on Cancer (IARC). Many other causes of cancer have also been identified by the IARC, which include: Sunlight, tobacco, pharmaceuticals, hormones, alcohol, parasites, fungi, bacteria, salted fish, wood dust, and herbs. The World Cancer Research Fund and the American Institute for Cancer Research have determined additional causes of cancer, which include beta carotene, red meat, processed meats, low fibre diets, not breast feeding, obesity, increased adult height and sedentary lifestyles. In brief, a historical review of the discoveries of the causes of human cancer is presented with extended discussions of the difficulties encountered in identifying viral causes of cancer.

Key words: Infections; Causes; Cancer; Carcinogens; Historical; Etiology; International agency for research on cancer

Core tip: The International Agency for Research on Cancer has worked for around 45 years evaluating the scientific literature, concerning the potential of around 1000 different agents to cause cancer. Those agents which were determined to definitively cause cancer in humans are reviewed from a historical perspective. It is reviewed how there were many complexities in identifying infectious agents as causes of cancer. The author incidentally discovered while writing this review that natural factors are an additional and relatively underappreciated cause of cancer.

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INTRODUCTION

The question of "what causes cancer" has intrigued people for generations. In 1950, the World Health Organization sponsored an international symposium, and the attendees were intrigued by the dramatic variations in the types of cancer found in different areas of the world[1]. It was learned that people who migrated to other countries, developed types of cancer common to their adopted countries, rather than their homelands. This implied that most cancers were caused by exposures in the environment, rather than inherited genetic factors. The symposium led to the creation of the International Agency for Research on Cancer (IARC) in 1965 which was instructed to conduct multidisciplinary investigations of the *causes* of human cancers[1,2]. The assessments of the IARC were initially based only on epidemiological evidence[3], and then later the criteria were extended to include experimental evidence[4].

There has been a widespread notion that synthetic agents are the cause of most cancers, so this review begins with a review of the discoveries of occupational and pharmaceutical agents which cause cancer, wherein it becomes evident how this opinion arose. The next section recounts how there has also been a strong suspicion that infectious agents cause cancer, and includes a description of the exhaustive search for viruses which cause cancer. This is followed by a section which discusses natural factors and non-viral infectious agents which have been demonstrated to cause cancer.

Numerous resources were frequently consulted, and influenced the selection of topics for discussion in this review. The historical treatise by Shimkin[5], the historic milestones outlined by Sirica[6], and the monograph by Ludwig Gross[7] were consulted many times. Most importantly, the monographs by the IARC were used to identify which agents have been determined to cause cancer in humans, and were frequently used to identify the earliest and most influential studies. "Food, Nutrition, Physical Activity and Prevention of Cancer: A Global Perspective" is an expert report published by the World Cancer Research Fund and the American Institute for Research on Cancer[8] which provided supplementary analyses for a variety of natural agents.

OCCUPATIONAL, PHARMACEUTICAL, AND TOBACCO

Early studies

Early epidemiological studies: The earliest carcinogens to be identified were generally associated with specific occupations. Bernardino Ramazzini[9] observed in 1713 that nuns suffered from high rates of breast cancer which he attributed to their celibate life. Percivol Pott[10] documented in 1775 that chimney sweeps frequently developed cancer of the scrotum which he deduced to be caused by their heavy exposure to soot. A century afterwards, reports emerged that a variety of other occupations were associated with increased rates of cancer. Richard von Volkmann[11] diagnosed three cases of scrotal cancer in 1875 among coal tar distillers in Germany, which was quickly followed by similar reports by other physicians[12]. Joseph Bell[13] described two cases of scrotal cancer among shale oil workers in Scotland in 1876, and commented that the cancer was quite common among shale oil workers. Harting and Hesse documented in 1879 that miners in the Black Forest regions of Schneeberg in Germany and Joachimsthal in Czechoslovakia suffered from a high mortality due to lung cancer[14,15]. Ludwig Rhen[16,17] reported in 1895 that long term dye workers in Germany frequently perished of bladder cancer. Wilhelm Conrad Röntgen[18] discovered X-rays in 1895, which were heralded as a phenomenal discovery, because they permitted the painless visualization of bones. The early radiologists routinely tested the performance of their equipment by exposing their hands. Then a few days after a prolonged exposure, an extremely painful skin condition termed radiodermatitis developed[19,20]. A decade after Röntgen's discovery of X-rays, case reports began emerging from many diverse areas of the world, that radiologists were succumbing to skin cancers[21,22].

A few non-occupational agents were also identified during this period. John Hill[23] reported in 1761 that immoderate use of tobacco snuff was associated with the occurrence of nasal cancers[24]. Sir Johnathan Hutchinson[25] observed in 1881 that patients who used a tonic which contained arsenic for extended durations frequently developed keratosis lesions which sometimes progressed to skin cancer.

Early experimental studies: In the late 1800s, there were three fundamental theories of the cause of cancer[26-28]. Virchow proposed that cancer was a product of chronic irritation[28,29]; Lobstein and Recamier, and later Cohnheim hypothesized that cancer was the result of displaced embryonal tissue[28,29]; others surmised that cancer was caused by an infectious (or parasitic) agent[27,28,30]. Numerous researchers attempted to induce cancer in experimental animals, based on one of these theories. However, experiments to produce tumors with irritating chemicals produced only benign growths[31]. Work to prove Cohnheim's theory by transplanting embryonal or fetal tissue into adult hosts similarly failed to induce malignant growths[32]. A broad range of microbes were identified in cancerous growths. However attempts to extract the microbes and produce cancers, could not induce cancers reproducibly[28]. Experimental induction of cancer was considered to be important, because this was expected facilitate the development of preventative measures and effective treatments[32].

In 1908, Ellermann and Bang[33,34] reported that a cell-free filtrate caused a leukemia in chickens, and Peyton Rous[35,36] reported that a cell-free filtrate produced

a sarcoma in chickens shortly afterwards. However, work with chickens seemed to be irrelevant to humans, so efforts to produce experimental cancer based on the other theories continued unabated.

Jean Clunet designed an experiment which simulated the procedures of early radiologists, who developed radiation burns after prolonged exposures to X-rays[37,38]. He administered X-rays to four rats, at dosages sufficient to induce epidermal ulcerations, then allowed the lesions to heal for a few days, and repeated the exposure[37,38]. Cancer developed in one of two surviving rats at the site of ulceration. However, the experiment was not widely accepted as a success for three reasons: Only one rat developed cancer, the tumor resembled spontaneous tumors of rats, and other experimenters had difficulty reproducing the experiment[39].

Katsusaburo Yamagiwa was a young associate professor, whom the Japanese government considered to have good potential. They sent him to Virchow's Institute in Germany, where he studied pathology from 1892-1894[26]. von Volkmann's study of skin cancers among coal tar workers had become very well known by the time of Yamagiwa's studies, with numerous other investigators reporting additional cases[40,41]. Yamagiwa was intrigued by these reports, so he devised to induce skin cancer in rabbits, by exposing them to conditions which resembled occupational exposure to coal tar. The ears of rabbit's were not known to be susceptible to spontaneous cancers, so he decided to apply tar to their ears. He reasoned that, since previous attempts to induce experimental tumors produced only benign lesions which regressed, then he should continue to apply tar when the benign lesions emerged. He surmised that the application of tar to benign lesions could promote further changes, which would progress to malignancy[40,41].

When Yamagiwa returned to Japan, he applied tar to the ears of 137 rabbits, and repeated the application every two or three days. Seven rabbits eventually developed cancerous lesions. The average cancer developed after five months of tarring; some cancers only emerged after a year of tar application[40,41]. Yamagiwa recorded the occurrence of metastasis in two of the rabbits, which confirmed the malignant nature of the tumors, and the experiment became widely regarded as the first successful experimental induction of cancer.

Numerous experimenters attempted to replicate Yamagiwa's experiment. Many endeavors to reproduce the experiment failed, which led investigators to decipher why Yamagiwa's experiment was efficacious. It became evident that many previous experimenters failed because they did not continue their treatments for a sufficient duration. Woglom colorfully reflected in 1926, that Yamagiwa and Ichikawa were possessed of "infinite patience", because they continued to apply tar for many months without evidence that cancers would develop[42]. Murray Shear[43] similarly conjectured that other investigators had terminated their experiments early, because they thought they were hopelessly "kicking a dead horse". Another reason for failure was because an insusceptible species had been chosen, since it was not appreciated that most carcinogens are species specific[42]. A further reason for Yamagiwa's success was that he chose to use many animals, because only seven of 137 rabbits developed cancer, and only two developed metastases.

1920-1950

Kennaway's experimental work: During 1920-1950, additional evidence emerged that synthetic agents were the cause of human cancer. Ernest Kennaway[44] studied many modifications of Yamagiwa's experiment. He was intrigued by observations that some fractions of coal tar induced cancer, while other fractions were ineffective. He suspected that this was due to an active component, which was present in only minute quantities, similar the vitamins in foods, or hormones in tissues which were first discovered during this period. An intense search was undertaken to identify "the cancer producing compound in coal-tar" under his direction[45,46]. Kennaway and Hieger[47] identified dibenz(a,h)anthracene in 1930 as the first pure chemical compound to induce cancer in experimental animals. This was followed by the isolation of benzo(a)pyrene as the major "cancer producing compound of coal-tar" in 1933[48]. Many other chemicals were identified that induced cancer in experimental animals during this period which are too numerous to describe, but were reviewed in 1947[49].

Occupational studies: A few additional reports of occupational carcinogens emerged during this period. During 1915-1929, young women in the United States were recruited to paint watch dials with a new florescent paint. The paint contained minute quantities of the newly discovered element named radium which illuminated the dials at night. The women ingested the isotope incidentally by pointing their paint brushes with their mouths. Shortly after they began the work, it was reported that many of them developed decreased levels of polymorphonuclear leukocytes and lymphocytes. A few years later, it was reported that numerous women were diagnosed with necrosis of the jaw bones which were frequently fatal[50]. After further follow-up, in 1929, it was recounted that the survivors commonly succumbed to osteosarcomas of the jaw bones[51,52]. X-rays, coal tar dyes and radium appeared to be astonishing discoveries when they were first introduced, but then horrendous diseases developed in those exposed to the new discoveries. The notion that synthetic agents were the cause of cancer was emerging.

During this period, the Report of the Chief Inspector of Factories and Workshops in England, described an increased incidence of nasal cancer among workers in a large nickel refining company in South Wales[53]. Machle

and Gregorius reported that men in the United States, whose employment involved industrial exposure to the fumes of chromate, developed lung cancer at a 25 fold higher rate compared to workers in other industries[54].

1950-1980

Numerous notable occupational studies: A strong groundswell of interest in cancer began to emerge in the 1950s and the disease received more systematic study. Some carcinogens were reported which caused very high rates of cancer. Robert Case worked to identify which of the 100s of chemicals used in the dyestuffs industry in England and Wales caused the high rates of bladder cancer among dye workers. Case analyzed the various combinations of chemicals had been used by persons who developed bladder cancer, and after a meticulous analysis, he deduced that 30%-50% of the workers who had long term exposure to β-naphthylamine developed bladder cancer[55]. He also estimated that about 10% of workers exposed to benzidine developed bladder cancer[55].

Another study reported that seventeen percent of a subset of workers involved in the production of 4-aminobiphenyl, (a chemical that was used as an antioxidant in the rubber industry), developed bladder cancer[56]. Six of eighteen (30%) of workers exposed to bis(chloromethyl)ether, (an important intermediate in the synthesis of organic compounds), were reported to have developed lung cancer after only six years of exposure[57,58]. A similar compound, chloromethyl methyl ether, induced lung cancer in 14 of 91 (15%) of exposed workers[59,60]. Thirty three cases of the extremely rare mesothelioma were reported among the residents of the asbestos mining area of North Western Cape Province in South Africa[61]. Karin is a small village in the Anatolian region of Turkey, which has high deposits of erionite, an asbestos-like mineral, which occurs naturally near the surface of the earth in the region. Erionite is easily cut into large blocks which have been traditionally used for construction of homes and multiple other purposes in the region[62]. Eighty-two of 179 (45.8%) deaths in the village of Karain have been reported to be due to mesothelioma[63,64].

There were discoveries of additional occupational exposures which cause cancer which were also significant, though less striking. Three cases of a very rare cancer, angiosarcoma of the liver, occurred among workers employed in the manufacture of vinyl chloride[65]. A case series of workers occupationally exposed to benzene, reported a high rate of hemocytoblastic leukemia[66]. The cause of the high rates of lung cancer among miners had been a mystery. Arsenic and cobalt were each been suspected, but during this period, it was deduced that radon emitted from uranium was the principal cause[67]. A follow-up of the British veterans of the 1914-1918 war was published. The report estimated that the veterans who were exposed to mustard gas had a twofold increase in death due to cancers of the lung and pleura over the expected number[68]. Follow-up of the survivors of the Hiroshima and Nagasaki atomic bomb explosions of 1945 was published, and it was reported that they had increased rates of leukemia, as well as other types of cancer[69].

The cigarette smoking and lung cancer controversy: Sophisticated methods for statistical analysis were developed to detect additional causes of cancer during this period. By the mid-1940s, it was evident that the rate of lung cancer was increasing at epidemic proportions, but the cause was unknown. Austin Bradford Hill sought to study medicine, but was unable when he contracted tuberculosis, so he completed a BSc in economics by correspondence while convalescing. Following this, Professor Major Greenwood mentored him in statistical methods[70,71]. Hill developed an interest in formulating mathematical methods to discern the health effects of exposure to chemicals introduced since 1900. He became involved in a committee, which designed a comprehensive epidemiological study to attempt to decipher the cause of the increasing rates of lung cancer[72]. Hill hired Richard Doll, a young physician who preferred to work with mathematics over patients[72]. A case-control study was designed which would investigate the effects of a wide variety of exposures that were new to the 1950s, which included automobile exhaust, road tars, atmospheric pollution, and cigarette smoking[72]. Doll and Hill[72,73] were surprised when their analysis showed that only cigarette smoking was correlated with the incidence of lung cancer. Doll himself was a cigarette smoker, as the harmful effects of smoking were generally unsuspected[74]. They decided to follow up with a prospective trial, in order to test for possible undetected flaws of the case-control study. After 29 mo of follow-up, thirty five lung cancer deaths occurred among 24, 389 men. The highest proportion of lung cancer cases occurred among the heaviest smokers in both the case-control and prospective studies which was interpreted as confirming that smoking was the cause[75]. Numerous other studies of smoking and lung cancer were published both before and after Doll and Hill's studies, which reported similar results. The studies were reviewed by the Royal College of Physicians of London in Great Britain in 1962[76], the United States Surgeon General in 1964[77] and more recently by Doll[78].

The studies of smoking and lung cancer were initially received with incredulity by the medical community. Firstly, because smoking was considered a benign activity, with some physicians recommending cigarette smoking because there were suggestions that smoking had various health benefits[74,79,80]. Secondly, the usefulness of mathematics to discern the cause of a disease, was a new discipline and not universally accepted[81-83]. The conventional approach to demonstrate disease causation was experimentation. The controversy concerning whether cigarette smoking causes lung cancer prompted extensive discussions of

the criteria to determine whether exposure to an agent causes a disease[81,84], and was the impetus for Hill to develop a seminal list of principles to discern whether epidemiological associations are causal[85]. The smoking and lung cancer controversy contributed strongly to the establishment of modern cancer epidemiology[1].

Pharmaceutical studies: Beginning in the late 1960s, pharmaceuticals began to be frequently identified as carcinogens. Users of high doses of analgesic mixtures containing phenacetin were reported to develop high rates of carcinoma of the renal pelvis[86]. Organ transplant recipients, who used the immunosuppressant drug azathioprine, were reported to develop high rates of lymphomas[87]. Studies of women who took diethylstilboestrol during pregnancy revealed that their female children had high rates of the extremely rare adenocarcinoma of the vagina in adulthood[88]. Post-menopausal women, who used estrogen replacement therapy, were reported to have a high risk of developing endometrial cancer[89]. Four of 5 patients who received high cumulative doses (200 g or more) of chlornaphazine, a chemical related to β-naphthylamine for treatment of Hodgkin's lymphoma, developed invasive bladder carcinomas[90,91].

Research concerning chemical warfare agents had shown that the sulphur and nitrogen mustards (β-chloroethyl sulphides and amines) exerted strong cytotoxic activity on rapidly proliferating tissue, especially lymphoid tissue, bone marrow and epithelium of the gastrointestinal tract[92]. Subsequently, numerous analogues were developed for use as therapeutic agents for the treatment of cancer. Melphalan[93], busulfan[94], and cyclophosphamide[95] were each shown to be associated with increased rates of acute nonlymphocytic leukemia (ANLL).

Medical radioisotopes were also found to be associated with high rates of cancer. Patients suffering polycythaemia vera were treated with radioactive phosphorus ($^{32}PO_4$) during this period. They developed high rates of leukemia[96]. Patients who were administered a contrast medium which contained radioactive thorium, for imaging purposes, developed high rates of the rare angiosarcoma of the liver[97,98]. The use of thorium was curiously preceded, by numerous cautions predicting its probable carcinogenic effects due to its radioactivity[99,100].

1980-present

Sophisticated occupational studies: The most obvious agents were already identified in previous periods, so many studies published after 1980 were frequently re-examinations of previously tested agents. Cogliano *et al*[101] described how the IARC relaxed the criteria which are required to classify agents as carcinogenic during this period. They recounted how agents which lacked epidemiologic evidence of carcinogenicity were permitted to be classified as group 1 carcinogens based only on mechanistic evidence. The author observed that epidemiological studies generally became larger, employed more sophisticated methods of analysis and frequently only reported modest increases.

Ortho-toluidine is an aromatic amine which belongs to the same class of chemicals as β-naphthylamine. It is used in the synthesis of dyes, herbicides, synthetic rubber and other chemicals[102]. Ward *et al*[103] reported that six of 73 workers exposed to ortho-toluidine for over ten years in a synthetic rubber manufacturing plant developed bladder cancer which yielded a standardized incidence ratio of 27.2.

A study of workers in Vermont granite manufacturing plants, reported that exposure to silica dust was associated with increased rates of lung cancer. A standardized mortality ratio (SMR) of 1.81 was reported for workers exposed to silica dust for over 30 years[104]. Exposure to diesel exhaust was also reported to be associated with a higher risk of lung cancer. A cohort of 54973 United States railway workers who were exposed to diesel exhaust for 24.8 years were estimated to have a relative risk of lung cancer of 1.40 based on 4351 lung cancer deaths[105].

Sulfuric acid has been used to remove oxides from the surfaces of steel in preparation for painting and other coating processes. Exposures to mists of sulfuric acid have been found to cause laryngeal cancer. A study of 879 steelworkers who were exposed to mists of sulfuric acid for a mean of 9.5 years reported a standardized incidence ratio of 2.30 for laryngeal cancer based on nine cases[106].

Exposure to formaldehyde was determined to cause nasopharyngeal carcinoma (NPC) during this period. Eight of 25619 workers succumbed to nasopharyngeal cancer after exposure to formaldehyde for a median of 35 years. A SMR of 2.10 was estimated from this study[107].

1,3-butadiene is a chemical used in the production of synthetic rubbers and polymers, which has been ascertained to cause non-Hodgkin's lymphoma. Four of three hundred and sixty-four men involved in 1,3-butadiene production died of non-Hodgkin's lymphomas for a SMR of 5.77[108,109].

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is a large complex molecule which occurs as a by-product of some industrial chemical reactions, which has received much media attention as a probable carcinogen. Fingerhut *et al*[110] conducted a study of mortality among twelve plants in the United States, which produced chemicals contaminated with TCDD. They reported a slight increase in all cancers combined, though the increase was limited to workers with the heaviest exposures to TCDD[110].

Trichloroethylene is a solvent which has been used in the dry cleaning industry and for degreasing metal machine parts that has been determined to cause renal cell cancer. A case-control study from France reported an odds ratio of 1.96 for the development renal cell carcinoma among workers with high cumulative expo-

sure, after adjusting for various factors[111].

Ethylene oxide is used mainly as a disinfectant and sterilizing agent in medical facilities for the manufacture of sterile disposable items, which has been determined to cause lymphatic and haematopoietic cancers. The IARC examined the epidemiological evidence concerning exposure to ethylene oxide, and concluded that mortality from lymphatic and haematopoietic cancers were "only marginally elevated"[112].

Beryllium is a metal with a high strength and excellent electrical conductivity which is commonly used in electronics, which has been concluded to cause lung cancer. A study of 689 patients with beryllium lung disease estimated a SMR of 2.0 for the mortality due to lung cancer based on 28 deaths[113]. The IARC has concluded that beryllium is a group 1 carcinogen[114], though various investigators have challenged the conclusion[115].

4,4'-Methylenebis(2-chlorobenzenamine) (MOCA) is a chemical used in the synthesis of some polyurethane products which has been designated as a bladder carcinogen. Screening of 540 workers exposed to MOCA detected two noninvasive papillary tumors of the bladder[116]. The IARC designated MOCA as carcinogenic to humans based on mechanistic rationale[117].

Some of the early nuclear industry personnel in Russia were exposed to high levels of plutonium. However, the reports were originally published in Russian in "classified" reports or journals, which were unavailable to western scientists. The 1990s witnessed a relaxation of this secrecy, and it was reported that workers exposed to high levels of plutonium developed high rates of lung, liver and bone cancers[118,119]. The Chernobyl accident in 1986 resulted in release of iodine-131 into the atmosphere, which was followed by increased rates of childhood thyroid cancers in the nearby areas of Belarus, Russian Federation and the Ukraine[120].

Additional pharmaceutical studies: Additional pharmaceuticals were identified as carcinogens. Patients who had been treated with MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) for Hodgkin's disease, were reported to have an increase in ANLL[121]. A randomized trial of polycythemia vera patients reported a thirteen fold increase in ANLL among those receiving the nitrogen mustard drug chlorambucil, which was strongly related to the dosage[122]. A dose-response relationship was reported between the use of the nitrosourea drug semustine and the occurrence of ANLL[123,124]. Patients who were treated with cyclosporine immunosuppressive therapy, developed increased frequencies of lymphoma, Kaposi's sarcoma and skin cancer[125]. Patients who used tamoxifen were reported to have an increased risk of endometrial cancer[126], but to have a reduced risk of recurrence of breast cancer[127].

Etoposide is a semi-synthetic derivative of an extract of the roots and rhizomes of species of the genus Podophyllin[128]. Ratain *et al*[129] reported that 4 of 21 lung cancer patients who survived longer than one year, of a chemotherapy regimen which included etoposide, developed ANLL. Thiotepa and treosulfan are alkylating agents used in chemotherapy which have each been shown to increase the risk of various types of leukemia[130-132].

The use of combined (estrogen/progestin) oral contraceptives was reported to be associated with decreases in ovarian and endometrial cancers[133,134]. Epidemiological studies concerning the association between oral contraceptive use and breast cancer produced inconsistent results which were difficult to interpret. Some studies reported higher rates, others reported lower rates, and still others reported no effect. A reanalysis of 53297 women with breast cancer and 100239 controls from 54 epidemiological studies reported a relative risk of 1.24 (95%CI: 1.15-1.33) among current users, and a relative risk of 1.07 (95%CI: 1.02-1.3) among previous users who discontinued usage for 5-9 years[135].

Emerging studies of possible new synthetic carcinogens

Having considered the past and present advancements in our understanding of the causes of cancer, it is interesting to consider a few promising areas which are presently under investigation. The effect of prenatal exposures on the rates of cancers in both childhood and also in adulthood is generating interest. The concept of prenatal exposure has been well demonstrated in principle. Prenatal exposure to diethylstilboestrol has been demonstrated, to cause the rare adenocarcinoma of the vagina in the female offspring of mothers who used the drug during pregnancy[88]. A considerable amount of literature indicates that many chemicals can have biological effects at extremely low levels, at levels far below those currently recognized by government regulatory agencies such as the United States Environmental Protection Agency (EPA)[136-138]. Evidence is emerging that *in utero* exposure to xenoestrogens causes reproductive cancers. Bisphenol A (BPA) is a xenoestrogen which has been widely used in the manufacture of polycarbonate plastics, which are used as food storage containers, and epoxy resins which are used to line food and beverage cans[139,140]. BPA has been shown to leach into food products at very low levels[141,142], and to accumulate in the amniotic fluid of pregnant women[143]. When BPA was administered to pregnant nonhuman primates at levels similar to human exposure levels, the histology of the mammary glands of the newborn females was altered[144]. Rats which received *in utero* exposure of BPA, at 1/20000 the level currently estimated by the EPA as the lowest observable adverse effects level, developed mammary gland ductal hyperplasia and carcinoma *in situ*[145]. Male rat fetuses which were exposed to low levels of BPA displayed an increased propensity to develop prostatic intraepithelial neoplasias[146]. It will be interesting to follow the research concerning *in utero* exposure to BPA, as well

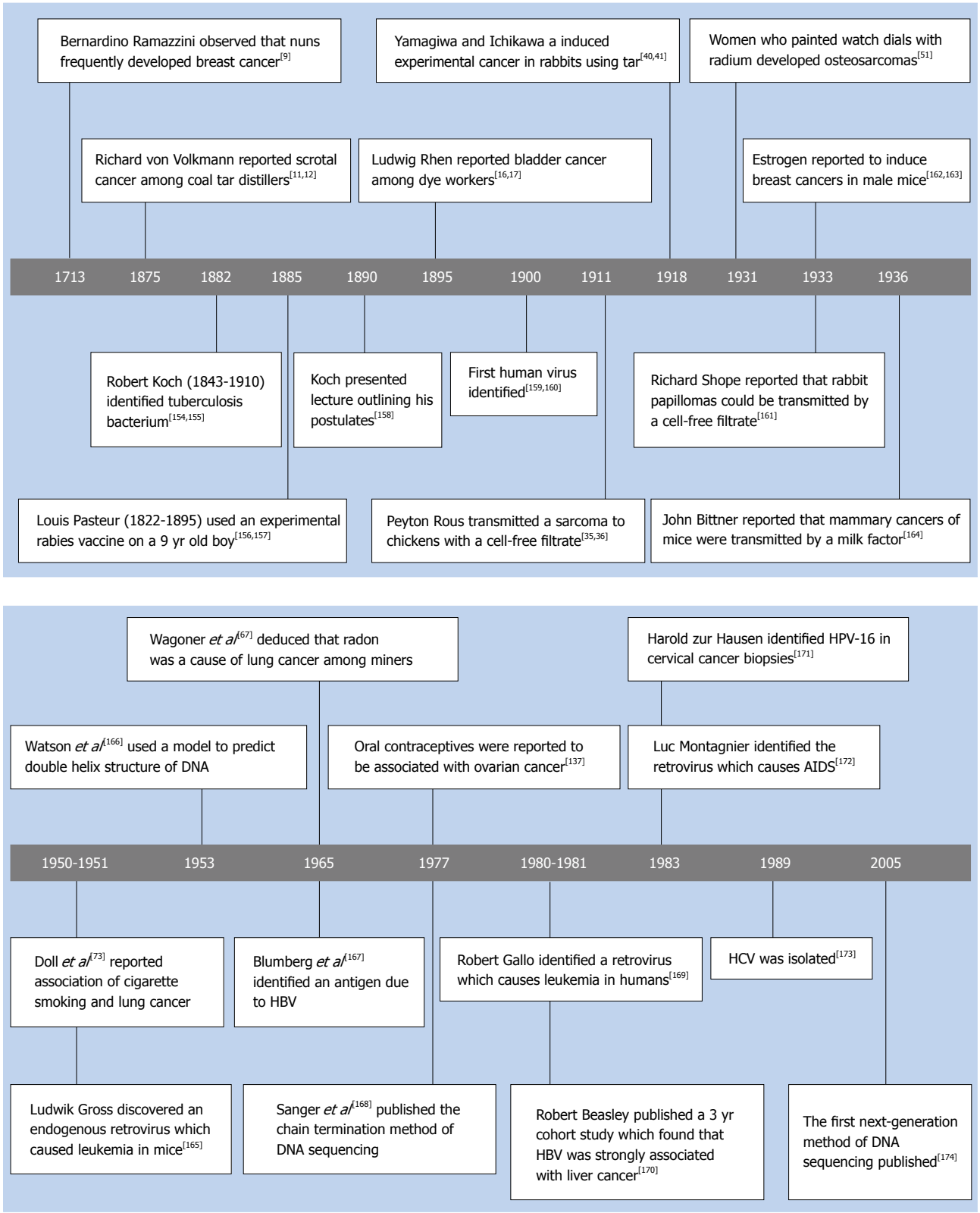

Figure 1 Some milestone publications concerning the causes of cancer, microbiology, and technology.

as other xenoestrogens as possible contributors to the high rates of breast and prostate cancers in developed countries[147-149].

