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EDITORIAL

Transanal total mesorectal excision: Myths and reality

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Abstract

Transanal total mesorectal excision (TaTME) is a new and promising approach for the treatment of rectal cancer. Whilst the experience is still limited, there are growing evidences that this approach might overcome the limits of standard low anterior resection. TaTME might help to decrease the conversion rate especially in difficult patients, and to improve the pathological results, while preserving the urogenital function. Evaluation of data from large registries and randomized studies should help to draw firmer conclusions. Beyond these technical considerations, the next challenge seems to be clearly the safe introduction of this approach, motivating the development of dedicated courses.

Key words: Transanal total mesorectal excision; Bottom up; TAMIS; Laparoscopy; Robotic; Outcomes; Rectal cancer

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Core tip: The experience and evidences regarding the use of transanal total mesorectal excision is still scarce but promising. Preliminary data showed excellent results, without sacrificing the pathological and oncological outcomes. Whilst still in its infancy, further investigations should be encouraged. Data from large registries and randomized trials are awaited before to draw definitive conclusions.

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There is no doubt that low anterior resection (LAR) and total mesorectal excision (TME) have revolutionized the management of rectal cancer and improved its oncological outcomes[1]. On the other hand, the introduction of

minimally invasive surgery for oncological rectal resection has not yet completely convinced the most skeptical open surgeons. Whilst potentially better short-term outcomes have been published favoring laparoscopic approach[2,3], the recent ALaCaRT and ACOSOG Z6051 trials failed to show the non-inferiority of laparoscopic LAR in comparison to open surgery[4,5]. Indeed, there is still a degree of uncertainty, notably regarding the risk of incomplete TME specimen, positive margins, and worse long-term oncological outcomes. To fuel the debate further, other large randomized series did not show inferior pathological or oncological outcomes following laparoscopic LAR[6]. Meanwhile, even the amazing introduction of robotics has not significantly improved the outcomes[7].

To overcome the challenges posed by abdominal TME surgery, a transanal approach has been developed over the last decade, with promising early outcomes. There is growing evidence available including our recent review of transanal TME (TaTME) showing excellent results[8]. However, TaTME is still in its infancy and definitively requires more robust data and longer follow-up. Since the first description of TaTME, a number of relatively large series have been published, showing not only the feasibility of the approach, but also its safety[9,10] even in challenging patients. In our own experience, we have recently shown a low conversion rate, low R1 rate, and an excellent completeness of TME[11-13].

Several parameters and factors pose technical challenges and need special consideration when considering planning TME surgery: (1) dealing with "difficult anatomy" (male, obese, narrow pelvis, post radiation); (2) increasing the sphincter-preserving rate; (3) performing a safe distal rectal stapling; (4) avoiding positive margins; (5) reducing the risk of incomplete TME; (6) improving the oncological outcomes; and (7) offering adequate functional outcomes.

TaTME seems to offer a solution for most of these parameters/factors. The narrow pelvis with a bulky irradiated specimen in an obese male patient is no longer a relative contra-indication to laparoscopic surgery. Starting the most difficult part of the dissection (the lowest part of the pelvis) from the distal end offers obvious advantages. First of all, the distal margin can be assessed precisely and secured with a purse-string before performing the rectotomy. This in turns avoids the need for distal cross-stapling, which can be laparoscopically challenging due to the limited angle of the endoscopic stapler and the pelvic morphology. This often results in multiple firing to complete the transection with the associated risk of anastomotic leak after more than 2 reloads[14]. With TaTME, this is no longer a challenge. Different anastomotic techniques have been proposed, guaranteeing a safe and efficient way to rejoin the bowel[15]. Although, this may increase the rate of sphincter-preserving surgery, it is at the cost of a higher rate of coloanal anastomosis.

Beyond these technical considerations, the interest to proceed with a complete TME is important.

The threat of incompleteness of mesorectal excision was recently shown to be significant after LAR and APE (36% and 13% respectively)[16]. The lowest part of the mesorectum is at risk of being left behind, which is unacceptable from an oncological point of view. Again, starting the dissection from below might help to obtain a more complete TME specimen. Moreover, comparative studies have shown better pathological outcomes after TaTME in comparison to laparoscopic TME[17,18]. The awaited results from the large multicenter registry study (LOREC) should hopefully help to draw more definitive conclusions.

The main challenges for the future of TaTME can be summarized in three different categories: (1) the long-term oncological outcomes; (2) the functional outcomes; and (3) the safe introduction of this approach.

Obviously, the technique is still in its infancy and long-term outcomes are not yet available. Early oncological data seem promising[13], but it is too early to draw definitive conclusions. The COLOR Ⅲ study[19], evaluating TaTME *vs* laparoscopic TME, should provide a more comprehensive overview of the added value of the transanal approach. In addition, quality of life and functional outcomes will be assessed. Based on previous reports[20-22], adequate function has been reported. However, still a high rate of coloanal anastomosis is performed and the risk of worse functional outcomes is possible.

As for any new surgical technique, the danger of widespread rapid and unmonitored adoption without proper training exists. The development of a dedicated curriculum should be established in order to avoid unnecessary preventable complications during the early phase of a surgeon-s learning curve. As already mentioned for robotic surgery and other surgical innovations, training is probably the biggest challenge[23]. Dedicated theoretical and practical courses including cadaver workshops as well as live cases proctoring are key to ensuring the safe introduction of a new surgical technique[24].

In conclusion, TaTME is a promising approach, aiming to overcome the limitations of laparoscopic TME. So far, the published data support its use. Excellent pathological and acceptable short-term clinical outcomes have been reported, however long-term oncological and functional data are still awaited. There is no doubt that TaTME will play a significant role in the evolution of rectal surgery as the drive to perfecting TME and improving outcomes continues.

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REVIEW

Oncogenic fingerprint of epidermal growth factor receptor pathway and emerging epidermal growth factor receptor blockade resistance in colorectal cancer

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Abstract

Epidermal growth factor receptor (EGFR) has been an attractive target for treatment of epithelial cancers, including colorectal cancer (CRC). Evidence from clinical trials indicates that cetuximab and panitumumab (anti-EGFR monoclonal antibodies) have clinical activity in patients with metastatic CRC. The discovery of intrinsic EGFR blockade resistance in Kirsten RAS (KRAS)-mutant patients led to the restriction of anti-EGFR antibodies to KRAS wild-type patients by Food and Drug Administration and European Medicine Agency. Studies have since focused on the evaluation of biomarkers to identify appropriate patient populations that may benefit from EGFR blockade. Accumulating evidence suggests that patients with mutations in EGFR downstream signaling pathways including KRAS, BRAF, PIK3CA and PTEN could be intrinsically resistant to EGFR blockade. Recent whole genome studies also suggest that dynamic alterations in signaling pathways downstream of EGFR leads to distinct oncogenic signatures and subclones which might have some impact on emerging resistance in KRAS wild-type patients. While anti-EGFR monoclonal antibodies have a clear potential in the management of a subset of patients with metastatic CRC, further studies are warranted to uncover exact mechanisms related to acquired resistance to EGFR blockade.

Key words: Epidermal growth factor receptor; Oncogenic signature; Kirsten RAS; BRAF; Cetuximab; Panitumumab; Epidermal growth factor receptor blockade resistance

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Core tip: Epidermal growth factor receptor (EGFR) blockade treatment is a well-established targeted therapy in metastatic colorectal cancer (CRC) patients. However, a limited number of patients benefit from EGFR inhibition, with limited time duration of response. This review article

discusses the most recent updates from the current-state-of-the-science related to molecular pathways of EGFR signaling, the mechanism of action and efficacy of EGFR blockade treatment, and possible molecular pathways related to EGFR blockade resistance in CRC. We further discuss potential mechanisms contributing to targeted EGFR inhibition. Lastly, future perspectives are discussed to shed some light on efforts to overcome this potential challenge in the era of targeted treatment.

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INTRODUCTION

Colorectal cancer (CRC) with an incidence of 1.2 million cases per year is now the third most common cancer in males and second in females[1]. Although there is a minor trend "towards" a decrease in the incidence of the disease, it is yet one of the major health care burden in United States[2]. Despite extensive research, a limited number of targeted agents have been shown to be active in CRC.

Cetuximab and Panitumumab are two new generation monoclonal antibodies targeting epidermal growth factor receptor (EGFR) recently approved by the Food and Drug Administration (FDA) for the management of metastatic CRC in the United States. Although their exact mechanism of action is unknown it is hypothesized that the direct interaction of these antibodies with EGFR results in apoptosis. Herein, we discuss the molecular signature of the EGFR pathway, the possible mechanism of action of anti-EGFR monoclonal antibodies, the clinical consequences of these cell-based interactions in treatment of metastatic CRC, along with emerging resistance to these agents during the treatment of CRC.

ONCOGENIC SIGNATURE OF EGFR PATHWAYS IN CRC

EGFR, also known as ErbB, is a member of the receptor tyrosine kinase family; it stimulates multiple intracellular proto-oncogenic signaling mediators including Kirsten RAS (*KRAS*)[3]. As a 170 kDa transmembrane glycoprotein expressed on cell surface, EGFR has physiological roles in many organs including the epithelium of the gastrointestinal system, bronchial tract and cutaneous tissue[4]. In normal homeostasis, EGFR is activated by binding with external ligands such as growth factors (EGF, epiregulin or amphiregulin). This interaction initiates homodimerization and heterodimerization processes *via* a diverse combination of identical and different members of the family such as ErbB2 (HER-2), ErbB3 (HER-3) and ErbB4 (HER-4)[5]. The ensuing phosphorylation of tyrosine kinase domain results in activation of oncogenic pathways including mitogen activated protein kinase (MAPK) and phosphotidylinositol-3-kinase (PI3KCA) pathways (Figure 1). These signaling axes have been shown to function in many critical pro-survival cellular reactions in cancer cells including protein synthesis, cell growth, cell cycle progression, transformation and invasion. KRAS, a critical growth signal response in cancer cells, is an upstream activator of the MAPK pathway[6] (Figure 1). KRAS-driven MAPK translocates into the cell nucleus, initiates a transcription cascade and promotes cell growth[7]. For example, KRAS activation leads to upregulation of c-myc which fuels proliferation of human colon cancer cells and provides a survival advantage[8]. Signal cascades of KRAS also induce cell cycle progression *via* activation of the transcription factor Elk-1, which ultimately increases the expression of cell cycle promoting proteins such Cyclin D1[9]. Moreover, as a part of the complex network of EGFR signaling, the KRAS driven MAPK pathway interacts with JNK signaling to modulate cellular stress responses which enhance cellular plasticity. This response helps malignant cells to adapt to dynamic microenvironmental changes[10]. In transformed cancer cells, *KRAS* mutations abolish regulation *via* the upstream EGFR loop; the MAPK and PI3KCA pathways, and other pro-survival cascades are continuously activated, leading to distinct cellular behavior[11,12].

Phosphatidylinositol 3-kinase (PIK3CA) is another well-studied signal transducer of the EGFR pathway. In normal homeostasis, activation of PIK3CA by EGFR leads to induction of Akt-mTOR pathway which has been shown to be crucial signal for protein synthesis and cell cycle progression[13]. Activation of PIK3CA also abrogates apoptosis and cellular senescence in cancer cells[14]. PIK3CA-driven mTOR activates Bcl-2 and ultimately inhibits apoptosis[15], indicating that PIK3CA signaling may have an important role in the immortality of transformed cells. PIK3CA activation has also been shown to be related to elevated expression of COX-2 which enhances angiogenesis in CRC[16]. Consistent with evidence from preclinical observations, mutant *PIK3CA* is associated with development of various cancers including CRC[17]. Current thinking suggests that the changes in the gene expression profile caused by activating mutations of PIK3CA may culminate in changes in the proteome of cancer cells and that this transformation enhances cellular growth and invasion by creating distinct oncogenic signatures[18].

BRAF, a member of the RAF kinase family, functions as a serine/threonine protein kinase, and gets activated by the upstream Ras oncogene (Figure 1)[19]. Activating mutations of the BRAF oncogene occur in the kinase domain and the V599E mutation accounts for the vast majority of point mutations (approximately 80%)[20]. Mutant *BRAF* propagates Raf-MAPK signaling in the absence of upstream stimulation and ultimately induces cell growth and proliferation in malignant clones[21].

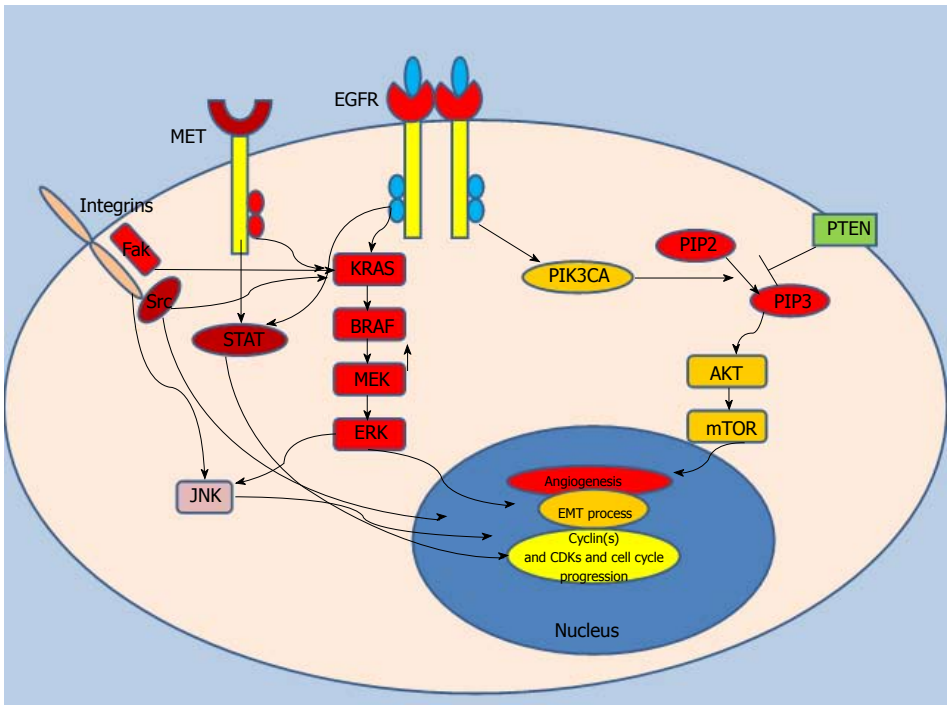

Figure 1 Epidermal growth factor receptor signaling along with co-activated other receptor tyrosine kinases. EGFR: Epidermal growth factor receptor; PIK3CA: Phosphatidylinositol 3-kinase; mTOR: Mechanistic target of rapamycin.

Similar to *PIK3CA* mutations, *BRAF* mutations also transform the protein expression profiles of cancer cells and alter internal metabolism. For example, CRC cells with mutant *BRAF* were found to be more resistant to apoptosis compared to those carrying wild-type BRAF[22]. Moreover, *BRAF* may increase the expression of cell cycle promoting proteins which further enhance the expansion of selected clones[23]. *BRAF* mutations have also been shown to be associated with constitutively activated NF-κB[24], leading to tumor angiogenesis that optimizes the microenviroment for cancer cells[24]. All this evidence suggests that activation of the BRAF oncogene may add further distinct characteristics to the cancer cells' genomic fingerprint.

Src and *STAT*, two other critical oncogenes, have been demonstrated to be involved in the development and progression of epithelial tumors along with cancer angiogenesis, and both mediators operate in the signaling cascade of the EGFR pathway[25]. A study showed that EGFR overexpressing cancer cells bear a 10-fold increase in Src activity compared to low EGFR expressing cancer cells[26], and that increased Src activity has been associated with highly aggressive tumor behavior and metastatic potential in CRC cells[27]. Oncogenic Src, as a non-receptor tyrosine kinase (nRTK), turns on many downstream survival pathways such Ras-MAPK pathway. It further activates receptor tyrosine kinases which create continuous growth signals for cancer cells[28]. Activating mutations of *Src* are related to adverse outcomes in CRC[29,30]. STATs are also activated by EGFR[25] and function as transcriptional factors in downstream pathways of receptor tyrosine kinases and cytokine receptors[31]. Induction of STATs through EGFR signaling[32] may also fuel angiogenesis in the tumor microenvironment[33]. Although activation of STATs has shown to be related to enhanced proliferation in CRC cancer cells[34], the exact role of STATs in development and progression of CRC remains to be elucidated.

Altogether, current evidence indicates intricate EGFR signaling. Variant alterations in the downstream signal transducers of EGFR are likely responsible for the change in expression profiles and molecular behavior of cancer cells.

EMERGENCE OF MONOCLONAL CHIMERIC ANTIBODIES TO EGFR

Considering the diverse oncogenic pathways activated by EGFR, it has become a promising target for therapy in various epithelial tumors[35,36]. The aforementioned studies led to research targeting EGFR signaling *via* different approaches, including small molecule inhibitors of receptor tyrosine kinases such as erlotinib and monoclonal antibodies to neutralize the receptor *via* an internalization and degradation process.

The initial investigations in this field were conducted using murine anti-EGFR antibodies. In these studies, both agonist and antagonist antibodies were tested[37], and antagonist antibodies inhibited the proliferation of the malignant clones with high affinity binding[38]. Since murine antibodies are recognized as foreign antigens in humans, human-mouse chimeric antibodies were generated to further study this effect and they were shown to have superior biological efficacy compared to

murine antibodies in human tumor xenograft models[39]. Cetuximab, a chimeric antibody against EGFR, was demonstrated to have 10 times higher affinity to EGFR compared the murine antibodies (M225)[40], and limited toxicity was observed in phase Ⅰ clinical studies[41].

The mechanism of action of anti-EGFR antibodies was at first attributed to internalization of receptors bound by the anti-EGFR antibodies[42]. Further studies demonstrated that the EGFR blockading agents not only catalyze the removal of the receptors but also inhibit receptor tyrosine kinase activity, and induce apoptosis *via* cell cycle arrest in colonic adenocarcinoma cell lines[43]. Besides its-single agent activity, an enhanced anti-cancer effect of cetuximab was observed in combination with the topoisomerase inhibitor (irinotecan) in human CRC xenograft models[44]. These discoveries led to further studies to elucidate the role of EGFR blockade in modern cancer treatment, and clinical trials were opened for enrollment in different clinical settings.

CLINICAL TRIALS TO ASSESS THE ROLE OF EGFR BLOCKADE IN CRC TREATMENT

To further investigate the promising results observed in preclinical studies, early phase and clinical efficacy trials were designed (Table 1). A phase Ⅰ safety trial was conducted to determine the optimal biological dose, and the doses achieved at maximal systemic clearance were well tolerated in both monotherapy and combined treatment models[45]. In a phase Ⅱ clinical trial of 120 irinotecan-resistant CRC patients with positive EGFR expression, cetuximab reversed the resistance to irinotecan with a 22.5% major objective response rate and 17% radiologic response rate in a combination treatment model[46]. The promising radiological response rate had already resulted in FDA approval for the cetuximab treatment for metastatic CRC patients. In order to solidify the response observed in the original study, cetuximab was investigated as a single agent treatment in patients with advanced stage refractory CRC[47]. In this study, 9% partial response rate along with a median survival of 6.4 mo was reported after cetuximab monotherapy in CRC patients expressing EGFR. To further investigate these findings and to test the superiority of combination treatment to monotherapy, a randomized phase Ⅱ clinical trial was conducted[48]. Patients were randomized to receive cetuximab and irinotecan combination *vs* cetuximab alone. Patients in the combination arm demonstrated a 22.9% partial response compared to 10.8% in the cetuximab monotherapy arm (P = 0.007). While progression free survival (PFS) was significantly improved in patients receiving combination therapy there was no overall survival (OS) difference between the groups. A subsequent study demonstrated limited survival benefit in patients treated with cetuximab compared to best supportive care (6.1 mo *vs* 4.6 mo respectively)[49]. Although EGFR expression was considered as a predictor of EGFR blockade response in early clinical trials, this concept has since changed and EGFR expression is no longer considered a biomarker in CRC patients[50].

The observed improvement in response rate in the treatment of irinotecan-refractory cases led to further studies in treatment-naïve patients. The CRYSTAL study showed significantly improved PFS in metastatic CRC patients who received a cetuximab plus FOLFIRI (bolus 5-fluoruracil/leucovorin chemotherapy plus irinotecan) regimen compared to patients who underwent FOLFIRI treatment alone (Table 1). However, there was no significant difference in OS between the two groups[51]. Subsequent analysis of the study demonstrated improved OS (approximately 3.5 mo) in patients with wild-type KRAS who underwent cetuximab treatment. No significant response was observed in *KRAS*-mutant patients[52]. This study led to the FDA approval of cetuximab as a first line treatment in combination with FOLFIRI in patients with metastatic CRC. In the OPUS study, cetuximab was combined with FOLFOX-4 and an improved PFS [hazard ratio (HR) 0.567, P = 0.0064] was observed in KRAS wild-type metastatic CRC patients who received combination therapy[53]. In order to assess the role of cetuximab in earlier stages of the disease, N0137 trial was conducted: Stage Ⅲ CRC patients were enrolled to receive cetuximab plus FOLFOX-6 combinations *vs* FOLFOX-6 in adjuvant settings after initial resection[54]. After a of median 28 mo follow-up in both wild-type and mutant *KRAS* groups, results of this trial showed no benefit of cetuximab when added to FOLFOX-6 regimen in the setting of locally advance disease. In order to compare the efficacy of cetuximab in combinations with FOLFOX-6 and FOLFIRI regimens as a neoadjuvant treatment in unresectable metastatic CRC patients, a phase Ⅱ clinical trial (CELIM trial) was conducted[55]. Although there was no significant survival difference between the two groups, addition of cetuximab resulted in higher response rates compared to historical controls (FOLFOX and FOLFIRI). A total 36 of 116 patients were able to receive R0 resection. The result of this study was also consistent with previous studies demonstrating improved outcomes limited to patients with wild type KRAS.

Panitumumab, another monoclonal antibody for EGFR blockade, has also been investigated in CRC patients. This agent received FDA approval after prolonged PFS was demonstrated in an open-label phase Ⅲ clinical trial in which patients were enrolled to receive single-agent panitumumab *vs* best supportive care (median PFS, 8 wk *vs* 7.3 wk; HR, 0.54; 95%CI: 0.44-0.66)[56]. Follow-up analysis of this study showed that the survival benefit was again, limited to wild-type KRAS CRC patients[57]. A retrospective study suggested potential resistance in patients with BRAF V600E mutation and requirement of wild-type BRAF for clinical benefit, along with wild-type KRAS[58]. Currently this drug is also approved as a first line agent in combination with FOLFOX4 in metastatic CRC patients with wild-type KRAS due to observed

Table 1 Clinical trials investigating the impact of epidermal growth factor receptor blockades in colorectal cancer patients

Ref.	Year	Sample size	Mutation status	Treatment groups	Special considerations	Summarized findings
Saltz *et al*[46]	2001	120	EGFR +	Cetuximab plus Irinotecan		22.5% major objective response rate 17% radiologic response rate
Saltz *et al*[47]	2004	57	EGFR +	Cetuximab		9% (CI: 3% to 19%) partial response Median survival of 6.4 mo. *P* value ??
Cunningham *et al*[48]	2004	329	EGFR +	Cetuximab plus irinotecan *vs* cetuximab alone		22.9% partial response in combination arm, 10.8% partial response in the cetuximab monotherapy arm No difference in OS
Jonker *et al*[49]	2007	572	EGFR +	Cetuximab compared to best supportive care		6.1 mo OS in treatment arm *vs* 4.6 mo with supportive care. $P = 0.005$ Quality of life was better preserved in the cetuximab group ($P < 0.05$)
Van Cutsem *et al*[56]	2007	463	EGFR+	Panitumumab *vs* best supportive care		Median PFS 8 wk in treatment arm compared to 7.3 wk in patients receiving supportive care (HR 0.54; $P < 0.0001$)
Amado *et al*[57]	2008	427	EGFR+ Wild type KRAS *vs* mutant *KRAS*	Panitumumab *vs* best supportive care	Reanalysis of Van Cutsem 2007	In patients with wild type KRAS median PFS was 12.3 wk for panitumumab *vs* 7.3 wk for supportive care Response rates to panitumumab was 17% for patients with wild type KRAS compared to 0% in patients with mutant *KRAS*
Van Cutsem *et al*[51] CRYSTAL Trial	2009	1198	EGFR +	Cetuximab plus FOLFIRI *vs* FOLFIRI alone		HR for PFS in combination therapy 0.85 ($P = 0.048$) when compared to FOLFIRI alone There was no difference in OS (HR 0.93; $P = 0.31$) Although not significant PFS was improved with cetuximab in patients with wild-type-KRAS (HR 0.68; $P = 0.07$)
Folprecht *et al*[55] CELIM trial	2010	113	Wild type KRAS *vs* mutant *KRAS*	Cetuximab plus FOLFOX *vs* Cetuximab plus FOLFIRI *vs* historical controls	Neoadjuvant setting	No survival difference between the two groups Higher response rates compared to historical controls (FOLFOX and FOLFIRI) Total 36 of 116 patients were able to receive R0 resection Improved outcomes limited to patients with wild type KRAS
Van Cutsem *et al*[52]	2011	1198	EGFR+ Wild type KRAS *vs* mutant *KRAS*	Cetuximab plus FOLFIRI *vs* FOLFIRI alone Wild type KRAS *vs* mutant *KRAS*	Reanalysis of data from CRYSTAL trial	Patients with wild type KRAS had improvements in OS from 20 to 23.5 mo PFS from 8.4 to 9.9 mo and response rates 39.7% to 57.3% with addition of Cetuximab to FOLFIRI
Bokemeyer *et al*[53] OPUS study	2011	315	Wild type KRAS/BRAF *vs* mutant *KRAS/BRAF*	Cetuximab plus FOLFOX		Improved PFS (HR 0.567) and response (OR 2.55) in patients with KRAS wild-type tumors
Alberts *et al*[54] N0137 trial	2012	2686	Wild type KRAS *vs* mutant *KRAS*	Cetuximab plus FOLFOX *vs* FOLFOX alone	Locally advanced disease	No additional benefit of Cetuximab when added to FOLFOX-6 regimen in the setting of locally advance disease

EGFR: Epidermal growth factor receptor; PFS: Progression free survival; OS: Overall survival.

improvement in PFS in a randomized phase Ⅲ clinical trial (PRIME trial; median PFS, 9.6 mo *vs* 8.0 mo; $P = 0.02$)[59]. A small phase Ⅱ trial evaluated Panitumumab in 33 wild-type KRAS metastatic CRC patients whom were deemed ineligible for chemotherapy. The study demonstrated a PFS rate of 36.4%, objective response rate of 9.1% and stable disease in 54.5% of patients. The median PFS was 4.3 mo and OS was 7.1 mo, indicating a role for Panitumumab monotherapy in patients who are not eligible for chemotherapy[60].

EMERGING RESISTANCE TO EGFR BLOCKADE

The promising results observed with monoclonal antibodies either as monotherapy or in combination treatments in metastatic CRC patients were encouraging. However the relatively small magnitude of improvement in PFS was an early sign of emerging resistance to cetuximab therapy. Therefore, further clinical and translational studies were conducted to elucidate possible underlying mechanisms related to EGFR blockade resistance.

EGFR mutations were considered as a possible mechanism for EGFR blockade resistance in CRC patients. However, in a clinical study, only one out of the 293 CRC patients was found to be harboring an EGFR mutation[61]. Considering the rarity of EGFR mutations, they are unlikely to be a common cause of resistance to EGFR blockade in CRC patients.

As mentioned above, a retrospective study first revealed that the *KRAS*-mutant CRC patients did not

benefit from cetuximab therapy[52]. In a study by De Roock *et al*[62], KRAS 13D mutation was reported to be associated with cetuximab response unlike other *KRAS* mutations. However another retrospective study did not confirm this favorable outcome[63]. Whether *KRAS* 13D-mutant CRC tumors have a distinct clinical behavior from other *KRAS* mutations warrants further study. Similar to KRAS, a clinical study found NRAS to be a biomarker of anti-EGFR antibody resistance in CRC patients[64]. This association of NRAS mutations with EGFR blockade resistance needs to be validated in future studies.

Since disruptions downstream of EGFR cause autonomous activation of the signaling pathway and potential intrinsic resistance to EGFR blockade, clinical studies also investigated the utility of *BRAF* mutations as a biomarker in CRC patients (Figure 2). In two clinical trials, patients with CRC harboring *BRAF* mutations were reported to have a poor response to cetuximab; indicating *BRAF* mutations could also limit the efficacy of anti-EGFR treatment[58,65]. A large retrospective multicenter study looking at survival in patients with metastatic CRC and their mutation status; also reported similar findings[64]. Although current evidence strongly suggests that *BRAF* mutations might be a negative biomarker for monoclonal anti-EGFR treatment, guidelines do not currently preclude such patients from this targeted treatment approach.

A study of 23 *PIK3CA*-mutant metastatic CRC patients reported no resistance to anti-EGFR treatment[66]. However, in another cohort of 15 *PIK3CA*-mutant CRC patients, no objective response to panitumumab and cetuximab treatment was observed[67]. A recent study of patients with metastatic CRC harboring wild-type KRAS showed a trend towards improved PFS in patients with wild-type PIK3CA compared to *PI3KCA*-mutant patients who received cetuximab either as a monotherapy or in combination with chemotherapy (P = 0.06)[68]. Furthermore, a meta-analysis of 576 CRC patients reported a significantly lower PFS in patients harboring a *PIK3CA* mutation compared to patients with wild-type PIK3CA when treated with EGFR blockade[69]. Although a majority of the aforementioned studies suggest resistance to EGFR blockade in *PIK3CA*-mutant metastatic CRC patients with wild-type KRAS, the absence of a consensus precludes making a clear conclusion in this specific patient group. A recent study investigated the role of PTEN expression in anti-EGFR blockade therapy and reported potential resistance to cetuximab treatment in metastatic CRC patients with low PTEN expression[70]. Another study also demonstrated a significantly diminished response and worse PFS in patients with loss of PTEN function (32 wk *vs* 14 wk, P < 0.0001)[71]. Loss of PTEN expression was also studied in patients with KRAS wild-type CRC which again suggested a lack of benefit from EGFR blockade[68]. While accumulating evidence supports the hypothesis that PTEN loss may result in potential intrinsic resistance to EGFR blockade in CRC patients, further studies are needed to understand exact role of dysregulation of this gene.

The *MET* proto-oncogene, a receptor tyrosine kinase that belongs to the hepatocyte growth factor receptor, is also related to EGFR blockade resistance in CRC patients[72]. A mechanistic study suggested that, resistance essentially arises in cancer stem cells *via* increased rebound activity of the *MET* oncogene upon inhibition of EGFR signaling by monoclonal antibodies[73]. Another study identified increased Met activity driven by oncogenic Src and this upregulation was found to be related to cetuximab resistance[74]. Increased TGF-α expression mediated by the Met oncogene could be also another oncogenic pathway related to cetuximab resistance[75]. A mechanistic study reported that inhibition of both the Met and EGFR pathways by a bispecific monoclonal antibody may abrogate EGFR blockade resistance[76]. Although these studies suggest that upregulation of this tyrosine kinase pathway may be related to EGFR blockade resistance, the exact mechanism of resistance with Met activity is yet to be elucidated.

POSSIBLE MECHANISMS OF RESISTANCE IN WILD TYPE KRAS PATIENTS

The response to EGFR blockade even in metastatic CRC patients with wild-type KRAS is unfortunately limited: Following an initial honeymoon period, a majority of patients develop disease progression within months of initiation[77]. Researchers investigated pathways possibly related to resistance in wild-type KRAS patients. An animal model study demonstrated that human epidermal growth factor receptor 2 (HER-2) amplification was associated with cetuximab resistance in a subset of metastatic CRC patients with wild-type KRAS, NRAS, BRAF and PIK3CA[78]. The authors also reported prolonged responses with concurrent inhibition of HER-2 and EGFR in their model. Another mechanistic cell line study corroborated the association of aberrant ERBB2 (HER-2) signaling with EGFR blockade resistance[79]. In a retrospective cohort study, HER-3 overexpression was also suggested to be a predictor of EGFR blockade resistance in metastatic CRC patients who underwent cetuximab and irinotecan treatment[80]. Similar to the aforementioned studies, all patients included in this study were also harboring wild-type KRAS. A recent translational study examined the emergence of resistance in CRC cells sensitive to EGFR blockade[81]. The authors examined the ultimate effect of continuous cetuximab treatment on the development of cetuximab-resistant clones. One of the cell lines developed a rescue mechanism by *KRAS* amplification and the other acquired a *KRAS* mutation. In the same study, the authors studied human samples and reported acquisition of *KRAS* mutation in 6 of 10 cases and *KRAS* amplification in another patient who developed resistance to cetuximab after an initial response. In another recent translational study, authors tested their theory with mathematical modeling; they hypothesized that *KRAS*-mutant CRC subclones might exist prior to initiation of anti-EGFR

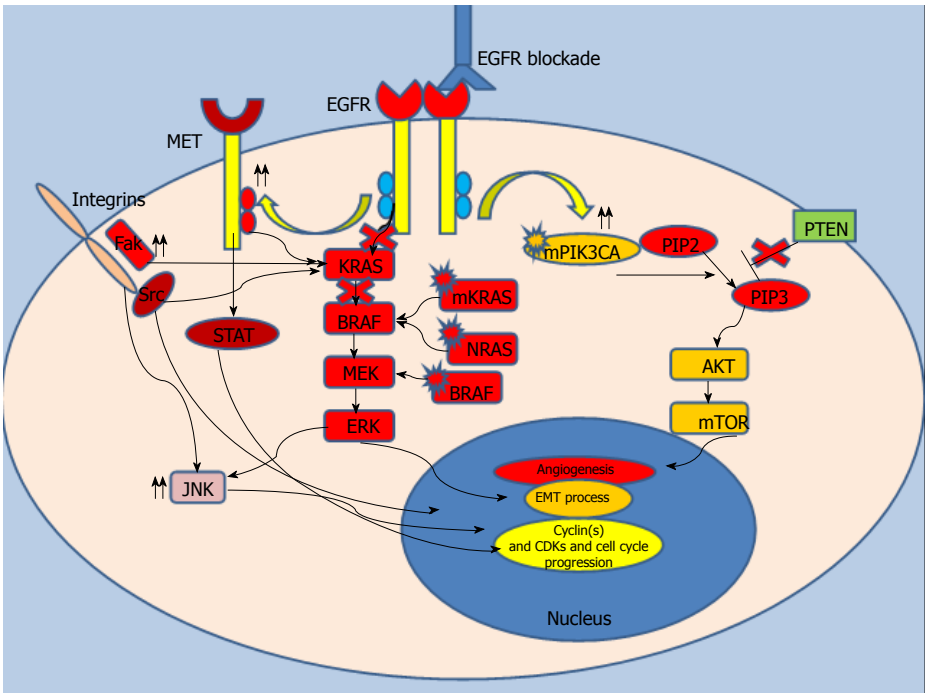

Figure 2 Possible resistance pathways to epidermal growth factor receptor blockade; gain of mutations in KRAS, BRAF, and PIK3CA and upregulation of other receptor tyrosine kinases. EGFR: Epidermal growth factor receptor; mTOR: Mechanistic target of rapamycin; PI3KCA: Phosphotidylinositol-3-kinase.

treatment[82]. According to this model, anti-EGFR monoclonal therapy results in the selective proliferation of resistant subclones. The time required for these subclones to become radiologically detectable corresponds to the time of disease progression and development of EGFR blockade resistance. This hypothesis is also supported by tumor heterogeneity, a consequence of variant clones arising from a single parental clone due to continuous diverse genetic alterations throughout carcinogenesis and metastasis. These diverse genetic signatures have also been demonstrated to cause mixed clinical responses to cytotoxic chemotherapy. Moreover, a recent whole-exome sequencing study of 129 wild-type KRAS CRC tumor samples identified potential resistance mechanisms resulting from mutations in other growth factor pathways such as FGFR, and PDGFRA[83]. This data suggests activation of potential bypass pathways related to other receptor tyrosine kinases abrogating the inhibitory effect of EGFR blockade.

Another possible resistance mechanism to anti-EGFR treatment could be attributed to ineffective structural interaction between the drug and its receptor[84]. Mutations in the extracellular domain of EGFR hindering the binding of cetuximab to EGFR were demonstrated in 2 of 10 study participants with resistance to cetuximab. Moreover, one of those two cases responded to panitumumab. Since the number of the patients in the study was limited, this specific mutation should be further studied in larger populations.