A very recent review has highlighted the fact that most research has analyzed the effects of single agents, while human exposures involve complex combinations of agents, and that combinations of agents may have synergistic effects. They encourage researchers to investigate the effects of low doses of combinations of chemicals[150].

VIRAL

Early experimental studies

Belief in a contagious cause of cancer began in classical times, when it was hypothesized that a single infectious organism caused every kind of cancer. A tremendous amount of work was performed searching for a contagious cause, but nothing was found which could be confirmed[151-153]. In the late 1800s Pasteur and Koch demonstrated the contagious origin of many diseases (see Figure 1)[9,11,12,16,17,35,36,40,41,51,67,73,137,154-174] which prompted many more searches for a microbial cause of cancer[151-153]. In 1900, the first human virus was identified, with the isolation of the yellow fever virus[159,160]. Shortly afterwards, Borrel[175,176] hypothesized that cancer had a viral etiology. Following this, Vilhelm Ellermann and Oluf Bang[33,34,177] reported that a cell-free filtrate induced leukemia in chickens, and Peyton Rous[35,36] reported that another cell-free filtrate caused a sarcoma in chickens. However, these early reports kindled little interest, because many orthodox pathologists did not accept the notion that leukemia was a form of cancer. Furthermore, the chicken seemed to be too different from humans for the work to be relevant[153,178].

A well studied observer reflected in 1903[27] that the number of reports which claimed to have identified a microbial cause of cancer peaked in 1887, and then steadily decreased each year thereafter. Interest faded when none of the results could be confirmed[28,179]. The search for an infectious cause of cancer became so intense, and extended for so long, that it eventually "led to wide acceptance of the dogma that cancer did not, and could not have an infectious agent as its cause"[153,180]. An infectious cause came to be considered as ruled out by most well respected investigators when the first studies of chicken cancers were published[181-183]. Furthermore, numerous reports had emerged that a variety of industrial exposures, such as chimney soot, coal tar, dye chemicals, and X-rays caused cancer in humans when these studies emerged, which further eroded interest in the virus studies.

1920-1950

Additional experimental studies: Rous worked with the chicken virus for a decade, and then became discouraged so he abandoned the work[180]. Following this, William E Gye published an independent investigation of the virus, which incited fierce controversies[184-186]. He argued that the work was an embarrassment to both sides. Those in favour of the parasitic theory found little support in the work, because it was not evident how the agent could provide a unifying concept for the tremendous variety of tumors which occur in so many different species[184,186]. The virus only induced one type of cancer in one species. Those opposed to the parasitic theory argued that the Rous sarcoma virus (RSV) was purifiable by chemical methods which no living organism could survive[186]. Other opponents argued that the sarcoma was a disease peculiar to chickens, not really a cancer, and therefore not relevant[184,185].

Methods to successfully transplant cancerous tissue were developed in the late 1800s. Carol O Jensen and Leo Leob independently produced a considerable amount of research on the nature of transplanted cancers by 1903[187]. They each searched for evidence to prove/disprove the infectious cause of cancer theory. Jensen reported that cancers which were transplanted as whole cells frequently survived in a new host, but when cancer cells were crushed in a mortar, the inoculation was never able to produce cancers in the recipients[188,189]. This seemed to be definitive evidence that the transplanted cancers were not the product of transmission of an infection.

The first crude electron microscope (EM) was only developed in 1933[190], and the RSV was not visualized until 1947[191]. The EM only became consistently reliable for the examination of biological specimens in the 1950s[192]. Hesitation to accept the discoveries of viral induced cancers in chickens is understandable.

A few additional viruses were identified which induced cancer during this period. Shope demonstrated that rabbit papillomas could be transmitted with a cell-free filtrate in 1933[161]. Bittner reported that mammary carcinomas of mice could be induced by an infectious agent transmitted in the milk in 1936[164]. Lucke reported in 1938 that a virus caused renal adenocarcinoma in leopard frogs[193]. The reports of Shope and Bittner were the first evidence that viruses could induce tumors in mammals, but interest in synthetic industrial agents as causes of cancer had become overwhelmingly convincing.

1950-1980

A golden era for animal experimental studies: Endogenous viruses were discovered in the 1950s. Mice had been systematically bred for genetic inheritances of high and low frequencies of leukemia in the 1920s and 1930s[194]. In 1951 Gross[165] reported that a cell-free extract from the high frequency Ak strain, induced leukemia in the low frequency C3H strain. In related works, normal chicken embryos were reported to contain an antigen, which was indistinguishable from an antigen of a virus which caused leukemia in chickens in 1967[195]. There was an intriguing report, that either ionizing radiation or chemical carcinogens could induce the replication and spontaneous release of an avian leukemia virus from normal uninfected cells in 1971[196], and another report indicated that the murine leukemia virus could be similarly induced[197,198]. These curious reports became comprehensible when it was found in 1972, that retroviruses frequently integrate into the host cell DNA[199]. Selective breeding for high rates of cancer using the principles of genetics had unintentionally selected for latent endogenous retroviruses which were transmitted vertically[200].

In 1964 Jarrett *et al*[201,202] discovered a retrovirus which caused lymphomas in common domestic cats,

which has been estimated to account for as many as 70% of the lymphomas occurring in domestic cats[203]. During this period, virus particles were detected in cultured human Burkitt's lymphoma cells[204]. An antigen was discovered in a hemophiliac patient, which would later be found to be produced by the hepatitis B virus (HBV)[167]. Breast milk of women who were at high risk of developing breast cancer, was reported to contain particles which were morphologically similar to the MMTV, and to have reverse transcriptase activity[205].

The virus cancer program: The discovery of the mouse leukemia virus[165], along the chicken leukemia virus[33,34], and the feline lymphoma virus[201,202], made it seem reasonable that similar agents could induce leukemia in other species[206]. There were many other discoveries leading up to, and during this period, and additional reasons which fuelled interest in a possible role of viruses in human cancers[7,207]. Some were predicting that viruses would be found to have a universal role in all cancers, including those induced by radiation and chemicals. Others advocated a more bridled enthusiasm. They accepted that viruses were involved in some cancers, but they did not accept the hypothesis that viruses were involved in all cancers[207].

In 1968 the Congress of the United States considered the evidence that viruses induce cancers in animals to be overwhelming. They expected the viruses responsible for human cancers to be found soon, so they began investing ten million dollars a year in the virus cancer program at the National Cancer Institute[208,209]. Much work focussed on searching for human oncogenic retroviruses, analogous to the endogenous retroviruses which were found in animals. A decade was spent searching for the suspected viruses, but very few were found, and those which were found could not be grown in culture. Adding further to the disillusionment, some agents were eventually shown to be contaminants[200-211], while other agents could not withstand the increasingly sophisticated methods of analysis which were being developed[212,213]. The notion became widespread again that human cancers were not caused by viruses. The mood became analogous to the scepticism of the early 1900s[214]. Suspected cancer causing viruses had previously been frequently termed "tumor viruses". Naysayers became so confident that a virus would never be found which caused human cancer, that they mockingly called them "rumor viruses"[212,215].

1980-present

Major advances in the establishment of the roles of viruses in human cancer occurred during this period for numerous reasons. Laboratory methods were improved which enabled the analysis of much lower levels of viruses. The epidemiological methods which had been developed to evaluate the association of tobacco smoking with lung cancer were revised for analysis of infectious agents with other types of cancer. However each virus presented unique difficulties, as described below.

Human T-cell leukemia virus: Robert Gallo's only sibling was diagnosed with leukemia when she was only five years of age, and Robert was eleven. Influenced by his sister's physician, Gallo enrolled as a medical student in 1960, and studied the biochemistry of blood cells[216]. In 1970, it was discovered that RNA viruses replicate using the reverse transcriptase enzyme[217,218], and Gallo decided to work developing enzyme assays to detect viral reverse transcriptase, as a more sensitive method of detecting possible cancer causing viruses[219] (The EM had become the gold standard, but it was tedious and time consuming).

Gallo *et al*[219] were determined to search for a retroviral cause of human leukemia when other researchers were abandoning the search. They were provoked by the discoveries of a retrovirus which caused leukemia in nonhuman primates[220], and another retrovirus which caused leukemia in cattle[221]. The discovery of the bovine leukemia retrovirus intrigued them because it induced leukemia with only very low levels of replication. They reasoned that human leukemia could have similarly low levels of replication, and therefore be difficult to isolate[210].

They eventually found a retrovirus in a T-cell line established from a patient with a cutaneous T-cell lymphoma, which they termed the human T-cell leukemia retrovirus (HTLV-1) in 1980[169,210]. Hinuma *et al*[222] reported in 1982 that 140 of 142 patients with adult T-cell leukemia (ATL) were positive for the virus from certain areas of Japan. Southern blot analysis showed that all cases contained the virus, and that it was integrated into the host genome in monoclonal form, indicating that infection and integration occurred before clonal expansion of the tumor cells[223]. The discovery of HTLV-1 has been reviewed[210,224,225].

The virus causes only a particular type of leukemia, which is common in some areas of Japan, but it is rare in the United States. Parkin *et al*[226] estimated that the global fraction of leukemia due to HTLV-1 is only about 1%, but the principle that viruses could cause human cancer, was finally proven. Convincing evidence that a retrovirus causes common forms of leukemia has still not emerged, though evidence concerning other infectious agents continues to be intriguing[227].

The mechanism by which HTLV-1 causes leukemia is only partially understood. The virus integrates into the host genome as a component of its regular replication cycle. It is found as a single integrated provirus in around 80% of ATL patients, with the remaining ATL patients displaying either two integration sites or multiple clones[228]. The virus codes for a protein termed Tax, which has multiple effects which are predicted to induce transformation[229]. However, it is perplexing that about 60% of patients with ATL have lost expression of the *tax* gene[230], so investigators have been searching for another mechanism to account for how HTLV-1 causes ATL. It has been reported that the HTLV-1 basic leucine

zipper factor *(HBZ)* gene is transcribed in all cases of ATL[231]. *HBZ* RNA promotes T cell proliferation[231], and transgenic mice which express the *HBZ* gene in $CD4^+$ cells develop lymphomas[232]. For recent reviews of HTLV-1 replication and ATL see[233,234].

Human immunodeficiency virus: In the summer of 1981, the first reports of a mysterious new syndrome emerged. Young male homosexuals in New York City and California were reported to have phenomenally high rates of *Pneumocystis carinii* pneumonia[235], and unprecedented rates of Kaposi's sarcoma (KS)[236].

The etiology of the acquired immunodeficiency syndrome (AIDS) perplexed the medical community and many bizarre theories were suggested. Max Essex suggested to Gallo that some feline leukemia retrovirus variants caused immunosuppressive syndromes[224,237]. Gallo reasoned that HTLV sometimes caused immunosuppression, and that the geographic distribution of HTLV across Africa resembled the distribution of AIDS in Africa[238]. He wondered if AIDS could be caused by a retrovirus closely related to HTLV. He decided to abandon the search for retroviral causes of human leukemia/lymphoma, in order to investigate whether the cause of AIDS was a retrovirus, and possibly intercept an impending worldwide epidemic[215]. Luc Montagnier *et al*[239] at the Pasteur institute in France had also been searching for oncogenic human retroviruses, when the AIDS epidemic emerged. Inspired by Gallo's theory, they decided to redirect their resources to search for a retroviral cause of AIDS as well. Using a reverse transcriptase assay Luc Montagnier and Francois Barre-Sinoussi reported identification of a retrovirus in the lymph node biopsy of a patient in the early stages of AIDS in May of 1983[172].

Montagnier *et al*[240] reported detection of antibodies to the retrovirus in the serum of 60% of patients with the pre-AIDS lymphadenopathy syndrome, but in only 20% of patients with AIDS, so the significance of their discovery was initially uncertain. Following this, Sarngadharan *et al*[241] reported that they had developed an improved method to detect antibodies to the virus, and that they found around 90% of cases with AIDS were seropositive in May of 1984. This was followed a few months later by two independent reports of detection of antibodies to the virus in around 90% of patients with AIDS[242,243], which confirmed the virus as the cause of AIDS. Shaw *et al*[224] also developed a cell line capable of yielding sufficient quantity of the virus to be studied and they produced a commercial blood test for the virus in May of 1984[244]. Montagnier *et al*[245] reported development of a comparable method of production of the virus in July of 1984, and a reliable method to detect antibodies in Oct of 1984[246]. Of course the virus was later termed the human immunodeficiency virus (HIV).

Luc Montagnier and Francoise Barre-Sinoussi received the prestigious Nobel Prize for their identification of the virus in 2008 (http://nobelprize.org). A succinct review of the chronology of events concerning the discovery of HIV has been published[247]. Gallo and Montagnier have both written detailed accounts of their personal reflections of this period[248,249]. Robin Weiss has also written a concise informative review of the discovery of HIV[250].

Around 50% of patients diagnosed with AIDS, were also diagnosed with KS at the time of their AIDS diagnosis in the early 1980s[251,252], which would seem to be unequivocal proof that HIV causes KS. However, KS cells were found to be curiously not infected with HIV[253]. Large trials also demonstrated that non-Hodgkin's lymphoma occurred in around 4% of patients with AIDS at the time of their diagnosis, and is the second most common cancer among AIDS patients[254]. However, B cells of AIDS related lymphomas have also been reported to be devoid of the virus[255,256].

The mechanism of how HIV causes cancers is not straightforward. The virus infects T cells and macrophages which release an HIV encoded protein known as Tat, which is taken up by other cell types in the microenvironment. Tat has numerous effects which are also predicted to promote angiogenesis and carcinogenesis[257-259]. It appears that immunosuppression caused by HIV is a potent cofactor in KS and lymphomas, rather than a cause of these cancers, since they also occur at increased rates in transplant patients [Also see the discussion of human herpes virus 8 (HHV-8) below for additional discussion of KS].

The IARC concluded in 2012 that HIV causes not only Kaposi sarcoma and non-Hodgkin's lymphoma, but also Hodgkin's lymphoma, and cancers of the cervix, anus, and conjunctiva[260].

HBV: Baruch Blumberg was formally educated as a physician in the United States, and relates that his education left him "woefully ignorant about viruses"[261,262]. In the early 1960s he was searching for genetic polymorphisms which could account for differences among individuals in their susceptibility to diverse diseases. Harvey Alter was assisting him in this, when they found a precipitin in a serum which they were hoping was caused by a genetic polymorphism[167,263]. Following this, Blumberg became intensely interested in studying every detail about the precipitin. After many months of study, they noticed that a couple of patients who were negative for the antigen became positive, and that the patients coincidentally developed hepatitis[264-266]. Blumberg collaborated with some experienced electron microscopists and reported visualization of small particles in the serum of the patients which were later identified as the surface antigen of HBV[267].

It was not evident that HBV caused hepatocellular carcinoma (HCC) when it was discovered. The geographical correlation between the distributions of the rates of HBV infection and HCC became recognized a decade after the discovery of the antigen[268]. However, aflatoxin had been previously demonstrated to induce

liver cancers in rodents[269] and it was proven to cause liver cancers in primates as well[270], so aflatoxin seemed more likely to be the cause of HCC in humans. Furthermore, serum antigens of the virus were not reproducibly detectable at the time of diagnosis of HCC using the methods that were available in the 1970s[271,272]. Moreover, viral antigens were only present at much reduced levels in the tumor tissue compared to the surrounding normal liver tisue[273-275]. In spite of this evidence, Robert Beasley was determined to definitively test whether HBV caused HCC. He decided to study a healthy population without HCC, and to test them for serum HBV antigens using newly developed and highly sensitive radioimmunoassay techniques. He devised to follow subjects prospectively to determine which individuals developed HCC. It was difficult to get funding for a large study when the evidence seemed to indicate that aflatoxin was the cause of liver cancer. He recruited 22707 healthy male government employees in Taiwan, and tested them for a variety of indicators of HBV infection[170]. After three years of follow-up, forty one deaths due to HCC occurred. Only 15% of the employees were seropositive for hepatitis B surface antigen (HBsAg), but forty of the 41 men who developed HCC were seropositive for the antigen. The relative risk of HCC among men who were seropositive for HBsAg was calculated to be 223 (95%CI: 28-1479)[170]. This was evidence which could not be dismissed[276]. Vaccination of Taiwanese children has resulted in a pronounced decrease in the incidence of childhood HCC[277,278], which is confirmation that HBV causes HCC.

While epidemiological studies have provided convincing evidence that HBV causes HCC, investigations of the mechanisms of how it causes cancer have been less straightforward. A variety of mechanisms have been proposed, which can be divided into three general categories; viral proteins, inflammation and genetic instability. The virus produces oncogenic proteins; HBsAg and HBx have received the most intensive investigations. Mice which are transgenic for HBsAg or HBx have been reported to develop liver cancers[279,280]. The second general mechanism involves the immune response to the virus which results in a state of chronic inflammation, which produces cirrhosis that deteriorates into HCC[281,282]. The third general mechanism involves the integration of the virus into the host genome, which generates genetic instability. The normal replication cycle of HBV does not include integration into the host cell genome. However, the virus has been found integrated into the host cell genome in around 80% of HCC cases associated with HBV[283]. The integrated virus is fragmented and rearranged, so that it cannot produce infectious particles[284-286]. However, the integrated virus is attributed to induce chromosomal instability of the host cell[287]. Each of these mechanisms likely causes a few cases of HCC, with most cases involving two or all three of the above mechanisms. Cases of HCC which develop without cirrhosis are caused by viral integration and viral proteins. Cases which develop without viral integration are caused by inflammation/cirrhosis and viral proteins.

Human papillomavirus: Harold zur Hausen was intrigued by the fact that epidemiological evidence indicated that the risk of cervical cancer was correlated with sexual promiscuity, which suggested an infectious cause[288,289]. Herpes simplex virus type 2 seemed like a plausible agent, so he analyzed cervical cancer biopsies for the virus. However, the samples were found to be devoid of the virus, so he decided to investigate papillomaviruses[289]. The early methods of analysis for human papillomavirus (HPV) were crude and did not produce convincing evidence of a causal role. In the mid-1970s recombinant DNA technology was developed, and zur Hausen's group used the technology to sequence the virus. They discovered that the HPV exists as a variety of different types[290,291]. They identified HPV-16 and HPV-18 in cervical cancer biopsies[171,292]. Following this, the early epidemiological studies still did not consistently identify HPV DNA in cervical cancers, because the methods of analysis of HPV DNA varied substantially in sensitivity and specificity[293,294]. As the sensitivity of the techniques increased, HPV DNA became detectable in 97%-98% of cervical cancer biopsies[295,296].

HPV-16 and HPV-18 produce a number of proteins (E5, E6, and E7) which have various oncogenic activities[297,298]. E6 induces chromosomal instability by binding to the tumor suppressor protein p53, interfering with its normal function and inducing its degradation[299]. E7 also induces chromosomal instability by interfering with the normal functioning of the retinoblastoma family of proteins and inducing their degradation[300,301]. HPV replicates as an episome, in a cycle that does not involve integration into the host's cell genome[302]. However, the virus has been frequently found integrated into the host cell's genome. It is not known precisely how the virus becomes integrated, but it likely results when DNA breaks occur, which are likely promoted by episomal E6 and E7[303,304]. The integrated virus is found in a truncated form, though E6 and E7 usually remain intact, and continue to be transcribed. Numerous reports have found that E6 and E7 are transcribed at higher levels when it is integrated into the host genome[305], and the proteins produced by the integrated virus may have increased stability[306]. Studies which have analyzed the frequencies of integration in premalignant and invasive cancers have consistently reported higher rates of integration among invasive cancers[307,308]. It has also been consistently reported that the infectious episomal form of the virus is detected less frequently in invasive cancers than in premalignant lesions[308].

Recent prospective cohort studies have reported strong associations among women in whom HPV was detected on multiple occasions, which is consistent with cervical cancer developing from persistent infections. Analysis of ten years of follow-up of a cohort

in Copenhagen, reported that 13.6% (167/1229) of women who were positive for high risk HPV at enrolment developed high grade lesions (severe dysplasia or carcinoma *in situ*), while 20.0% (83/414) of women who tested positive at enrolment and tested positive again two years later developed high grade lesions[309].

Confirmation of the causative role of HPV in cervical cancer is expected to emerge, with the successful clinical trials of vaccines for HPV. Vaccine efficacy for a bivalent vaccine (HPV 16, 18) was tested in over 10000 HPV naïve women who were followed for 4 years. The efficacy was 64.9% (95%CI: 52.7-74.2) for protection against CIN2 irrespective of HPV DNA in the lesion, and 93.2% (95%CI: 78.9-98.7) for prevention of CIN3 irrespective of HPV DNA in the lesion. Seven cases of adenocarcinoma in situ occurred in the unvaccinated controls, whereas zero cases occurred in the vaccinated women[310]. Results of the efficacy of HPV vaccination against cervical cancer are expected to emerge in 5-10 years[311].

Initial studies with HPV focussed on identification of the cause of cervical cancer. Further studies have shown that mucosotrophic HPV types also cause cancers of the vulva, vagina, penis, oropharynx, oral cavity, and tonsil[312,313]. Zur Hausen was awarded the Nobel Prize for his work with HPV in 2008 (http://nobelprize.org). Robin Weiss has written a concise reflection of zur Hausen's work[250].

Hepatitis C virus: After the discoveries of HAV and HBV, it was found that most cases of transfusion associated hepatitis were caused by neither HAV nor HBV[314]. A non-A non-B (NANB) infectious agent was suspected of causing transfusion associated hepatitis. The NANB infectious agent was also suspected of causing HCC, based on case reports of transfusion recipients who developed chronic hepatitis, which progressed to cirrhosis, and then HCC[315,316]. However, the identification of hepatitis C virus (HCV) was excruciatingly difficult. The NANB hepatitis agent could be transmitted to chimpanzees; however efforts to isolate the virus using traditional methods, based on antigens and antibodies were without success for over fifteen years. Numerous tests were published, but none could be confirmed by independent laboratories[317,318]. The results were so perplexing and inconsistent, that it was speculated that the agent might only be present at very low levels[318], and/or the immune response could be very weak and therefore difficult to identify[318,319].

Finally, a massive systematic search was initiated by Michael Houghton *et al*[320] at the Chiron Corporation together with Daniel Bradley at the Centers for Disease Control. Their approach was to clone the agent before isolating it, using a "blind" immunoassay. The identification began in the early 1980s, and utilized the recombinant DNA techniques which had been recently developed. Michael Houghton *et al*[320] searched intensely for the elusive NANB hepatitis agent(s), using many different approaches to screen hundreds of millions of cDNA clones. After seven years, they identified the elusive agent, as a single stranded RNA molecule of about 10000 nucleotides, and termed it HCV[173]. Choo *et al*[321] have written a detailed review of the discovery of HCV.

After the identification of the clone, the development of an ELISA test for the detection of circulating antibodies was promptly published[322], and many reports of high rates of detection of antibodies to HCV in the serum of patients with HCC soon followed[323,324].

HCV is considered to cause HCC, though conclusive proof has been elusive. HCV induced HCC evolves through a progression of chronic hepatitis, to cirrhosis, to HCC which generally requires 20-30 years, or longer to develop, so prospective trials have been few. A meta-analysis of HCV positive cirrhotics reported that 17%-30% of patients developed HCC over five years of observation[325]. An eleven year prospective study followed 925 patients with antibodies to HCV. They reported a cumulative risk of HCC of 1.1% among those with undetectable HCV RNA levels and 14.7% among those with the high serum levels of HCV RNA[326].

Confirmation that HCV causes HCC has also been elusive. Treatment of HCV infection with α-interferon (or α-interferon plus ribavirin) results in decreased rates of HCC[327]. However, the demonstration that α-interferon reduces the incidence of HCC is complicated by the evidence that α-interferon has anti-carcinogenic effects independent of its antiviral effects[328-330]. A meta-analysis by Kimer *et al*[331] reported that the rate of HCC was reduced in both sustained virological responders [risk ratio (RR) = 0.15, 95%CI: 0.05-0.45] and also in non-responders (RR = 0.57, 95%CI: 0.37-0.85). The improved prognosis among non-responders is consistent with α-interferon having an anti-carcinogenic effect in addition to its anti-viral effect. Interferon-free treatment regimens are very recently becoming widely prescribed[332], so the long term effect of viral clearance by these treatments should provide a definitive confirmation of the causative role of HCV in HCC.

HCV has an interesting and complex life cycle. The virus replicates using an error prone polymerase which lacks proofreading activity. Consequently, HCV circulates in an individual as a heterogeneous population of sequences or quasispecies, which is very complex for the immune system to clear[333]. A systematic review estimated that 75% of individuals fail to eradicate the virus, because their immune response is only partially effective[334]. The principal mechanism by which HCV causes HCC is considered to be the chronic immune inflammatory response[335]. Around 40% of patients with chronic HCV develop cirrhosis after 30 years[336]. While HCC develops mostly among cirrhotics, it also develops at low rates among patients devoid of cirrhosis[337], which is interpreted as evidence that the virus may possess some directly carcinogenic effects. The virus replicates in the cytoplasm and does not integrate into the host genome as a component of its replication cycle or incidentally, unlike some of the viruses discussed above.

Epstein-barr virus

Burkitt's lymphoma: Dr Burkitt was an English missionary physician posted in Uganda, when he became impressed by the unusually high incidence of a very aggressive jaw and abdominal tumor among the local children. He published an account of it in 1958, then returned home to England in 1961, and presented public lectures on his recent studies[338]. A young Tony Epstein was performing electron microscopy studies of tumor viruses during this period, when he happened to decide to sit in on a lecture of Dr Burkitt's. Afterwards, Epstein requested Dr Burkitt to ship biopsy samples to him from Uganda[339], and a few years later Epstein *et al*[204] reported that they detected virus particles in cell cultures of the biopsy samples.

Henle *et al*[340] reported in 1969, that patients with BL had higher antibody titres to the Epstein-Barr virus (EBV) viral capsid antigen (VCA) than controls. This was followed by a seven year prospective study of 42000 Ugandan children, 14 of whom developed BL in 1978. This study found that high antibody titres to EBV VCA preceded the development of BL[341]. The virus was found to be present in monoclonal episomes in BL cells, which is consistent with the infection of a single normal cell occurring before the transformation and expansion of the tumor[342].

The above discussion has been limited to the endemic BL, which occurs principally in certain areas of Africa, and is strongly associated with malaria[343]. Malaria is discussed further in the natural factors section below. A sporadic form of the tumor occurs outside of Africa, in areas where malaria is rare, at much lower rates. The sporadic form of BL usually lacks detectable levels of EBV in the tumors. For detailed reviews of BL see the following references[344-346].

NPC: The incidence of NPC varies widely throughout the world; however EBV is consistently present in the undifferentiated form of NPC in various geographic regions[347,348]. High serum EBV antibodies have been found to precede the development of NPC[349], and to be informative in predicting the onset of NPC[350]. The virus is found in monoclonal episomes in every tumor cell, indicating that infection of the cells occurred prior to clonal expansion[342,351]. Administration of EBV-specific cytotoxic T-lymphocytes has induced remissions of cases of NPC which are refractory to conventional radiotherapy and chemotherapy[352,353].

Deciphering the mechanism of how EBV causes NPC has been complex. The virus has a dual tropism for B cells and epithelial cells. The life cycle is furthermore complicated by possessing a variety of different latency cycles as well as a lytic cycle[354]. The virus is present in NPC cells in a latent cycle. The virus produces a latent membrane protein 1 which has strong carcinogenic effects, but is only present at extremely low levels in most NPC biopsies[355]. NPC biopsies are characterized by high levels of untranslated EBV RNA which are termed EBER 1 and EBER 2[355]. NPC biopsies also have high levels of rightward transcripts derived from the *Bam*HI A region (BARTs) of the EBV genome[356]. The *Bam*HI A transcripts are multiple spliced which yield numerous microRNAs[357]. It is unclear precisely how EBERs and *Bam*HI A transcripts might induce malignant changes, but in recent years evidence has emerged that untranslated RNA, especially microRNAs, can have strong effects on cellular metabolism[358]. The mechanisms whereby EBV could induce malignancy, have been recently reviewed[355,359].

EBV has also been demonstrated to cause non-Hodgkin lymphomas among immunosuppressed patients, and some cases of Hodgkin lymphoma[360]. While EBV was discovered before HBV, HCV, HPV-16, HPV-18, and HIV, work to prevent cancers caused by EBV has dawdled behind achievements to prevent cancers caused by other viruses. A commercial vaccine for EBV is only in the early stages of development[361].

HHV-8: The initial identification of HHV-8 consisted of only a few epidemiological observations at the Centers for Disease Control, which were followed up by two or three scientists with very limited laboratory experience, who worked for only around three months[362]. Using polymerase chain reaction-based technology, the causative agent was identified as a new herpes virus, with homology to both EBV and herpesvirus saimiri[363]. However, definitive evidence that it causes KS has been challenging.

HHV-8 DNA is present in 91%-96% of KS biopsies[364]. Prospective studies which followed patients with AIDS (or transplant recipients) reported that 20%-50% of persons with detectable levels of HHV-8 in their peripheral blood develop KS[365-368]. Two studies reported that low levels of HHV-8 specific T-cell responses are associated with a high risk of developing KS among immunosuppressed patients[369,370].

Studies of antiherpes drugs usage for cytomegalovirus retinitis and other cytomegalovirus induced disease among HIV patients provide additional evidence that HHV-8 causes KS. Three large studies conducted before the widespread use of HAART, reported decreases in the number of cases of KS among HIV patients who were treated with the antiherpes drugs ganciclovir or foscarnet for cytomegalovirus infections (Reviewed by Casper *et al*[371]).

Not everyone has agreed that HHV-8 has been established as the cause of KS, or that KS is a form of cancer. It has been argued that KS is polyclonal reactive process, rather than a true malignancy. Furthermore, studies concerning the clonality of the virus indicate that some are monoclonal, but most are oligoclonal[372]. Many objections to whether KS is a true cancer, and whether HHV-8 is the cause are settled if it is considered that most KS lesions are preneoplastic with a propensity to progress to cancer[373]. HHV-8 is also attributed to cause a rare cancer termed primary effusion lymphoma[374]. For recent reviews see the following[374,375].

Emerging studies of possible new viral carcinogens

Tremendous progress has been made in understanding the causes of liver and cervical cancers since the 1980s. However, our understanding of breast, prostate, and colon cancers has made relatively little progress. It is striking that the rates of breast cancer are so high, and that few if any, agents have been identified which strongly increase or decrease the risk. The author hypothesizes that an initiating event occurs in all females during fetal development, which renders human breast tissue susceptible to the promoting effects of estrogen and a diet high in protein and calories.

It was decided during the early drafts of writing this review, that discussions of genetics would be beyond the scope of this review. However, it is ambiguous whether some elements of the human genome should be considered as infectious or genetic. The International Human Genome Sequencing Consortium has reported that only around 1.5% of the human genome consists of protein coding sequences. Around 50% of the human genome consists of repeat sequences which have been traditionally dismissed as uninteresting "junk". Most of the repeat regions are classified as transposable elements, which consist of; short interspersed elements, long interspersed elements (LINEs), long terminal repeat (LTR) retrotransposons, and DNA transposons[376]. These elements appear to be ancient with uncertain origins, though they have considerable homology with viruses[377]. The LTR retrotransposons resemble ancient retroviruses, and are commonly termed human endogenous retroviruses (HERV). Most HERV have multiple mutations and deletions which render them unable to replicate for general reviews see[212,378,379]. However, a few HERV copies appear to be complete, and sometimes produce viral proteins, though complete viral particles are only rarely produced[380]. Many women with breast cancer have increased expression of HERV in both their serum and cancers[381,382]. Melanoma biopsies have also been repeatedly reported to have high levels of expression of HERV[212,383].

LINEs are much more active than HERV. LINEs make copies of themselves which are inserted into random regions of the genome with deleterious effects[384]. Recent studies have reported a high frequency of novel insertions of LINEs in lung and colon cancer samples[385,386]. Transposable elements could cause cancer by mutagenesis and altering regulation of host genes[387,388].

LINEs and HERVs are silenced by epigenetic mechanisms, though they are frequently active during early development[389,390]. Do altered activation patterns of LINEs and HERVs during early development increase the risk of breast and other cancers?

NATURAL FACTORS AND NON-VIRAL INFECTIOUS AGENTS

When high rates of cancer were reported in a variety of occupations in the early 1900s, it seemed self-evident the cause of cancer was synthetic agents. Then research since the 1980s firmly established viruses as another important cause of cancer. In addition, there are a variety of other agents which cause cancer which the author has categorized as "natural factors and non-viral infectious agents".

Hormones

Interest in the notion that natural factors cause cancer is generally dated to have commenced in the 1930s. Estrone was identified in the urine of pregnant women in 1929[391], which was followed by an extensive research effort which resulted in the elucidation of the structures of cholesterol, bile acids, and the sex hormones[392]. In the early 1930s, Lacassagne administered weekly injections of estrone to three castrated male mice, and reported that every mouse injected with estrone developed mammary adenocarcinoma[162,163]. The experiment drew widespread interest among scientists, because estrone is a natural hormone which is produced endogenously in females. The experiment particularly was striking because male mice are not susceptible to spontaneous mammary tumors, and the cancers developed in each of the treated mice. The renowned Alexander Haddow considered Lacassagne's study to be the first report of a "natural" carcinogen[49]; though a variety of natural agents were recognized to cause cancer in animals by some well-studied cancer researchers when Lacassagne's publication appeared[393].

Bernardino Ramazzini was an Italian physician and professor who observed that each occupation was characterized by a distinct pattern of diseases. Ramazzini observed that nuns have high rates of breast cancer in 1713[9], but the reasons for this have been complex to decipher. Lane-Claypon[394] performed a case-control study which concluded that women who had multiple childbirths were less likely to develop breast cancer[395]. MacMahon *et al*[396] performed an international collaborative study and concluded that a younger age at the first childbirth was associated with a decreased risk. Breast feeding was also suspected of decreasing the risk, but it was difficult to definitively decipher. An international collaborative group recently estimated that the risk of breast cancer decreases by 4.3% for every 12 mo of breast feeding[397]. There is strong evidence that all of the above factors affect the risk of breast cancer, however the interaction of these factors has not been deciphered, and there is only a consensus for breast feeding reducing the risk[398].

Ultraviolet radiation

Paul Unna was a general physician who took a strong interest in skin diseases[399]. He built a clinic in order to diagnose and treat patients with skin diseases, and published a textbook of dermatology. In the publication he described the histological changes which accompanied the clinical conditions which were encountered by dermatologists. He described a "diffuse cyanotic redness" which occurred on the face and hands of

sailors after long term exposure to the "weather" which is implied to be due to ultraviolet light. He described both the clinical and histological changes as the lesions progressed to become cancerous. Following this, he compares the course of the sailor's skin disease with xeroderma pigmentosum in adults who had been exposed to the sun and developed skin cancer[400,401].

Parasites

Schistosoma haematobium (*S. haematobium*) is a parasitic fluke, which is endemic in Africa and Southwestern Asia. It is spread through human skin contact with infected water[402]. Ferguson reported a case series of *S. haematobium* and bladder cancer in Egypt in 1911[403]. He documented that 40% of males over 5 years of age were infected with the parasite, and described 40 patients with bladder cancer with ova of the fluke present mostly in the portal vein, bladder or tumor. *Opisthorchis viverrini* (*O. viverrini*) is another parasitic fluke, which is contracted by eating raw fish. The infection rates are endemic in Thailand and other east Asian countries. *O. viverrini* was suspected of being a carcinogen by Stewart[404] in 1931; however the first case series did not appear until the report by Bhamarapravati and Viranuvatti[405] in 1966, who reported that the infection was associated with the bile duct cancer known as cholangiocarcinoma in Thailand[406].

Dr. Burkitt began working in a teaching hospital in Kampala Uganda in the mid-1950s, when he encountered a high number of children with very rapidly growing lymphomas. The lymphomas were frequently characterized by massive malignant growths that occurred in the abdomen and jaw areas. With funds of only £ 25 ($75), he prepared leaflets and photographs, which were sent to health care workers across Africa. The leaflets described the clinical features of the lymphoma, in order to survey the geographical distribution of the cancer. This was followed up with a 10000 mile safari, for which he described his research resources as: A photograph album illustrating the tumor; a second hand Ford stationwagon; the companionship of Dr. Clifford Nelson, "a Canadian doctor"; and Dr. Ted Williams "a mission doctor with a life-time of African experience and an expert car mechanic"[338]. The lymphoma rates were found to be high in a belt stretching from 10° north to 10° south of the equator, and rare at altitudes of over 5000 feet above sea level. This corresponded to the distribution of the areas which were holoendemic for malaria which is attributed to cause the lymphoma[343].

Fungus

In 1960, turkey poults in England were dying at high rates of a mysterious acute hepatic necrosis, which resulted in dramatic economic loses. A Brazilian groundnut component of the diet was identified as the cause of the illnesses[407]. Lancaster *et al*[269] reported that rats were peculiarly resistant to the acute necrosis, but after extended feeding they developed liver tumors without cirrhosis. Adamson *et al*[270] reported that the toxin induced liver tumors in primates as well. The carcinogenic factor was identified as aflatoxin B1 (AFB_1), a toxin which is synthesized by the fungus *Aspergillus flavus*[408]. Following this, it was been difficult to ascertain whether aflatoxins are carcinogenic to humans. The rates of liver cancer are high in hot humid tropical regions of the world where aflatoxin contaminates stored foodstuffs. However, the prevalence of aflatoxin contamination is highly heterogeneous in areas of high contamination. It was necessary to determine the exposure of specific individuals, which was not performed in early studies. Furthermore, areas with high levels of aflatoxin contamination frequently have high rates of HBV infection, which also required consideration[409,410].

A study from Shanghai, China collected blood and urine samples from 18000 middle aged men and followed them prospectively for four years[411,412]. A nested case-control analysis of the cohort estimated a relative risk of 3.4 for men with urinary metabolites of aflatoxin only, a relative risk of 7.3 among men with seropositivity for HBsAg only, and a relative risk of 59 for men who had both urinary metabolites of aflatoxin and were seropositive for HBsAg. They concluded that aflatoxin is carcinogenic, and that there is a strong positive interaction between aflatoxin and HBV. Wu *et al*[413] have written a succinct review of the evidence that aflatoxin causes liver cancer.

Bacteria

Robin Warren was a pathologist at the Royal Perth Hospital who possessed an attention for detail. He was examining gastric biopsy specimens in 1979, when he thought he saw small spiral shaped bacteria growing on the surface[414,415]. Dogma stated that the stomach is sterile, so there was much opposition to the notion of viable bacteria growing in the stomach[414]. The editors at the Lancet wanted to publish the findings, but were delayed for months because no reviewer could be found who thought the work was credible[414,415]. The bacteria were reported to be present in 80% of patients with gastric ulcers and in 100% of patients with duodenal ulcers[416]. It was later found that *helicobacter pylori (H. pylori)* survives in the stomach by residing in the gastric mucosa which provides a partially effective physical barrier from the acidic pH and by synthesizing small amounts of ammonia which neutralizes hydrochloric acid[417,418]. *H. pylori* curiously have a trophy for healthy mucosal epithelium. Among patients with duodenal ulcers, the bacteria is interestingly present at considerably low frequencies in the ulcer crater and acutely inflamed edges, compared to the healthy gastric antrum where it is present at very high frequencies[419]. As gastric tissue progresses through atrophic gastritis to intestinal metaplasia and carcinoma, the bacterium is similarly detected at decreasing frequencies in metaplasias and carcinomas compared to the healthy gastric tissue[420-422].

Patients with gastric cancer have been reported to have a higher rate of seropositivity for *H. pylori* than normal persons. A meta-analysis of 19 studies (5

cohort and 14 case-control) reported a summary odds ratio of 1.92 (95%CI: 1.32-2.78) for the occurrence of gastric cancer among those who were seropositive for *H. pylori*[423]. The association was reported to be considerably stronger when the samples for serology were collected more than ten years before the diagnosis of cancer[424].

Wood dust, alcohol

Woodworkers in the furniture industry of the Oxford region of England were reported to have high rates of nasal adenocarcinoma[425]. The increased rates were observed mostly among persons exposed to hardwood rather than softwood dust[426]. Men who worked in trades which consumed high amounts of alcohol, such as brewers and inn keepers, were reported to have high rates of cancer of the oesophagus[427].

Food preparation technique

It was reported that the incidence of NPC was high in areas of Southern China, where the consumption of salted fish was high[428]. It was later found that the process of preparing salted fish in the high incidence areas is susceptible to bacterial contamination[429], and that nitrosamines are produced during the salting process[430,431]. Rats which were fed Cantonese style salted fish developed cancers of the nasal and paranasal regions[432,433]. Some studies have reported that the consumption of Cantonese-style salted fish during weaning is associated with a higher risk of development of NPC than consumption during adulthood[434].

Herb

A group of middle aged women were involved in a weight loss regimen in Belgium that involved consumption of a mixture of Chinese herbs which included the *Aristolochia* species. They developed high rates of renal fibrosis that frequently progressed to urothelial carcinomas of the renal pelvis and ureter after a very short latency[435]. In a series of ten patients who received renal transplants for nephropathy due to *Aristolochia* consumption, four were diagnosed with a multifocal high grade carcinoma *in situ* of the renal pelvis and ureter[435]. The exposure had lasted for an average of 20 mo, and the cancers were diagnosed after only 2-6 years after ceasing the regimen[435]. In another series, 17 of 39 patients with end stage nephropathy were diagnosed with upper urinary tract urothelial carcinomas[436]. Upper urinary tract urothelial carcinomas had been previously reported to occur at high rates in phenacetin analgesic abusers[86], and were characteristic of those living in the Balkans where *aristolochic* species contaminate wheat fields[437,438] and in Taiwan where consumption of *aristolochic* herbs is common[439,440]. Urothelial carcinomas of the upper urinary tract were rare in other regions of the world[441], so the imported herb was promptly identified as the cause.

WCRF/AICR analyses of diet, obesity, exercise, and supplements

The World Cancer Research Fund and the American Institute for Research on Cancer (WCRF/AICR) undertook comprehensive meta-analyses of the potential of foods, nutrition, and physical activity to prevent cancer (www.wcrf.org). Experimental work in the 1940s and 1950s demonstrated that caloric restriction dramatically reduced the incidence of most spontaneous and induced tumors of mice[442,443]. However it is difficult to accurately estimate caloric intake of humans on an individual level, and the epidemiologic studies yielded unclear results. Body weight is much more reliably measured, and is considered a reflection of total energy balance; the sum of energy intake and energy expenditure. Cancers of the oesophagus, kidney and endometrium have been consistently reported to be associated with overweight and obesity[444,445]. There is also convincing evidence that a sedentary lifestyle increases the risk of colon, and probably breast and endometrial cancers[446,447]. Consumption of red and processed meats has been estimated to cause around a 20% increase in risk of colorectal cancer with daily consumption of moderate amounts[448,449]. Increased adult height is also associated with an increased risk of colorectal cancer[450]. Consumption of a high fibre diet has been estimated to cause around a 10% decrease in risk of colorectal cancer[451].

There has been much interest in the potential of nutritional supplements to prevent cancer[452]. Epidemiological studies have shown that populations with higher serum levels of β carotene have reduced rates of lung cancer. However, investigators were confounded when a large randomized double-blind study reported that smokers who took β carotene supplements had higher rates of lung cancers compared to those who took a placebo[453]. Numerous studies confirmed that consumption of β carotene supplements increased the risk of lung cancer in smokers, though there is no evidence that β carotene supplements increase the rate among non-smokers[454].

The WCRF/AICR used the designation of "convincing" to indicate their highest level of certainty that an agent increases or decreases the risk of cancer[455]. The discussion above was limited to factors which received the designation of "convincing". Consensus concerning the effect of diet and nutritional supplement usage is an evolving area. As new studies are published which include additional patients and are better designed, the WCRF/AIRC expects their consensus to further evolve. They concluded that there was "convincing" evidence that diets high in fruits and vegetables decreased the rates of a variety of cancers, based predominantly on evidence of case-control studies in a 1997 publication. However a number of cohort studies have been published since 1997 which have weakened the evidence, such that the evidence is no longer considered to be "convincing", so it has been revised in subsequent publications[456,457]. There

is good evidence that consumption of dairy products reduces the risk of colorectal cancer, however there is also fairly consistent evidence that consumption of dairy products increases the risk of prostate cancer[458]. This is considered to be a complex problem, so dairy products have not been designated. Recent analysis has concluded that consumption of coffee reduces the risk of liver cancer among men, though the evidence is designated as "probable" rather than "convincing"[459].

Natural verses synthetic

It is evident that numerous natural agents cause cancer in humans. Many natural components of foods have been reported to be carcinogenic to rodents[460,461]. A variety of toxins are well recognized to be produced by plants[462-464]. Bruce Ames *et al*[462] surveyed the natural chemicals which have been tested to cause cancer in rodents, and concluded that about half of those tested induced tumors[465]. Concerning the synthetic chemicals which have been tested, about half of them similarly induced tumors[462,465] (Brambilla *et al*[466] independently concluded that around 50% of pharmaceuticals cause cancer). Ames *et al*[462] concluded that natural chemicals are just as carcinogenic as synthetic chemicals.

It is frequently not obvious which substances are synthetic. Soot, coal tar, asbestos, erionite, arsenic, uranium, radon, and radium could each be considered natural because they occur naturally, though usually at very low levels. The author posits that the level of exposure is more important than whether a substance is natural or synthetic. The dose-response relationship is strongly supported by studies with both experimental animals and humans[467-469]. According to the dose-response relationship, when the exposure levels are low, the rates of cancers are low, and when the exposures are high, the rates of cancer are high. Whether the dose-response curve is linear is controversial, and the shape of the curve may change at low dosages, but the principle of increasing rates of cancer for increasing levels of exposure is well established for most exposures[138].

Recognition of soot as a carcinogen only emerged when young boys were exposed to high levels with the advent of chimney sweeping as an occupation. Radium occurs naturally in the mineral pitchblende. Its carcinogenic activity only became obvious when it was refined from pitchblende, ingested by watch dial painters, and then induced osteosarcomas of the jaw bones among dial painters. It could be argued that occupational levels of exposure are unnatural, though it is relevant to mention that a great number of occupational exposures are not associated with cancer[470].

Emerging studies of possible new natural carcinogens

The variety of natural agents which have been documented to cause cancer is considerable. While viruses have received an intense amount of research for their possible roles in cancer, bacteria have received considerably less. The colon contains 10^{11}-10^{12} bacteria per gram of fecal material[471]. The recent development of high-throughput technologies is permitting detailed analysis of the microbial composition of the feces as well as the mucosa of the colon[472,473]. Interesting associations of certain bacterial species with colorectal cancers have been reported[474-476]. The microbiota of the colon can be modified with antibiotics, prebiotics and probiotics, so it will be interesting to observe how this research develops.

CONCLUSION

Some occupational carcinogens were readily identified, because they were akin to natural experiments, in which a few people in the community were exposed to uncommon substances with very rare types of cancer occurring in some occupations. Scrotal cancer was practically unheard of, except among chimney sweeps, and then later among men with occupational exposure to coal tar or shale oil[12]. Mesothelioma was encountered so seldom, that before the report of 33 cases among asbestos miners in South Africa, some pathologists had argued that tumors of the mesothelium did not occur[61,477,478]. It had been argued that tumors ascribed to mesothelial origin were likely misdiagnosed tumors of other cell types, which had metastasized to the mesothelium. Occupational exposures which caused very rare cancers were promptly recognized.

Other occupational exposures caused excessively high rates of common cancers. Around 50% of the early miners of Schneeberg and Joachimsthal were estimated to have developed lung cancer[479]. A small group of men who worked distilling β-naphthylamine in England were reported to have 90%-100% incidence of bladder cancer[480-482]. Barling reflected in 1926, that with the exception of Hall-Edwards, all of the other early radiologists whom he knew, had succumbed to skin cancers[483]. Occupational exposures which caused extremely high rates of common cancers were also readily recognized.

The notion that synthetic agents are the cause of cancer evolved from the early identification of occupational (or synthetic) agents. Infectious agents, as well as other naturally occurring agents, were usually more widespread geographically. The exposure to naturally occurring agents generally extended temporally for generations, so their effects were not readily evident.

Proof of most infectious causes of cancer required both technical advancements to identify the agents and large epidemiological studies to determine whether the agent is associated with increased rates of cancer. A few of the earliest infectious agents were identified with simple methods, but others could not be elucidated until more advanced methods were developed. The ova of *S. haematobium* and *O. viverrini* were identified with simple laboratory techniques and low powered light microscopes[403,484]. EBV was discovered using the EM[339], which only became consistently reliable in the

1950s[192]. HTLV and HIV were discovered using enzyme assays for reverse transcriptase, which were developed following the discovery that RNA viruses replicate using this enzyme[217,218]. HPV-16, HPV-18 and HCV were identified using recombinant DNA technology shortly after it became available in the mid-1970s. Modern techniques, such as next generation DNA sequencing introduced in 2005[174] have the potential to lead to the identification of additional infectious causes of cancer.

Each of the early theories concerning the cause of cancer which were described in the introduction, have been demonstrated to have some validity. Cancers are now considered to be caused by both irritation and infectious agents. Cohnheim's theory of embryonic rests also appears to have some validity, as there is evidence that tumor cells originate from stem cells[485]. The range of identified human carcinogens now includes occupational exposures, pharmaceuticals, X-rays, natural factors, non-viral infectious agents, and viruses.

Important contributions were sometimes made by persons who were not formally well qualified and occasionally by researchers not specifically searching for causes of cancer. Those who made important contributions were usually characterized by meticulous attention to detail and those who investigated viruses often persisted in their study in the presence of adversity.

It is interesting to reflect, that contrary to the dogma of the early 1900s, that infectious agents did not and could not cause cancer, it is now evident that they do. Discoveries of the roles of infectious agents as causes of cancer have contributed significantly to our ability to prevent cancer since 1980. Cervical cancer is the second most common cancer among women in the developing world[486]. Essentially all cervical cancers are now attributed to HPV, as cervical cancer seldom occurs without exposure to HPV[487]. Few chemical carcinogens share this distinction of being essential, for the development of specific cancer. Cervical cancer accounts for 5.2% of the world cancer burden cancer[488]. Liver cancer is the third most common cancer among men in the developing world[486]. Liver cancers caused by HBV and HCV are estimated to account for 4.9% of the total world burden of cancer[488]. *H. pylorus* has been estimated to cause around 2/3 of the cases of gastric cancer[486], and is estimated to cause 5.5% of the world cancer burden[488]. The global burden of cancers due to infectious agents has been estimated to be 17.8%[488].

Infectious agents were expected to be present in the diseased tissue according to Koch's postulates, but liver cancer caused by HBV is often devoid of the virus, except for the integrated virus which is truncated and unable to produce infectious particles. HPV follows a similar pattern in cervical cancers, while H pylora is usually completely devoid in gastric cancer tissues. Infectious agents did not cause cancer in a manner predicted by microbiologists of the early 1900s.

The IARC designated 109 agents as group 1 carcinogens in their update of November 7, 2012. Group 1 is defined as having "sufficient evidence of carcinogenicity in humans". About half of these agents have been discussed in this review. Selection of carcinogens for inclusion/exclusion was designed to include the widest possible variety of agents without producing an excessively long list. If a mixture was found to be carcinogenic and later a component of the mixture was also reported to be carcinogenic, then usually only the mixture or the pure agent was reviewed. If one route of exposure was determined to be carcinogenic (*e.g.*, dermal exposure to coal tar), then usually other routes of exposure were not discussed (*e.g.*, inhalation of fumes of coal tar). About half of the agents listed as group 1 carcinogens were excluded based on these principles. The complete list of the 109 group 1 carcinogens is available at the IRAC website (www.iarc.fr) or (http://monographs.iarc.fr/ENG/Classification/index.php last accessed January 27, 2013).

Additional information concerning the above discussed carcinogens can be found in the IARC monograph series. Cumulative indexes are provided at the end of recent volumes of the monographs, and also at their website (http://www.iarc.fr). The National Toxicology Program has independently compiled a similar list of carcinogens (http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf). Foods, supplements, physical activity, and obesity have been comprehensively reviewed in the publication titled Food, Nutrition, Physical Activity and Prevention of Cancer: A Global Perspective which is available at the World Cancer Research Fund website (http://www.wcrf.org).

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REVIEW

Overview, prevention and management of chemotherapy extravasation

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Abstract

Chemotherapy extravasation remains an accidental complication of chemotherapy administration and may result in serious damage to patients. We review in this article the clinical aspects of chemotherapy extravasation and latest advances in definitions, classification, prevention, management and guidelines. We review the grading of extravasation and tissue damage according to various chemotherapeutic drugs and present an update on treatment and new antidotes including dexrazoxane for anthracyclines extravasation. We highlight the importance of education and training of the oncology team for prevention and prompt pharmacological and non-pharmacological management and stress the availability of new antidotes like dexrazoxane wherever anthracyclines are being infused.