Some recent studies indicate that certain signaling mediators which inhibit apoptosis could potentially rescue malignant cells during initiation of anti-EGFR monoclonal treatments[85,86]. A recent study investigated acquired EGFR blockade resistance by analyzing circulating cancer cells and reported progressive genomic alterations throughout the acquisition of resistance suggesting that genomic instability in cancer cells may execute an important role in EGFR blockade resistance[87]. Activation of other oncogenic tyrosine kinases by the local microenvironment with closed loop feedback might be another key mechanism of resistance in wild type KRAS patients[73]. Further studies are required to elucidate the impact of tumor stroma on EGFR blockade resistance.

FUTURE PERSPECTIVES

Given the limited duration of responses to EGFR blockade and the inevitable development of resistance in both *KRAS* mutant and KRAS wild-type patients, studies were enrolled to optimize treatment outcomes. Based on potential mechanisms of resistance to EGFR blockade identified in preclinical studies, clinical trials have been designed to combat resistance. Considering the role of *PIK3CA* mutations, a phase Ⅱ trial investigated the combination of cetuximab with PX-866, an irreversiblepan-isoform inhibitor of PI3KCA, in 86 patients with KRAS wild type metastatic CRC. The authors reported no improvement in PFS, objective response rate, or OS with the addition PX-866[88]. Combinations of cetuximab with HER-2 and HER-3 targeting agents are currently being studied in clinical trials. The combination of cetuximab with pertuzumab (a monoclonal antibody that disrupts the dimerization of HER-2) showed clinical activity, but produced intolerable side effects and toxicity[89]. Studies

combining cetuximab with HER-3 antagonists have demonstrated promising preliminary data, although final results are yet to be reported[90].

A xenograft study investigated a new agent to overcome *EGFR* mutation-related resistance. Sym004, a novel mixture of two nonoverlapping anti-EGFR monoclonal antibodies, has demonstrated binding and ligand-dependent activation in patients with *EGFR* mutations conferring resistance to conventional anti-EGFR antibodies in functional studies[91]. Phase Ⅰ trials of Sym004 in metastatic CRC patients with wild type *KRAS* mutation and previous EGFR blockade response showed tumor radiologic regression in 17 of 39 patients (44%) and partial response in 5 patients (13%)[92]. Phase Ⅱ clinical trials of Sym004 and early phase studies of other targeting agents of the EGFR pathways are currently being investigated which may enlighten the utility of concurrent/simultaneous inhibition of prosurvival pathways to abrogate EGFR blockade resistance.

CONCLUSION

Current state-of-the-science endorses clinical activity of the EGFR blockade in selected subsets of patients with treatment naïve and refractory metastatic CRC. However, growing evidence has advanced our understanding of the limitations of anti-EGFR treatment.

Although cetuximab and other monoclonal anti-EGFR antibodies effectively inhibit EGFR signaling; their clinical activity is limited to a short period of time. Disease heterogeneity, created by continuous and dynamic genetic alterations, distinct mutational signatures in the EGFR downstream signaling pathways, and rebound activation of other growth signals appears to be the primary driving force rescuing cancer cells from apoptosis. New subclones with new oncogenic fingerprints arising as a consequence of genomic instability, appear to be one of the most challenging factors in the era of targeted treatment. Frequently, this dynamic process and the associated genetic plasticity overcome the inhibitory effect of targeted agents and ultimately, disease progression occurs despite optimal treatment. Moreover, the addiction of cancer cells to a single oncogenic pathway appears to be limited, and the co-activation of rebound survival pathways further limits the impact of a single targeted agent. The lack of durability of the clinical responses to EGFR blockade observed in the aforementioned studies also represents the phenotypic characteristics of dynamic changes in genotype. Whether combinations of targeted agents along with alternate treatment cycles can overcome this acquired resistance requires further clinical studies. Studies are warranted to better understand the underpinnings of dynamic genomic alterations and its role in acquired resistance to targeting agents including EGFR blockades.

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REVIEW

Role of Akt signaling in resistance to DNA-targeted therapy

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Abstract

The Akt signal transduction pathway controls most hallmarks of cancer. Activation of the Akt cascade promotes a malignant phenotype and is also widely implicated in drug resistance. Therefore, the modulation of Akt activity is regarded as an attractive strategy to enhance the efficacy of cancer therapy and irradiation. This pathway consists of phosphatidylinositol 3 kinase (PI3K), mammalian target of rapamycin, and the transforming serine-threonine kinase Akt protein isoforms, also known as protein kinase B. DNA-targeted agents, such as platinum agents, taxanes, and antimetabolites, as well as radiation have had a significant impact on cancer treatment by affecting DNA replication, which is aberrantly activated in malignancies. However, the caveat is that they may also trigger the activation of repairing mechanisms, such as upstream and downstream cascade of Akt survival pathway. Thus, each target can theoretically be inhibited in view of improving the potency of conventional treatment. Akt inhibitors, *e.g.*, MK-2206 and perifosine, or PI3K modulators, *e.g.*, LY294002 and Wortmannin, have shown some promising results in favor of sensitizing the cancer cells to the therapy *in vitro* and *in vivo*, which have provided the rationale for incorporation of these novel agents into multimodality treatment of different malignancies. Nevertheless, despite the acceptable safety profile of some of these agents in the clinical studies, with regard to the efficacy, the results are still too preliminary. Hence, we need to wait for the upcoming data from the ongoing trials before utilizing them into the standard care of cancer patients.

Key words: Phosphatidylinositol 3 kinase/Akt; Platinum; Taxane; Antimetabolite; Radiation

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Core tip: The Akt pathway plays an important role in resistance to several cytotoxic agents, targeted drugs and radiation. Exposure to these drugs will stimulate the Akt survival pathway leading to a decreased response to these drugs. In model systems inhibition of the Akt pathway enhanced the cytotoxicity of drugs like taxanes, antimetabolites, platinum analogs, several targeted drugs

and radiation. Akt inhibitors offer a new opportunity to increase the efficacy of currently used drugs and of radiotherapy.

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AKT PATHWAY SIGNALING OVERVIEW

The Akt signal transduction pathway controls most hallmarks of cancer, including metabolism, cell survival, cell cycle progression, regulation of apoptosis, protein synthesis, motility, and genomic instability by phosphorylation of the substrates[1]. Aberrant loss or gain of Akt activation has been associated with the development of various diseases, *e.g.,* diabetes, autoimmune diseases, and cancer[2-5].

The Akt pathway consists of phosphatidylinositol 3 kinase (PI3K), mammalian target of rapamycin (mTOR), and the transforming serine-threonine kinase Akt protein isoforms (further referred to as Akt), also known as protein kinase B (PKB) and phosphate and tensin homologue (PTEN) as a critical tumor suppressor. PI3K enzymes phosphorylate phosphatidylinositol-4,5-biphosphate (PIP2) to generate phosphatidylinositol-3,4,5-triphosphate (PIP3) at the cell membrane that are required for the recruitment and activation of Akt[6,7] (Figure 1). These phospholipids are constitutively elevated in most cancer cells. Docking of Akt to the cell membrane causes a conformational change, which in turn leads to phosphorylation of the two critical amino acid residues, threonine 308 and serine 473, and finally leads to the activation of Akt[8]. After the activation, Akt is translocated to intracellular compartments where it phosphorylates several substrate proteins. The downstream targets of Akt are numerous due to the multiple interactions with its consensus sequence[1]. In summary, the most important effects of Akt activation are: (1) cell survival through inhibition of BAD, caspase-9, and FOX[9-11]; (2) cell proliferation and gluconeogenesis through inhibition of GSK3, P21, P27, *etc.*[12]; and (3) protein synthesis and cell growth through activation of mTOR[13] (Figure 1).

To date, 3 Akt family members have been identified in mammals, *i.e.,* Akt1 (also known as PKBα), Akt2 (PKBβ) and Akt3 (PKBγ). Having shown highly conserved properties, these homologues may be activated by the same mechanism[14]. However, being encoded by three different regions at 14q32, 19q13, and 1q44, respectively, these three isoforms are distinct substrates with distinct physiological outcomes, and also opposing to each other. Accumulating evidence casts Akt1 and Akt2 function almost in contrary to each other in modulating phenotypes associated with migration and invasion. Akt isoforms contain an N-terminal pleckstrin homology (PH) domain, a central catalytic domain, and a C-terminal regulatory region. The PH domain can bind phosphatidylinositol lipids (*e.g.,* PIP3) with high affinity and targets Akt to the cell membrane[15].

Regulation of Akt, on the other hand, is mainly achieved through PTEN, which antagonizes PI3K. *PTEN* is a tumor suppressor gene that is frequently mutated in different types of cancer, and loss of PTEN leads to elevation of PI3K lipid products and thus activating the Akt pathway[16]. Thus, PTEN negatively regulates the Akt pathway, while loss of PTEN results in overactive Akt, which induces proliferation and promotes survival by inhibiting apoptosis[10,17]. Among the three Akt isoforms, Akt2, is exclusively having carcinogenic properties in PTEN-deficient solid tumors[18].

Despite many breakthroughs in elucidating the cancer behavior and possible mechanisms leading to developing different treatments, resistance is still a problem. The main goal of cytotoxic cancer therapy is to eliminate irregularly dividing cancer cells by targeting DNA synthesis or the mitotic apparatus. Different molecules, genes, proteins and signal transduction pathways are involved in this complicated process[1,19,20]. Resistance is often related to uptake, metabolism or alterations in the target. Besides, many studies demonstrated the modulation of key signaling pathways by the DNA-targeted therapies (reviewed in the following sections). The PI3K/Akt signaling pathway being mutated in a high percentage of malignancies[20] is widely implicated in tumor growth, which may also render tumor cells resistant to chemotherapeutic drugs[5]. Thus, inhibition of this pathway should foil local tumor growth. Many trials are underway to investigate whether adding inhibitors targeting PI3K/Akt pathway may improve the efficacy of the conventional regimen by reducing the apoptotic threshold[21]. Here, we review the literature on the potential value of modulating Akt pathway in view of improving the cytotoxicity of DNA-targeted anticancer drugs and radiotherapy.

METHODS: A SYSTEMATIC BEST EVIDENCE REVIEW

We looked for publications studying the effects of the approved or tested DNA-targeted cytotoxic agents on the Akt signaling using the Medline *via* PubMed database. The inclusion criteria consisted of studies on modulation of Akt signaling by DNA-targeted cytotoxic agents, *i.e.,* platinum agents (cisplatin, carboplatin, oxaliplatin), taxanes (paclitaxel, docetaxel), antimetabolites (gemcitabine, fluorouracil, pemetrexed), and radiation in glioblastoma, mesothelioma and lung, ovary, and pancreas cancers, including their synonyms, with no language restriction as of September 2014. Search terms related to the Akt modulation were "p-Akt" OR "pAkt" OR "phospho-Akt"

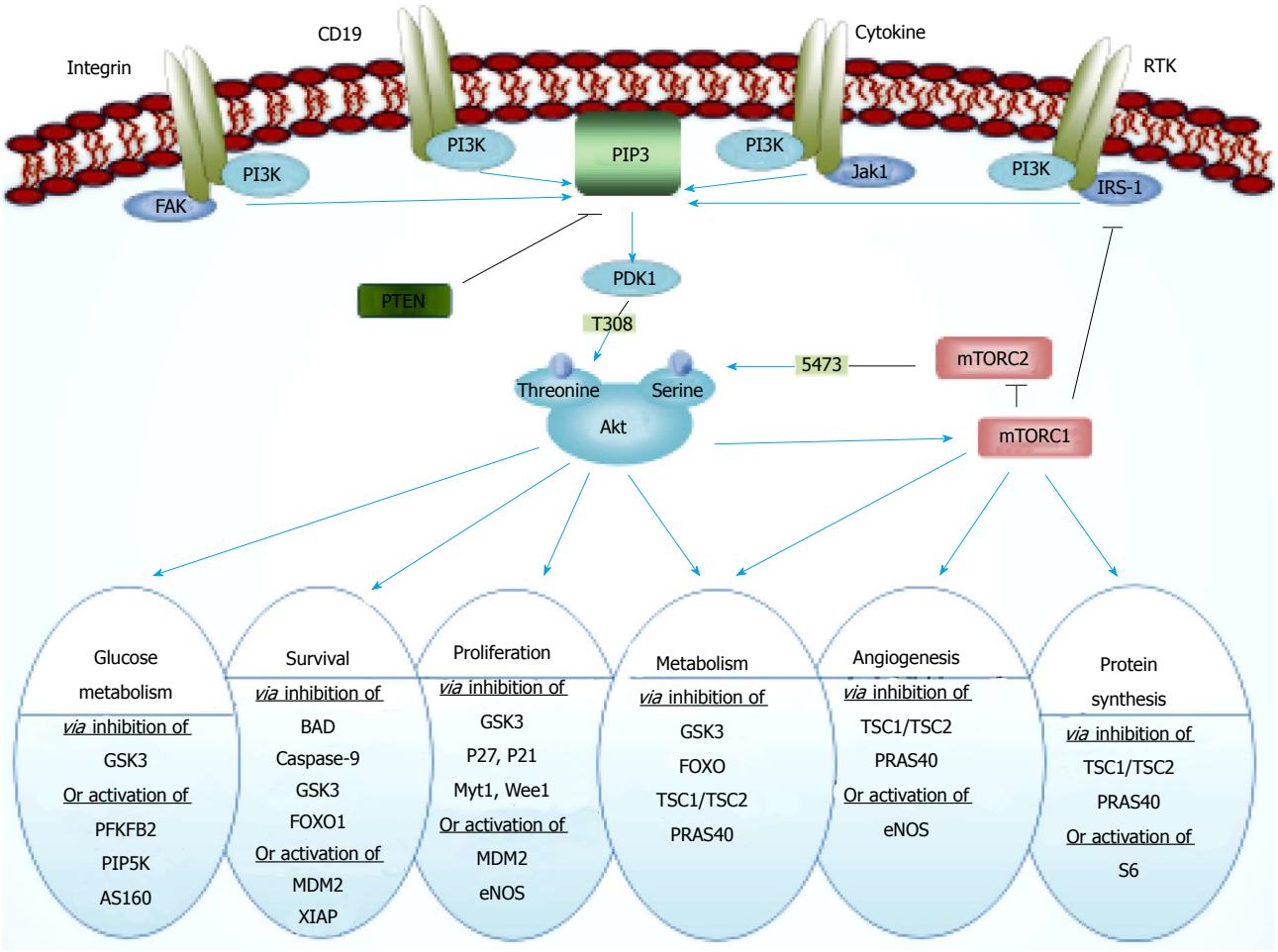

Figure 1 Phosphatidylinositol 3 kinase/Akt pathway. Activated RTKs activate PI3K through direct binding or through tyrosine phosphorylation of scaffolding adaptors, such as IRS1, which then bind and activate PI3K. PI3K phosphorylates PIP2 to generate PIP3, in a reaction that can be reversed by the PIP3 phosphatase PTEN. Activation of PI3K results in membrane recruitment and thus activation of Akt protein. Akt regulates cell growth and many other cellular processes through its effects on mTOR pathways and thus regulates glucose metabolism, protein synthesis, mitochondrial metabolism, lipid metabolism, adipogenesis, lipogenesis, angiogenesis, autophagy, proliferation and cell growth. Other targets of Akt include insulin receptor substrate-1 (IRS-1), glycogen synthase kinase 3 (GSK3), phosphodiesterase-3B (PDE-3), B cell lymphoma-2-associated death promoter (BAD), human caspase-9, Forkhead box (FOX) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) transcription factors, endothelial nitric oxide synthase (eNOS), Rapidly Accelerated Fibrosarcoma (Raf) kinases, P21CIP1/WAF1 (P21; a potent cyclin-dependent kinase inhibitor), P27Kip1, Tuberous Sclerosis Complex 2 (TSC2; also known as Tuberin), X-linked inhibitor of apoptosis protein (XIAP; also known as inhibitor of apoptosis protein 3 and baculoviral IAP repeat-containing protein 4), and Mouse Double Minute 2. Following the activation, Akt phosphorylates and blocks the molecules involved in the apoptotic pathway, including FOX, Caspase-9 and BAD. In addition to the inhibition of proapoptotic factors, Akt can activate the transcription of antiapoptotic genes through the activation of the transcription factor Rel/NFκB. Akt also phosphorylates and activates IκB, which results in IκB degradation by the proteasome. This allows NFκB to translocate from the cytoplasm to the nucleus and activate transcription of a variety of substrates including anti-apoptotic IAP genes, such as the *c-IAP1* and *c-IAP2*. IRS1: Insulin receptor substrate 1; PI3K: Phosphatidylinositol 3-kinase; PIP3: Phosphatidylinositol-3,4,5-trisphosphate; PTEN: Phosphate and tensin homologue; RTK: Receptor tyrosine kinase.

OR "phosphorylat* Akt" OR "Akt phosphorylation" OR "Akt inhibition" OR "Akt modulation" OR "inhibit* Akt" OR "inactivation of Akt" OR "Akt inactivation" OR "activation of Akt" OR "inactivating Akt". Because of a large body of data on this subject, here we narrowed the scope of the current review down to the preclinical and clinical data on the simultaneous administration of cisplatin, paclitaxel, gemcitabine, or pemetrexed with some of the most clinically relevant PI3K/Akt modulators, *i.e.,* PI3K inhibitors, (*e.g.,* LY294002, Wortmannin), PI3K/mTOR inhibitors (*e.g.,* BEZ235), or Akt inhibitors (*e.g.,* perifosine, MK2206), which were tested in combinations with DNA-targeted agents in any of the five types of cancers. We excluded papers not meeting the inclusion criteria as well as retracted publications, duplicates, or non-original papers, *i.e.,* review articles, letters and editorials, comments, and case reports.