Key words: Chemotherapy; Extravasation; Vesicant; Tissue damage; Dimethyl sulfoxide; Dexrazoxane; Antidote; Hyaluronidase

Core tip: Chemotherapy administration carries safety concerns, which include accidental extravasation, to patients. We review and update readers and health care providers on the risks of chemotherapy extravasation, prevention and management. We present the definitions, grading, classification and guidelines related to chemotherapeutic drugs and groups. We present an update on prevention and management and antidotes, particularly dexrazoxane for anthracyclines extravasation. We present summary statements of American Society of Clinical Oncology, European Society of medical Oncology, Oncology Nursing Society and European Oncology Nursing Society guidelines. We stress the importance of education and training of the entire oncology team members who share responsibility to ensure the safe

administration of chemotherapy and avoid extravasation.

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INTRODUCTION

Intravenous infusion is the principal modality of administration of anti-cancer drugs for most types of malignant disorders with numbers exceeding 1 million infusions each day worldwide[1]. Chemotherapy administration carries safety concerns to both patients and the medical team. These concerns include extravasation of chemotherapy, which is defined as the accidental infiltration of chemotherapy into the subcutaneous or sub-dermal tissue at the injection site[1-4], and can result in tissue necrosis[1,2,4,5]. The exact incidence of chemotherapy extravasation varies greatly due to the general lack of reporting and absence of centralized registry of chemotherapy extravasation events. While center-based guidelines and policies attempt to minimize its risk, chemotherapy extravasation still has a prevalence that can range from 0.1% to 6% when administered through a peripheral intravenous access[3] and from 0.26% to 4.7% when administered through a central venous access device (CVAD)[6-8]. Institution-based guidelines should be based on evidence, where available, but they are often vague and non-specific, if present[1,4].

In order to avoid additional chemotherapy adverse effects, every effort should be made to minimize the complications of chemotherapy administration. All the oncology team members share responsibility to ensure the safe administration of chemotherapy. In this article, we review the literature, provide clinical information on chemotherapy extravasation, and discuss guidelines and recommendations for its prevention and management. This article serves as a review of the clinical aspects of chemotherapy extravasation and latest advances in classification, prevention and management of chemotherapy extravasation.

This review includes a comprehensive literature search in the PubMed, Med-Line and Google Scholar databases was conducted for guidelines, case reports, clinical trials, retrospective studies and conferences on chemotherapy extravasation prevention and management. We used the following Medical Subject Headings terms: "Chemotherapy", "extravasation", "prevention", "management", "extravasation", and "guidelines" and combined them using boolean operators. Once we found a set of relative citations, we included citations using the "related articles" option as well. All references that we thought were relevant were printed and analyzed, and their main relevant ideas were paraphrased and noted. Literature review was focused on our research question: "What should a healthcare practitioner know about chemotherapy extravasation, its prevention, and its management based on the current literature?".

CLASSIFICATION

Classification of intravenously administered drugs

Intravenously administered drugs can be classified into five categories according to their damage potential: Vesicant, Exfoliants, Irritants, Inflammitants, and Neutrals. The drug damage from extravasation can range from skin erythema to soft tissue necrosis. We list below examples of intravenously administered drugs according to various categories and in decreasing order of damage potential[9-13].

Vesicants: Drugs that can result in tissue necrosis or formation of blisters when accidentally infused into tissue surrounding a vein[14]. They include Actinomycin D, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Mitomycin C, Vinblastine, Vindesine, Vincristine, and Vinorelbine.

Exfoliants (may have low vesicant potential): Drugs that can cause inflammation and shedding (peeling off) of skin without causing underlying tissue death[15]. Drugs may cause superficial tissue injury, blisters and desquamation[12,13]. They include Aclacinomycin, Cisplatin, Docetaxel, Liposomal Doxorubicin, Mitoxantrone, Oxaliplatin, and Paclitaxel.

Irritants: Drugs that can cause inflammation, pain or irritation at the extravasation site[14], without any blister formation. Clinicians use the term irritant also to refer to drugs that can cause a burning sensation in the vein while being administered: Bendamustine, bleomycin, carboplatin, dexrasoxane, etoposide, teniposide, and topotecan.

Inflammitants: Drugs that cause mild to moderate inflammation, painless skin erythema and elevation (flare reaction) at the extravasation site[14]. They include bortezomib, 5-fluorouracil, methotrexate, and raltitrexed.

Neutrals: Drugs that neither cause inflammation nor damage upon extravasation[14]. Monoclonal antibodies (rituximab and trastuzumab) are also listed under this category: Asparaginase, bevacizumab, bleomycin, bortezomib, cetuximab, cyclophosphamide, cytarabine, eribulin, fludarabine, gemcitabine, ifosfamide, melphalan, rituximab, and trastuzumab.

Table 1 summarizes the different types of the above mentioned drugs according to their vesicant potential.

Vesicants may be sub-classified into DNA binding drugs and non-DNA binding drugs[16]. DNA binding drugs are capable of producing more severe tissue damage and mainly include anthracyclines and alkylating agents such as mechloretamine and bendamustine. Non-DNA

Table 1 The different types of the above mentioned drugs according to their vesicant potential

Neutrals	Inflammitants	Irritants	Exfoliants (may have low vesicant potential)	Vesicants
Asparaginase Bevacizumab bleomycin Bortezomib cetuximab, Cyclophosphamide	Bortezomib	Bendamustine	Aclacinomycin cisplatin Docetaxel liposomal Doxorubicin mitoxantrone Oxaliplatin paclitaxel	Actinomycin D
Cytarabine eribulin Fludarabine gemcitabine Ifosfamide	5-Fluorouracil methotrexate raltitrexed	Bleomycin		Dactinomycin daunorubicin Doxorubicin epirubicin Idarubicin mitomycin C Vinblastine vindesine
Melphalan rituximab Trastuzumab		Carboplatin dexrasoxane Etoposide Teniposide Topotecan		Vincristine vinorelbine

binding compounds are mainly vinca alkaloids and taxanes[13].

Drugs do not always fall under the strict definitions, and case reports of different extravasation potentials have been described. For example, taxanes have a poorly defined delineation between vesicants or irritants[13]. Docetaxel, though usually refered to as an irritant, has exfoliant and low vesicant properties described in 12 case reports[5,12,16-25]. While vinorelbinecan cause severe irritation inside the vein site of infusion, it is a moderate vesicant if extravasated into the surrounding tissue[17,18,25]. Alkylating agents like cyclophosphamide, ifosfamide and andmelphalan are considered neutrals[16]. Although etoposide and teniposideare usually classified as irritants, they may have low vesicant potential if a highly concentrated infused drug is extravasated[9].

Flare reaction, vessel irritation and venous shock, are other events that should be differentiated extravasation. Flare reaction is a not uncommon transient painless skin streaking erythema looking like urticaria with skin elevation that may occur with anthracycline administration. It is usually associated with itching, burning sensation and pain and that resolves within 1 to 2 h[26,27]. Vessel irritation causes pain, discomfort and tightness along the infused vessel with possible accompanied erythema and dark skin discoloration[27]. While both flare reaction and vessel irritation do not usually present with erythema, the extravasation is usually associated with swelling of the tissue surrounding the infused veinand predominantly manifests by erythema. The patient will complain of aches and burning sensation at the administration site. Unlike flare reaction and vessel irritation, extravasation is usually manifested with no or minimal blood return at the infusion site. Venous shock is due to the administration of very cold agents into the vein causing the loss of blood flow return due to venous muscle spasm, and it is managed by the application of warm compressors which can help to relax the vein[27].

RISK FACTORS

Risk factors of chemotherapy extravasation

Risk factors are related to the chemotherapeutic agent infused, patient factor, and iatrogenic causes. Factors related to chemotherapeutic agent itself and that increase the risk of chemotherapy extravasation include the vesicant properties of the drug, its concentration, volume and duration in which the infusion extravasated[28]. Factors related to patients and that increase the risk of chemotherapy extravasation include small and/or fragile veins, lymphedema, obesity, impaired level of consciousness, and having had previous multiple venipunctures. Iatrogenic causes include lack of training of nurses, poor cannula size selection, poor location selection and lack of time. Extravasation can occur upon accidental puncturing of the vein or upon movement of the cannula itself due to movement of the patient or insecure fixing. Prolonged peripheral line infusions of vesicants carry an increased risk of extravasation and vesicants should not be infused as prolonged unsupervised infusions *via* a peripheral vein[3].

CLINICAL MANIFESTATIONS

Tissue damage

Chemotherapy extravasation is manifested by a wide range of symptoms that can be mild and can present as an acute burning pain, swelling, at the infusion site. Symptoms vary according to the amount and concentration of extravasated drug. Pain and erythema, induration and skin discoloration progresses over few days and weeks, and may progress to blister formation. Blister formation or necrosis can lead to invasion and destruction of deeper structures[1,3-5]. Damage can reach tendons, nerves, and joints[19] depending on the location of the vein where extravasation occurs.

Grading of severity of extravasation

According to the latest Common Terminology Criteria for Adverse Events (CTCAE), published by the United States Department of Health and Human Services, National Institutes of Health, Natinal Cancer Institute, and widely used in Clinical Trials[20] (Version 4.0, May 2009), extravasation can be divided into four grades (Table 1) ranging from 2, which is manifested by erythema with associated edema, pain, induration, and phlebitis,

Table 2 Grades of Infusion site extravasation according to common terminology criteria for adverse events (V4.0, May 2009)

Adverse event	Grade				
	1	2	3	4	5
Infusion site extravasation	-	Erythema with associated symptoms (*e.g.*, edema, pain, induration, phlebitis)	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death

to grade 5, which refers to extravasation that leads to death. There is no grade 1. Table 2 shows the four grades of extravasation of chemotherapy (CTCAE V4).

Factors that determine the extent of tissue damage from chemotherapy extravasation

Factors that determine the extent of tissue damage from chemotherapy extravasation include its pH, osmolarity, vasoconstrictive potential, and duration for which it remains in tissue. Infusion solution whose pH is far from the physiologic pH (7.35-7.40) and/or osmolarity (281-282 mOsm/L) can irritate the venous endothelium and vessel wall and can damage the cell proteins and cause cell death[21]. Hypertonic solutions can further increase tissue injury and lead to necrosis. Vesicants with high vasoconstrictive potential can result in tissue necrosis by severe vasoconstriction of capillary smooth muscles and reducing blood flow. Vesicants that are retained in extravasation tissue area for a long duration lead to a vicious cycle of direct cell injury. Typical examples are anthracyclines which enter the cells and bind to DNA causing immediate and continuous tissue injury. On the other hand, vesicants that are easily metabolized and are not retained in tissue include vinca alkaloids and taxanes. Despite their ability to cause direct tissue damage, they cannot bind to DNA and are easily metabolized[21].

Manifestations of some commonly used chemotherapeutic drugs

Anthracyclines: Although all vesicants can cause tissue damage upon extravasation, anthracyclines, such as daunorubicin, doxorubicin, epirubicin, and idarubicin, have the greatest vesicant potential when compared to other chemotherapeutic agents. While all chemotherapeutic agents cause similar signs upon extravasation, anthracyclines are characterized by causing immediate pain and burning sensation, which can last up to hours and can be severe. Lesions form slowly over weeks and expand over periods of months due to tissue retention of the extravasant vesicant[9]. Weeks after the extravasation episode, surrounding tissue may become red, firm and tender. The resolution of redness depends on the size of the extravasation area. If the area is small in size, redness will gradually resolve over the following weeks. If extravasation is significant, the center of the redness area becomes necrotic and painful. The accidental leak of anthracyclines can cause severe tissue damage. By cellular uptake and remaining for an extended period of time in tissue, they cause a continuous vicious cycle of tissue damage[22-24,29]. In two prospective, open-label clinical trials[22,30], the patient with anthracycline extravasation who developed tissue necrosis had a large extravasation area of 253 cm^2. If the necrotic area is painful, surgical debridement may be required to remove any damaged and possibly infected necrotic tissue. In case no debridement is indicated, necrosis can progress to result in a thick, leathery eschar surrounded by a band of red painful skin, and can ulcerate to the underlying neurovascular tissue and tendons and cause pain. Ulceration is usually progressive and can result in persistent burning pain, nerve damage, and joint stiffness all of which may compromise the function of the involved organ or even cause its permanent disability[29]. Spontaneous healing rarely occurs after anthracyclines extravasation. In addition to surgical debridement, split-thickness skin graft is usually required when the necrosis extends deep into the tissue. In case the periosteum of underlying bone was involved, the skin graft cannot survive on cortical bone and the area of injury should be covered instead by a pedicle skin flap[23]. Dexrazoxane hydrochloride was FDA-approved for anthracyclines extravasation and has been reported to produce significant extravasation wound healing[4].

Liposomal encapsulation of doxorubicin reduces the toxicity of doxorubicin extravasation by decreasing its diffusion capacity and hence its toxicity to healthy tissue[31]. In phase Ⅱ and Ⅲ clinical trials assessing liposomal doxorubicin efficacy, two extravasations were reported and caused only inflammation with complete recovery and no tissue damage[31]. In a few case reports of liposomal doxorubicin extravasation, patients had reported pain, erythema, and edema but no necrosis or ulceration of extravasation area nor there were need to undergo surgical debridement[31].

Vinca alkaloids: Vinca alkaloids, which include vinblastine, vincristine, and vinorelbine, can cause direct cellular damage upon extravasation. Extravasation is known to cause a mostly painful ulceration, local paresthesia and slow healing[9,32]. It can cause significant irritation and usually presents with intense pain around intravenous line or port site, erythema and tenderness[32]. Erythema may be delayed by 1-2 h and even 3 d depending on the dosage of the vinca alkaloid administered[32]. This is followed by blister formation, swelling and induration and can be complicated by sloughing, ulceration and tissue necrosis. Vinorelbine, which is a moderate vesicant, also causes common irritation and burning sensations which are prevented by proper dilution, short infusion time and use of an adequately large vein[33].

Taxanes: Taxanes, including docetaxel and paclitaxel, are most often classified by literature as vesicants although there is no clear delineation. Most reactions following extravasation of taxanes consist of erythema, tenderness and swelling[12]. There are case reports of patients who had necrosis and skin exfoliation[34-38]. It is rare that taxane extravasation requires surgical debridement. In a paper that combined 35 case reports, only three patients developed ulceration two of whom required skin closure[11].

Oxaliplatin: Platinum compounds have been classified as irritants. Oxaliplatin has been recently reported to have vesicant properties[9]. Extravasation usually begins with a palpable swelling and discomfort upon palpation[9]. Lesion usually progresses to erythematous painful lesions and resemble erysipelas[9,10]. Long-term outcome is usually healing and necrosis and surgical debridement are rarely needed. The harm caused by oxaliplatin extravasation is not comparable to that of anthracyclines and vinca alkaloids[10].

PREVENTION

Medical team continuing education and training

Education and training are basic elements for licensing health care professionals and for good clinical practice. They are essential to improve management and patient outcome. Education and training among nurses and physicians remains the mainstay of safe chemotherapy administration and emphasizes the importance of being *preemptive* instead of reactive to extravasation[1,27]. In fact, the Joint Commission International emphasizes the standards of proper chemotherapy administration[39]. Knowledge of literature and international guidelines is essential. Local institution policies should be available and stress proper administration of Ⅳ chemotherapy and prevention of accidental extravasation[19,37]. Education of the medical team about extravasation prevention includes ensuring knowledge of risk factors, signs and symptoms, guidelines for prevention and management. Compliance to manufacturer's recommendations for each drug should be ensured byboth, nurses and physicians, as well as clinical pharmacists.

Appropriate vascular access

Consideration of the appropriate vascular access is crucial for the prevention of chemotherapy extravasation. Chemotherapy infusion can be either through a central venous access or through an adequate peripheral vein. Central venous access can be accomplished through a CVAD that is placed either as an implanted port or as a peripherally inserted central catheter[1]. CVADs are also known as Port-a-cath[40] or polysite[41] catheters. Veins that are small and/or fragile should be avoided as they might not withstand the required flow and rate of infusion and may have a lower threshold for extravasation. Locations that are also generally avoided include the dorsum of the hand, the antecubital fossa, and the radial and ulnar aspects of forearm[2,20]. Patients who do not have adequate peripheral venous access should have a central venous catheter placed[16].

Peripheral arm assessment consists of: (1) assessing location and fragility of the patient's veins that can be reflected by the inspection and palpation of the vein. Veins that have a small caliber and/or are superficial are generally considered fragileand should be avoided. In addition, assessment; also consists of (2) patient's age; (3) presence of diabetes; (4) steroid use; (5) history of previous venipunctures; (6) presence or absence of ecchymosis; (7) prior hospitalization or blood drawing history of axillary lymph nodes dissection; (8) lymphedema; (9)vascular accident in an extremity, which is the accidental puncturing of a vein. In parallel to peripheral arm assessment, the level of consciousness of the patient should be also assessed for the purpose of assuring immobility and compliance during catheter insertion[1,2,16].

Appropriate cannula and needle selection

Selection of the appropriate cannula type and size play an important role in chemotherapy extravasation prevention. The ideal cannula is one that can remain patent to allow blood flow and that does not dislodge from its place. The recommended choice is to use the smallest size of adequate and appropriate cannula in the largest vein available. Use of 1.2-1.5 cm long small bore plastic cannula and a clear dressing that shows any possible extravasation beneath it are recommended[42]. A butterfly needle should never be used for vesicant chemotherapy administration[16,43].

Patient education

Since patients are the first to feel any symptoms of possible extravasation and are relied upon to report them, their education is a crucial step in chemotherapy extravasation prevention. Risk of chemotherapy extravasation should be clearly explained to patients. Physician and nurses should emphasize to the patient the importance of providing accurate history regarding previous manipulation in extremities, cooperation with the person performing the venipuncture, and reporting any symptoms that may arise during the infusion[1,27]. Patients should be instructed to report any discomfort, pain, redness or swelling at infusion sites. Nurses and physicians should never underestimate the significance of any patient symptom and check the infusion site and venous patency immediately. Patients should also be aware of the class of drug and options of venous access and understand the higher risk of extravasation associated with it should be explained if they choose peripheral venous access over central[1].

Guidelines for chemotherapy administration and extravasation prevention

Although there are no prospective randomized clinical trials to establish treatment of chemotherapy extravasation, management of chemotherapy extravasation

Table 3 Overall summary of guidelines for prevention of chemotherapy extravasation

Continuous education of the medical team about all policies and protocols regarding chemotherapy administration
Classification of chemotherapeutic drugs: Knowledge of characteristics of the drug and compliance to the manufacturer's recommendations
Appropriate vascular access
In case a central vascular access is not possible, an adequate peripheral vein is used[16]
Veins that are small and/or fragile should be avoided[2,20]
It is not recommended to use veins located at the dorsum of the hand, the antecubital fossa, and the radial and ulnar aspects of forearm[2,20]
Appropriate peripheral arm assessment[1,2,16]
Palpation of the vein
History of previous venipunctures
Available extremities where veins can be punctured
Level of consciousness of the patient
Appropriate equipment selection[42,43]
Use of the smallest size of cannula in the largest available vein
Use of 1.2-1.5 cm long small bore plastic cannula
Use of a clear dressing
Avoiding the use of a butterfly needle
Educating the patient about all risks associated with chemotherapy administration
Devising and updating standards and policies regarding chemotherapy administration at each healthcare center
Documentation and reporting of any extravasation incident

have been learnt through case reports, animal models and international clinical studies. We present relevant important statements from North American and European Guidelines published by the European Oncology Nursing Society (EONS), Oncology Nursing Society (ONS), American Society of Clinical Oncology (ASCO) and European Society of medical Oncology (ESMO)[16,44]. In addition to International published guidelines, local institutions should have their own adapted guidelines and pathways for chemotherapy administration and also management of accidental extravasation.

ASCO and ONS: The ASCO and the ONS published safety standards for chemotherapy administration in outpatient[19,44] and inpatient settings[45,46]. These standards outlined the important steps in chemotherapy administration, including defining the "extravasation management procedures"[44] prior to administration. ONS published extravasation prevention and management guidelines in the book "Chemotherapy and Biotherapy Guidelines and Recommendations for Practice", Polovich *et al*[11] (2009). Examples of guidelines provided are close monitoring of the infusion site every 5 to 10 min and avoiding infusion of vesicants for more than 30 to 60 min[1]. In addition, ONS has an online course, ONS/ONCC Chemotherapy Biotherapy Certificate Course[47] that reinforces important information to safe administration of chemotherapy and provides links to online courses, such as "Access Device: The virtual clinic", which helps better train nurses and physicians[47]. The ASCO has a special emphasis on chemotherapy administration. It launched in 2010 the Quality Oncology Practice Initiative (QOPI) Certification Program (QCP). In the QCP report published in the Journal of Oncology Practice in March 2013, Gilmore *et al*[48] measured implementation of chemotherapy administration safety standards in the setting of outpatient cancer patients. Extravasation management procedures were met by 40.47% of practices. The report emphasized the importance of availability of up-to-date extravasation management standards at the sites[48].

ESMO and EONS: The EONS published in 2007 guidelines that can help nurses better understand extravasation[49,50]. It conducted its sixth Spring Convention in 2008 in Geneva, Switzerland, where it launched the new guidelines for chemotherapy extravasation prevention and management. Guidelines included nursese ducation, assessment of venous access, assessment of equipment used, and the importance of patient education[42,50]. This was followed by publishing guidelines developed jointly with the ESMO in 2012[16]. Details of guidelines published are included in the following section "Management".

Local institution guidelines: These should be encouraged and include definition and diagnosis of extravasation, risk factors, guidelines for prevention, and management[27]. For example, Cleveland Clinic has standards of chemotherapy administration clearly stated in its "Chemotherapy/Biotherapy Safe Handling Guidelines (Policy NPM-127), which was initially published in 1996 and revised in 2007[51]. Any local incidence of extravasation should be reported. While documentation may differ among institutions, certain items remain essential and should be documented for every incident. In addition to date and time and patient's name, name of the drug, characteristics of the solution infused, the Ⅳ access used, description of the extravasation area, signs and symptoms and management should always be documented[16].

Table 3 summarizes guidelines for chemotherapy extravasation prevention.

MANAGEMENT

Continuous monitoring at the beginning and during the infusion is essential every 5 to 10 min. Cancers centers should ensure the availability of "Extravasation

Kits" at the treatment units. These kits should contain disposable syringes and cannulas, cold-hot packs, gauze pads, adhesive plaster, gloves, and antidotes that can be used in cases of extravasation and that will be discussed below[3]. Management according to EONS and ONS, and few available clinical studies, are outlined below.

Initial non-pharmacologic management

In case of chemotherapy extravasation and as soon as the patient complains of pain or swelling, the first step should be immediate cessation of the infusion while keeping the cannula or port needle in place. This is followed by attempts at aspiration of the chemotherapeutic agent and removing the cannula or port needle[3]. Aspiration of the drug is usually done by a 10 mL syringe, percutaneous needle aspiration, liposuction, simple squeeze maneuver, or by surgical fenestration and irrigation[3,21]. Catheter can then be removed if there are no antidotes that need to be infused at the extravasated site. Elevation of the affected limb and thermal application by either cold or hot packs should follow[28]. Elevation of the limb helps in reabsorption of the extravasated agent by decreasing capillary hydrostatic pressure and It is recommended to during the first 24 to 48 h of the incident[21]. It is also recommended that thermal application is performed approximately four times daily for 20 min each for 1-2 d[42]. In addition, saline dispersion can help in diluting the vesicantby infiltrating normal saline *via* a large catheter[21]. Taking a photo of the extravasation area helps for follow up of progress or healing process. Cold compresses can be used to reduce pain and local inflammation by causing vasoconstriction and reducing drug further spread. Cold compresses should not be used in the cases of extravasation of vinca alkaloids because it may cause further tissue damage[14]; warm compresses and heat can be applied in incidents of vinca alkaloids extravasation as they may cause vasodilatation and absorption of extravasated drug from tissue sites.

Pharmacologic management

Dexrazoxane hydrochloride for anthracycline extravasation: Dexrazoxane is a member of the bisdioxopiperazine family and is an FDA-approved antidote for intravenous anthracycline extravasation[52]. The exact mechanism by which it reduces tissue damage resulting from chemotherapy extravasation is unknown. There is general belief that it works through two main mechanisms. Being an analog of the iron chelatorethylenediaminetetraacetic acid that can strongly bind Iron and displace it fromanthracycline, it is thought that dexrazoxane helps to reduce the oxidative stress caused by complexes of metal ions and anthracyclines[4,53]. Also, it can exerta catalytic inhibition of topoisomerase Ⅱ,the main target of anthracyclines[53]. Dexrazoxane has been initially used to reduce the incidence of cardiomyopathy associated with anthracyclnes and is approved in patients with breast cancer responding to doxorubicin and requiring continued therapy after they exceed 300 mg/m^2[52]. Dexrazoxane is administered as a 1-2 h intravenous infusion (Ⅳ) for 3 consecutive days through a large caliber vein in a limb other than the affected one[3,4,21] as follows: It is usually given at a dosage of 1000 mg/m^2 within 5 h of extravasation and then at a dosage of 1000 mg/m^2 on second day and 500 mg/m^2 on the third day following extravasation[3,43]. To date, in addition to several case reports[4,52], there are two large prospective multicenter clinical trialsabout the use of dexrazoxane in anthracyclines vesicant extravasation[21,22,30,52]. The overall efficacy of dexrazoxanewas 98%[53]. Langer *et al*[41] also reported prevention of complications of doxorubicin and epirubicin extravasation by dexrazoxane. In a case of port-a-cath chest wall massive extravasation, El-Saghir *et al*[4] reported the successful use of dexrazoxane, for immediate relief of pain and slowing down of necrosis, along with local infiltration of granulocyte-macrophage colony-stimulating factor at the borders of the ulceration site to promote the acceleration of wound healing and reduce the need for skin grafting[4]. The two prospective open-label single-arm studies in patients with anthracyclines extravasation were published in 2007 by Mouridsen *et al*[30]. Dexrazoxane was given within 6 h and repeated at 24 and 48 h. Efficacy was noted in 53 of 54 patients (98.2%) and only one patient required surgical debridement. Toxicity was manageable and includes transient elevation of liver enzymes and neutropenia that may be also due to chemotherapy itself. The use of dexrazoxane as an antidote to anthracyclines extravasation is now recommended by NCCN, EONS, ONS, and ASCO and has been formulated in a new preparation and has level Ⅲ-B evidence (Evidence Level Ⅲ: Evidence obtained from well-designed controlled trials without randomization; "B": Moderate strength of recommendation)[16,54]. Doxorubicin is one of the most widely used drugs and hence has the highest potential and risk for extravasation, and, therefore, dexrazoxane should be made available at all centers that administer anthracyclines chemotherapy.

Hyaluronidase: Hyaluronidase is an enzyme that degrades hyaluronic acid in tissues and promotes diffusion of the extravasated agent. The usual dose consists of multiple subcutaneous injections of hyaluronidase 150-100 IU given as five 0.2 mL injections[42,55]. When used for chemotherapy extravasation, it is recommended for vinca-alkaloids, etoposide[56] and taxanes extravasation mainly[16,23] and has level Ⅴ-C evidence (Evidence Level Ⅴ: Evidence from systematic reviews of descriptive and qualitative studies; "C": Poor strength of recommendation)[16,54]. It is injected locally subcutaneously into the extravasation area.

Dimethyl sulfoxide: Dimethyl sulfoxide (DMSO) is an organosulfar solvent that is topically applied to improve absorption of the extravasated solvent[21,49].

It also has free-radical scavenging properties[3]. Its efficacy was observedin few studies. In a prospective study by Cassagnol *et al*[3], patients withanthracycline extravasation, DMSO 99% was administered twice daily for a period of 14 d and no ulcers were described[3]. In another prospective study by Bertelli *et al*[56], out of a total of 122 assessable patients with extravasation of doxorubicin, epirubicin, mitomycin, mitoxantrone, cisplatin, carboplatin, ifosfamide or fluorouracil, only one patient suffered an ulceration. Treatment with DMSO was generally well tolerated with the only side effect being mild local burning and breath odor[57]. The use of topical DMSO (99%) as an antidote to anthracycline extravasation and to Mytomicin C has level Ⅳ-B evidence (Evidence Level Ⅳ: Evidence from well-designed case-control and cohort; "B": moderate strength of recommendation)[16,54]. DMSO is available as a solvent, and a dropper is usually used to instill drops over the affected skin. It is used asa topical application of DMSO 99% of four drops per 10 cm^2 to twice the size of the extravasation area[3,22]. In cases of anthracyclines extravasation, the combination of DMSO and cooling are most commonly described initial therapy for minor anthracyclines extravasation, especially when dexrazoxane is not available.

Sodium thiosulfate: It is an antidote generally recommended for mechlorethamine (nitrogen mustard) extravasation. A study conducted by Doellman *et al*[21] showed that the use of sodium thiosulfate was associated with significantly improved healing time in 63 patients who had a variety of chemotherapy induced extravasation injuries, including doxorubicin, epirubicin, vinblastine, and mitomycin C[21]. The use of sodium thiosulfate as an antidote to mechlorethamine extravasation has level Ⅴ-C evidence (Evidence level Ⅴ: Evidence from systematic reviews of descriptive and qualitative studies; "C": poor strength of recommendation)[16,54]. It is usually subcutaneously locally injected in a 2 mL solution at a concentration of 0.17 mol/L[16].

Acceleration of wound healing

Local injection of corticosteroids has been hypothesized to accelerate wound healing and prevent ulcer formation. While *in vitro* animal experimental studies showed no prevention of ulcer formation after corticosteroid injection, it was reported to have clinical benefit on ulcer prevention when used on humans[58-61]. Variable results have been reported regarding the success of wound healing after the use of local corticosteroids, which depends on the amount of inflammatory cells generated at the site of extravasation[62]. Local injection of granulocyte macrophage colony-stimulating factor, which is a glycoprotein growth factor, has been reported to be beneficial to wound healing in cases of doxorubicin extravasation[4,62]. The mechanism is believed to be through stimulation of cellular components such as fibroblasts and endothelial cells[62]. Also, local injection of normal saline has been also mentioned as beneficial in prevention of wound ulceration after extravasation[62].

Surgery and skin grafting

Indications for surgery in chemotherapy extravasation include full-thickness skin necrosis, chronic ulcer, and persistent pain. It is crucial that all necrotic tissue be removed until bleeding occurs and only healthy tissue left for wound coverage. To ensure complete excision, some surgeons use intraoperative fluorescent dye injection to detect the doxorubicin HCl in the tissue to ensure complete excision. After this, either immediate or delayed surgical reconstruction and skin grafting can be performed[63].

Extravasation in the presence of CVADs

Accidental cases of extravasation in the presence of CVADs is very rare and reported in 0.24% of cases[16]. Extravasation may occur in the subcutaneous tissue of the chest wall or neck, or in the mediastinum. Physicians and nurses should make sure that infusion needles are properly inserted in the port or chamber. In cases of extravasation in the subcutaneous tissue, infusion should be stopped immediately when patient complains of pain or swelling. Pharmacological management, including the use of dexrazoxane for anthracyclines extravasation should be instituted as reviewed in the above sections[16]. A recent report indicated benefit from immediate removal of the CVAD along with Subcutaneous Wash-Out Procedure if extravasation is detected early, to help minimize the exposure of tissue to extravasated agent and the risk of tissue necrosis[64]. In cases of mediatinal extravasation, ESMO guidelines include stopping the infusion, use of dexrazoxane for cases of anthracyclines, and possible surgical draining procedures for the remaining solution, antibiotics, steroids and analgesics to control symptoms from mediastinitis or pleuritic[64].

Experimental non-pharmacologic methods

Negative pressure wound healing: Also called vacuum-assisted closure (VAC) dressing, this method applies a negative pressure to the wound, aids in aspiration of extravasated vesicant, and improves its environment. There are only few reports in which negative pressure wound healing (NPWH) was used for vesicant extravasation. Lucchina *et al*[65] reported a case where surgical VAC dressing was used for vinorelbine extravasation, in addition to hyaluronidase and DMSO, resulted in complete healing of the wound. In an experimental animal study conducted by Evren *et al*[66] on rabbits with doxorubicin extravasation, there was smaller extravasation areas in rabbits subjected to NPWH, but no histological difference compared to control rabbits.

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy (HBO) is defined by the Undersea and Hyperbaric Medical Society as a therapy consisting of intermittent breathing 100% oxygen in a

Table 4 Non-pharmacological management of chemotherapy extravasation

Institutions should always ensure availability of "extravasation kits" at floors in which chemotherapy can be given
Initial non-pharmacologic management
Continuous monitoring at the beginning and during the infusion is essential every 5 to 10 min
Aspiration of the vesicant by a 10 mL syringe, percutaneous needle aspiration, liposuction, simple squeeze maneuver, or by surgical fenestration and irrigation
Elevation of the affected limb and thermal application (cold or hot)

Table 5 Pharmacological management of chemotherapy extravasation

Dexrazoxane as an antidote to anthracyclines extravasation has level Ⅲ-B evidence[16]
Hyaluronidaseas an antidote to vinca-alkaloids and to taxanes extravasation has level Ⅴ-C evidence[16]
Topical DMSO (99%) as an antidote to anthracycline extravasation and to Mytomicin C has level Ⅳ-B evidence[16]
Sodium thiosulfate as an antidote to mechlorethamine extravasation has level Ⅴ-C evidence[16]

DMSO: Dimethyl sulfoxide.

chamber whose pressure is greater than atmospheric pressure[67]. Its role in chemotherapy extravasation is still unclear, but it is believed that HBO increases production of oxygen free radicals and thus can aid in extravasation wound healing. In an experimental animal study conducted by Aktas *et al*[67] on Wistar-Albino rats for adriamycin extravasation, there was complete wound healing for 16 animals out of the 36 animals in the HBO group but no complete wound healing in any of the control group[68].

Use of biologically synthesized nanoparticles

Recent advances in the development of chemotherapeutic agents that incorporates biologically synthesized nanoparticles have been associated with less toxicity to surrounding tissue[31]. Nanodrugs are based on the combination of chemotherapeutic molecules with nanoparticles carriers, which include liposome, polymer, and micelle[69]. Chemotherapeutic molecules which have been used so far for synthesis of nanoparticles include cisplatin, carboplatin, doxorubicin, 5-fluorouracil, paclitaxel, vinblastin and etoposide[69]. For example, the use of liposomal forms of chemotherapeutic agents such as doxorubicin was associated with decreased diffusion capacity of the drug and hence less toxicity to surrounding tissue[31,69].

Tables 4 and 5 summarize non-pharmacological and pharmacological management of chemotherapy extravasation, respectively.

CONCLUSION

Safe administration of chemotherapy and prevention of extravasation is a shared responsibility among medical team members. Education of patients about risks and manifestations are essential. Prevention of chemotherapy extravasation is an important quality indicator for certification of chemotherapy infusion centers (QOPI, ASCO). International guidelines have been published by ASCO and ONS in the United States and ESMO and EONS in Europe. While only some healthcare institutions devise their own policies and guidelines regarding extravasation prevention and management, there is a need to have local institution education, training and guidelines. All institutions that administer intravenous chemotherapy should have known antidotes available. In spite of all efforts to prevent, accidental extravasation still occurs and more research for antidote for many drugs is needed.

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MINIREVIEWS

Intensive care outcomes in adult hematopoietic stem cell transplantation patients

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Abstract

Although outcomes of intensive care for patients undergoing hematopoietic stem cell transplantation (HSCT) have improved in the last two decades, the short-term mortality still remains above 50% among allogeneic HSCT patients. Better selection of HSCT patients for intensive care, and consequently reduction of non-beneficial care, may reduce financial costs and alleviate patient suffering. We reviewed the studies on intensive care outcomes of patients undergoing HSCT published since 2000. The risk factors for intensive care unit (ICU) admission identified in this report were primarily patient and transplant related: HSCT type (autologous *vs* allogeneic), conditioning intensity, HLA mismatch, and graft-versus-host disease (GVHD). At the same time, most of the factors associated with ICU outcomes reported were related to the patients' functional status upon development of critical illness and interventions in ICU. Among the many possible interventions, the initiation of mechanical ventilation was the most consistently reported factor affecting ICU survival. As a consequence, our current ability to assess the benefit or futility of intensive care is limited. Until better ICU or hospital mortality prediction models are available, based on the available evidence, we recommend practitioners to base their ICU admission decisions on: Patient pre-transplant comorbidities, underlying disease status, GVHD diagnosis/grade, and patients' functional status at the time of critical illness.

Key words: Stem cell transplantation; Intensive care; Mechanical ventilation; Comorbidity; Outcome prediction

Core tip: The outcome of hematopoietic stem cell transplantation (HSCT) patients admitted to intensive care remains poor but not "futile". While risk factors for intensive care unit (ICU) admission are mostly patient and transplant related, prognostic factors for HSCT patients admitted to ICU are primarily related to patients' functional status and interventions in ICU. Based on the available evidence, we recommend patient

selection for ICU to be based on patient pre-transplant comorbidities, underlying disease status, graft-versus-host disease diagnosis/grade, and patients' functional status at the time of critical illness.

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INTRODUCTION

The role of hematopoietic stem cell transplantation (HSCT) has been established in the treatment of various high-risk malignancies and non-malignant conditions. Due to the intense conditioning prior to HSCT and slow post-transplant immune recovery, patients undergoing HSCT are prone to develop infectious and other complications that may lead to death. In fact, transplant related mortality (TRM) is a significant cause of HSCT failure and TRM rates as high as 50% have been reported in high-risk transplants/patients[1]. The factors related to the patient (performance status, comorbidities) and the transplant (conditioning regimen intensity, donor and graft type) determine post-transplant immune recovery and organ damage extent, hence influence the risk and severity of the treatment complications[2-7]. While the pace of post-transplant immune recovery is variable; its pattern is more predictable and may be divided into 3 phases: Pre-engraftment, early post-engraftment, and late post-engraftment. The primary immune defects leading to infections are innate immunity in the pre-engraftment phase; cell-mediated immunity in the early post-engraftment phase; cell-mediated and humoral immunity in the late post-engraftment phase.

Post-transplant complications may be life-threatening and require intensive care due to respiratory failure, shock, organ failure, and others. Even though intensive care unit (ICU) outcomes have improved over the last few decades, ICU admission after allogeneic HSCT (AlloSCT) is still associated with poor prognosis. Consequently, the benefit of intensive care has been challenged in this patient population. In this manuscript, we review the outcomes of intensive care in adult patients undergoing HSCT with emphasis on the factors leading to intensive care and outcome prediction. As practices of intensive care and HSCT have evolved over years, we will focus on seventeen reports published since 2000 summarized in Table 1.

FACTORS ASSOCIATED WITH ICU ADMISSION RATES

In cohorts including only AlloSCT patients, ICU admission rates have been consistently reported to range between 9%-20%[8-12] with only two outliers[13,14]. Naeem *et al*[14] reported an ICU admission rate of 57% among patients who received umbilical cord blood grafts. However, the advent of double-cord blood transplants has improved the immune recovery since that study was published; the ICU admission rate after cord blood transplants is very likely lower now. The small variation in ICU admission rates among the rest of the studies may be explained by different patient selection criteria and varying patient/disease characteristics of the study cohorts.

The ICU admission rate reported in the only study including exclusively autologous HSCT (AutoSCT) patients was 3.3%[15]. Similarly, the reported ICU admissions rates in cohorts including both Allo and AutoSCT[12,16] are lower than those reported in exclusively AlloSCT cohorts[8,10,11,17,18]. The lower admission rates after AutoSCT are likely due to less frequent pulmonary post-transplant complications compared to AlloSCT[19].

The reported risk factors for ICU admission among AlloSCT patients are myeloablative conditioning, acute graft-versus-host disease (GVHD), and HLA mismatch between donor and recipient[9,13,14]; all of which are transplant-related and not surprisingly also increase TRM[6,20,21]. In the only recent study that methodologically assessed the ICU admission risk factors, Benz *et al*[9] did not find patient age, gender, disease type or stem cell source to affect ICU admission risk. While the association between patient pre-transplant comorbidities and ICU admission risk has never been evaluated, comorbidities have been shown to significantly increase patient's risk for critical illness as they influence both TRM[7] and ICU outcomes[8].

The most common reasons for ICU admission after HSCT are respiratory failure and septic shock; pulmonary infections can cause both simultaneously. Nevertheless, non-infectious pulmonary diseases, *i.e.*, diffuse alveolar hemorrhage and acute respiratory distress syndrome, may also lead to respiratory failure after HSCT. Other reported reasons for ICU admission include cardiac dysfunction, neurological disorders, and gastrointestinal bleeding. These may arise due to treatment itself, *i.e.,* busulfan induced seizures and alkylator induced congestive heart failure; development of GVHD; and patients' comorbidities.

PROGNOSTIC FACTORS AND ICU OUTCOMES

Despite the improvement in general ICU outcomes, prognosis for HSCT patients admitted to ICU is still poor with reported hospital mortality ranging from 46% to 84% in series published between 2000 and 2015 (Table 1). The wide range is likely due to inclusion of AutoSCT patients in some of the cohorts and different patient selection criteria between centers. In cohorts including only AlloSCT patients, hospital mortality and overall

Table 1 Summaries of the studies of adult hematopoietic stem cell transplantation patients admitted to intensive care unit published between 2000-2015

Ref. (study period)	No. of patients admitted to ICU [total N of HSCTs (%)], ICU admission risk factors	Reasons for ICU admission (%)	Interventions (%)	Outcomes	Factors evaluated for outcome prediction	Predictors of outcome on mutlivariate analysis	Notes
Boyaci *et al*[31] (2007-2010)	48 patients (7 Auto and 41 AlloSCT)	Respiratory failure 86%, sepsis/septic shock 75%, renal failure, liver failure, AMS	MV 75%	Mortality: 79% in hospital	Age, gender, underlying disease, remission status, HSCT type, HLA match, conditioning intensity, cause of ICU admission, GVHD, SOS, APACHE Ⅱ, GCS, SOFA, # of organ failures, various vitals and lab values, VA, MV	APACHE Ⅱ score and VA in ICU a/w higher mortality	
Bayraktar *et al*[8] (2001-2010)	389 AlloSCT patients a/to ICU within 100 d of HSCT [Of 3039 patients (13%)]	Respiratory failure 61%, septic shock 12%, AMS 9%, arrhythmia 5%, non-GI, non-CNS bleeding 4%	N/R	Mortality: 64% in hospital	Age > 55, underlying disease, year of HSCT was, HSCT period at ICU admission, graft source, HLA match status, donor relation, conditioning intensity, aGVHD at ICU admission, HCT-CI score	HCT-CI ≥ 2, ablative conditioning, aGVHD at ICU admission a/w higher mortality. ICU admission during conditioning regimen a/w lower mortality	HCT-CI score, a measure of pre-transplant comorbidities, can be calculated even prior to HSCT
van Vliet *et al*[17] (2004-2009)	49 AlloSCT [Of 319 (15%)]	Infectious complications 86%, respiratory failure 67% Ablative conditioning and unrelated donor grafting a/w increased risk for ICU admission	N/R	1-yr OS: 15% Mortality: 33% in ICU, 53% in hospital		NR	Univariate analyses demonstrated improved 100-d survival between 2004-2005 to 2008-2009
Agarwal *et al*[30] (1998-2008)	123 HSCT patients (73% AlloSCT)			Mortality: 41% in ICU, 62% in hospital. OS @ 1yr: 24%	Age, underlying disease, type of HSCT, GVHD, neutropenia, hospital admission-ICU interval, organ failures, sepsis type, APS, APACHE Ⅱ, MV	Fungal infection and number of organ failures a/w higher ICU mortality	Hard to explain why GVHD was a/w lower ICU mortality
Depuydt *et al*[33] (2000-2007)	44 AlloSCT	Bacterial infections 32%, non-bacterial infections 30%, non-infectious causes 39%. Overall, pulmonary related causes 39%	MV (73%), RRT (27%)	Mortality: 61% in ICU, 75% in hospital, 80% @ 6 m	Age, gender, bacterial infection, GVHD grade, HSCT-ICU interval, SOFA	Bacterial infection as the cause of ICU admission a/w lower hospital mortality	Improvement in SOFA score by 5th d of ICU was sig better in patients with bacterial infections
Benz *et al*[9] (1998-2007)	33 AlloSCT [Of 250 (13%)] ICU admission risk factors: aGVHD grade Ⅱ-Ⅳ and HLA mismatch	Pulmonary complications 33%, sepsis 24%, neurological disorders 18%, cardiovascular problems 6%,	MV 64%, VA 42%, RRT 27%	OS @ 1yr: 28%		NR	SAPS Ⅱ and SOFA scores did not reliably predict survival
Townsend *et al*[13] (1996-2007)	164 AlloSCT (majority TCD) [Of 552 (30%)]. ICU admission risk factors: Ablative conditioning	Sepsis 67%, respiratory failure 55%	MV 50%	Survival: 32% in ICU OS @ 1yr: 19% overall, 61% in patients who survived ICU	Donor type, conditioning intensity, reason for ICU admission, NIV, MV, VA, RRT, various labs, APACHE Ⅱ, duration of ICU stay, duration of MV	MV, raised BUN at admission and ablative conditioning a/w worse ICU survival	
Trinkaus *et al*[15] (2001-2006)	34 AutoSCT patients admitted within 100 d of SCT [Of 1013 (3.3%)]	Sepsis 32%, respiratory failure 29%, cardiovascular failure 26%		ICU mortality: 38%		NR	

Neumann *et al*[18] (1999-2006)	64 AlloSCT [Of 319 (20%)]	Pulmonary complications 53%, Sepsis 22%, renal failure 9%, bleeding 3%, status epilepticus 3%		ICU mortality: 66%	Age, gender, underlying disease, remission status, conditioning intensity, HLA match status, GVHD, ICU admission indication, HSCT-ICU interval, SOFA, various labs, SOS	SOFA ⩾ 12 and BUN > 60 a/w higher ICU mortality	
Gilli *et al*[10] (1995-2005)	91 AlloSCT (29% < 18 yrs old) [Of 661 (14%)]	Respiratory failure 41%, septic shock 31%, neurological events 12%	MV 48%, RRT 5%, VA 58%	Mortality: 58% in ICU, 70% @1m	Conditioning intensity, reason for ICU transfer APACHE II, SOFA, VA, RRT, IMV	SOFA score a/w 30 d mortality	APACHE Ⅱ underestimated mortality
Naeem *et al*[14] (1998-2003)	25 UCBT [Of 44 (57%)] ICU admission risk factors: Ablative conditioning	Pneumonia 52%, GI bleeding (12%), Sepsis 8%, renal failure 8%	MV 48%	ICU mortality: 72%		NR	
Pène *et al*[11] (1997-2003)	209 AlloSCT [Of 1025 patients (20%)]	Repiratory 67%, hemodynamic 23%, neurologic 18%, renal 17%, other 5%	MV (58%), RRT (28%), VA (47%)	Survival: 48% in ICU, 32% in hospital, 27% @ 6 m, 21% @ 1 yr MV patients: 18% in ICU, 16% in hospital	Age, gender, underlying disease, remission status, conditioning intensity, graft source, HSCT-ICU interval ⩽ 30 d, corticosteroid Rx, serum bilirubin level, MV, VA, RRT	Corticosteroid Rx, serum bilirubin level at ICU admission, MV	None of the 35 patients with admission LOD score > 10 survived the hospital stay
Scale *et al*[41] (1992-2002)	504 patients (264 AlloSCT) who were admitted to ICU following the BMT hospitalization [Of 2653 (19%)]		MV 51%, RRT 7%	1-yr mortality: 67%		NR	
Kim *et al*[42] (1999-2001)	18 AlloSCT [Of 210 (9%)]	Respiratory failure 50%, renal failure 39%, septic shock 11%		ICU mortality: 94%			
Soubani *et al*[16] (1998-2001)	85 HSCT patients (45 AlloSCT) [Of 745 (11%)]	Respiratory 48%, Sepsis 23%, cardiac 19%, neurologic 6%, bleeding 2%	MV in 60%	Mortality: 39% in ICU, 59% in hospital, 72% @ 6 m CU mortality 63% among patients with MV	Age, gender, smoking history, race, underlying disease, HSCT type, HLA match, HSCT-ICU interval, GVHD, various labs	High lactate level, MV, > 2 MOFs during ICU stay a/w higher ICU mortality	
Kew *et al*[12] (1992-2001)	37 HSCT patients (28 AlloSCT) [Of 440 (9%)]	Respiratory failure 65%, hemodynamic instability 57%,	MV in 68%	29 patients died within 1 yr	Pre-ICU patient characteristics, MV, VA	VA a/w shorter OS	
Afessa *et al*[32] (1996-2000)	111 patients (62 Auto, 50 AlloSCT)	Respiratory failure 40%, cardiac reasons 26%, sepsis 14%, CNS dysfunction 5%, GI bleeding 5%	MV 55%	Mortality: 33% in ICU, 46% in hospital 30-d mortality was 78% among AlloSCT patients	Type of HSCT, graft source, post-transplant days @ ICU admission, GVHD, APACHE III, APACHE II, ARDS, MOF, sepsis, MV, VA	Higher APACHE III score @ ICU admission, AlloSCT, MV, ARDS, MOF, sepsis, VA a/w higher 30-d mortality	AUC of receiver operating characteristic curve for APACHE III and hospital mortality was 0.704

A/to: Admitted to; a/w: Associated with; AlloSCT: Allogeneic HSCT; AutoSCT: Autologous HSCT; AMS: Altered mental status; APACHE: Acute Physiology and Chronic Health Evaluation; APS: Acute physiological score; ARDS: Adult Respiratory Distress Syndrome; BUN: Blood urea nitrogen; CNS: Central nervous system; GCS: Glasgow Coma Score; GI: Gastrointestinal; GVHD: Graft-versus-host disease; HCT-CI: Hematopoietic cell transplantation-specific comorbidity index; HSCT: Hematopoietic stem cell transplantation; LOD: Logistic Organ Dysfunction score; m: Months; MOF: Multiorgan failure; MV: Invasive mechanical ventilation; NIV: Non-invasive ventilation; NR: Not reported; OS: Overall survival; pts: Patients; RRT: Renal replacement therapy; Rx: Treatment; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment; SOS: Sinusoidal obstruction syndrome; UCBT: Umbilical cord blood transplantation; VA: Vasoactive drug treatment.

survival (OS) at one year range from 53% to 75% and from 15% to 28%, respectively.

The ICU outcomes in HSCT patients were even more dismal prior to 2000. In reports published prior to 2000,

Table 2 Possible reasons for improved outcomes in patients who are admitted to intensive care unit after hematopoietic stem cell transplantation

Improvements in HSCT
Reduced intensity conditioning
Better antimicrobial prophylaxis
Pre-emptive therapy of cytomegalovirus infections
Improved antifungal therapy
Improvements in intensive care
Early use of non-invasive ventilation
Early goal-directed therapy for septic shock
Better patient selection
Improved recognition of clinical deterioration and earlier ICU admission
Use of palliative care for patients with a slim chance of recovery

HSCT: Hematopoietic stem cell transplantation; ICU: Intensive care unit.

Table 3 Factors that were found to be associated with outcomes of intensive care among hematopoietic stem cell transplantation patients

Patient/disease related factors
Pre-transplant comorbidities
Transplant related factors
Type of HSCT (allogeneic *vs* autologous)
Conditioning regimen intensity
Graft-*vs*-host disease
Patient functional status at ICU admission
Serum bilirubin level
Serum lactate level
Blood urea nitrogen level
APACHE Ⅱ / Ⅲ scores
SOFA
Type of infection (bacterial *vs* fungal)
Post-ICU admission factors
Mechanical ventilation
Vasopressor support

APACHE: Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; HSCT: Hematopoietic stem cell transplantation; ICU: Intensive care unit.

hospital mortality ranged from 77% to 98% and long-term survival ranged from 3% to 10%[22-29]. Similarly, Agarwal *et al*[30] reported higher ICU mortality rates between 1988-1998 compared to 1998-2008 in a single center. The improvement in ICU outcomes over the last few decades may have been due to improvements in intensive care, HSCT, and patient selection (Table 2).

Poor outcomes among HSCT patients admitted to ICU is not universal. Moreover, HSCT patients surviving ICU stay do not necessarily perform worse in the long-run compared to those who never require intensive care[13]. On the other hand, non-beneficial intensive care has costs to patients, families, and the healthcare system overall. Preventing non-beneficial intensive care may provide comfort and dignity for the patient, increase bed availability for those who would benefit from intensive care, and reduce the economic burden. To identify the factors affecting outcomes in HSCT patients admitted to ICU and guide patient selection for intensive care, various small retrospective cohort studies have been performed (Table 1). These factors may be divided into four categories (Table 3).

Patient/disease related factors

Patient age, gender, and underlying disease type were not found to be associated with ICU outcomes. The only patient related factor that has been shown to affect ICU outcomes is the presence of pre-transplant comorbidities. In one of the largest cohorts reported to date, we have shown that an Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) of more than or equal to 2 was significantly and independently associated with increased hospital mortality[8]. A higher HCT-CI score, an index of pre-transplant comorbidities, was also associated with shorter OS. Available at the time of HSCT planning, HCT-CI may help providers and patients make informed decisions regarding intensive care before the need arises.

Transplant related factors

Conditioning regimen intensity and the type of HSCT (autologous *vs* allogeneic) affects a patient's risk for critical illness[13]. Whether they affect the ICU outcomes is not as clear. Of the few studies that included both allogeneic and autologous HSCT[30-32], only one demonstrated significantly worse prognosis after AutoSCT compared to AlloSCT[32]. However, these included small cohorts, and in the only recent study including exclusively AutoSCT patients[15], ICU mortality was numerically lower than that in most AlloSCT patient cohorts. Similarly, while Townsend *et al*[13] and our group have observed that ablative conditioning is associated with higher hospital mortality, other researchers have not found such association[10,11,18,31]. Additionally, Pène *et al*[11] and we observed that patients who had active GVHD or were being treated with corticosteroids at the time of ICU admission had worse prognosis than those who did not. There likely is a genuine cause-effect relationship between GVHD and ICU mortality as GVHD causes tissue damage and its treatment suppresses immune system cultivating severe infections. Finally, none of the recent studies found an association between ICU outcomes and patient age, underlying hematological diagnosis, donor type, HLA match or stem cell source.