For clinical data, we searched for all the registered trials, being planned or performed to study the effect of PI3K/Akt inhibitors, *i.e.,* MK2206, perifosine, LY294002, Wortmannin, BEZ235, in combination with any of the nine DNA-targeted modalities discussed above, *i.e.,* carboplatin, cisplatin, oxaliplatin, paclitaxel, docetaxel, fluorouracil, gemcitabine, pemetrexed, and radiation. All published full text papers or abstracts as well as those with preliminary results in any languages fulfilling selection criteria were

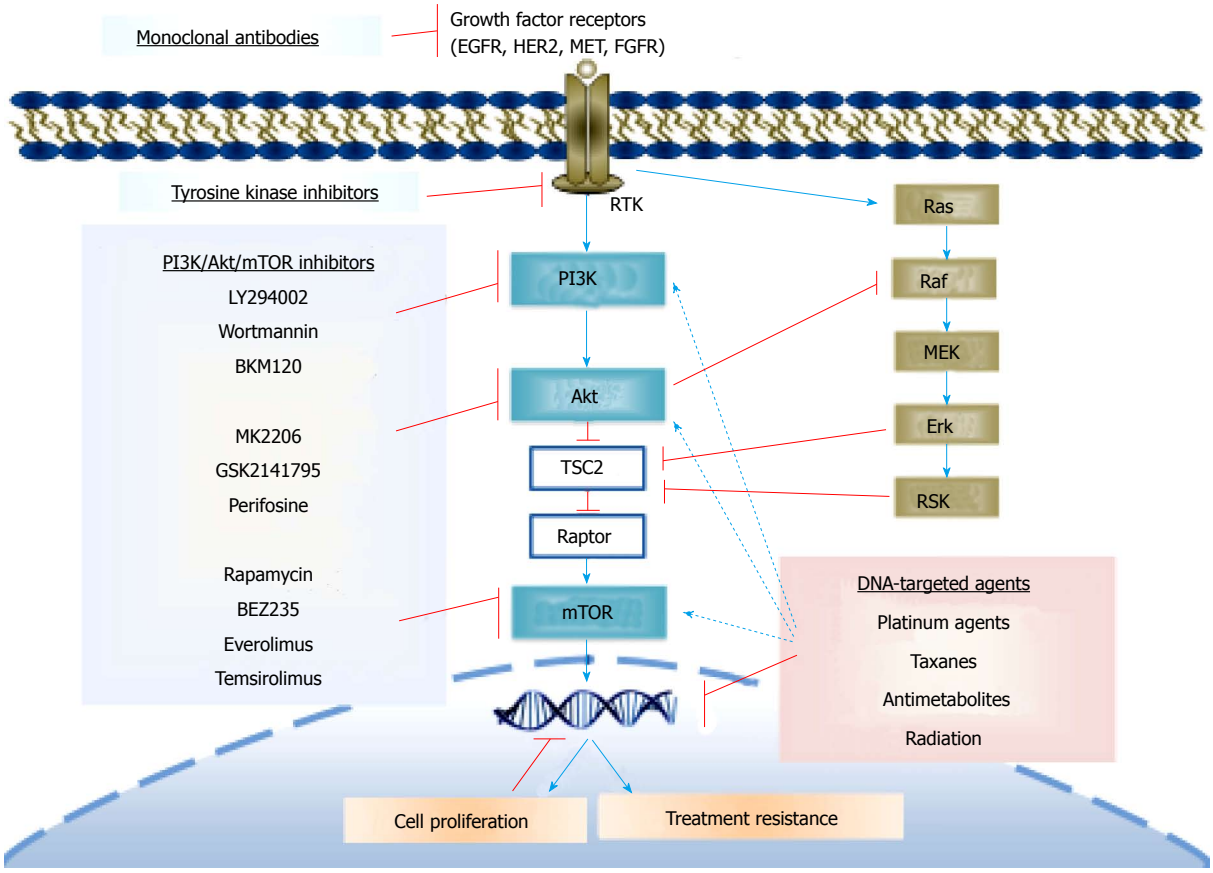

Figure 2 A schematic figure showing the complementary effects of phosphatidylinositol 3-kinase/Akt inhibitors with platinum agents, taxanes, antimetabolites, tumor antibiotics, and radiation resulting in a better cytotoxic profile.

included with no time period restriction. Studies with prior administration of anticancer medications, or combination of drugs targeting other signaling pathways other than Akt, PI3K, and mTOR were excluded. The databases that were used in this phase included the US clinical trials registry (clinicaltrials.gov), NIH clinical research studies (clinicalstudies.info.nih.gov), worldwide clinical trials listings (www.clinicaltrialssearch.org/5-cancer-clinical-trials.html), and the WHO International Clinical Trials Registry Platform (apps.who.int/trialsearch). The latter included 13 registries (Nationalities: Australian, New Zealand, Chinese, American, Canadian, European, Dutch, Brazilian, Indian, Korean, Cuban, German, Iranian, Japanese, Pan African, Sri Lankean, and Thai clinical trials).

AN OVERVIEW ON PI3K/AKT INHIBITORS

Many compounds have been developed to inhibit PI3K, Akt, and mTOR signaling, among which only few were tested in clinical settings (Figure 2). However, they did not yet have significant clinical benefit, except for idelalisib (GS-1101, a p110δ-selective inhibitor), which is the first Food and Drug Administration approved PI3K inhibitor[22], and some mTOR inhibitors, *e.g.,* rapamycin and its analogs. There are six general classes of these agents targeting the Akt network: Pan-class Ⅰ PI3K inhibitors, isoform-selective PI3K inhibitors, rapamycin analogues (rapalogues), active-site mTOR inhibitors, pan-PI3K-mTOR inhibitors and Akt inhibitors[23] (Table 1). Isoformspecific PI3K inhibitors targeting PI3Kβ, inhibitors of ribosomal protein S6 kinase β1 (S6K), PDK1 inhibitors and isoform-selective Akt kinase inhibitors (Akt1 and 2) are also under investigations soon to be tested in the clinic[23].

PI3K is upstream of Akt pathway, and with its inhibition all the subsequent signaling will potentially be down-regulated. Emerging clinical data show limited single-agent activity of inhibitors targeting PI3K. However, the drugs targeting PI3K pathway usually modulate the myriad substrates, including Akt and/or mTOR. LY294002, Wortmannin, and perifosine as PI3K/Akt inhibitors, BEZ235 as dual PI3K/mTOR inhibitor, and MK2206 as a specific Akt inhibitor are some of the commonly used drugs in this category that modulate Akt signaling. LY294002 is a morpholine-containing chemical compound that is a potent reversible inhibitor of PI3K signaling. Wortmannin is a steroid metabolite of the fungi Penicillium funiculosum with an irreversible inhibitory effect on PI3K. Perifosine is an orally active alkylphospholipid analog, which targets cell membrane and modulates different

Table 1 Drugs targeting phosphatidylinositol 3 kinase/Akt/mammalian target of rapamycin pathway

PI3K/Akt/mTOR subgroups	Agents	Clinical Stage
Pan-PI3K inhibitors	XL147	Phase Ⅱ
	BKM120	Phase Ⅲ
	GDC0941	Phase Ⅱ
Rapalogues (mTORC1 inhibitors)	Sirolimus	Phase Ⅲ
	Everolimus	Approved
	Temsirolimus	Approved
	Ridaforolimus	Phase Ⅲ
mTORC1/2 inhibitors	INK128	Phase Ⅱ
	AZD8055	Phase Ⅰ
	OSI027	Phase Ⅰ
PI3K–mTOR inhibitors	BEZ235	Phase Ⅱ
	XL765	Phase Ⅱ
	GSK1059615	Phase Ⅰ
Isoform-specific PI3K inhibitors	CAL-101 (p110δ)	Phase Ⅲ
	INK1117 (p110α)	Phase Ⅰ
	BYL719 (p110α)	Phase Ⅱ
Akt inhibitors	Perifosine	Phase Ⅲ
	MK-2206	Phase Ⅱ
	GDC0068	Phase Ⅱ
	GSK690693	Phase Ⅰ

PI3K: Phosphatidylinositol 3 kinase; mTOR: Mammalian target of rapamycin.

signaling pathways, and Akt in particular[24,25].

INDIRECT ALTERATION OF AKT SIGNALING AND ITS MODULATION

We finally selected 65 papers being suited to this review (Figure 3; Table 2) by which combination of a PI3K/Akt inhibitor with any of cisplatin, paclitaxel, gemcitabine, or pemetrexed was studied. The results are precisely discussed in the following sections.

Effect of platinum analogs on Akt signaling

Cisplatin, carboplatin and oxaliplatin are the 3 most commonly used anticancer platinum analogs. The main antitumor properties of cisplatin are attributed to the formation of platinum-DNA adducts causing DNA bending[26], which interferes with DNA replication, transcription and other nuclear functions leading to the inhibition of cellular proliferation and tumor growth. Intrinsic and acquired resistance limits the efficacy of platinum drugs in cancer treatment. Decreased drug uptake along with increased influx or inactivation by sulfhydryl molecules, such as glutathione, or increased DNA adduct repair can result in platinum resistance[27,28]. Wang and Lippard[29] suggested that induction of signaling pathways might be an alternative mechanism of resistance to platinum analogs.

Some data suggest that cell death induced by cisplatin may occur through regulation of cell cycle[30-32]. Mitsuuchi *et al*[32] demonstrated that PI3K, Akt1, and Akt2 are required for p53 protein expression and the full induction of p21 in ovarian cancer cells treated with cisplatin. Liu *et al*[33] suggested that Akt1 expression regulates cisplatin resistance in lung cancer cells through mTOR pathway, and its inhibition may sensitize cells to cisplatin. Moreover, amplification of a catalytic subunit of PI3K (PIK3CA) was also found to be associated with the risk of resistance to platinum-based chemotherapy in a group of patients with ovarian cancer, but not with the overall survival[34]. Thus vailable data support the probable role of Akt amplification/overexpression in platinum-resistance *in vitro* and *in vivo*[33,35-43].

The PTEN (encoding PIP3 phosphatase) and PIK3CA (encoding the PI3K catalytic isoform p110α) are the two most frequently altered mutated tumor suppressor and oncogenes, respectively[23]. Moreover, a low level of the PTEN expression is associated with amplified PIK3CA expression and finally PI3K/Akt activity[42]. Other data suggest that loss of the FHIT (fragile histidine triad; an inhibitor of Akt signaling) and overexpression of Redd1 (an inhibitor of mTOR signaling) are associated with cisplatin resistance in lung cancer cell lines[44-46]. Furthermore, overexpression of ADAM17 (a disintegrin and metalloproteinase-17) has been found to be associated with hypoxia-induced cisplatin resistance in hepatocellular carcinoma cells through activation of EGFR/PI3K/Akt pathway *in vitro*[47]. ADAM17 is a member of the metalloproteinase superfamily involved in the cleavage of ectodomain of many transmembrane proteins. Besides, prostate apoptosis response-4 (a proapoptotic tumor suppressor protein) downregulation was associated with cisplatin resistance in pancreatic cancer cells through upregulation of PI3K/Akt signaling *in vivo*[31]. Given that cisplatin activates PI3K/Akt signaling, downregulation of this pathway may bypass cisplatin-resistance. Akt pathway overactivation may decrease cisplatin sensitivity and cause treatment resistance even in platinum sensitive cells, whereas downregulation of Akt can boost the drug sensitivity and resistance to platinum compounds like cisplatin[36,39].

Effect of Akt-inhibition on platinum sensitivity

Some studies did not find treatment sensitization by adding LY294002 to cisplatin[48,49]. However, the PI3K/Akt inhibitors, LY294002, Wortmannin, or BEZ235 in combination with cisplatin showed synergistic or additive effects against malignant mesothelioma and lung cancer[44,50-55], pancreatic cancer[30], ovarian cancer[41-43,56-61], as well as glioblastoma[62,63] *in vitro* and *in vivo* (Table 3).

Perifosine increased the antineoplastic activity of cisplatin in ovarian[25,87], endometrial[91], and lung[44,54,55,92] cancer cells by activating apoptotic pathways and thus enhancing the cytotoxicity of cisplatin. Likewise, the specific Akt inhibitor MK-2206 showed synergism when combined with cisplatin in lung cancer[51,55], gastric cancer[93], and nasopharyngeal carcinoma cells[94] *in vitro* and *in vivo*. It can be concluded that activation of the Akt survival pathway plays a pivotal role in platinum resistance, and inhibition of Akt may enhance the effect of this type of anticancer drug.

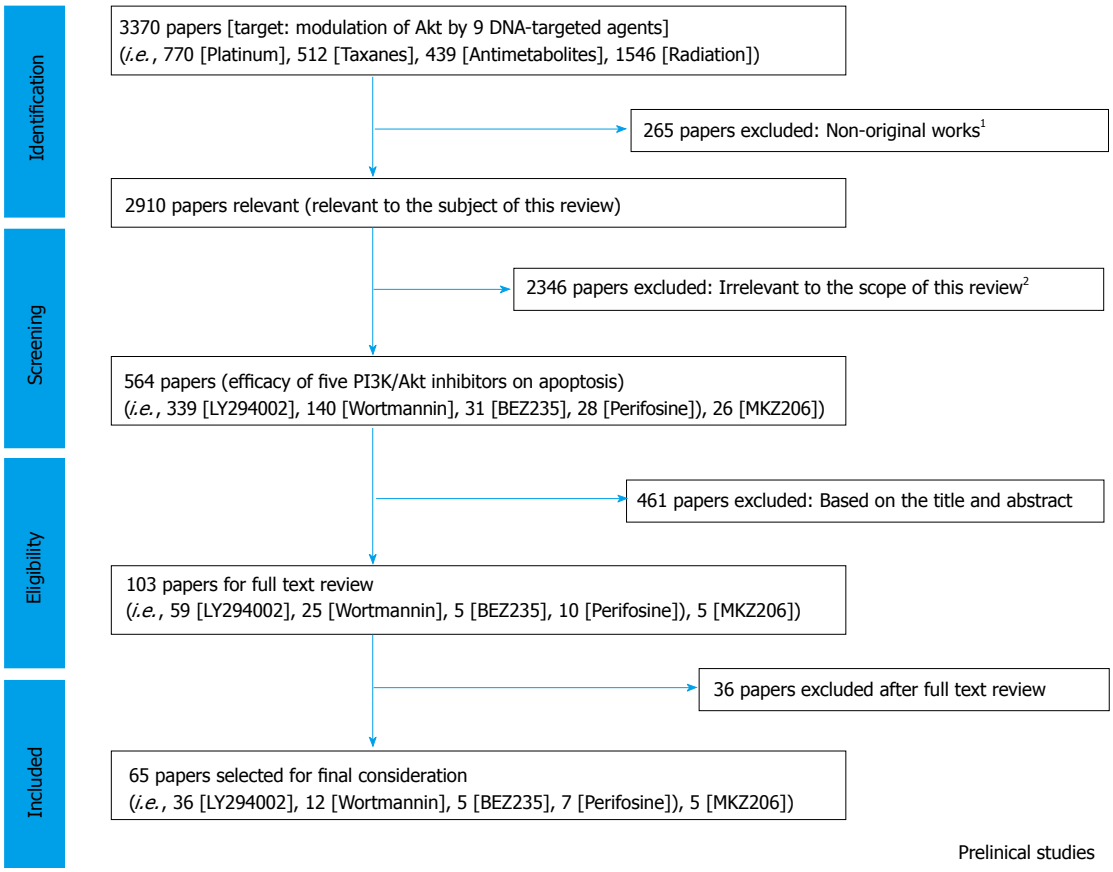

Figure 3 Review flow diagram of the publication selection in preclinical category. [1]Exclusion criteria include the retracted publication, duplicate publication or non-original papers, including review articles, letters and editorials, comments, case reports, *etc.*; [2]According to the scope of the current review: Efficacy of Akt modulation by 9 DNA-targeted agents in 5 types of cancer, including lung cancer, malignant mesothelioma, pancreatic cancer, ovarian cancer, and malignant glioma. PI3K: Phosphatidylinositol 3 kinase; EGFR: Epidermal growth factor receptor; mTOR: Mammalian target of rapamycin.

Table 2 Systematic chart of searching methodology and the results based on PubMed

DNA-targeted therapies	Agents	Akt modulation							
		All papers[1]	Non-original[2]	Relevant papers[3]	PI3K/Akt inhibitors[4]				
					LY294002	Wortmannin	BEZ235	Perifosine	MK2206
Platinum	Carboplatin	68	6	51	3	1	0	0	4
	Cisplatin	631	15	589	85	22	8	9	5
	Oxaliplatin	71	5	59	5	2	0	0	0
Taxane	Docetaxel	149	13	127	9	2	3	1	3
	Paclitaxel	363	18	331	47	15	4	2	5
Antimetabolite	Fluorouracil	191	5	174	18	6	0	2	3
	Gemcitabine	213	14	191	16	8	3	1	1
	Pemetrexed	35	3	32	5	2	1	0	0
Radiation	Irradiation/ radiation	1649	186	1356	151	82	12	13	5
Total articles	(9 agents)	3370	265	2910	339	140	31	28	26

[1]Terms used in the search: Query agent/radiation, and Akt, within whole the article with no language limitation, as of September, 2014; [2]Including the retracted publication, duplicate publication or non-original papers, including Review articles, Letters and Editorials, Comments, Case Reports, *etc.*; [3]Relevant with regard to the modulation of Akt by the query agent. [p-Akt OR pAkt OR "phospho-Akt" OR (phosphorylat* Akt) OR "Akt phosphorylation" OR "Akt inhibition" OR "Akt modulation" OR (inhibit* Akt) OR "inactivation of Akt" OR "Akt inactivation" OR "activation of Akt" OR "inactivating Akt"]; [4]Suppressing Akt cascade by a PI3K/Akt inhibitor to sensitize the query agent. PI3K: Phosphatidylinositol 3 kinase.