Patient functional status at the time of ICU admission

The patient's severity of illness at the time of ICU admission is known to be predictive of general ICU survival, hence various predictive scoring systems, such as Acute Physiology and Chronic Health Evaluation (APACHE) and Sequential Organ Failure Assessment (SOFA) score, based on organ function are commonly used in clinical practice. It is evident that organ function at the time of ICU admission is also prognostic for HSCT patients. However, as results have been discrepant, there is no agreement on which measures are optimal. Elevated serum lactate[16], bilirubin[11], and blood urea nitrogen[13,18] have been inconsistently

found to be associated with worse prognosis. As for the organ function indexes, APACHE Ⅱ was not found to be predictive of ICU outcomes in HSCT population in various studies[10,13,30] except one[31]. In a cohort of 112 patients, Afessa *et al*[32] did not find APACHE Ⅱ to have prognostic value while they found APACHE Ⅲ to have a moderate discrimination and good calibration in predicting hospital mortality. Gilli *et al*[10] observed that APACHE Ⅱ underestimated mortality while higher SOFA scores were associated with higher hospital mortality. In their cohort, none of the 20 patients with a SOFA score > 11 survived. On univariate analyses, Trinkaus *et al*[15] also found SOFA to be predictive of mortality. Similarly, Neumann *et al*[18] found a SOFA score > 12 to be significantly and independently associated with higher mortality; of 45 patients with SOFA >11, only 4 survived. On the other hand, Boyaci *et al*[31] and Benz *et al*[9] did not find SOFA to be predictive of mortality in multivariate analyses. Finally, Depuydt *et al*[33] reported that patients who were admitted to ICU with bacterial infection had a better prognosis than others, likely due to the more rapid improvement of bacterial infections with antibiotic treatment. In contrast, Agarwal *et al*[30] found fungal infections to be associated with higher mortality.

Post-ICU admission factors

Several authors demonstrated that short-term outcomes are worse in patients who required endotracheal intubation[11,13,16,32]. In fact, mechanical ventilation (MV) is the most consistently shown prognostic factor in HSCT patients admitted to ICU. Unfortunately, 48%-78% of HSCT patients require MV during their ICU stay (Table 1). Similar to MV, vasopressor support has also been found to be associated with worse short-term prognosis in a few studies[12,31]. Although significantly associated with outcomes, events that happen in ICU cannot be used for outcome prediction before a patient's ICU admission.

Overall, it can be deduced that: (1) patient/disease related factors do not play a major role in determining ICU outcomes with the exception of patient comorbidities; (2) while transplant related factors affect ICU admission risk, they do not necessarily influence ICU outcomes with the exception of transplant type and GVHD diagnosis at the time of ICU admission; (3) the major determinant of ICU outcome seems to be the patients' functional status at the time of ICU admission; (4) the value of traditional prognostic indexes has not been validated in HSCT patients but may be useful in identification of patients with a very slim chance of survival.

FUTURE OBJECTIVES AND RECOMMENDATIONS

As physicians and researchers continue improving the HSCT process and outcomes, optimization of the delivery of a comprehensive intensive care plan should become an important component of the overall patient management. This goal, requires establishment of adequate communication channels among patients, relatives, transplant physicians and intensivists. In addition, we believe that there is a need for further development of clinical algorithms to assess benefits and risks of intensive care, alternative palliative care, and appropriateness of life support and resuscitation at multiple points in time: (1) prior to HSCT; (2) when early warning signs of critical illness appear; (3) upon development of critical illness; (4) every third to fifth day of intensive care; and (5) prior to initiation of life supportive measures such as endotracheal intubation and MV.

Prior to HSCT

Transplanters should assess patients' comorbidities, calculate their HCT-CI scores, and talk to the patient about the possibility and prognosis of intensive care/intubation beforehand.

Early warning signs

Retrospective studies demonstrated HSCT patients admitted to ICU demonstrated early warning signs that could be detected by early warning score systems (EWSS) consisting of nursing observations[34] and suggested improvements in ICU outcomes among HSCT patients after such systems and early outreach teams were implemented[35]. Accordingly, we recommend implementation of EWSS and outreach teams in transplant centers.

Upon development of critical illness

In the literature, there is a lack of agreement on which factors should be used to predict ICU outcomes of HSCT patients. We believe patient pre-transplant comorbidities, underlying disease status, GVHD diagnosis/grade, and patient's functional status at the time of critical illness should be taken into account while deciding on benefits of intensive care. Although none of the previous studies showed the underlying disease or its remission status to affect short-term ICU outcomes -similar to the studies done in cancer patients[36,37]; the remission status significantly affects the long-term survival of HSCT patients[20,21], and likely would affect the long-term outcome after ICU admission. To establish a clinical algorithm for patient selection, transplanters and intensivists need a prognostic index specific for critical HSCT patients. Hence, more studies on large HSCT cohorts with multi-center validation are needed. We believe hospital mortality should be the primary outcome assessed in such future studies as long-term outcomes of HSCT patients surviving ICU is similar to those of patients who never required intensive care[13] and, in our experience, the number of patients who died on their second ICU admission but during the same hospitalization is not insignificant.

Every third to fifth day of intensive care

Although none of the recent studies showed the length of ICU stay to affect short-term ICU outcomes, in our experience the longer the patient stays in ICU, the less likely he/she is to survive. Therefore, we believe the intensivist and the transplant physician should review the benefits of intensive care every three to five days in ICU.

Prior to endotracheal intubation

Initiation of MV is a turning point in the intensive care of HSCT patients. MV is associated with shorter survival and also suffering of patient and the family[11,16,32]. The combination of hepatic and renal failure in mechanically ventilated patients is almost universally fatal[38-40]. Therefore, for HSCT patients with renal and/or hepatic failure requiring MV, a frank discussion should be made with the patients' family prior to intubation and initiation of ventilation.

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MINIREVIEWS

Role of copper transporters in platinum resistance

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Abstract

Platinum (Pt)-based antitumor agents are effective in the treatment of many solid malignancies. However, their efficacy is limited by toxicity and drug resistance. Reduced intracellular Pt accumulation has been consistently shown to correlate with resistance in tumors. Proteins involved in copper homeostasis have been identified as Pt transporters. In particular, copper transporter receptor 1 (CTR1), the major copper influx transporter, has been shown to play a significant role in Pt resistance. Clinical studies demonstrated that expression of CTR1 correlated with intratumoral Pt concentration and outcomes following Pt-based therapy. Other CTRs such as CTR2, ATP7A and ATP7B, may also play a role in Pt resistance. Recent clinical studies attempting to modulate CTR1 to overcome Pt resistance may provide novel strategies. This review discusses the role of CTR1 as a potential predictive biomarker of Pt sensitivity and a therapeutic target for overcoming Pt resistance.

Key words: Resistance; Cisplatin; Copper transporter receptor 1; Copper transporter; Copper transporter receptor 2; ATP7A; ATP7B

Core tip: Platinum (Pt)-based chemotherapy is the backbone of treatment for various solid malignancies in both curative and palliative settings. However, the efficacy of Pt is limited by toxicity and inevitable resistance. Hence, it is important to understand the mechanisms of Pt resistance to not only identify treatment non-responders, but more importantly to help develop strategies to overcome resistance and improve efficacy. We herein discuss our current understanding of the mechanisms of Pt resistance, with a particular emphasis on the role of copper transporter receptor 1 in Pt resistance.

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INTRODUCTION

Role of platinum chemotherapeutics in cancer

Cisplatin, also called the "penicillin of cancer", has

remained the mainstay of treatment for a variety of solid tumors over the last four decades and is an essential component of both curative-intent and palliative chemotherapy regimens[1,2]. First described in 1845 as Peyrone's salt and subsequently noted to inhibit binary fission in *Escherichia coli* bacteria, cisplatin is platinum (Pt)-based alkylating agent that binds to DNA and causes intra/inter strand crosslinking which interferes with cell division and causes apoptosis. Carboplatin and oxaliplatin are newer members of the Pt family of compounds with similar mechanisms of action as cisplatin but with different toxicity profiles.

Pt agents have a number of toxicities that limit their clinical use. The most common adverse effects from cisplatin include nephrotoxicity, ototoxicity, neurotoxicity and myelosuppression. Cisplatin is also highly emetogenic. Carboplatin is less emetogenic and has a lower risk of nephrotoxicity and ototoxicity; however, it is more myelosuppressive than other Pt compounds. Oxaliplatin which is significantly neurotoxic has the lowest risk of nephrotoxicity and ototoxicity amongst Pt compounds.

Despite the same class, each Pt drug has a unique role in the management of individual cancers, and in most circumstances these agents are not interchangeable. Cisplatin is the most active Pt agent against testicular, lung, ovarian and bladder cancers, and is the only Pt drug recommended in curative-intent treatment for these malignancies. In contrast, carboplatin may be substituted for cisplatin in the palliative setting for advanced solid tumors where cisplatin may not be tolerated due to adverse effects. In general, oxaliplatin is the Pt of choice for colon cancer.

Pt resistance is an inevitable occurrence with rare exception. Aside from germ cell tumors, metastatic solid tumors are generally thought to be incurable with cytotoxic chemotherapy due to the development of resistance and subsequent disease progression. While advances in molecular biology and genomic (personalized) medicine have driven an exponential increase in therapeutic options and improved outcome in various malignancies, Pt-based chemotherapy remains the backbone of treatment for a variety of solid tumors. Therefore, it is crucial to understand mechanisms of Pt resistance in order to develop strategies to overcome this nearly universal phenomenon.

Mechanisms of Pt resistance

The clinical utility of Pt agents is limited by both intrinsic and acquired resistance. For example, cisplatin-based treatment is associated with up to 80% response rates in patients with limited stage small cell lung cancer; however, the median overall survival is less than a year due to lack of durable response[3]. Understanding the mechanisms of Pt resistance may improve clinical outcomes. Pt resistance is complex and is regulated by a cascade of events that interfere with any of the multiple steps involved in its cytotoxic actions, from initial drug entry into the cell to the final stages of apoptosis. While not fully understood, identified mechanisms of resistance include: Increased glutathione and metallothionein, which inactivates the reactive forms of Pt[4-6], activation of nucleotide excision repair pathway and other pathways associated with DNA repair[7,8], and dysregulation of the tumor suppressor *p53* gene that is required for apoptosis[9-12]. Dysregulation of the Ras and MAPK pathway[13,14] and the heat-shock proteins[15] have also been implicated in Pt resistance.

Despite the multifactorial nature of Pt resistance, reduced intracellular drug accumulation is one of the most consistently identified features of cisplatin-resistant cell lines[4,16]. Reduced influx or increased efflux of the drug is associated with decreased intracellular accumulation. Pt drug influx has been attributed to both non-saturable as well as energy-dependent active transport processes[17,18]. Currently identified Pt influx transporters include copper transporter receptor 1 (CTR1) and organic cation transporters belonging to the soluble carrier (SLC) SLC22A2 family. On the contrary increased levels of the multidrug resistance associated transporter protein MRP2 (cMOAT), adenosine triphosphate (ATP) binding cassette (ABC) multidrug transporters, CTR2 and copper-transporting P-type adenosine triphosphates (ATPase's) have been observed to confer resistance[19,20]. In this review we will focus on the importance of intratumoral Pt levels in promoting chemosensitivity and the role of CTRs, specifically CTR1, in contributing to Pt resistance.

INTRACELLULAR PT AND TUMOR PT-SENSITIVITY

It has been hypothesized that reduced intracellular Pt concentration may confer resistance to Pt-based chemotherapy. Both *in vitro* and *in vivo* studies provide data to support this hypothesis.

In vitro studies

Lanzi *et al*[21], demonstrated that a reduction of drug accumulation in cisplatin-resistant (A431/Pt) human cervix squamous cell carcinoma compared to Pt-sensitive squamous cancer cells directly correlated with the extent of cisplatin-induced DNA damage. Mann *et al*[22], noted that, in human ovarian cancer cell lines, decreased Pt drug accumulation is associated with resistance. Several other investigators observed similar positive correlations between accumulation of Pt and cytotoxicity in cancer cell lines derived from ovarian, leukemia and lung cancer tissues[23-26]. All these studies support drug accumulation as a contributing factor to Pt resistance. However, cell line studies represent only a single phenotype and do not take into account complex tumor- host interaction that may allow for other mechanisms of chemoresistance.

In vivo studies

It has been demonstrated that the elimination of Pt compounds is triphasic in nature, with a terminal

plasma half-life of 5.4 d for cisplatin. In contrast, Pt has a long half-life in human tissue that is yet to be quantified[27]. Pt and DNA adducts were detectable in autopsy tumor samples from patients who had received Pt up to 15 mo ante mortem[28,29]. In a prospective study of two groups of advanced non-small cell lung cancer (NSCLC) patients receiving cisplatin at two different doses, plasma Pt concentration correlated with the dose of cisplatin administered, however tissue Pt concentration did not. In this study there was a weak correlation between simultaneous plasma and tumor tissue concentration[30]. In 44 patients with NSCLC who had received neoadjuvant Pt-based therapy and subsequently underwent surgical resection, tissue Pt concentrations in resected tumor specimens significantly correlated with percent reduction in tumor ($P < 0.001$). The same correlations were seen irrespective of the Pt drug utilized, number of cycles and histologic subtype. Patients with higher intratumoral Pt concentrations also had longer time to recurrence ($P = 0.034$), progression-free survival ($P = 0.018$), and overall survival ($P = 0.005$). This was the first clinical study to establish a relationship between tissue Pt concentration and tumor response, and supports Pt accumulation as an important mechanism of resistance even in the clinical setting[31]. In another study of 19 patients with muscle invasive bladder cancer who had received Pt-based neoadjuvant therapy, total Pt concentration in normal adjacent bladder tissue significantly differed by tumor pathologic response ($P = 0.011$). Specimens with pathologic complete responses had the highest Pt concentrations compared to those with a down-staging to non-muscle invasive disease ($P = 0.0095$) or no response/progression ($P = 0.0196$)[32]. These findings suggest that intratumoral Pt accumulation may be an important determinant of Pt sensitivity and tumor responses across tumor types.

CTRS

Pt chemotherapeutics cross the cell membranes by passive diffusion and transporters. Various ion pumps and transporters have been implicated in the transport of Pt agents, some of which are well-characterized[33]. More recently, transporters involved in copper homeostasis have been identified as important in Pt influx and efflux. Copper is an essential micronutrient and a cofactor for many enzymes. However, its intracellular form is highly toxic, and hence, a complex network of proteins have evolved to chaperone copper to the copper-dependent proteins. Chaperone proteins include CTR1, CTR2, antioxidant protein (ATOX 1), ATP7A and ATP7B. All of the above discussed transporters possess a metal binding sequence that binds both copper and Pt[34].

CTR1 is the most extensively studied Pt influx transporter and will be described in detail in the next section. CTR2, another copper uptake protein, has a substantial structural homology to CTR1but functions as a Pt efflux transporter. Higher CTR2 levels correlated with Pt resistance in ovarian cancer cell lines[35]. It was also noted that in a human 2008 epithelial cancer cell model, higher expression of CTR2 was noted to be associated with increased intracellular copper and Pt resistance[36]. Further studies are needed to better understand the role of CTR2 in cisplatin resistance in human cancers.

ATP7A and ATP7B are two copper transporting P-type ATPase that also maintain copper homeostasis and have been implicated in Pt efflux[37,38]. ATP7A is thought to regulate Pt accumulation, primarily by sequestering Pt intracellularly, whereas ATP7B located in the Trans Golgi network mediates Pt drug efflux *via* a process that involves its transport into vesicles involved in the secretory pathway[39]. In human epidermoid carcinoma KB-3-1 cell(a derivative of HELA–cervical cancer line), transfection with ATP7B conferred cisplatin resistance[40]. Similarly in prostate cell lines, overexpression of ATP7B correlated with Pt resistance[40]. The observation that human tumor cells transfected with ATP7B acquire resistance to cisplatin lends credence to the hypothesis that drug efflux plays a role in resistance[41]. Several cell line studies, including one of fibroblasts derived from a patient with Menkes disease, which is characterized by copper deficiency, confirmed the role of efflux proteins in enhancing Pt resistance[42,43]. ATP7B silencing resulted in enhanced cisplatin sensitivity and increased DNA adducts formation in cisplatin-resistant cells; however this was not observed with ATP7A silencing[44]. In both NSCLC xenografts exposed to cisplatin and colorectal cancer patients treated with oxaliplatin, increased levels of ATP7B were associated with Pt resistance[45,46]. In the only study to simultaneously assess influx and efflux transporters, expression of CRT1, ATP7A and ATP7B were measured in three pairs of parent cell lines and cisplatin-resistant cell lines derived from various types of invasive oral squamous cell carcinoma. ATP7B expression correlated with the acquisition of cisplatin resistance more closely than either CTR1 or ATPP7A[39].

PROFILE OF CTR1

Structure and localization

CTR1 is a 190 amino acid (aa) protein with three trans-membrane domains, a approximately 67 aa extracellular N-terminal (ecto) domain, and a approximately 15 aa C-terminal cytosolic tail[47,48]. Crystallographic analysis of human CTR1 noted that the permeation conduit formed by the association of three CTR1 molecules involves a series of rings of methionines capable of chelating copper in a trimeric configuration[48,49]. Two rings each containing three methionines are stacked on top of each other in the narrowest part of the pore, and a ring of three cysteines is located at the bottom of the pore. The aperture has a truncated cone shape measuring approximately equal 8 Å at the external entrance and approximately equal 22 Å at the intracellular end[50]. The expression of CTR1 is ubiquitous and localizes to the plasma membrane in some cell lines and perinuclear vesicles in others[51].

Role in copper transport

CTR1 is the primary influx transporter of copper in human cells. Transport of copper by CTR1 is energy-independent[52] and results in conformational changes in CTR1[53]. Knockout of both CTR1 alleles results in an embryonic lethal phenotype thought to be secondary to deficiency of copper[54]. Organ-specific knockout of CTR1 in the intestine and liver confirms the role of CTR1 as an important copper transporter[55,56]. The exact mechanism of copper transport across CTR1 is not yet completely understood and further studies are warranted.

ROLE OF CRT1 AS A PT TRANSPORTER

Despite the "narrowest opening" of trimetric CTR1 being smaller than the molecular size of cisplatin, studies suggest that prior to entering a cell, cisplatin is activated by interacting with the extracellular methionine clusters of CTR1, which results in the formation of an intermediate that is smaller than the radius of the narrowest opening CTR1[57,58].

In vitro studies

Ishida *et al*[59], described CTR1 as a significant uptake transporter of cisplatin. They used a mutagenized wild type yeast cell library to select for mutants that grew in the presence of toxic doses of cisplatin. Cells with a CTR1 mutation that decreased CTR1 cell expression were noted to have profound Pt resistance compared to other mutants. In order to determine the mechanistic role of CTR1 in cisplatin resistance, cisplatin - DNA adducts were measured. They observed that decreased Pt uptake is responsible for lower Pt adduct levels and resistance. They also demonstrated that cisplatin, similar to copper, down-regulated CTR1 expression in yeast cell lines.

CTR1 knockout in intestinal epithelial mouse cell lines also led to a decrease in intracellular Pt levels and resistance[60]. Similarly, overexpression of CTR1 was associated with cisplatin sensitivity in ovarian and colorectal cancer cell lines[61,62]. In cisplatin-resistant small cell lung cancer cells, sensitivity was restored when CTR1 was introduced into these cells[63]. Ivy *et al*[64] also noted that higher intracellular Pt correlated with higher CTR1 levels in human embryonic kidney cells and mouse embryonic fibroblasts. However contrary to other studies, the investigators noted that in ovarian tumor cells uptake of Pt was linear and non-saturable, suggesting that there could be other mechanisms besides CTR1 involved in Pt transport, including proteins involved in copper homeostasis[64].

In vivo studies

In rat dorsal root ganglion, CTR1 expression by immunohistochemistry (IHC) and RT-PCR correlated with Pt uptake and treatment-induced cell body atrophy[65]. Similarly, in a murine model utilizing mouse embryo fibroblasts, CTR1 knockout completely eliminated responsiveness of cells to Pt agents[66]. In a mouse model of human cervical cancer (HPV16/E), the levels of cisplatin-induced DNA adducts correlated with CTR1 mRNA in most organs tested, including skin, lung, liver, pancreas, and uterus[67].

CTR1 EXPRESSION AND CLINICAL OUTCOME

To date, several human studies have investigated the role of CTR1 in Pt sensitivity. In 15 patients with stage Ⅲ/Ⅳ serous epithelial ovarian tumors who underwent optimal cytoreductive surgery (residual masses 1 cm or less) and subsequent Pt-based therapy, tumor CTR1 mRNA correlated with Pt sensitivity. Patients with no evidence of disease progression within 6 mo had higher CTR1 mRNA levels than in patients with refractory or resistant disease. Using clinical and array based expression data from the cancer genome atlas; the same investigators were able to independently validate the correlation of CTR1 m RNA levels with clinical outcomes in patients with advanced ovarian tumors who also underwent Pt-based therapy[67]. Higher CTR1 expression by IHC in patients with stage Ⅲ endometrial cancer who had received carboplatin also correlated with longer disease free and overall survival (P = 0.009)[68].

In a study of 30 patients with NSCLC who had received neoadjuvant Pt-based chemotherapy, patients with undetectable CTR1 expression in their tumors had reduced intratumoral Pt concentrations and tumor response[69]. In another study of 54 patients with stage Ⅲ non-small lung cancer who received Pt-based combination chemotherapy, higher CTR1 expression correlated with longer progression free survival and overall survival (P = 0.01 and 0.047, respectively)[70]. More recently, we demonstrated that tumor CTR1 expression in cystectomy specimens of patients with muscle invasive bladder cancer correlated significantly with pathologic downstaging after Pt- based neoadjuvant chemotherapy[71].

REGULATION OF CTR1

CTR1 expression has been shown to be regulated at both transcriptional and post-translational levels by various factors including transcription factor specificity protein 1 (Sp1) as well as copper and other heavy metals such as Cd, Zn and Ag[72,73]. Sp1 is a zinc finger transcription factor that binds to GC-rich motifs in promoters and is involved in many cellular processes including cell differentiation, cell growth, apoptosis, immune responses and response to DNA damage. Song *et al*[74] demonstrated that three binding sites in the CTR1 promoter of Sp1 are involved in the basal and copper concentration-dependent regulation of CTR1 expression. The zinc-finger domain of Sp1 serves as a sensor of copper that regulates CTR1 expression in response to fluctuations in copper concentration[74,75]. In addition,

modulation of Sp1 levels also affected the expression of CTR1. Cisplatin competes with copper for CTR1-mediated transport and trigger the rapid degradation of CTR1. It has been postulated that this mechanism serves to limit the toxic accumulation of the metal that it transports[76,77]. The down-regulation of CTR1 expression after Pt exposure has been confirmed in various cell lines and is considered functionally significant as subsequent copper uptake, despite Pt absence, is noted to be decreased[49,77].

In a study of 282 Chinese patients with NSCLC who received Pt-based therapy, genetic polymorphisms of CTR1 at reference single nucleotide polymorphism (rs) rs7851395 and rs12686377 were associated with Pt resistance and poor clinical outcomes. Patients with a GT haplotype had increased susceptibility to Pt resistance, whereas AG haplotype conferred longer overall survival[78]. In a second study of 204 Chinese patients, CTR1 polymorphism (rs10981694 A > C) correlated with Pt toxicity in patients with advanced stage NSCLC and could be potentially used for pretreatment evaluation of toxicity. However, the survival times of patients with different rs10981694 genetic polymorphisms were not significantly different[72]. Functional implications of these polymorphisms are not clear.

CTR1 AS A THERAPEUTIC TARGET

Cisplatin-induced degradation of CTR1 was noted to be reversible with the proteasome inhibitor bortezomib in both mouse fibroblast and human ovarian carcinoma cell lines. This in turn correlated with increased cellular uptake and the cytotoxicity of cisplatin in a synergistic manner[73]. Cells lacking CTR1 had no change in cisplatin uptake with bortezomib suggesting that bortezomib may act primarily through blocking CTR1 degradation. NCT01074411 is an ongoing phase 1 trial of intraperitoneal bortezomib and carboplatin that tests this hypothesis in patients with recurrent ovarian cancer.

Ag and zinc have been noted to induce CTR1 expression. In CTR1-transfected or nontransfected HEK293 cells Ag, Zn inhibited CTR1-mediated copper uptake[52]. Also in IGROV1 and SKOV-3 cells treated with different concentrations of Zn, and Ag, there was a concentration-dependent increase in expression of CTR1 and Sp1[79]. In a double-blind, placebo-controlled study of 34 patients with stage Ⅲ/Ⅳ nasopharyngeal carcinoma receiving cisplatin-based chemotherapy, zinc supplement (75 mg/d) was associated with longer overall survival, local-free survival and disease-free survival compared with placebo (P = 0.044, P = 0.007, and P = 0.033, respectively)[77]. More recently in ovarian cancer cells and xenograft mice, (-)-epigallocatechin-3-gallate (EGCG), a major polyphenol from green tea was noted to increase CTR1 m RNA and protein expression. These findings translated into EGCG enhancing the sensitivity of ovarian cancer SKOV3 and OVCAR3 cells to Pt through increased Pt accumulation and DNA-Pt adducts[80]. Copper chelators are a class of compounds that preferentially bind either cuprous or cupric forms of copper and potentially modulate copper redox-activity without removing copper from the system. They are characterized as either membrane-permeable or - impermeable and serve as an organ-selective copper delivery or deprivation system to manipulate the biological function of copper. Tetrathiomolybdate (TTM), a specific and effective copper chelator was initially developed as a therapeutic agent to treat Wilson's disease, which is characterized by excessive copper accumulation in liver and brain[81]. TTM demonstrates antiangiogenic, antifibrogenic, and anti-inflammatory actions in preclinical studies. While TTM has a good safety index, most of its toxicity in animals is due to copper deficiency that is easily reversible with acute copper supplementation[82]. Daily treatment with TTM has been shown to safely reduce bioavailable copper in 2-4 wk in humans and mice, likely through formation of a high-affinity tripartite complex with copper and proteins[83]. Liang *et al*[76] demonstrated that in cisplatin-resistant ovarian cancer cell lines derived from patients, resistance associated with reduced expression of the CTR1 could be overcome by copper-lowering agents (TTM, D-penicillamine and trientine) which enhanced CTR1 expression. In a murine model of human cervical cancer, combined therapy with TTM and cisplatin enhanced therapeutic efficacy by increasing tumor-specific uptake of Pt[67]. Similarly, in oxaliplatin-resistant cell lines derived from human cervical carcinoma, D-penicillamine in combination with cisplatin and oxaliplatin overcomes resistance through increased CTR1 expression by up regulation of Sp1[84]. These studies provided the mechanistic rationale for using copper chelation to overcome Pt resistance in cancer patients. Trientine was combined with carboplatin in ovarian cancer patients[85]. NCT01837329 is an ongoing phase 1 study combining TTM with Pt-doublet in advanced NSCLC patients. Further studies are needed to validate these findings in order to use the above agents as adjuncts to conventional Pt based therapy and improve outcomes.

CONCLUSION

Pt-based chemotherapy is the backbone of both curative and palliative treatment for numerous malignancies. Copper transporters, in particular CTR1, play a significant role in intracellular Pt accumulation and have the potential to be used as predictive biomarkers of Pt sensitivity. In addition, modulation of copper transporter expression may be a novel therapeutic strategy to enhance the efficacy of Pt chemotherapy by increasing intratumoral Pt concentration. Specifically, copper chelators and agents that prevent degradation of CTR1, such as bortezomib, are currently being studied in combination with Pt in a variety of solid tumors known to develop Pt resistance.

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MINIREVIEWS

Recent advances in microvascular autologous breast reconstruction after ablative tumor surgery

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Abstract

Breast cancer is a ubiquitous disease and one of the leading causes of death in women in western societies. With overall increasing survival rates, the number of patients who need post-mastectomy reconstruction is on the rise. Especially since its psychological benefits have been broadly recognized, breast reconstruction has become a key component of breast cancer treatment. Evolving from the early beginnings of breast reconstruction with synthetic implants in the 1960s, microsurgical tissue transfer is on the way to become the gold standard for post oncology restoration of the breast. Particularly since the advent of perforator based free flap surgery, free tissue transfer has become as safe option for breast reconstruction with low morbidity. The lower abdominal skin and subcutaneous fat tissue typically offer enough volume to create an aesthetically satisfying breast mound. Nowadays, the most commonly used flap from this donor site is the deep inferior epigastric artery perforator flap. If the lower abdomen is not available as a donor site, the gluteal area and thigh provide a number of flaps suitable for breast reconstruction. If the required breast volume is small, and there is enough tissue available on the upper medial thigh, then a transverse upper gracilis flap may be a practicable method to reconstruct the breast. In case of a higher amount of required volume, a gluteal artery perforator flap is the best choice. However, what is crucial in addition to selecting the best flap option for the individual patient is the timing of the operation. In patients with confirmed post-mastectomy radiation therapy, it is advisable to perform microvascular breast reconstruction only in a delayed fashion.

Key words: Breast cancer; Microsurgery; Autologous tissue transfer; Breast reconstruction; Flap; Transverse musculocutaneous gracilis; Fasciocutaneous infragluteal; Deep inferior epigastric perforator

Core tip: Mastectomy is a frequent sequela of the treatment and prophylaxis of breast malignancies. Autologous microvascular breast reconstruction is becoming the gold standard in correcting these disfiguring interventions. The lower abdomen, as well as the gluteal and thigh area, is the source of multiple flaps usable for breast reconstruction. With the advent of perforator flap surgery, today's reconstructive surgeons are able to minimize donor site morbidity whilst maximizing patient outcomes. If timed and performed correctly, microsurgical breast reconstruction is a safe procedure with low donor site morbidities and excellent aesthetic results.

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INTRODUCTION

Breast cancer is a ubiquitous disease and one of the leading causes of death in women in western societies. While breast conservation techniques together with postoperative radiation often are successful treatment strategies for local control, mastectomy often remains unavoidable in breast cancer therapy[1-3]. Reconstructive options are divided into implant based reconstruction and reconstruction using autologous tissue or a combination of both. Spanning from the early beginnings of breast reconstruction with implants in the 1960s to the autologous microvascular breast restoration of today, plastic surgery continues to offer a great variety of options for women having suffered from mastectomy. Today, with many different methods available, it is important to find a procedure that fits the specific needs of every individual patient[4-6].

HISTORICAL PERSPECTIVES

The history of breast reconstruction dates back to Vincenz Czerny, who was the first ever to successfully accomplish breast augmentation in 1895, by using a removed lumbar lipoma[7]. One year later, the latissimus dorsi myocutaneous flap was the first described autologous muscle flap for post-mastectomy breast reconstruction[8]. However, for the following decades autologous breast reconstruction was not the gold standard. Surgeons used paraffin injections and polyvinylic alcohol sponge implantation for breast augmentation, with terrible outcomes for the patients[9]. Silicone implants were first introduced in 1961 by Cronin *et al*[10] for aesthetic breast augmentation. Henceforth silicone implants became the leading option in breast reconstruction. Despite their considerable risk for capsular fibrosis, alloplastic implants are still used in the majority of breast reconstruction procedures worldwide at this day[12].

The rediscovery of the latissimus dorsi flap in the late seventies led to a renaissance of autologous breast reconstruction[13]. The rather limited amount of soft tissue volume that is available by this technique, however, often is not enough to replace the excised breast tissue sufficiently. Therefore, reconstruction of the breast mound with the latissimus dorsi musculocutaneous flap usually demands the addition of a silicone implant.

Striving to overcome the volume deficit of the latissimus dorsi flap and to be able to solely rely on autologous tissue, Scheflan *et al*[14] pioneered a pedicled abdominal flap with a transverse skin island in 1982. Not only its robust vascularization and the large arc of rotation, but also the concomitant contouring benefits of an abdominoplasty, made this technique popular among surgeons and patients alike[11]. On the contrary, herniation of the abdominal wall, bulk in unwanted regions due to its muscular pedicle as well as venous congestion are well known disadvantages of the pedicled transverse rectus abdominis muscle (TRAM) flap[15]. With the advent of perforator flaps, Koshima *et al*[16] revolutionized breast reconstruction by completely sparing the rectus muscle in 1989. This new technique, the deep inferior epigastric perforator (DIEP) flap, allowed the raising of large flaps without weakening of the abdominal wall. Consisting exclusively of fat tissue and skin, this autologous flap resembles the real breast tissue more closely and produces a more natural look of the reconstructed breast mound. However, experience has shown that technical challenges, variable anatomy and certain patient characteristics are limiting its applicability. Therefore, alternative flap donor sites also had to be taken into consideration.

Gluteally-based flaps such as the superior gluteal artery perforator (SGAP), the inferior gluteal artery perforator (IGAP) and the fasciocutaneous infragluteal (FCI) flap, as well as medial thigh based-flaps such as the transverse musculocutaneous gracilis (TMG) flap or the profunda artery perforator (PAP) flap have emerged as valuable alternatives if the use of abdominal flaps is precluded. Each of these flaps has particular advantages and disadvantages and indications must be assessed carefully. However, microvascular transplanted flaps should no longer be considered as a last resort, but rather begin to represent the first choice for post mastectomy breast reconstruction. Their ability to resemble a very natural breast mound has made autologous flaps an invaluable addition to the armamentarium of the reconstructive plastic surgeon.

FLAPS FORM THE LOWER ABDOMEN

Of all donor sites available, the lower abdomen has evolved as the gold standard in microsurgical breast reconstruction. The lower abdominal skin and subcutaneous fat tissue typically offer enough volume to create

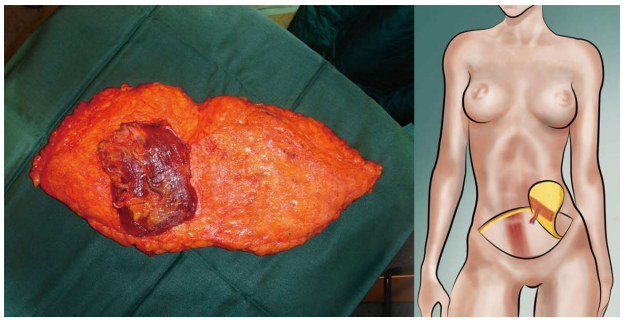

Figure 1 Typical example of a free transverse rectus abdominis muscle-flap showing the undersurface with its vascular pedicle and the completely excised rectus abdominis muscle.

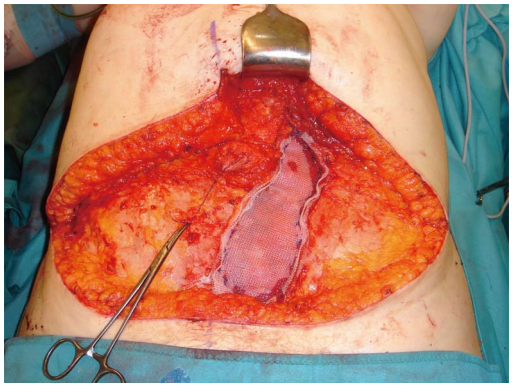

Figure 2 After harvest of a full transverse rectus abdominis muscle-flap, the fascial defect of the rectus fascia usually has to be closed with a synthetic mesh.

an aesthetically satisfying breast mound. Nowadays, the most commonly used flap from this donor site is the deep inferior epigastric artery perforator (DIEAP) flap[17]. Prerequisites for the usage of lower abdominal flaps are enough surplus tissue in this area to match the volume of the remaining breast and the acceptance of a relatively long scar.

TRAM flap

Both, the pedicled TRAM-flap by Scheflan *et al*[14] and the free TRAM flap by Holmström[18] have been widely used for autologous breast reconstruction in the past decades. The free TRAM flap is composed of a lower abdominal skin island overlying usually one rectus abdominis muscle (Figure 1). The overlying skin island and its subcutaneous fat tissue is supplied by the perforating vessels from the deep inferior epigastric artery (DIEA) travelling through the muscle. It covers approximately the area typically excised in an aesthetic abdominoplasty procedure. The DIEA is dissected together with a 5-6 cm strip of rectus muscle and anastomosed microscopically to the recipient vessel, which is typically the internal mammary artery (IMA) or, less frequently, the thoracodorsal artery. The fascial defect in the lower abdominal wall is typically repaired with an alloplastic mesh (Figure 2) and the wound is closed similarly to an abdominoplasty incision. Significant donor-site morbidity

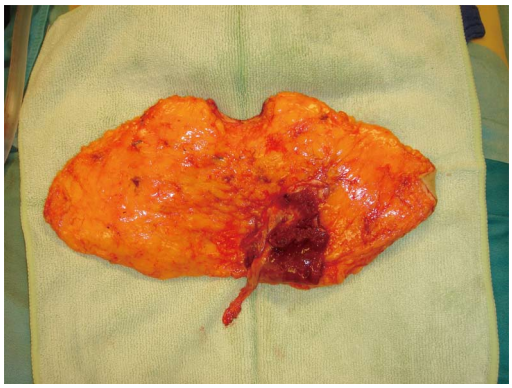

Figure 3 In a muscle-sparing free transverse rectus abdominis muscle, only a portion of the rectus muscle is included in the flap.

remained a major concern with this technique and resulted in an effort towards conserving as much muscle as possible[19]. Over time, the free TRAM flap sacrificing the whole muscle evolved into the muscle-sparing (ms-) TRAM flap and in further consequence into the DIEP flap with the main goal to minimize donor-site morbidity by harvesting less muscle and fascia. The ms-TRAM flap, in which only a small cuff of muscle around the vascular pedicle is harvested (Figure 3), is still a widely used method in breast reconstruction as it is technically less challenging than the DIEP flap with a comparable donor site morbidity[20].

DIEAP flap

The popularity of the DIEP flap has increased widely over the last decade. Currently it is the gold standard in autologous breast reconstruction not only because of its low donor site morbidity but also because of the satisfying aesthetic outcomes it can achieve. The DIEP flap utilizes the same skin island as the TRAM flap without harvesting the abdominal rectus muscle and anterior rectus sheath. Harvesting DIEP flaps requires considerable expertise in perforator pedicle dissection. Especially during the intramuscular part of the pre-paration one must dissect carefully to avoid damaging of the perforator vessels, which would subsequently lead to compromised flap circulation[21]. The DIEP flap is generally based on 1-3 perforating vessels and their connection to the DIEA (Figure 4). Unlike the TRAM flap dissection, these perforating vessels are carefully traced through the abdominal rectus muscle to their connection with the DIEA. Prior to surgery, the major perforating vessels can be identified *via* computed tomographic (CT)-angiography (Figure 5), Doppler ultrasonography or, if available, magnetic resonance imaging-angiography. Preoperative CT-angiography has been shown to reduce operative time, and can help to avoid injuries of the vulnerable pedicle[20]. DIEP flaps are typically outlined similar to aesthetic abdominoplasty procedures and surgical marking takes place in a standing position.

Simultaneous raising of the flap and preparation

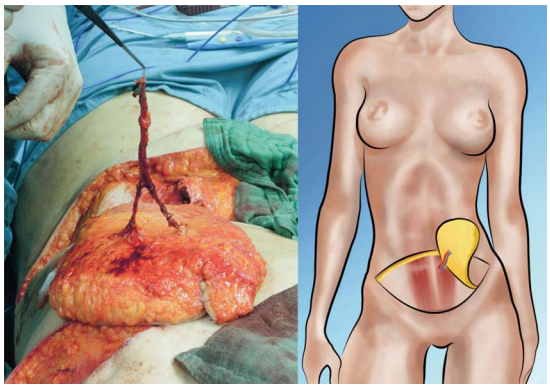

Figure 4 Example of a deep inferior epigastric perforator-flap with 2 perforators merging into the deep inferior epigastric vessels.

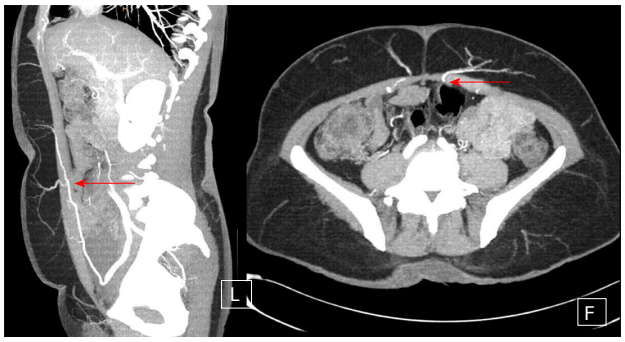

Figure 5 Computed tomographic-angiography to better elucidate the exact anatomy of perforators of the deep inferior epigastric perforator-vessels. The red arrow points out the piercing of the perforator through the rectus abdominis muscle on the sagittal (left) and transverse (right) view.

of the recipient vessels typically requires a two team approach. The IMA and vein are also here the preferred recipient vessels, while the thoracodorsal artery and vein remaining second choice. The IMA is typically approached in the second or third intercostal space. In case of a tight rib interspace, a small part of the rib cartilage can be removed for a better insetting of the pedicle.

After the skin incisions are made the superficial inferior epigastric artery (SIEA) and vein are first approached. In case of sufficient size and quality, they can be dissected down to their origin from the common femoral artery and a SIEA flap can be performed (see below). In case of an unsuitable SIEA, the abdominal skin island is raised carefully from lateral to medial until the lateral row of perforators is reached. Vessel diameter is the key factor in selecting a perforator. Once a perforator has been chosen, the anterior rectus fascia is incised and the perforator is dissected through the muscle until sufficient caliber of the pedicle is reached. Additional perforators in the same row may also be dissected out and included for further blood supply. However, intercostal motor nerves supplying the rectus muscle have been shown to enter the muscle just medial to the lateral row perforators and therefore harvesting of the lateral row perforators may decrease abdominal wall stability.

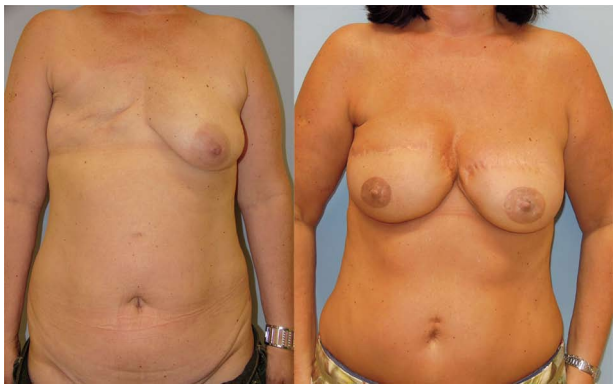

Figure 6 Pre- and post-operative pictures of a patient who underwent delayed reconstruction on her right side with a hemi-deep inferior epigastric perforator-flap and immediate reconstruction of her left side with the second hemi-deep inferior epigastric perforator-flap. She already had reconstruction of the nipple-areola-complex with tattooing and a local skin flap.

After completion of dissection, the flap is divided from its pedicle and passed to the chest for anastomosis and inset. The recipient vein and the vein of the flap are typically connected under magnification using an anastomotic coupling device. The arterial anastomosis is generally performed with a 9.0 suture. Upon completion of the anastomosis, the flap is checked for capillary refill. Finally, the incision in the anterior rectus fascia is closed directly without the need of a mesh.

DIEP flaps can be used in uni- and bilateral post-mastectomy breast reconstructions (Figure 6) and may also be used for reconstruction of congenital thoracic defects. There are only a few contraindications for the use of a DIEP flap. In the past, absolute contraindications included history of previous abdominoplasty, liposuction, or active smoking. Large transverse or oblique abdominal incisions were regarded as relative contraindications. Today, as a result of technical refinements, the only real absolute contraindications to DIEP flap reconstructions are non-compliant patients and/or a poor general condition. Obesity itself is not a contraindication, although it may lead to less aesthetically pleasing outcomes[22-24].

Complications occur infrequently. Partial flap loss has been reported as low as only 2.5%, total flap loss with less than 1%[25]. Problems regarding the venous anastomosis appear much more likely than problems with the arterial anastomosis. The most common complications include necrosis of the fat tissue of the flaps and seroma formation. The risk of bulging in DIEP flaps is reported as two thirds lower in comparison to TRAM flap reconstructions. Abdominal herniation occurred in fewer than 1% of all DIEP flaps compared with 3.9% in TRAM flaps[20,24,25]. However, current literature fails to prove a significant difference in the risk of developing abdominal bulge/hernia between ms-TRAM flap and DIEP flap based reconstructions[26]. Additionally, there is no significant difference in the risk of donor site morbidity between bilateral and unilateral DIEP flap breast reconstruction[26].

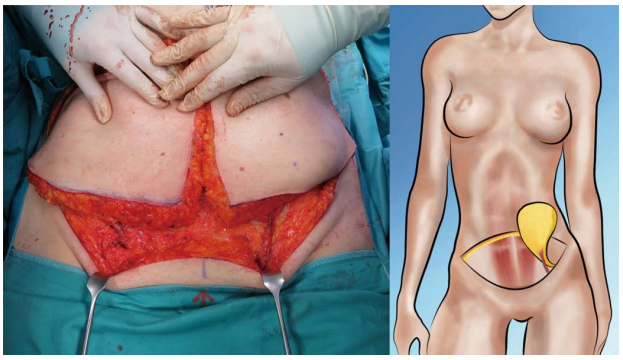

Figure 7 Example of a split superficial inferior epigastric artery-flap with both pedicles clearly visible. Since there is no injury to the rectus fascia, this the most desirable choice of flap from the lower abdomen.

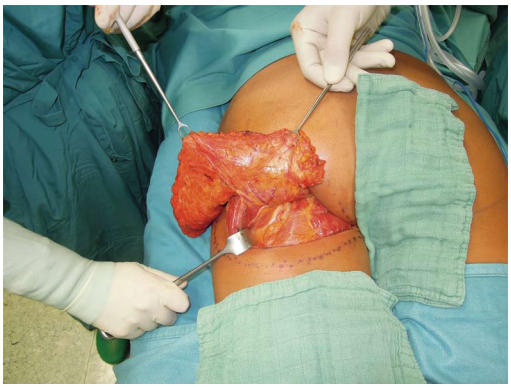

Figure 8 Example of a fasciocutaneous infragluteal-flap almost completely raised. At the undersurface of the flap, the flap's main vessels, *i.e.*, the descending branch of the inferior gluteal vessels, can be seen clearly. Additionally, a branch of the posterior femoral cutaneous nerve is visible and spared.

SIEA flap

The SIEA flap is the least invasive option of free abdominal tissue transfer for breast reconstruction. It provides the same tissue as the DIEP flap, without injuring the anterior rectus sheath and no vessel dissection needs to be performed through the muscle (Figure 7). Consequently, the SIEA flap causes the least donor site morbidity of all abdominal flaps, eliminating the risk of developing unwanted abdominal bulk or herniation[27]. However, the dissection of the vascular pedicle requires a high level of expertise, mainly because of its variability in existence, location, and caliber. The superficial inferior epigastric vessels have noted to be absent in 13%-42% of dissections. Furthermore, vessels are often not in appropriate caliber to sufficiently support the perfusion of the tissue volume needed for breast reconstruction. As a consequence, the amount of skin and fat tissue, which may be transferred utilizing a SIEA flap, is limited[22,24,28].

GLUTEAL AND THIGH FLAPS

If the lower abdomen is not available as a donor site, the gluteal area and thigh provide a number of flaps suitable for breast reconstruction. If the required breast volume is small, and there is enough tissue available on the upper medial thigh, a transverse upper gracilis flap may be a practicable method to reconstruct the breast. In case of a higher amount of required volume, a gluteal artery perforator flap is the best choice[22,29].

Other more exotic examples of free flaps in use for breast reconstruction, which will not be reviewed here in detail, include the anterolateral thigh flap[30,31] and the recently described PAP flap[32].

Gluteal artery perforator flaps and the FCI flap

The first free gluteal myocutaneous flap for breast reconstruction was performed by Fujino *et al*[33] in 1975. As the development of the perforator flap technique revolutionized the applicability of soft tissue transfer, gluteal flaps became popular in the 1990s[11]. Gluteal perforator flaps offer a great alternative for breast reconstruction in women who have more tissue available in the buttock area than in the lower abdomen. Similar to the DIEP and SIEA flaps, no sacrifice of muscle is required using this technique.

The superior and inferior GAP (S/I-GAP) flaps have been specified and modified by Allen *et al*[34-36]. The surgical procedure is similar to the DIEP flap. The gluteal vessel pedicle is dissected throughout the gluteus maximus muscle to their origin in the internal iliac artery. The pedicle is generally about 5-8 cm long, which provides sufficient length for a comfortable anastomosis and inset at the chest wall. A clear disadvantage of this method is in the necessary rearrangement of the patient from abdominal to supine position during surgery. GAP flaps are generally a safe reconstructive option. Vascular complications are reported in approximately 6%, total lap loss in 2% of all cases. However, approximately 4% of all patients require revisions because of donor site problems, such as contour deformity after SGAP, or scar issues after IGAP flaps[12,22,24].

Another, in our opinion more valuable flap of the gluteal region is the FCI flap. The FCI flap is based on the descending branch of the inferior gluteal artery with a very reliable location, good caliber and a pedicle length ranging up to 18 cm (Figure 8). Unlike other gluteal flaps, the vessels curl around the lower border of the gluteus maximus muscle. Therefore, no perforator dissection through the muscle is necessary. Studies demonstrated that even thin patients offer enough tissue to allow breast reconstruction with adequate volume[37], making the FCI flap a convincing alternative to standard free tissue breast reconstruction in certain cases. The tissue from this region is more compact and not as soft compared to the lower abdomen making it more suitable for reconstruction of firm breasts in younger patients (Figure 9).

Gracilis flaps

Since its first description for free tissue transfer in 1976[38] the gracilis flap has become a workhorse in autologous breast reconstruction. The TMG flap is an alternative method for patients who do not have adequate abdominal tissue, or seek to avoid abdominal

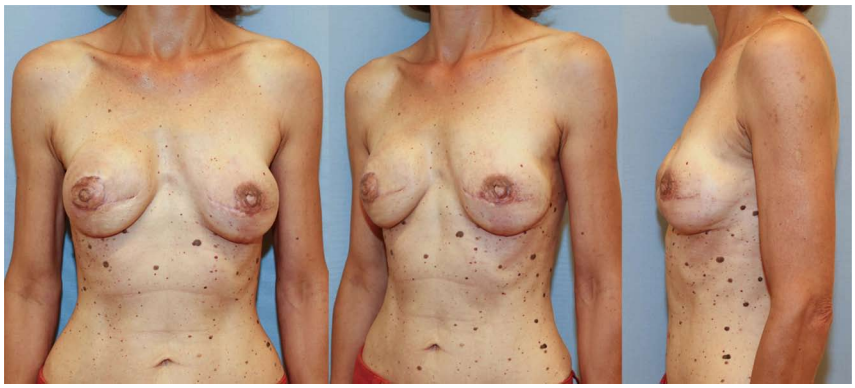

Figure 9 A 49-year-old patient after bilateral nipple-sparing prophylactic mastectomy and immediate breast reconstruction with bilateral fasciocutaneous infragluteal-flap.

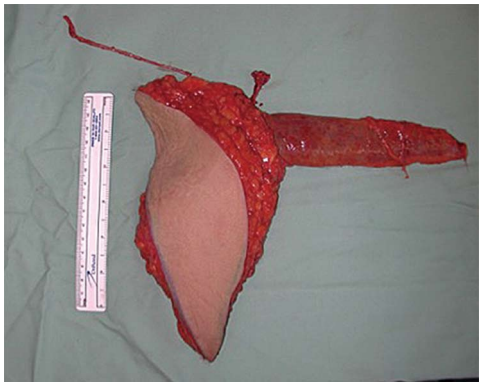

Figure 10 Intraoperative image of a completely harvested transverse myocutaneous gracilis flap with flap pedicle (short) and the relatively long saphenous vein as a venous supercharge.

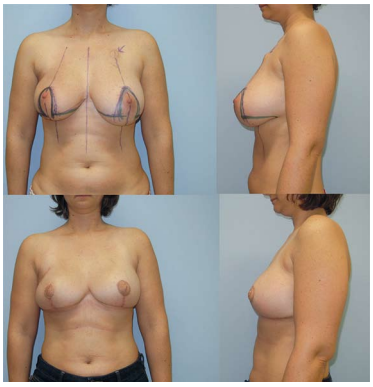

Figure 11 Pre- and post-operative pictures after immediate bilateral skin-reducing breast reconstruction with a transverse musculocutaneous gracilis-flap and reconstruction of the nipple-areola-complex.

scarring. The TMG flap is ideally suited for reconstruction of small to moderate sized breasts, providing excellent contour and projection[39]. The donor site is well hidden and harvesting the flap has no functional consequences[40].

The flap is designed with a semilunar skin island in the inner thigh with a mean volume of around 300 cc. The raising of the skin island is started close to the groin and harvested with the underlying fascia. The average pedicle length is reported as 6-8 cm, with an arterial diameter of 1.2 mm and a venous diameter of 2.8 mm. The artery is usually slightly smaller than the IMA, which usually does not preclude a safe anastomosis[41] (Figure 10).

Performing a unilateral TMG flap may cause asymmetry. The use in bilateral reconstruction is reported as one of the strongest advantages of the flap and even recommended as first choice, except for patients that would clearly benefit from an abdominoplasty (Figure 11). As with all flaps including a muscular component, the TMG flap is prone to volume loss resulting from neurogenic atrophy, but a true impact on cosmetic appearance has yet to be demonstrated[22,42]. A true disadvantage of the gracilis flap is the small size of the skin island. A single TMG flap may provide not enough tissue for sufficient reconstruction of a large breast. In this case, two TMG flaps or the usage of a combined infragluteal-transverse myocutaneus gracilis flap can be a viable option[43].

TIMING OF RECONSTRUCTION

Breast reconstruction can be divided into 3 approaches: Immediate, delayed-immediate and delayed[22]. The advantage of immediate breast reconstruction is the preservation of the breast skin and inframammary fold, which results in optimized aesthetic outcomes and minimized psychological burden of the patient by eliminating a period without a breast. The disadvantage is the uncertainty regarding radiotherapy and chemotherapy and their effects on autologous breast reconstruction. An increased incidence of fat necrosis and a higher rate of surgical revisions are reported in patients with free tissue transfer and further anti cancer therapy[44].