Effect of taxanes on Akt signaling

Taxanes, *e.g.*, paclitaxel and docetaxel, are frontline therapy for several cancers. They stabilize microtubules, leading to cell cycle arrest through centrosomal impairment, induction of abnormal spindles and suppression of spindle microtubule dynamics, finally triggering apoptosis[95]. Microtubules are critical for the integrity of the segregated DNA during mitosis. However, inherent or acquired resistance to taxanes

Table 3 Studies evaluating the efficacy of phosphatidylinositol 3 kinase phosphatidylinositol 3 kinase/Akt modulators on the apoptotic profile of cisplatin, paclitaxel, gemcitabine and pemetrexed

PI3K/Akt inhibitor and DNA-targeted agent combination	Akt modulation (phosphorylation)							
	Lung cancer and mesothelioma		Pancreatic cancer		Ovarian cancer		Malignant glioma	
	Synergistic	Antagonistic	Synergistic	Antagonistic	Synergistic	Antagonistic	Synergistic	Antagonistic
LY294002/Cisplatin	1[51]a	1[50]	2[30,31]	-	6[42,56-60]	1[49]	2[62,63]b	1[48]
LY294002/Paclitaxel	2[64,65]	2[50,66]	-	-	7[32,56,67-71]b	-	1[63]	-
LY294002/Gemcitabine	1[72]	1[73]	4[74-77]b	1[78]	1[56]	-	-	-
LY294002/Pemetrexed	1[79]	1[50]	-	-	-	-	-	-
Wortmannin/Cisplatin	1[52]		-	-	3[41-43]	-	-	-
Wortmannin/Paclitaxel	1[80]	-	-	-	1[70]	-	1[80]	-
Wortmannin/Gemcitabine	-	-	5[74,81-84]	-	-	-	-	-
Wortmannin/Pemetrexed	1[79]	-	-	-	-	-	-	-
BEZ235/Cisplatin	1[53]	-	-	-	1[61]	-	-	-
BEZ235/Paclitaxel	-	-	-	-	1[61]	-	-	-
BEZ235/Gemcitabine	-	-	1[85]	-	-	-	-	-
BEZ235/Pemetrexed	1[86]	-	-	-	-	-	-	-
Perifosine/Cisplatin	2[44,54]	-	-	-	2[25,87]	-	-	-
Perifosine/Paclitaxel	-	-	-	-	1[88]	-	-	-
Perifosine/Gemcitabine	1[54]b	-	-	-	1[89]	-	-	-
Perifosine/Pemetrexed	-	-	-	-	-	-	-	-
MK2206/Cisplatin	2[51,55]	-	-	-	-	-	-	-
MK2206/Paclitaxel	-	-	-	-	-	-	-	-
MK2206/Gemcitabine	2[54,90]b	-	-	-	-	-	-	-
MK2206/Pemetrexed	-	1[54]c	-	-	-	-	-	-
Total	17	6	12	1	24	1	4	1

[a]Chowdhry *et al*[51]. Reporting an increased sensitivity, but synergism not evaluated; [b]Shingu *et al*[62,63], Kawaguchi *et al*[69], Pinton *et al*[54] additive enhancement of proliferative inhibition; [c]Holcomb *et al*[76] LY294002 combination with gemcitabine showed additive effects on proliferative inhibition in PANC-1 and synergistic in $PaCa^{2+}$ pancreatic cancer cell lines. However, pAkt levels rebounded at later time points. PI3K: Phosphatidylinositol 3 kinase.

may compromise their therapeutic efficacy[96,97].

Resistance to taxanes includes increased efflux, and modification in tubulins. Akt pathway activation contributed to an increased resistance to paclitaxel or docetaxel in epithelial ovarian cancer, prostate cancer, and breast cancer cells[98-101]. Activation of Akt1 by HER2/PI3K may also lead to taxane resistance in breast adenocarcinoma cells[102]. Moreover, *PIK3CA* gene, encoding a catalytic subunit of the PI3K, is mutated and/or amplified in various neoplasms, such as ovarian cancer. Its amplification strongly decreased the sensitivity and thus response to platinum with/without taxanes in patients with ovarian carcinoma[34].

There are also crosstalks between PI3K/Akt pathway with BAD and ERK[41,68], and inhibition of these cascades sensitized ovarian cancer cells to taxanes. Therefore, in order to sensitize taxane treatment, PI3K/Akt cascade can be considered as a suitable target.

Effect of Akt-inhibition on taxane sensitivity

LY294002, Wortmannin, BEZ235, or perifosine-mediated inhibition of the PI3K/Akt-dependent survival pathway enhanced paclitaxel cytotoxicity in various cancers, *e.g.*, malignant glioma[63,80], lung[50,64-66,80], esophageal[64,80], and ovarian cancer cells[32,56,61,67-70,88] (Table 3). However, there are some data not in favor of the combination. LY294002 did not potentiate cisplatin, pemetrexed, or paclitaxel in A549 lung adenocarcinoma cells harboring K-ras mutation and wild-type EGFR[50]. Likewise, inactivation of PI3K/Akt signaling by LY294002 did not result in significant alteration of sensitivity of human ovarian carcinoma A2780 cells to paclitaxel[48]. Similarly, the combination of paclitaxel with LY294002 was antagonistic *in vitro* when dexamethasone was also administered; although dexamethasone did not alter the Akt activity[66].

Activation of NFκB is linked to Akt-dependent transcription of pro-survival genes[103]. Thus, LY294002-mediated suppression of the PI3K/Akt survival pathway with secondary inhibition of NFκB transcriptional activity is associated with enhancement of paclitaxel cytotoxicity in lung, esophageal and ovarian cancer cells[64,104,105], which indicates that NFκB may be the crucial intermediary step connecting Akt to the intrinsic susceptibility of cancer cells to paclitaxel.

Additionally, the Akt inhibitor MK-2206 augmented the efficacy of paclitaxel and carboplatin combination in gastric cancer[106], breast cancer[107], and melanoma cells[108]. However, addition of MK-2206 to paclitaxel alone had no additive inhibitory effect on growth of nasopharyngeal carcinoma cells *in vitro*[90]. Furthermore, Hirai *et al*[90] found that synergy of MK-2206 with docetaxel was dependent on the treatment sequence, in which a schedule of MK-2206 before docetaxel was not effective in terms of growth inhibition. Dual inhibition of PI3K and mTORC1/2 by BEZ235 may overcome docetaxel resistance in human castration resistant prostate cancer *in vitro* and *in vivo*[109]. Thus, modulation of the PI3K/Akt signaling may increase the efficacy and potency of taxanes according

to *in vitro* and *in vivo* data. However, the effect may have been masked by inclusion of platinum in several studies, indicating that in some studies, the effect might be *via* platinum.

Effect of antimetabolites on Akt signaling

Antimetabolites are a large group of anticancer drugs widely used in combination therapy of various leukemias and solid tumors. They interfere with DNA and RNA synthesis and therefore the growth of tumor[110]. Antimetabolites are categorized as pyrimidine analogs [*e.g.,* 5-fluorouracil (5-FU), gemcitabine], purine analogs (*e.g.,* azathioprine, mercaptopurine), and antifolates (*e.g.,* methotrexate, pemetrexed). In the present review, we mainly focused on two commonly used antimetabolites gemcitabine and 5-FU, as well as the novel anti-folate pemetrexed.

Effect of gemcitabine on Akt signaling and effect of Akt inhibition: Gemcitabine is used in the treatment of various carcinomas, such as lung cancer, bladder cancer, breast cancer, pancreatic cancer, and lymphomas[111]. A substantial number of potential biomarkers for sensitivity or resistance to gemcitabine have been characterized, including ribonucleotide reductase, deoxycytidine kinase, cytidine deaminase and human equilibrative transporter-1[112,113]. Additional mechanisms of resistance may exist, possibly not involving metabolism and direct targets[74,75,112,114].

Gemcitabine resistance in breast cancer cells may also be mediated by activation of the PI3K/Akt signaling pathway through phosphorylated Akt[115], so that inhibitors of PI3K/Akt might reverse the resistance to gemcitabine. Moreover, involvement and overexpression of PI3K and phosphorylated Akt in pancreatic carcinoma tissues has been reported in gemcitabine-resistant cells *in vitro*[73,78,84]. Rad51 overexpression may also mediate gemcitabine resistance through Akt or ERK1/2 activation in non-small cell lung cancer (NSCLC) cells, which could be overcome by downregulation of Rad51 or inhibition of Akt and ERK1/2 proteins[72]. Although Akt phosphorylation status is tailored as a predictive biomarker for gemcitabine resistance in NSCLC patients[116], gemcitabine may also reduce Akt phosphorylation without affecting the Akt overall expression[117]. Wilson *et al*[73] reported a weak correlation between phosphorylated S6K and phosphorylated Akt, suggesting the existence of Akt-independent regulation of mTOR-mediated resistance to apoptosis. Overall, inhibition of PI3K/Akt signaling may enhance the gemcitabine cytotoxic profile.

Wortmannin enhanced the efficacy of gemcitabine by a 5-fold increase of apoptosis in murine pancreatic xenografts[81]. A synergistic effect of Wortmannin, LY29 4002, and BEZ235 with gemcitabine was also reported in ovarian cancer[56] and pancreatic carcinoma[74-77,81-85] *in vitro* and *in vivo* (Table 3). Although gemcitabine induces cell cycle arrest at the G1 and early S phases, PI3K/Akt activation does not seem to influence gemcitabine-induced cell cycle arrest[84]. Likewise, perifosine has shown additivity in combination with gemcitabine by inhibiting Akt1 and Akt3 axis, and interfering Akt upstream, EGFR, and MET phosphorylation[54]. Perifosine also enhanced the gemcitabine-mediated antitumor effect on pancreatic cancer cells through blocking p70S6K1 (S6K1) activation, and thus disrupting S6K1-Gli1 association and subsequent Gli1 activation[89]. Besides, Akt[118], mTOR[119], and MAPK[120] may also activate Gli1. Likewise, the Akt inhibitor MK2206 enhanced the effect of gemcitabine on growth inhibition *in vitro* and *in vivo*[90]. In the contrary, Arlt *et al*[78] found that NFκB, rather than PI3K/Akt, activity conferred resistance to gemcitabine in a panel of five pancreatic carcinoma cell lines, which was strongly diminished by NFκB inhibitors, and not by LY294002. Overall, the PI3K/Akt inhibitors have been efficacious in improving gemcitabine cytotoxicity.

Effect of FU on Akt signaling and effect of Akt inhibition: 5-FU is an antimetabolite that acts by inhibition of thymidylate synthase (TS) and can be incorporated into RNA and DNA altering the cancer cell replication and proliferation[121]. 5-FU-based regimens are often used in adjuvant chemotherapy regimens and treatment of various advanced malignancies, such as colon cancer, head and neck cancer, breast cancer, but depending on the disease and stage of the tumor, intrinsic resistance to 5-FU can be as high as 50%[122]. Resistance to 5-FU has often been associated with an increased TS expression, both transient and permanent[123]. Other factors, such as enzymes involved in pyrimidine metabolism, *i.e.,* increased dihydropyrimidine dehydrogenase, decreased orotate phosphoribosyltransferase, or altered folate metabolism have been associated with 5-FU resistance[121,124,125]. Moreover, 5-FU has major effects on glycosylation pathways as well[126], which may indirectly have effects on signaling pathways. Hence, evidence is accumulating that 5-FU resistance is associated with altered signaling.

Smad4 deficiency may also contribute to 5-FU resistance through upregulation of vascular endothelial growth factor expression, which is associated with increased vascular density[127,128]. Zhang *et al*[129] found that loss of Smad4 in colorectal cancer patients may induce resistance to 5-FU through activation of Akt pathway. Akt can interact with Smad molecules to regulate transforming growth factor beta (TGF-β) signaling that is involved in transmitting chemical signals from the cell surface to the nucleus[130-132]. In summary, suppression of PI3K/Akt signaling may potentiate 5-FU.

The combination of LY294002 with 5-FU was synergistic *via* downregulation of PI3K/Akt signaling in Smad4-deficient colorectal cancer cells[129]. Likewise, sequential combination of 5-FU and LY294002 induced synergistic cytotoxicity and overcame intrinsic and acquired resistance of 5-FU *via* downregulation of Akt and mitochondria-dependent apoptosis in an Epstein-Barr virus positive gastric cancer cell line[133]. Wortmannin also promoted 5-FU antitumor activity in oral squamous cell carcinoma[134] and breast cancer cells[135]. In colorectal cancer cell lines, preclinical

studies indicate that perifosine and BEZ235 may enhance the cytotoxic effects of 5-FU, likely through the NFκB and thus PI3K/Akt pathway[136]. As a result, PI3K/Akt pathway is a rational target for sensitizing the tumor cells to 5-FU.

Effect of pemetrexed on Akt signaling and effect of Akt inhibition: Pemetrexed (Alimta; formerly known as LY231514), a multitargeted antifolate, inhibits thymidylate synthase (TS), dihydrofolate reductase, and the de novo purine nucleotide synthesis[137]. Pemetrexed is currently used as a single agent, but more often in combination with cisplatin for first line treatment of non-squamous NSCLC and malignant pleural mesothelioma[138-140]. Resistance to pemetrexed has been associated with TS upregulation in a colon cancer cell line[123,141], a transport deficiency, decreased activation by folylpolyglutamate synthetase and increased efflux[139]. Members of the ATP-binding cassette (ABC) transporters including P-glycoprotein (Pgp/ABCB1), multidrug resistance proteins (MRPs/ABCC) as well as breast cancer resistance protein (BCRP/ABCG2) as ATP-dependent drug efflux transporters may play roles in pemetrexed resistance[142]. The PI3K/Akt pathway regulates ABCG2-mediated drug efflux, which induces drug resistance[86,143-145].

Pemetrexed can also activate Akt signaling[79,143,146,147], although its molecular mechanisms is not completely understood. Chen *et al*[79] have observed a pemetrexed-induced cell apoptosis and a parallel increase in sustained Akt phosphorylation and nuclear accumulation in the NSCLC A549 cell line, and postulated that the activated Akt may play a proapoptotic role, while Giovannetti *et al*[147] observed that pemetrexed increased EGFR phosphorylation and slightly reduced Akt phosphorylation and enhanced apoptosis in six NSCLC cell lines[143,146] and malignant pleural mesothelioma (MPM) cells, particularly when combined with EGFR inhibitor erlotinib or carboplatin.

Adding a PI3K/Akt inhibitor may further increase pemetrexed antineoplastic effect. LY294002 and Wortmannin decreased the pemetrexed-stimulated Akt and GSK3β phosphorylated activation in the NSCLC A549 cell line[79] (Table 3). Perifosine antagonized the effect of pemetrexed in MPM cells by interfering upstream of Akt, affecting EGFR and MET phosphorylation[54]. Likewise, BEZ235 enhanced the antineoplastic effect of pemetrexed in malignant pleural mesothelioma by decreasing ABCG2-mediated drug efflux at the cell surface[86], which may be of therapeutic value in combination regimens. These data suggest that combining pemetrexed with a PI3K/Akt inhibitor may result in a better antineoplastic effect in various tumors.

Effects of irradiation and chemoradiation on Akt signaling: The combination of radiation with cytotoxic chemotherapy has become a standard treatment option for the majority of inoperable, locally advanced cancers, including brain, head and neck, lung, and gastrointestinal malignancies[148]. However, resistance to irradiation compromises therapeutic efficacy leading to tumor recurrence or metastasis. Tumors that recur after a successful radiation are often associated with radioresistance[149]. Resistance to radiotherapy is predominantly related to efficient repair of the DNA damage induced by X-ray. Both normal and neoplastic cells have several types of repair pathways, usually starting with the recognition and excision of the lesion, and then insertion of a new nucleotide. Regulation of several of these repair enzymes is mediated through methylation of the gene or activation of various protein kinases[150]. Given the complex biology underlying the interactions between the targeted agent and chemoradiation, comprehensive preclinical investigations are critical to design the rational combination[148].

Different combinations of drugs and radiation have been studied to improve efficacy and lessen toxicity. Chemotherapeutic drugs that perturb nucleotide metabolism have the potential to produce substantial sensitization of tumor cells to radiation treatment. Redistribution of cells into S-phase of the cell cycle and depletion of deoxynucleotide pools are probable mechanisms for gemcitabine and 5-FU, which made them potent radiosensitizers[151,152].

Radiation can activate multiple signaling pathways in cells[153], such as EGFR and several downstream proteins, *i.e.,* PI3K/Akt, MAPK JNK, p38, NFκB, *etc.,* stimulating DNA repair and thus causing radioresistance and survival of tumor cells. Loss of PTEN[154], as well as *KRAS* mutations[155,156] and NF-κB activation[157,158] also are associated with radioresistance, making the DNA less susceptible to ionizing radiation. Additionally, the ability of radiation to activate signaling pathways may depend on the expression of growth factor receptors, *RAS* mutation, and autocrine or paracrine ligands such as TGF-α, TGF-β, HB-EGF, neuregulins, and interleukin 6[153].

Effect of Akt-inhibition on radiation sensitivity: Alkylphosphocholines may potentiate the effect of radiation if given before or together with radiotherapy[159]. Targeting the PI3K pathway by LY294002 led to radiosensitization in glioblastoma[154] and human bladder cancer cell xenografts[160] *in vivo.* BEZ235 has also shown a modest antitumor response *in vivo*, while the combination of BEZ235 and ionizing radiation provided a longer survival and led to a smaller tumor volume when compared to radiation alone[161]. Likewise, the PI3K inhibitor BKM120 inhibited the radiation-induced activation of Akt[162]. This induced suppression of DNA-double-strand breaks repair and increased apoptosis, which resulted in increased sensitivity of liver cancer cells to irradiation[162]. Perifosine showed some radiosensitization in squamous cell carcinoma[163-165], malignant glioma[166], lymphoma[167], and prostate cancer[168]. In contrast, one study failed to show any favorable results with perifosine in terms of increasing its anticancer potency, despite a significant reduction in the level of phosphorylated Akt as well as Akt activity *in vitro* and *in vivo*[169]. Overall, given the activation of PI3K/Akt pathway by radiation, addition of a PI3K/Akt inhibitor may potentiate the therapeutic index of the conventional

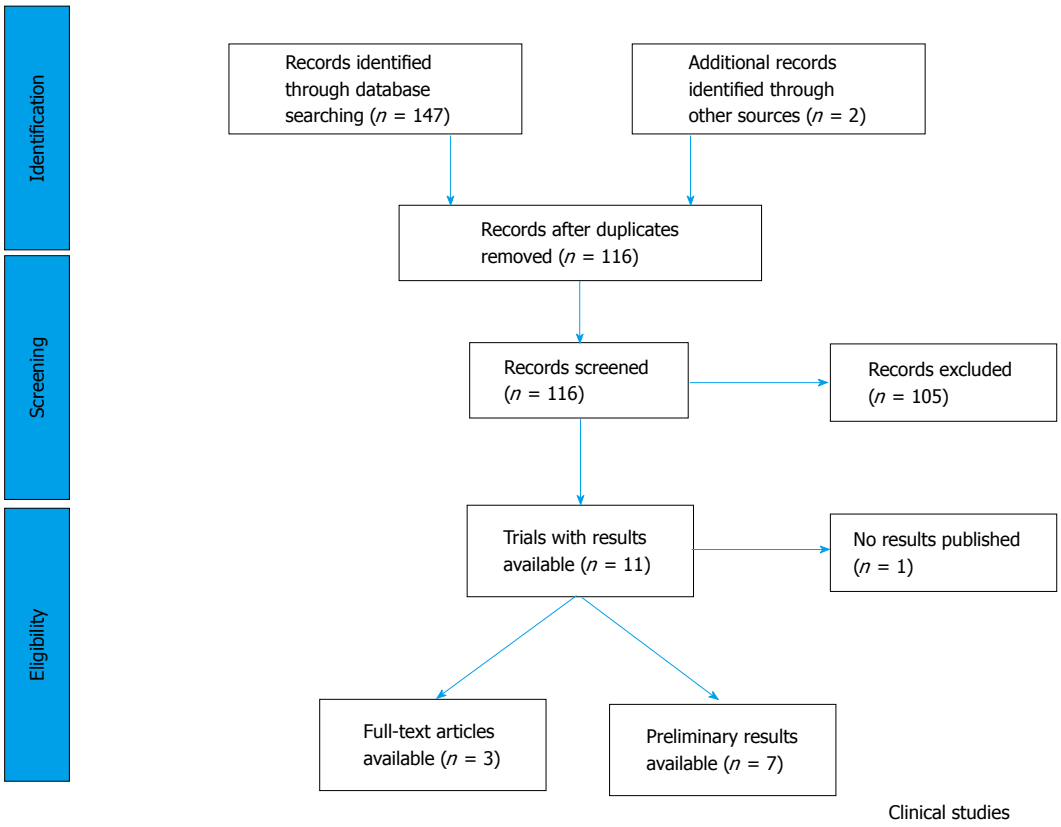

Figure 4 Review flow diagram of the publication selection in clinical category.

chemoradiation therapy.

PI3K/AKT INHIBITORS IN THE CLINIC

In the trial databases, we found 147 studies (Figure 4), which were designed to test the clinical profile of the six PI3K/Akt inhibitors. Only 11 trials - three published - were planned to assess the efficacy and safety of any of the PI3K/Akt inhibitors in combination with DNA-targeted agents (Table 4). We could not find any clinical trials being reported or registered to study the safety and efficacy of LY294002 or Wortmannin.

MK-2206

From 36 trials on MK-2206, registered between April 2008 (the first trial) and May 2013 (the last trial), to evaluate the safety and efficacy of MK-2206; only three suited our selection criteria (Table 4).

In a phase Ⅰ study with 72 patients with advanced solid tumor, Molife *et al*[170] (clinicaltrials.gov; NCT00848718) demonstrated that MK-2206 (45 or 60 mg every other day) plus carboplatin [area under the curve 6.0 mg/mL (AUC 6)] and paclitaxel (200 mg/m^2), docetaxel (75 mg/m^2), or erlotinib (100 or 150 mg daily) was well-tolerated, with early evidence of antitumor activity. The main dose-limiting toxicities included skin rash, febrile neutropenia, tinnitus, and stomatitis. Common drug-related toxicities included fatigue, nausea, and rash.

In a phase Ⅰ trial (NCT01235897) on 17 patients[171], MK2206 in combination with weekly paclitaxel (80 mg/m^2 weekly) with or without trastuzumab (2 mg/kg weekly after a 1-time loading dose of 4 mg/kg) has been tested in patients with human epidermal growth factor receptor 2 (HER2)-overexpressing solid tumor malignancies (11 breast, 3 gastric, 1 esophageal). The highest safe dose of MK-2206 was found to be 135 mg weekly [The Best Disease Response by Response Evaluation Criteria in Solid Tumor (RECIST) scoring was used for evaluation of the treatment toxicity in 15 patients]. Two patients experienced dose-limiting toxicities, while 64% showed tumor response and 29% had no disease progression[171]. Although all patients experienced different adverse events due to the treatment, serious or life threatening adverse events were reported in 5/17 (29.41%) participants. Based on these results, the authors concluded that MK2206 weekly at a dose of 135 mg in combination with weekly paclitaxel and trastuzumab was safe and well tolerated.

A phase Ⅰ trial (NCT01263145) is ongoing to determine the maximum tolerated dose, safety and antitumor activity of MK2206 and paclitaxel combination in patients with locally advanced or metastatic solid tumors or metastatic breast cancer. Thus, based on this scant evidence, we cannot conclude for or against administration of MK-2206 in combination with the available DNA-targeted agents.

Perifosine

From 42 trials on perifosine, registered between June

Table 4 Clinical trials on phosphatidylinositol 3 kinase/Akt inhibitors in combination with DNA-targeted agents

PI3K/Akt inhibitor	Target(s)	Combination arm(s)	Condition	Trial phase/status	Trial/registration
MK-2206	Akt	Carboplatin + paclitaxel, docetaxel, erlotinib	Locally advanced, metastatic solid tumors	Ⅰ/completed (published)	NCT00848718/February 2009[170]
		Paclitaxel, trastuzumab	HER2-overexpressing advanced solid tumors	Ⅰ/completed (abstract is published)	NCT01235897/ November 2010[171]
		Paclitaxel	Adult solid neoplasm, recurrent or metastatic breast cancer	Ⅰ/ongoing (unpublished)	NCT01263145/ December 2010
Perifosine	PI3K/Akt	Docetaxel, prednisone	Neoplasms	Ⅰ/completed (abstract is published)	NCT00399087/ November 2006[172]
		Docetaxel	Recurrent ovarian cancer	Ⅰ/completed (abstract is published)	NCT00431054/February 2007[173]
		Paclitaxel	Neoplasms	Ⅰ/completed (abstract is published)	NCT00399126/ November 2006[174]
		Gemcitabine	Neoplasms	Ⅰ/completed (abstract is published)	NCT00398697/ November 2006[175]
		Radiation	Solid tumors	Ⅰ/published	Vink *et al*[176]
		Radiation	Biochemically recurrent, hormone-sensitive prostate cancer with previous prostatectomy and/or radiation therapy	Ⅱ/published	Chee *et al*[177]
BEZ235	PI3K/ mTOR	BEZ235 + paclitaxel, BKM120 + paclitaxel, BEZ235 + paclitaxel + trastuzumab, BKM120 + paclitaxel + trastuzumab	Metastatic or locally advanced solid tumors	Ⅰ/completed (abstract is published)	NCT01285466/January 2011[178]
		Paclitaxel	Inoperable locally advanced breast cancer, metastatic breast cancer	Ⅰ and Ⅱ/completed (abstract is published)	NCT01495247/ September 2011[179]

HER2: Human epidermal growth factor receptor 2; PI3K: Phosphatidylinositol 3 kinase; mTOR: Mammalian target of rapamycin.

2000 (first trial) and August 2014 (last trial), only four studies as well as two published papers met our selection criteria for the current review.

Three trials evaluated the safety and efficacy of docetaxel or paclitaxel with perifosine (50, 100, and 150 mg/d). However, the results, despite the completion of the studies, have not yet been published (Table 4). In the first open-label study (NCT00399087) on 39 patients[172], daily doses up to 150 mg perifosine in combination with 75 mg/m^2 per 3 wk docetaxel with/without prednisolone was tolerated. Furthermore, the maximum tolerated dose for the weekly perifosine was 1200 mg. In the second trial (NCT00431054)[173] the safety and efficacy of the combination of docetaxel and perifosine were studied on 22 patients with epithelial cancer of the ovary, fallopian tube cancer or gynecologic primary peritoneal cancer, which were platinum resistant or refractory. The third phase Ⅰ trial (NCT00399126)[174] studied the gastrointestinal toxicity and cancer progression on the combination of daily perifosine with weekly or every 3-wk paclitaxel in 12 cancer patients. The preliminary results showed that the combination was well tolerated without increasing the toxicities being expected from using each drug alone.

Perifosine in combination with gemcitabine has also been studied in a non-randomized open-label phase Ⅰ trial (NCT00398697) on 22 patients[175]. The preliminary results showed that 150 mg daily perifosine might have some manageable toxicities without affecting the pharmacokinetic of perifosine.

The feasibility and tolerability of daily perifosine and radiation combination have also been studied in two independent successive trials. Vink *et al*[176] tested this combination in 21 radio-naive patients with solid tumors, of which 17 had NSCLC. Dysphasia and pneumonitis were the main complications of radiotherapy, and nausea, fatigue, vomiting, diarrhea, and anorexia as major drug-related toxicities in the population. One hundred and fifty milligram daily perifosine combined with fractionated radiotherapy was considered a safe modality. Chee *et al*[177] conducted a phase Ⅱ trial in 25 patients with biochemically recurrent, hormone-sensitive prostate cancer with previous prostatectomy and/or radiation therapy. However, only 20% of patients met the primary endpoint of prostate-specific antigen reduction, defined as a decrease by $\geqslant$ 50% from the pretreatment value. Accordingly, we should wait for the results of ongoing and future studies before coming to the conclusion if perifosine may add to the potency of DNA-targeted therapies.

BEZ235

From 23 trials on BEZ235, registered between February 2008 (first trial) and December 2013 (last trial), only two met our selection criteria for the final review, evaluating the safety and efficacy of the combination with any of the named DNA-targeted agents.

A phase Ⅰ multi-center, open-label, 4-arm dose-escalation study (NCT01285466)[178] is ongoing to evaluate the safety and efficacy of oral BEZ235 and

BKM120 in combination with weekly paclitaxel in patients with advanced solid tumors and weekly paclitaxel/trastuzumab in patients with HER2$^+$ metastatic breast cancer. The preliminary results[178] showed that of 35 patients who received BEZ235 at 400-800 mg/d and paclitaxel at 70-80 mg/m^2 per week dose-limiting toxicities occurred in 5 patients, 29% discontinued due to adverse effects and 63% due to progressive disease. Thus, they concluded that the safety over efficacy of this regimen would be questionable.

Additionally, a dose-finding phase Ⅰ followed by an open-label, randomized phase Ⅱ trial (NCT01495247)[179] of oral BEZ235 given twice daily (bid) with paclitaxel in patients with HER2 negative, locally advanced or metastatic breast cancer have recently been completed. The preliminary results with 18 patients showed that the determined maximum tolerated dose of BEZ235 (200 mg bid) in combination with paclitaxel (80 mg/m^2 per week) was not reached, and the trial has been terminated[179]. Thus, based on these two preliminary results, BEZ235 seems not safe in combination with paclitaxel for patients with solid tumors.

CONCLUSION

The Akt pathway is clearly important for the regulation of cell proliferation and survival. Its activation is an additional resistance mechanism for current chemoradiotherapy. Therefore, modulation of Akt activity is an attractive strategy to enhance the efficacy of treatment. However, insight in the mechanism of protection is incomplete and warrants further research. This lack of knowledge hampers to properly evaluate combinations in clinic, while current clinical trials are too preliminary to draw conclusions, despite having several drugs that are relatively safe and efficacious. Thus, the Akt modulation is an attractive target to improve the toxicity and safety profile of classical antitumor compounds and irradiation. Nevertheless, studies were inadequate and therefore inconclusive regarding the additive effect of PI3K/Akt inhibitors to the standard regimens. Accordingly, more in-depth preclinical and clinical studies as well as a critical appraisal are warranted to find congruent rational avenues to designing solid studies on any of the combinations.

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Accelerated partial breast irradiation: Past, present, and future

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Abstract

Accelerated partial breast irradiation (APBI) focuses higher doses of radiation during a shorter interval to the lumpectomy cavity, in the setting of breast conserving therapy for early stage breast cancer. The utilization of APBI has increased in the past decade because of the shorter treatment schedule and a growing body of outcome data showing positive cosmetic outcomes and high local control rates in selected patients undergoing breast conserving therapy. Technological advances in various APBI modalities, including intracavitary and interstitial brachytherapy, intraoperative radiation therapy, and external beam radiation therapy, have made APBI more accessible in the community. Results of early APBI trials served as the basis for the current consensus guidelines, and multiple prospective randomized clinical trials are currently ongoing. The pending long term results of these trials will help us identify optimal candidates that can benefit from ABPI. Here we provide an overview of the clinical and cosmetic outcomes of various APBI techniques and review the current guidelines for selecting suitable breast cancer patients. We also discuss the impact of APBI on the economics of cancer care and patient reported quality of life.

Key words: Breast cancer; Intracavitary brachytherapy; Accelerated partial breast irradiation; Interstitial brachytherapy

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Core tip: Given that accelerated partial breast irradiation (APBI) is becoming increasingly utilized in the management of early breast cancer patients, it is crucial to address the evolution of studies that led to the current guidelines in identifying the suitable group of patients who obtain the most benefit clinically and cosmetically. We, herein, discuss the available clinical and cosmetic outcomes of different APBI techniques in addition to details of ongoing phase Ⅲ randomized clinical trials. We also discuss the effects of APBI on breast cancer patient quality of life.

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INTRODUCTION

Breast conservation surgery (BCS) has been offered to newly diagnosed breast cancer patients as early as the 1950s[1]. BCS with adjuvant whole-breast irradiation (WBI), collectively referred to as breast conservation therapy (BCT), is one of the acceptable standard of cares. Numerous prospective randomized studies, with long term follow-up, have shown the equivalence of BCT to modified radical mastectomy in overall survival (OS) and disease-free survival (DFS)[2-5].

Standard WBI usually consists of 6-7 wk of daily radiation treatments to the whole breast with doses of 45 to 50 Gy. WBI typically includes a 10 to 16 Gy boost to the lumpectomy cavity for many patients to further reduce local recurrence. Local recurrence can also be reduced by tamoxifen or aromatase inhibitors in estrogen receptor positive breast cancer patients[6-9]. Hypofractionated WBI has recently been accepted as a treatment option in BCT, with local control (7.5% 10-year local recurrence rate)[10] and treatment toxicities comparable to conventional fractionation.

RATIONALE FOR ACCELERATED PARTIAL BREAST IRRADIATION

Accelerated partial breast irradiation (APBI) delivers radiation to the tumor bed at a higher dose per fraction. The radiobiologic model of the linear quadratic equation serves as the basis for APBI. A shorter radiation treatment course, given at higher dose per fraction, could achieve the same therapeutic effect as a longer treatment course, given at lower dose per fraction, based on the concept of radiobiologic equivalence. Ipsilateral breast tumor recurrences (IBTR) develop in and around the tumor bed in 44%-86% of cases[11-14], and treatment to the whole breast may be unnecessary. Therefore, by focusing the radiation to the area of potential recurrence, much of the surrounding tissues (including the lung, heart, uninvolved ipsilateral breast, contralateral breast, and skin) could be spared, reducing toxicity and improving cosmetic outcome[15-19].

EARLY APBI TRIALS

In the earliest prospective, randomized study, Christie Hospital (Manchester, United Kingdom) enrolled 708 patients, 355 of which were treated with wide-field (WF) irradiation and 353 treated with limited-field (LF) irradiation, from 1982 to 1987[20]. The study included patients younger than 70 years with tumor size $\leqslant$ 4 cm, and all women underwent tumorectomy "with gross or macroscopic clearance" only. The WF group received 40 Gy in 15 fractions over 21 d to the whole breast through parallel opposed tangent fields with a single matched anterior field covering the axillary, infraclavicular, and supraclavicular regions. The accelerated, partial breast LF group received 40 to 42 Gy in 8 fractions delivered over 10 d to the tumor bed only. At 8-year median follow-up, the survival in the two groups was the same (72%); however, the LF group showed a local recurrence rate of 25% *vs* 13% in the WF group (P = 0.00008)[21]. The authors concluded that APBI was possible, but would need more stringent selection of patients.

The next APBI trial was conducted by Guy's Hospital (London, United Kingdom) beginning in the late 1980s and used low dose rate (LDR) brachytherapy to deliver focal radiation. Twenty-seven non-randomized patients received BCS and axillary clearance immediately followed by placement of brachytherapy needles in a multi-planar arrangement around the surgical cavity. Iridium-192 seeds were loaded into the needles to deliver 55 Gy over 5 d to a 2 cm margin around the tumor bed[22]. Results showed good to excellent cosmesis in 80%-96% of patients at 27 mo of median follow-up; however, 37% of patients suffered local regional failure at 72 mo of median follow-up[23]. The high rate of local regional recurrences was attributed to the inclusion of subjects with recognized risk factors, such as positive margins and node positive disease.

Three additional trials explored dose escalation using interstitial brachytherapy for APBI at the Careggi Hospital (Florence, Italy), Royal Devon and Exeter Hospital (Exeter, England), and, again, Guy's Hospital (London, United Kingdom). Similarly, these studies included patients with unknown or positive margins, resulting in high local recurrence rates[24,25]. Around the same time period, the Milan group reported a much lower IBTR rate of 4.8% with WBI[26]. In summary, these studies demonstrated the feasibility of APBI and provided a basis for the design of subsequent APBI trials with young age, positive margin status, larger tumors, high nuclear grade, extensive ductal carcinoma *in situ*, invasive lobular carcinoma, involved nodes, and lymphovascular invasion (LVSI) established as risk factors for recurrence.

TRIALS WITH MODERN APBI TECHNIQUES

Brachytherapy

Multicatheter interstitial brachytherapy: Investigators at Ochsner Medical Institutions conducted a pilot trial, enrolling 50 patients from January 1992 to October 1993 in a phase Ⅰ/Ⅱ study of multicatheter interstitial brachytherapy (MIB), after segmental mastectomy, for invasive or intraductal tumors $\leqslant$ 4 cm with negative inked margins and $\leqslant$ 3 involved axillary lymph nodes[27]. Patients were treated to the target volume with continuous

LDR brachytherapy of 45 Gy over 4 d or fractionated HDR brachytherapy of 32 Gy in 8 fractions, given twice daily over 4 d. Cosmetic evaluation at median follow-up of 20 mo showed good to excellent cosmetic result in 75% of patients in both arms. At 75-mo median follow-up, there were 4 local-regional failures (8%). In another study, William Beaumont Hospital accrued patients between 1993 and 1999 for an APBI trial with stringent patient selection criteria: Tumor size ≤ 3 cm, age ≥ 40 years, and no extensive DCIS or lobular histology[28]. All patients had lumpectomy and axillary node dissection with ≥ 2 mm clear microscopic margin of the lumpectomy cavity. Patients with 1-3 involved nodes were initially included but were later excluded. The early phase of the trial delivered 50 Gy of continuous LDR brachytherapy over 5 d with iodine-125 sources[29]. The later phase of the trial used HDR brachytherapy with iridium-192 to deliver 32 Gy in 8 twice daily fractions or 34 Gy in 10 twice daily fractions[30]. The planned treatment volume was the lumpectomy cavity with additional 1 to 2 cm margin. With 5.7 years of median follow-up, 90% (total 199 patients) of patients had good to excellent cosmesis with comparable complications to matched WBI treated patients. The 5-year actuarial recurrence rate was 1.2%. These studies, using multicatheter interstitial brachytherapy, were followed by other successful, non-randomized studies listed in Table 1, and ultimately led to multi-institutional trials.