Therefore, a delayed-immediate reconstruction may be a good alternative. Expander implants are inserted as a temporary placeholder to maintain the breast skin envelope. Breast reconstruction is performed once radiotherapy is complete. In contrast, a fully delayed reconstruction offers the advantage of a typically very motivated patient with completed oncological treatment. The obvious disadvantage is the reduction of skin tissue

and the presence of significant scarring[45].

The optimal timing of breast reconstruction in relation to radiation therapy is controversially discussed. Therefore, it is advisable to perform autologous microvascular breast reconstruction in patients, who are confirmed for post-mastectomy radiation therapy, only in a delayed fashion. If post-mastectomy radiation therapy is likely but not certain, delayed-immediate breast reconstruction may be the better choice. Nonetheless, because of the mostly satisfying aesthetic outcome and the contradictory literature regarding this topic, immediate autologous breast reconstruction should at least be considered and discussed with the patient, even when post-mastectomy radiotherapy is anticipated.

CONCLUSION

Autologous microvascular breast reconstruction is becoming more and more accepted as a key component of breast cancer treatment after mastectomy. The abdominal wall or, alternatively, the gluteal and thigh areas provide numerous flaps suitable for breast reconstruction with appealing results. Recent advances in surgical technique enable today's reconstructive surgeons to minimize donor site morbidity whilst maximizing patient outcomes. If timed and performed correctly, microsurgical breast reconstruction is able to provide the best aesthetic outcomes with low complication rates and donor site morbidity.

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MINIREVIEWS

Targeting metabolism in breast cancer: How far we can go?

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Abstract

Adjuvant therapies for breast cancer have achieved great success in recent years and early breast cancer is now a curable or chronic disease. Targeted therapies, including endocrine therapy and human epidermal growth factor receptor-2 targeted therapy, marked a new era of breast cancer treatment. However, except for chemotherapy, an efficient drug treatment to improve the overall survival of breast cancer patients is still lacking for triple negative breast cancer. Furthermore, a certain proportion of breast cancer patients present with resistance to drug therapy, making it much more difficult to control the deterioration of the disease. Recently, altered energy metabolism has become one of the hallmarks of cancer, including breast cancer, and it may be linked to drug resistance. Targeting cellular metabolism is becoming a promising strategy to overcome drug resistance in cancer therapy. This review discusses metabolic reprogramming in breast cancer and the possible complex mechanism of modulation. We also summarize the recent advances in metabolic therapy targeted glycolysis, glutaminolysis and fatty acids synthesis in breast cancer.

Key words: Breast cancer; Targeted therapy; Metabolism; Drug resistance; Chemotherapy

Core tip: Breast cancer cells display distinct metabolic characteristics according to different molecular phenotypes. There may be crosstalk with the estrogen receptor and human epidermal growth factor receptor-2 signal pathways in the metabolic regulation in breast cancer cells that make it more complex to evaluate the efficiency of an anti-metabolic drug. On the other hand, the research on target metabolism in breast cancer will also largely help us to understand the complicated mechanism by which an anti-metabolic drug improves the efficacy of cancer therapy or overcomes drug resistance.

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INTRODUCTION

Breast cancer now has the highest incidence of cancer in women. This is attributed to the molecular classification of breast cancer based on the hormonal receptor and human epidermal growth factor receptor-2 (HER-2), targeted therapy and other adjuvant therapies that prolong the overall survival and greatly decrease the mortality of this disease. However, mortality remains high for locally advanced and metastatic cancer. We still lack effective methods for treatment when drug resistance occurs and recurrence and metastasis develop, especially in triple negative breast cancer (TNBC).

Females have a specific energy metabolic pattern compared to males[1]. Estrogens, progesterone-to-estrogen ratio and androgen levels affect the energy material transporter and metabolic enzyme expressions in cells[2]. Estrogens may increase the expression of peroxisome proliferator activated receptor, Akt and activate AMP-activated protein kinase (AMPK), which consequently influence the metabolic process, including glucose utility, lipid uptake, storage, lipogenesis and lipid oxidation[3,4]. Endocrine therapy plays a pivotal role in estrogen receptor (ER) positive breast cancer treatment. Rapamycin, which inhibits the mammalian target of rapamycin (mTOR), is a downstream target of Akt and enhances the susceptibility of breast cancer cells to endocrine therapy[5]. However, there is still a certain proportion of breast cancer patients that present with primary resistance to endocrine therapy and some patients could develop secondary resistance which makes it much more difficult to control the disease progress[6]. A similar condition occurs in chemotherapy and HER-2 targeted therapy in breast cancer. Therefore, researchers are looking for new strategies or compounds to reduce drug resistance and enhance the efficacy of therapy.

Metabolic reprogramming is the primary and basic factor during cell transformation[7,8]. Foreign stress forces tumor cells to accommodate new circumstances through metabolic reprogramming caused by epigenetic change and gene mutation. Altered energy metabolism has become one of the hallmarks of cancer[7]. Mounting evidence also attributes the drug resistance to dysregulated cellular metabolism[9,10]. Recently, much more interest has focused on targeting metabolic enzymes for cancer therapy or reversing drug resistance[11-13]. Cancer cells have distinct metabolic properties, including enhanced aerobic glycolysis, fatty acid synthesis and glutaminolysis, to sustain immortal proliferation[7,14]. This review will discuss the metabolic reprogramming and advances in metabolic targeted therapy in breast cancer.

METABOLIC REPROGRAMMING IN BREAST CANCER

To meet the abundant requirement of energy and materials for proliferation, most malignant cells present with increased aerobic glycolysis, fatty acid synthesis and glutaminolysis, which are distinctive from normal cells[15] (Figure 1). In 1956, Warburg[16] first postulated that cancer cells had a significantly higher rate of glycolysis than normal cells to produce ATP for proliferation. He also hypothesized that due to the defective function of mitochondria (this was proved wrong afterwards), pyruvate produced from glycolysis was converted to lactate more than acetyl CoA through the tricarboxylic acid (TCA) cycle. This phenomenon is now called the Warburg effect and it exists regardless of oxygen availability. For the adaption of the Warburg effect, cancer cells exhibit altered expression of different glucose transporters and glycolysis enzymes. Glucose crosses the plasma membrane *via* glucose transporter proteins (GLUTs) and fourteen types have been identified. Although little is known about the role of glucose transporters in cancer biology, GLUT1, GLUT2, GLUT3, GLUT4, GLUT5 and GLUT12 have been detected in breast cancer cells[17-20]. Different expression patterns of GLUT isoforms in breast cancer may have an association with pathological grade, cancer cell differentiation and prognosis. According to the molecular subtype of invasive breast cancer, HER-2 positive and TNBC mostly exhibit higher levels of glycolysis which need higher levels of expression of GLUT[21]. As the most invasive type in breast cancer, TNBC had the highest expression of GLUT-1 when compared to other types[21]. Increased activity of enzymes involved in glycolysis, like hexokinase (HK) and lactate dehydrogenase-A (LDHA), have also been studied and their expression may affect cancer cell growth[22,23].

Increased glutamine metabolism is another alternative energy origin for cancer cells, including breast cancer, and is thought to be a central metabolic pathway cooperating with glycolysis[24,25]. Most cancer cells cannot proliferate without a glutamine supply and glutamine addiction provides intermediates for amino acid and lipid synthesis[26]. Under hypoxic conditions, proliferating cells, including breast cancer cells, mostly employ reductive metabolism of glutamine-derived alpha-ketoglutarate to synthesize acetyl CoA for lipid synthesis that normally enters into the canonical TCA cycle. That pathway is isocitrate dehydrogenase 1 dependent[27,28]. Intermediate metabolites derived from glutamine metabolism, such as antioxidants NADH, glutathione and ammonia, could change the reduction-oxidation status in cancer cells, promote stromal cell autophagy and increase tumor growth and drug resistance[25,29]. Cell studies showed that a high glutamine supply protected MCF7 cells from tamoxifen-induced apoptosis[30]. Amino acid transporter-2, glutaminase 1 (GLS) and glutamate dehydrogenase are three key enzymes involved in glutamine metabolism[31]. Immunohistochemical staining of breast cancer tissues indicated that HER-2 positive and TNBC exhibited the most frequent expression of glutamine metabolism related proteins than other types[32]. Glutamine produces glutamate under the catalytic effect of glutaminase, thus the ratio of glutamate

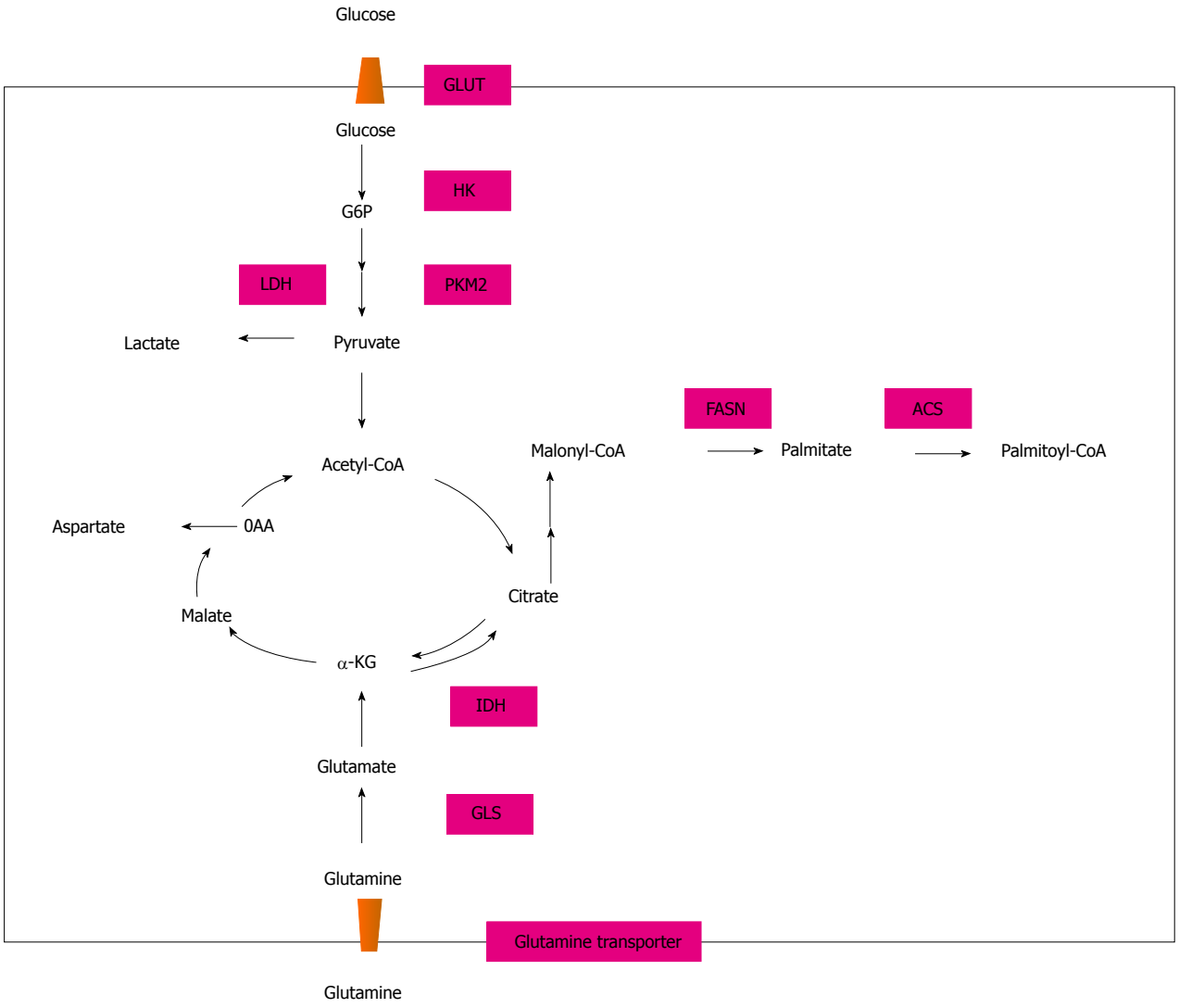

Figure 1 Metabolic reprogramming in malignant cells. Most malignant cells present with increased aerobic glycolysis, fatty acid synthesis and glutaminolysis. The pink circles in the figure show the possible metabolic targets of enzymes or receptors. GLUT: Glucose transporter proteins; HK: Hexokinase; PKM: Pyruvate kinase M; LDH: Lactate dehydrogenase; FASN: Fatty acid synthase; ACS: Acetyl-CoA synthase; IDH: Isocitrate dehydrogenase; GLS: Glutaminase.

to glutamine may indicate the glutamine metabolic activity[33]. Asiago *et al*[34] reported that an elevated level of glutamate was associated with disease outcome in breast cancer patients. Metabolomic analysis of 270 clinical breast cancer samples and 97 normal breast samples showed that breast cancer cells had a higher glutamate-to-glutamine ratio than normal cells, particularly ER- tumor cells[35]. A cell study showed that highly invasive and drug-resistant breast cancer cells were characterized by increased glutamine metabolism with an increased glutamate-to-glutamine ratio and greater expression of glutaminase compared with noninvasive breast cancer cells[36].

Under normal conditions, breast cells utilize circulating lipids for the synthesis of new structural lipids, while breast cancer cells mostly synthesize fatty acids by themselves. The biosynthetic enzyme fatty acid synthase (FASN) is the key enzyme required for the synthesis. FASN expression in breast cancer was first explored during the 1980s when its expression was increased after progestin treatment[37]. Recently, FASN expression has been recognized as an oncogene for its role in carcinogenesis. Upregulation of FASN has been reported in many different tumors, including breast cancer, and it may be associated with tumor development, recurrence and prognosis[38]. Immunohistochemistry staining revealed the highest FASN expression in HER-2 breast tumors and lowest in TNBC tumors, with the studies in breast cancer cells obtaining the same results[39,40]. Vazquez-Martin *et al*[41] postulated a "HER2-FASN axis" that indicated the bidirectional regulation mechanism between FASN and HER2 which could enhance cancer cell proliferation, survival, chemoresistance and metastasis in breast carcinomas.

MODULATION OF METABOLIC REPROGRAMMING IN BREAST CANCER

Breast cancer is classified into four molecular subtypes: Luminal A, luminal B, HER-2 overexpression and basal

types, with type luminal A accounting for about 70%[42]. The estrogen and HER-2 signal pathways play critical roles in breast cancer carcinogenesis, progression and prognosis. They may interact with each other as well as other signal pathways. Since most cancer cells have a high nutrition intake requirement to accommodate cell proliferation and altered metabolism may be a hallmark of cancer development, different molecular subtypes of breast cancer should exhibit distinct metabolic phenotypes. However, to date, we still know much less about the modulation mechanism of tumor-specific metabolic changes, especially in breast cancer[43]. We also know less about how these changes may change molecular phenotypes of breast cancer and affect response to drug treatment.

Although scientists are trying hard to find how signal pathways control the energy metabolism of cancer cells, little is known about the complex network. Hypoxia-inducible factors (HIF) and the proto-oncogene c-Myc are two major regulators in energy metabolism, including glucose, protein and fatty acid metabolisms[44]. Other genes, including *Akt*, *Ras*, *Raf*, *Src* and *EGFR,* may also be involved in glycolysis and activating these genes could cause increased glucose uptake. mTOR inhibitor rapamycin may inhibit cancer cell glucose metabolism by downregulating pyruvate kinase M2 and restoring the susceptibility of breast cancer cells to tamoxifen treatment effectively may be one mechanism of rapamycin[45]. On the other hand, estrogen-induced HIF-1 accumulation in breast cancer cells stimulates glucose uptake *via* the PI3K/Akt signaling pathway[19,46] which also leads to increased mTOR phosphorylation[47]. Another clinical study found that HIF-1 had the highest expression in HER-2 positive breast cancer[21]. It indicated that HIF-1 has crosstalk with the ER and HER-2 signal pathways.

The *c-MYC* gene controls cancer cell glutaminolysis through several targeted genes. MYC is overexpressed in 30%-50% of high-grade breast tumors[48,49]. Increased MYC expression often indicates increased dependency on glutamine and glucose for survival, may have a correlation with drug resistance in breast cancer cells and inhibition of MYC could reverse the drug resistance[50-52]. In antiestrogen resistant breast cancer cells, MYC could activate an unfolded protein response through glucose-regulated protein-78 (GRP78/HSP5A/BiP) and inositol-requiring enzyme-1α (IRE1α/ERN1) and increase c-Jun N-terminal kinase activation and spliced X-box protein-1 to support cell survival[45]. The inhibition of MYC was shown to decrease glutaminase activity, although there were different results in drug resistant breast cancer cells and other cells[50,53,54]. Inhibition of glutaminase reversely could decrease MYC expression[51]. Activation of the Akt/mTOR signal pathway also stimulates uptake of glutamine through increased glutaminase activity[55] and the underlying mechanism may be through eIF4B dependent control of c-Myc translation[56]. In both ER and HER-2 positive breast cancer cells, upregulation of HER-2 is one possible mechanism for endocrine treatment resistance. The crosstalk between ER and HER2 may regulate MYC-mediated glutamine metabolism[52]. ER downregulator fulvestrant may decrease glutamine consumption through inhibition of MYC and glutaminase and consistent expression of MYC may abrogate the effect of rapamycin on glutaminase[52,56], although the highest glutamine metabolic activity was seen in HER2-type breast cancer, which meant a possible correlation between glutamine activity and the HER-2 signal pathway[32].

Although the mechanism of overexpression of FASN in breast cancer cells is still uncertain, it has been proved that the potent lipogenic transcription factor sterol regulatory element-binding protein 1 (SREBP-1), can regulate FASN expression through binding with the site of the FASN promoter with co-activating transcription factors such as NF-γ Sp1 and Spot14[57,58]. Dietary polyunsaturated fatty acids suppress FASN expression through the modulation of NF-γ binding to the FASN promoter by SREBP-1c[59]. PI3k-Akt and the MAPK signal transduction pathway are also thought to be involved in FASN modulation[60,61]. Under hypoxic conditions, *FASN* gene is upregulated *via* the activation of Akt followed by the induction of the *SREBP-1* gene[62]. Inhibition of MAP kinase also decreases transcription from the FASN promoter and reduces FASN expression in MCF7 cells[63]. The mTOR inhibitor rapamycin may also inhibit FASN in breast cancer cells[64]. Recently, a "HER2-FASN axis" is thought to exist which indicates the bidirectional regulation mechanism between FASN and HER2. The highest level of FASN expression in the HER-2 positive breast cancer type also confirms this hypothesis. FASN could also be regulated by estrogen in ER-positive breast cancer cells. Estrogen stimulates FASN expression and inhibiting FASN augments E2-stimulated transcriptional activity and enhances the E2-mediated ER expression synergistically[65].

TARGETING GLYCOLYTIC ENZYMES

As a basic energy resource for cancer cells, many enzymes are involved in glucose metabolism. The efficiency of target metabolism therapy has been proved in enhancing anticancer treatments or overcoming drug resistance in breast cancer cells, including chemotherapy resistance, endocrine therapy resistance and HER-2 targeted therapy resistance. Besides searching for a new agent to block glucose metabolism or induce a switch from glycolysis to mitochondrial respiration, researchers are also making much effort to find the underlying effect of existing agents on metabolic changes. Sorafenib is a multikinase inhibitor and may downregulate GLUT-1 expression in breast cancer cells through AMPK-dependent inhibition of the mTORC1 pathway, inhibit cell proliferation and induce apoptosis[66].

The glucose transporter family consists of 14 sodium-independent facilitative glucose transporters (SLC2A1-14 or GLUT1-14). GLUT1 appears to be the predominant glucose transporter in many types of cancer cells, inclu-

ding breast cancer[67]. A small compound, WZB117, has shown its inhibitory activity on GLUT1 in MCF-7 breast cancer cells[68]. Synergistic anticancer effects of combined WZB117 with other anticancer drugs, cisplatin or paclitaxel, were also observed. Added to the mitochondrial inhibitor, WZB117 was more efficient in inhibiting cell proliferation, which indicated WZB117 may be more effective in aggressive cancer cells that invariably had mitochondrial dysfunction[68].

HK-2, the first regulatory enzyme in glycolysis, has an important role in glycolysis. 2-DG, a glucose analog, binds with HK competitively and inhibits glycolysis. Although as a single agent the antitumor effect was not significant, a study showed that 2-DG combined with trastuzumab inhibited trastuzumab-sensitive and resistant breast cancers in *in vitro* and *in vivo* models of HER-2 positive breast cancers with more efficient inhibition of glycolysis *via* downregulation of heat shock factor 1 and LDHA[69].

LDHA is the enzyme that catalyzes the conversion of pyruvate to lactate. LDHA knockdown stimulates the switch of HER-2-initiated breast cancer cells to mitochondrial oxidative phosphorylation, decreases cell proliferation to hypoxic conditions and interferes with tumorigenicity[70]. Dichloroacetate (DCA), an inhibitor of pyruvate dehydrogenase kinase (PDK), may activate pyruvate dehydrogenase, which is governed by PDK, and facilitate the conversion of pyruvate to acetyl Co-A, which demonstrates the antiproliferative properties in highly metastatic diseases of DCA[71]. The inhibitor of LDH-A selectively inhibits the growth of HER-2-overexpressing cells and enhances the sensitivity of trastuzumab-resistant breast cancers to trastuzumab treatment[69,23]. Furthermore, downregulation of LDH-1 by oxamate shows a synergistical inhibitory effect on taxol-resistant breast cancer cells by promoting apoptosis when combined with taxol[9].

TARGETING GLUTAMINE METABOLISM

In many cancer cells, glutamine is used to replenish the TCA cycle and oxidative phosphorylation instead of glucose to produce enough ATP to support cell proliferation[72]. Glutamine addiction is a common strategy for some cancer cells like breast cancer cells to escape drug treatment. Glutamine transporters or glutaminolysis are becoming a potential pharmacological target to revert resistant cancer cells to respond to the initial therapy. An amino acid transporter SLC6A14, also known as $ATB^{0,+}$, is upregulated specifically in ER-positive breast cancer. Blockade of SLC6A14 in ER-positive breast cancer cells could inhibit mTOR activity, cause cell apoptosis and activate autophagy[73].

Glutaminase, the enzyme that catalyzes glutamine to glutamate has attracted much interest for targeted cancer therapy recently. Two novel glutaminase inhibitors have been discovered: CB-839[74] and 968[51]. CB-839 showed the most potent antiproliferative activity in a TNBC cell line, while no antiproliferative activity was observed in an ER–positive cell line. In xenograft models, CB-839 displayed significant antitumor activity, both as a single agent and in combination with paclitaxel. Compound 968 showed the greatest cytotoxic effect in MDA-MB-231 breast cancer cells. Genome analysis proved that compound 968 could induce changes in many anti-apoptotic and/or promote metastasis-related gene expression and histone modifications as well, which subsequently activate apoptosis and decrease the invasiveness of MDA-MB-231 cells. It also enhanced chemotherapy sensitivity of breast cancer cells when combined with the chemotherapeutic drug doxorubicin.

TARGETING FATTY ACID METABOLISM

FASN is the key biosynthetic enzyme in the fatty acid synthesis pathway that synthesizes long-chain fatty acids palmitate from malonyl-CoA. Acetyl-CoA carboxylase (ACC) carboxylates acetyl-CoA to malonyl-CoA. Upregulation of FASN has been reported both in premalignant lesions and most human cancers. In normal cells, fats are absorbed freely and FASN is downregulated, except in the lactating breast and cycling endometrium. The unique distribution of FASN in different tissues makes FASN an attractive target for cancer therapy. The inhibition of FASN causes depletion of the end product long chain fatty acids and the accumulation of the substrate malonyl-CoA. Evidence showed that inhibition of ACC did not induce cancer cell apoptosis, which meant the accumulation of malonyl-CoA may be the reason for the antitumor effect of FASN inhibition[75,76].

A bidirectional regulation mechanism between FASN and HER2 was illustrated[41,77]. FASN blockade suppresses HER2 overexpression at the transcriptional level with the upregulation of the expression of PEA3, a transcriptional repressor of HER-2. HER-2 overexpression stimulates FASN expression and fatty synthesis and this HER-2 mediated induction can be inhibited by trastuzumab. The combination of FASN inhibitor and trastuzumab stimulates MDA-MB-231/HER-2 cell apoptosis and re-sensitizes trastuzumab-resistant breast cancer through the downregulation of HER-2 expression[78,79]. Menendez *et al*[77] hypothesized that FASN inhibition would result in major changes in the synthesis of phospholipids, which should increase the degradation of HER-2 and enhance the action of the anti-HER-2 antibody trastuzumab.

Furthermore, FASN inhibitor cerulenin demonstrated a strong synergism with docetaxel in HER-2 overexpressing and docetaxel-resistant SK-Br3 cells, which indicated the role of FASN in HER-2-induced breast cancer chemotherapy resistance[80]. FASN blockade also could induce a synergistic chemosensitization of breast cancer cells to other chemotherapy agents, such as paclitaxel, adriamycin, 5-FU and vinorelbine[81-84].

CONCLUSION

Breast cancer is a heterogeneous group of neoplasms

originating from epithelial cells that can be divided into various molecular phenotypes. Targeted therapy, such as endocrine therapy and HER-2 targeted therapy, has achieved great success in breast cancer treatment. However, like chemotherapy resistance, resistance to endocrine therapy and HER-2 targeted therapy can produce discouraging results. Recently, cancer research has focused on dysregulated metabolism in cancer cells and metabolic reprogramming is now considered a hallmark of cancer. More and more evidence supports the idea that dysregulated cellular metabolism may be associated with drug resistance in cancer therapy. In breast cancer, many agents that target specific enzymes in the metabolic pathways, including glycolysis, glutaminolysis and fatty acid synthesis, have been developed or proposed. Some of them have shown the ability to enhance the efficacy of current therapies and resensitize resistant cancer cells and have now been progressed to clinical trials. However, to date, none have been put into routine clinical practice for a couple of reasons. The main reason may be the extremely complex modulation of metabolism and their crosstalk with other signal pathways. Hence, there are three key problems that need to be elucidated: (1) energy pathways may be employed by cancer cells as well as normal cells. The influence or toxicity of metabolic drugs on normal cells should be evaluated carefully besides its antitumor effect. This question is prominent when combining metabolic drugs targeting different pathways to avoid insufficient effects or drug resistance; (2) for breast cancer, different molecular types may possess a specific metabolic phenotype. Even a "good" molecular type of breast cancer, like luminal A, may have recurrent metastasis caused by drug resistance in a relatively short period and so it is critical to find which specific enzymes for specific molecular phenotypes could be promising targets. This understanding will help us better distinguish which altered metabolic phenotypes may have a poorer prognosis and higher invasiveness than other types; (3) it has been postulated that metabolic regulation may have crosstalk with ER and HER-2 signal pathways. The genetic regulators such as c-myc, PI3k/Akt /mTOR and MAPK regulate metabolism as well as ER and HER-2 signal pathways. They form a complex framework, like the "FAS-HER-2 axis" and "c-myc-mTOR axis", which determines the growth, apoptosis and drug resistance of cancer cells. Completely understanding the framework for breast cancer is still a challenge for developing a successful metabolic therapy. Nevertheless, much effort and progress has been made in this field and we hope that, in the near future, targeting tumor metabolic pathways may become an important component of the comprehensive treatment of breast cancer.

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