Radiation Therapy Oncology Group (RTOG) 9517 was opened as a multi-institutional phase Ⅰ/Ⅱ MIB-based APBI trial, and enrolled patients with unifocal tumors < 3 cm, negative margins, and axillary lymph-node sampling, with involvement of ≤ 3 involved nodes with no extra-capsular extension[31]. One hundred patients were accrued between 1997 and 2000, and 99 patients were evaluated. Thirty-three patients received 45 Gy in 3.5-5 d with LDR, and 66 patients to 34 Gy in 10 twice-daily fractions with HDR. In both cases, the target volume was the lumpectomy cavity with 2 cm margin peripherally and 1 cm superficially and deep. The 5-year actuarial in-breast failure rates were 6% and 3% for LDR and HDR brachytherapy, respectively[32]. Acute toxicities, including pain, tenderness, erythema, edema, and infection, were followed, and 3 of 33 patients receiving LDR APBI and 2 of 66 patients receiving HDR APBI experienced grade 3 or 4 adverse effects. These rates of toxicity were similar to earlier single institution trials. Reported late toxicities included breast tenderness, skin thickening, and fibrosis, and the LDR group suffered more frequent late toxicities than the HDR group (18% *vs* 4%)[31].

The first phase Ⅲ trial included patients treated with MIB-based APBI[33]. A total of 258 patients, with T1N0-1mi, grade 1-2 non-lobular breast cancer with negative resection margins and no extensive intraductal component, were randomized to partial breast irradiation (PBI) or WBI between 1998 and 2004. PBI included either LF external-beam irradiation of 50 Gy in 25 fractions for patients who were technically unsuitable for HDR MIB or HDR MIB of 5.2 Gy for 7 fractions. One hundred thirty-three patients were accrued in WBI group and 128 in PBI group (88 HDR MIB and 40 LF external-beam PBI). The 10-year actuarial local recurrence rate (5.9% PBI *vs* 5.1% WBI) was similar for the two arms (P = 0.77). The rates of good to excellent cosmetic outcome were 81% in the PBI groups together and 63% in the WBI group (P < 0.01). HDR MIB APBI demonstrated superior cosmesis compared to LF external-beam PBI, with 85% *vs* 72.5% good to excellent cosmesis[34].

A collaborative effort in Europe recently reported a phase Ⅲ, randomized, non-inferiority trial, using solely MIB[35]. A total of 1184 patients between April 2004 and July 2009, with favorable invasive carcinoma and DCIS, were randomized to either WBI (551 patients) or MIB APBI (633 patients). The primary endpoint was local recurrence. Five patients in WBI group and 9 patients in APBI group had local recurrence at 5-year follow-up. The cumulative incidence of local recurrence of APBI was 1.44% *vs* 0.92% with WBI. The 5-year rate of grade 2-3 late toxicities to the skin was 5.7% with WBI *vs* 3.2% with APBI (P = 0.08), and the 5-year rate of grade 2-3 subcutaneous tissue late side-effects was 6.3% *vs* 7.6% (P = 0.53). The incidence of severe grade 3 fibrosis was 0.2% with WBI at 5 years and 0% with APBI (P = 0.46). There were no grade 4 late toxicities. The study concluded that the 5-year LC, DFS, and OS were similar for MIB APBI and WBI after BCS for patients with early breast cancer.

Intracavitary brachytherapy (balloon and hybrid applicators): The success of MIB APBI is highly dependent on center expertise; therefore, it is not easily accessible to the general population. This led to the development of a more user-friendly brachytherapy approach with flexible balloon catheter. The Mammo Site® (Hologic Inc., Marlbourough, MA) intracavitary breast brachytherapy applicator was approved by the FDA in 2002 and simplified APBI administration. The deflated, single-channel balloon catheter is positioned into the lumpectomy cavity after resection at the time of surgery or post-operatively *via* a subsequent procedure. The balloon is then inflated with a mixture of saline and radio-opaque contrast to fill the lumpectomy cavity. CT imaging is used for assessment of catheter positioning and to assure appropriate skin spacing of at least > 5 mm or > 7 mm optimally. A computer-controlled remote after-loader is used to insert iridium-192 source into the balloon catheter to deliver 34 Gy in 10 twice daily fractions (prescribed to 1 cm from the balloon surface). The catheter is removed after the final fraction and deflation of the MammoSite® balloon.

The MammoSite® Breast Brachytherapy Registry Trial enrolled 1449 patients and had a median follow-up of 63.1 mo with 5-year actuarial rate of IBTR of 3.8%. Tumor size and the lack of estrogen receptor expression were found to be associated with IBTR. At 84 mo, 90.6% of patients had good to excellent cosmesis[36].

William Beaumont Hospital enrolled 45 patients in a phase Ⅰ/Ⅱ study using MammoSite balloon brachytherapy with an alternative fractionation schedule[37]. A total

Table 1 Additional selected, non-randomized clinical experience with interstitial brachytherapy with more than 5 years follow-up

Ref.	No. of patients	Follow-up interval (yr)	Modality	Scheme	Total dose (Gy)	5-yr LR (%)	Good/excellent cosmesis
Strnad *et al*[57]	274	5.25	PDR/HDR	PDR = 0.6 Gy/h HDR = 4 Gy × 8	PDR = 50 Gy HDR = 32 Gy	2.9%	90%
Rabinovitch *et al*[32,58]	98	11.3	LDR/HDR	LDR = 3.5-5 d HDR = 3.4 Gy × 10	LDR = 45 Gy HDR = 34 Gy	4%	68%
Shah *et al*[59,60]	199	12.0	LDR/HDR	LDR 0.52 Gy/h × 96 h HDR = 4 Gy × 8 HDR = 3.4 Gy × 10	LDR = 50 Gy HDR = 32 Gy HDR = 34 Gy	5% (12-yr 5%)	99%
King *et al*[27]	51	6.25	LDR/HDR	LDR = 4 d HDR = 4 Gy × 8	LDR = 45 Gy HDR = 32 Gy	3.9%	75%
Ott *et al*[61,62]	274	5.33	PDR/HDR	PDR = 0.6 Gy/h HDR = 4 Gy × 8	PDR = 49.8 Gy HDR = 32 Gy	2.3%	92%
Polgár *et al*[63]	45	11.1	HDR	4.33 Gy × 7 5.2 Gy × 7	30.3 Gy 36.4 Gy	4.4% (12-yr 9.3%)	78%

HDR: High-dose rate; LDR: Low dose rate; LR: Local recurrence; PDR: Pulsed-dose rate.

dose of 28 Gy in 4 fractions were given in 2 d. At ≥ 6 mo, 2% had grade 2 induration, radiation dermatitis, or hyperpigmentation and 2% grade 3 breast pain. There were 4 cases of fat necrosis. Cosmesis was good to excellent in 96% of cases. The investigators concluded that the 2-d dose schedule resulted in acceptable toxicity rates.

Efforts were made to improve the conformity of radiation delivered *via* balloon applicators with a multi-catheter design. The SAVI® (Strut Assisted Volume Implant), which was FDA approved in 2006, is a bundle of flexible, tiny catheters that can be expanded uniformly to conform to the size and shape of tumor cavity. Fisher *et al*[38] compared outcomes for 117 patients; 77 of whom received APBI *via* MammoSite® device and 40 patients *via* the SAVI® APBI device. None of the patients implanted with the SAVI device required explantation due to skin proximity. This compared to 57% of the patients implanted with the Mammo Site® device, whose skin to target distance was < 7 mm, had explantation. The closest target-to-skin distance treated with the SAVI® device was 2 mm. Good to excellent cosmesis was reported in the 12 patients who had limited skin spacing treated with SAVI®. Contura is another commercially available multilumen balloon breast brachytherapy catheter device, and investigators conducted a multi-institutional phase Ⅳ registry trial for this device, enrolling 342 evaluable patients between January 2008 and February 2011. The median follow-up was 36 mo, and the 3-year local recurrence-free survival was 97.8% and good to excellent cosmesis in 88% of the patients. The incidence of infection was 8.5%, and 4.4% of patients suffered symptomatic seroma[39]. Patients treated at high-volume centers had a superior cosmetic outcome, with 95% of those patients with good to excellent overall cosmesis, indicating that cosmetic outcome is variable among centers.

External beam radiation therapy

External beam radiation therapy (EBRT) includes 3D-conformal radiation therapy (3D-CRT) and intensity modulated radiation therapy (IMRT) defined by the inverse planning of radiation fields. EBRT delivers radiation to a clinical target volume, which for APBI is the tumor bed with 10 to 15 mm. An additional 5 to 10 mm margin was added for set-up errors and target motion. Patients receiving APBI can be set up either supine or prone and are typically treated with four or five non-coplanar beams. A potential advantage of EBRT is that it is widely available. RTOG 0319, a phase Ⅰ/Ⅱ trial, sought to evaluate the efficacy and toxicity of 3D-CRT APBI. The trial enrolled 52 evaluable patients with tumors ≤ 3 cm, ≤ 3 positive nodes, and negative margins. Patients received 38.5 Gy in 10 twice daily fractions. With median follow-up of 4.5 years, the 4-year estimates of IBTR, DFS, and OS were 6%, 84%, and 96%, respectively. Only 4% of patients suffered grade 3 toxicities[40]. RTOG 0319 demonstrated the feasibility of 3D-CRT APBI, and the effectiveness of EBRT was further explored in subsequent trials.

The phase Ⅲ study, NSABP B39/RTOG 0413 is the largest ongoing randomized trial of WBI *vs* APBI. The APBI techniques utilized in the trial are multicatheter brachytherapy (34 Gy), MammoSite (34 Gy), and EBRT (38.5 Gy), given twice daily for 10 fractions, with at least 6 h in between.

While the oncology community waits for the results of NSABP B39/RTOG 0413, interim results from other randomized studies of EBRT APBI have been presented. The Ontario Clinical Oncology Group sponsored RAPID, a randomized trial of APBI using 3D-CRT *vs* WBI. The study enrolled 2135 patients between 2006 and 2011, and an interim cosmetic and toxicity report demonstrated increased adverse cosmesis at 3 years for patients receiving APBI as compared with WBI evaluated by trained personnel (29% *vs* 17%, $P < 0.001$), by patients (26% *vs* 18%, $P = 0.0022$), and by review of imaging by physicians (35% *vs* 17%, $P < 0.001$). Grade 3 toxicities were uncommon in the 2 treatment arms (1.4% for APBI *vs* 0% for WBI)[41]. In another study, the University of Florence (Florence, Italy) recently reported the result

of a phase Ⅲ randomized controlled trial comparing IMRT *vs* WBI. A total of 520 patients were randomized with 260 patients in each arm between March 2005 and June 2013[42]. At a median follow-up of 5.0 years, the IBTR rate was 1.5% in the APBI and WBI groups. The 5-year OS was 96.6% for the WBI group and 99.4% for APBI group. Patients treated with APBI demonstrated significantly less acute and late toxicity and better cosmetic outcome.

Other groups are investigating alternative external beam fractionation regimens. The ACCEL Trial (NCT02681107), sponsored by AHS Cancer Control Alberta, is a phase Ⅱ study evaluating patients treated with EBRT APBI to a prescribed dose of 27 Gy over 5 fractions delivered daily. The Mayo Clinic is sponsoring a phase Ⅱ trial evaluating APBI given in 3 fractions of 7.3 Gy using EBRT or 7 Gy using catheter-based brachytherapy (NCT02453737).

ADDITIONAL APBI TECHNIQUES

Intraoperative radiation therapy

Intrabeam: Intraoperative radiation therapy (IORT) refers to radiation treatment of the tumor bed in a single treatment delivered in the operating room after resection and prior to closure. The rationale for IORT is that a single fraction delivered at the time of surgery makes post-operative radiotherapy unnecessary. In the past, the popularity of IORT was limited because of the expense and impracticality of the specialized radiation delivery devices, but more recently advances in technology have made IORT devices more mobile and available[43]. The first widely available IORT device, Intrabeam®, was first used introduced 1998. Since then, at least two mobile IORT-capable linear accelerators, the Mobetron and Novac-7 systems have become available. While Intrabeam® is a kilovoltage photon system, Mobetron and Novac-7 generate megavoltage electrons.

Intrabeam® (Oberkochen, Germany) uses spherical applicators to deliver kilovoltage photons once inserted into the surgical cavity for uniform dose deposition. The estimated time required to deliver APBI using this device is 20 to 35 min in a single application (this is comparable to the treatment times for each of the 10 fractions delivered for EBRT) making this type of treatment more convenient in some setting. In addition, it has been hypothesized that single fraction IORT has a better therapeutic index[44].

The TARGIT-A trial randomized 3451 patients to either EBRT or TARGIT-A (20 Gy IORT with 50 kV photons). Patient eligibility criteria included age ⩾ 45 years, tumor size ⩽ 3.5 cm, N0-1, M0, and unifocal invasive ductal carcinoma[45]. TARGIT-A patients with adverse risk factors identified on final pathology were given an additional 50 Gy equivalent of EBRT. At 29 mo of median follow-up, the 5-year recurrence rates for patients treated with TARGIT-A and WBI were 3.3% and 1.3%, respectively (P = 0.042). Wound complication rates between the 2 groups were similar; however, grade 3 or 4 skin complications were lower with TARGIT-A *vs* EBRT (P = 0.029). Twenty-one percent of prepathology TARGIT-A patients received 5 wk of EBRT. Patients who received only TARGIT-A had 3 times the recurrence rate of those who received TARGIT-A plus EBRT (2.7% *vs* 0.9%). Breast cancer mortality was similar between two groups; however, the number of non-breast cancer deaths was lower in the TARGIT-A group (1.4% *vs* 3.5%, P = 0.0086). The study concluded that longer follow-up is needed, but the results are promising, given the good survival rate and low recurrence rate. Importantly, some of the patients included in the trial might not be suitable for APBI according to current guidelines.

Mobetron: The Mobetron consists of a mobile robotic arm linear accelerator with multiple electron energies. The Mobetron device is inserted into the surgical cavity for the delivery of electron radiation. An acrylic resin-copper disk may be placed between the breast tissue and the underlying muscle to protect the thoracic wall. A phasee Ⅰ/Ⅱ single arm dose-escalation study treated patients with 19 to 21 Gy at the 90% isodose line[46]. Selection criteria for the study included age > 50 years, tumors < 2.5 cm, surgical margins > 1 cm, no extensive intraductal component, no prior chest irradiation, and free surgical margins by intraoperative pathology. The target volume is lumpectomy cavity with 2 cm margin. 6-12 MeV electrons were used for treatment. With only 9 patients and an average follow-up of 11.3 mo, conclusions are limited; however, the largest dose of 21 Gy seemed to be well-tolerated. The authors used Common Terminology Criteria for Adverse Events v3.0 for reporting toxicities and reported grade 1 hematoma in 1 of 3 patients, grade 1 soft tissue infection in 1 of 3 patients, and grade 2 soft tissue necrosis in 2 of 3 patients[46].

Novac7: Novac7 (Hitesys, Latina, Italy) is also a mobile linear accelerator with electrons of multiple energies delivered *via* a cylindrical perspex applicator with a diameter of 4 to 10 cm. The unit is mounted on a robotic arm for positioning. The phase Ⅲ Electron IntraOperative Therapy (ELIOT) trial randomized 1305 patients, who were ⩾ 48 years with tumors ⩽ 2.5 cm, to either a single intraoperative dose of 21 Gy or to EBRT of 50 Gy WBI with a 10 Gy boost all delivered over 6 wk[47]. The trial employed the Novac7, as well as a similar device, the Liac. At 5.8 years of median follow-up, the 5-year recurrence rates for ELIOT and EBRT were 4.4% and 0.4% respectively (P = 0.0001). A low risk ELIOT group had a 5-year recurrence rate of 1.5%. The ELIOT group had significantly less skin toxicity (erythema, dryness, hyperpigmentation, or itching), but a higher incidence of fat necrosis.

Proton therapy

Bush *et al*[48] reported the 5-year results of a phase Ⅱ trial using proton beam radiation to deliver APBI in patients with invasive nonlobular carcinoma with a

Table 2 Accelerated partial breast irradiation patient selection criteria according to American Society for Radiation Oncology consensus statement[52]

Factors	Suitable	Cautionary	Unsuitable
Age (yr)	> 60	50-59	< 50
BRCA1/2 mutation	Not present	NS	Present
Tumor size	< 2 cm	2.1-3.0 cm	> 3 cm
T stage	T1	T0 or T2	T3-4
Margins	Negative (> 2 mm)	Close (< 2 mm)	Positive
Grade	Any	NS	NS
LVSI	No	Limited/focal	Extensive
ER status	Positive	Negative	NS
Multicentricity	Unicentric only	NS	Present
Multifocality	Clinically unifocal with total size < 2 cm	Clinically unifocal with total size 2.1-3.0 cm	Microscopically multifocal > 3 cm in total size or if clinically multifocal
Histology	Invasive ductal or other favorable subtypes	Invasive lobular	NS
Pure DCIS	Not allowed	< 3 cm	> 3 cm
EIC	Not allowed	< 3 cm	> 3 cm
Associated LCIS	Allowed	NS	NS
LN status	pN0 (i-, i+)	NS	pN1, pN2, pN3, or if not evaluated
Neoadjuvant therapy	Not allowed	NS	If used

DCIS: Ductal carcinoma *in situ*; EIC: Extensive intraductal component; ER: Estrogen receptor; LCIS: Lobular carcinoma *in situ*; LVSI: Lymphovascular space invasion; LN: Lymph node; NS: Not specified.

maximal dimension of 3 cm, negative axillary lymph nodes on sampling, and negative surgical margins. Proton therapy was given to the surgical bed with 40 Gy in 10 fractions, once daily over 2 wk, using skin-sparing techniques. The study enrolled 100 patients. At median follow-up of 60 mo, the 5-year actuarial rates of IBTR-free survival, DFS, and OS were 97%, 94% and 95%, respectively. There were no grade 3 or higher acute skin reactions, and patient- and physician-reported cosmesis was good to excellent in 90%[48]. In addition, Chang *et al*[49] reported results of prospective study of 30 patients treated with 30 cobalt gray equivalent in 6 fractions delivered daily over 5 consecutive days. At 59 mo of median follow-up, no patients had local or metastatic recurrence, and all patients were alive at the last follow-up. Qualitative physician cosmetic assessments of good to excellent were 69% at 3 years[49].

CyberKnife stereotactic APBI

With technological advances in stereotactic radiotherapy, CyberKnife has been investigated as a method to deliver APBI. CyberKnife provides for real-time tracking, respiratory motion management, and submillimeter accuracy and allows for treatment intensification while reducing dose to surrounding normal structures[50]. Georgetown University Hospital treated 10 patients, who were $\geqslant$ 48 years with DCIS or invasive non-lobular carcinoma $\leqslant$ 2 cm in maximum diameter and $\geqslant$ 2 mm of negative margin, using CyberKnife[51]. The planning target volume was delineated on CT scans with 5 mm expansion, and 30 Gy was delivered in daily fractions for 5 consecutive days to the planning target volume. All 10 patients experienced good to excellent cosmetic outcomes with no breast events recorded at median follow-up of 1.3 years. The authors concluded that CyberKnife was reliable in delivering APBI that was well-tolerated; however, the study was limited by its small sample size and brief follow-up.

CURRENT PATIENT SELECTION GUIDELINES

The initial APBI trials have demonstrated the importance of patient selection. With more strict criteria, APBI has been shown to have comparable local recurrence rates in addition to better cosmetic outcome. The most recent American Society for Radiation Oncology (ASTRO) consensus guidelines were published in 2009[52]. Patients were classified into three groups: Suitable, cautionary, and unsuitable. The specific criteria are listed in Table 2. In addition, Table 3 compared the guidelines from different task groups.

OTHER CONSIDERATIONS

Patient reported quality of life

Quality of life is a vital consideration when patients are choosing their breast cancer treatments. Bitter *et al*[53] analyzed self-reported cosmetic outcomes for the treated breast and quality of life for patients treated with WBI or APBI *via* single and multilumen HDR brachytherapy. Two hundred and forty-two patients between 2004 and 2014 with early breast cancer treated with APBI were compared to 59 matched patients treated with WBI from 2012 to 2014. They were evaluated with modified Functional Assessment of Chronic Illness Therapy breast quality of life questions which measured pain, lymphedema, energy level, self-consciousness, and breast cosmesis. Compared to APBI eligible patients treated with WBI, the APBI cohort experienced significantly better lymphedema ($P = 0.0002$), self-consciousness ($P = 0.0004$), and energy level ($P = 0.009$) scores during the first year after treatment. The APBI group reported significantly better breast cosmesis

Table 3 Accelerated partial breast irradiation patient selection criteria from selected organizations

Organization	Age	Tumor size	Margin	Histology	LN status
American Brachytherapy Society[64]	> 50	< 3 cm	Negative (at inked margin)	Invasive ductal carcinoma	pN0; by SLN or axillary dissection
American Society of Breast Surgeons[36]	> 45	< 2 cm	Negative (> 2 mm)	Invasive ductal carcinoma or DCIS	pN0; by SLN or axillary dissection
ASTRO[52]	> 60	< 2 cm	Negative (> 2 mm)	Invasive ductal or other favorable subtypes (mucinous, tubular, and colloid)	pN0; by SLN or axillary dissection

ASTRO: American Society for Radiation Oncology; DCIS: Ductal carcinoma *in situ*; SLN: Sentinel lymph node.

Table 4 Phase III prospective randomized trials evaluating the equivalence or non-inferiority of accelerated partial breast irradiation with whole-breast irradiation

Trial	No. of patients	Follow-up interval (yr)	Inclusion criteria	APBI technique	5-yr LR APBI *vs* WBI (%)
TARGIT-A[45]	3451	2.4	Age ⩾ 45 yr; T1, small T2, N0, N1; ductal; non-lobular and no EIC	20 Gy in 1 fraction, IORT low energy X-rays (50 kV)	3.3 *vs* 1.3
ELIOT[47]	1305	5.8	Age ⩾ 48 yr; T ⩽ 2.5 cm, N0; invasive carcinoma; quadrantectomy	21 Gy in 1 fraction, IORT, electrons up to 9 MeV	4.4 *vs* 0.4
RAPID (OCOG)[41]	2135	Pending	Age > 40 yr; T ⩽ 3 cm, N0; DCIS or invasive carcinoma; negative margins	38.5 Gy in 10 fractions (5-8 d) using 3D-CRT	Pending
GEC-ESTRO[35]	1184	5.0	Age ⩾ 40 yr; T ⩽ 3 cm, pN0-Nmi; stage 0, I, II; DCIS, ductal or lobular carcinoma; margin ⩾ 2 mm	32 Gy in 8 fractions or 30.3 Gy in 7 fractions MIB HDR or 50 Gy MIB PDR (1 pulse/h, 24 h/d; 0.6-0.8 Gy/h)	1.4 *vs* 0.9
Florence (NCT02104895)[42]	520	5.0	Age > 40 yr; T < 2.5 cm; clear margins > 5 mm	IMRT 30 Gy in 5 daily fractions	1.5 *vs* 1.5
IMPORT-LOW	2018	Pending	Age ⩾ 50 yr; T ⩽ 3 cm, node negative; invasive adenocarcinoma; margin ≥ 2 mm	IMRT; Arm 1: 40 Gy in 15 fractions to primary tumor region + 36 Gy in 15 fractions to low-risk region (EBRT) Arm 2: 40 Gy in 15 fractions to primary tumor region (EBRT)	Pending
IRMA (NCT01803958)	3302 (Currently Enrolling)	Pending	Age ⩾ 49 yr; T < 3 cm, N0; invasive carcinoma; margins ⩾ 2 mm	38.5 Gy in 10 fractions using 3D-CRT, BID	Pending
SHARE (NCT01247233)	1006	Pending	Age ⩾ 50 yr; invasive carcinoma; T ⩽ 2 cm; margin⩾ 2 mm; pN0 (i+/-)	3D-CRT 40 Gy in 10 fractions, BID	Pending
NSABP B-39/RTOG 0413	4300	Pending	Age ⩾ 18 yr; DCIS or invasive adenocarcinoma; stage 0, I, II (T < 3 cm); lumpectomy; margins free of tumor; ⩽ 3 positive nodes	34 Gy in 10 fractions using MIB or MammoSite® /MammoSite® ML/SAVI® or 38.5 Gy over 10 fractions using 3D-CRT	Pending

3D-CRT: 3D conformal external-beam radiation; BID: Twice daily; DCIS: Ductal carcinoma *in situ*; EBRT: External beam radiation therapy; EIC: Extensive intraductal component; HDR: High-dose rate; MIB: Multicatheter interstitial brachytherapy; ML: Multilumen; IMRT: Intensity modulated radiotherapy; IORT: Intraoperative radiotherapy; PDR: Pulsed-dose rate; WBI: Whole-breast irradiation.

during the second year after treatment. There were no significant differences in the recurrence rates ($P > 0.05$)[53]. Moreover, analyses of late toxicities and cosmesis of patients treated with APBI on RTOG 0319 demonstrated good to excellent cosmesis in 82% and 64% of patients at 1 year and 3 years, respectively. When questioned at 3 years, 31 patients were satisfied with their treatment, 5 were not satisfied but would choose 3D-CRT again, and no patients would elect standard radiation therapy[54].

Economics of treatment

In addition to identifying the group of patients with the appropriate breast cancer biology, it is important to consider other factors, such as socioeconomic issues. Shah *et al*[55] reported results of cost-efficacy of multiple APBI techniques compared with WBI. Their analyses included cost minimization, incremental cost-effectiveness ratio (ICER), and cost per quality adjusted life year (QALY) analyses. For 1000 patients treated, the cost savings would be $6.0 million (APBI 3D-CRT), $2.0 million (APBI IMRT), and $0.7 million (APBI interstitial) with the utilization of APBI compared to WBI 3D-CRT. The cost per QALY was $54698 and $49009 for APBI multilumen and APBI 3D-CRT, respectively, when incorporating the cost of recurrences and non-medical costs[55].

CONCLUSION

APBI has gained acceptance for appropriately selected cases of early stage breast cancer, as outlined by cu-

rrent guidelines. Shaitelman *et al*[56] showed increased utilization of APBI from 3.8% of breast cancer radiation in 2004 to 10.6% in 2011 ($P < 0.0001$), with most of the APBI given *via* brachytherapy. The proliferation of APBI demonstrates its acceptance by patients in the modern era owing in part to its increased convenience and potential for reduced toxicities. As the use of APBI expands, the need for patient selection guidelines and consensus statements becomes even more important. There are many ongoing phase Ⅲ trials that are testing the non-inferiority and equivalence of various forms of APBI compared to WBI (Table 4). Some of these ongoing studies have reported results of interim analyses. As the data matures, we will be able to more appropriately select the specific patients benefiting most from APBI. Furthermore, as patient reported outcome measures, such as quality of life, gain traction in parallel to outcome studies, this data should be incorporated into shared decision making with patients.

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Granulocyte colony-stimulating factor-producing hepatocellular carcinoma with abrupt changes

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Abstract

Granulocyte colony-stimulating factor (G-CSF)-producing tumor is one of the rare types of cancer clinically characterized by an elevated fever and white blood cell (WBC) increment. Although G-CSF producing tumors have been reported in several types of cancer including those of the lungs, cervix and bladder, G-CSF producing hepatocellular carcinoma is extremely rare. Here, we report the case of a rapidly growing and poorly differentiated hepatocellular carcinoma producing G-CSF. The patient showed symptoms of continuous high fever, stomach pain and cough, and high serum WBC counts, C-reactive protein (CRP) and G-CSF levels were found in laboratory tests. After a radical hepatectomy, the patient completely recovered from the above symptoms and inflammatory state. The serum levels of G-CSF were reduced to normal levels after radical surgery. An immunohistochemical analysis revealed the overexpression of G-CSF in the cytoplasm of certain hepatocellular carcinoma (HCC) cell. The patient's serum WBC, CRP and G-CSF levels remained within normal levels in the six months after surgery without recurrence. This is the 9th case report of G-CSF producing hepatocellular carcinoma in English literature. We review the clinical characteristics of the G-CSF producing HCC and discuss a possible treatment strategy.

Key words: Granulocyte colony stimulating factor; Granulocyte colony-stimulating factor producing tumor; Hepatocellular carcinoma; Immunohistochemistry; Sarcomatous changes

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Core tip: Granulocyte colony-stimulating factor (G-CSF)-producing tumor is one of the rare types of cancer clinically characterized by an elevated fever and white blood cell increment. Although G-CSF producing tumors have been reported in several types of cancer including those of the lungs, cervix and bladder, G-CSF producing hepatocellular carcinoma (HCC) is extremely rare. This is the 9th case report of G-CSF producing HCC in English literature. We report our case and review reported literatures with special reference to the clinical characteristics of the G-CSF producing HCC and a possible treatment strategy.

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INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) is a naturally produced glycoprotein that is synthesized by stromal cells in bone marrow. G-CSF stimulates progenitor cells to differentiate and enhances the functions of neutrophils. The G-CSF producing tumor is characterized by leukocytosis without infection and high serum G-CSF levels. In 1977, the G-CSF producing tumor was first reported in lung cancer[1]. After that, several G-CSF producing tumor cases were reported for cancers of the bladder[2,3], lung[4], thyroid[5], gallbladder[6] and uterine cervix[7]. Among them, the G-CSF producing HCC is extremely rare and is generally reported as having a poor prognosis because of its dramatic tumor progression. Liver cancer including hepatocellular carcinoma (HCC) is the second cause of cancer death worldwide[8]. It is common that HCC develops in the patient with chronic hepatitis caused by viruses, especially hepatitis B virus (HBV). The development of the HCC is driven by the genetic factor, epigenetic factor, environmental factor and viruses. Although, the novel factors such as hematopoietic stem cells and non-coding RNA are reported in the recent researches to be involved in hepatocarcinogenesis[9-11], the mechanisms of the carcinogenesis of G-CSF producing HCC remains unclear.

We report a G-CSF producing HCC that was radically resected and diagnosed by pathological and serological findings. We review previous reports regarding the clinical behaviors of the G-CSF producing HCC, including our case.

CASE REPORT

A 79-year-old man was admitted to our hospital with a continuous fever, cough and high degree of serum C-reactive protein (CRP). A physical examination revealed a hard, fixed mass palpable on the right upper quadrant of the abdomen. Laboratory tests showed an increased degree of serum CRP (17.3 mg/dL) and white blood cell (WBC) counts, and a worsening of anemia compared with the patient's initial examination. In addition, a higher level of serum G-CSF (42 pg/mL) was detected. A preoperative computed tomography (CT) examination revealed an irregular mass in segment Ⅳ of the liver, approximately 60 mm in diameter with peripheral enhancement (Figure 1A). Tumor markers, such as the absence of protein-induced vitamin K or antagonist (PIVKA)-Ⅱ level, α-foetoprotein (AFP) level, carcinoembryonic antigen (CEA) level and carbohydrate antigen 19-9 (CA19-9) levels, were within the normal range. Further evaluations of the liver mass were performed.

Detailed CT examination during arterial portography (CTAP), computed tomography during hepatic arteriography (CTHA), magnetic resonance cholangio pancreatography (MRCP), and gadoxetic acid-enhanced MRI (Gd-EOB-MRI) revealed that the liver mass was a poorly differentiated carcinoma, rather than a liver abscess. The tumor partially occupied segment IV of the liver and protruded toward the abdominal cavity (Figure 1A and B).

Four days after admission, the patient continued to have an intermittent fever (Figure 2A) and the tumor size became drastically enlarged within a short period; therefore, we decided to perform surgery. The surgery was a complete resection with a segment Ⅳ partial hepatectomy. There was ascites around the tumor in the abdominal cavity, but a cytological analysis revealed that was no malignant cells in it.

After the radical hepatectomy, the patient's fever gradually dropped to a normal temperature and the other symptoms, such as cough and abdominal pain, ceased (Figure 2A). The laboratory data, such as WBC count and neutrophil percentage returned to the normal range by postoperative day 5 (from 13020/μL to 6180/μL, 88.3% to 68.5%, respectively). The serum CRP level dropped gradually from 28.7 mg/dL to 1.5 mg/dL by postoperative day 12. The patient had an uneventful postoperative recovery and was discharged on postoperative day 12. Afterward, serum WBC counts, CRP and G-CSF returned to normal levels (Figure 2B and C).

The pathological findings of the resected specimen showed that the tumor size was 12.0 cm × 10.0 cm × 10.0 cm, and the gallbladder and partial greater curvature were also resected with the main tumor (Figure 1C). The cut surface of the tumor was white with an irregular margin and vast necrotic tissue was observed inside the tumor (Figure 1D). Microscopic findings revealed that the tumor was mainly composed of poorly differentiated hepatocellular carcinoma (Figure 3A) and partially sarcomatous spindle-shaped malignant cells (Figure 3B) were detected. Moreover, a drastic neutrophil infiltration within the hepatocellular carcinoma cells was

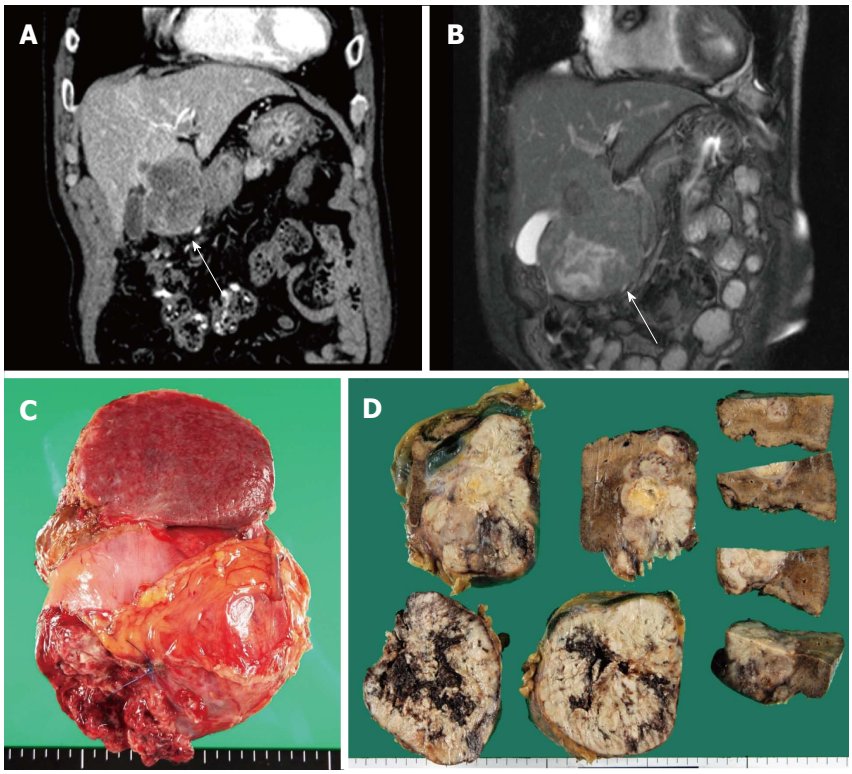
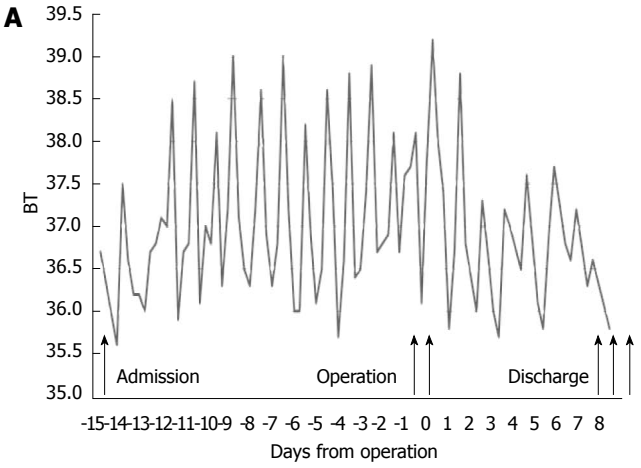
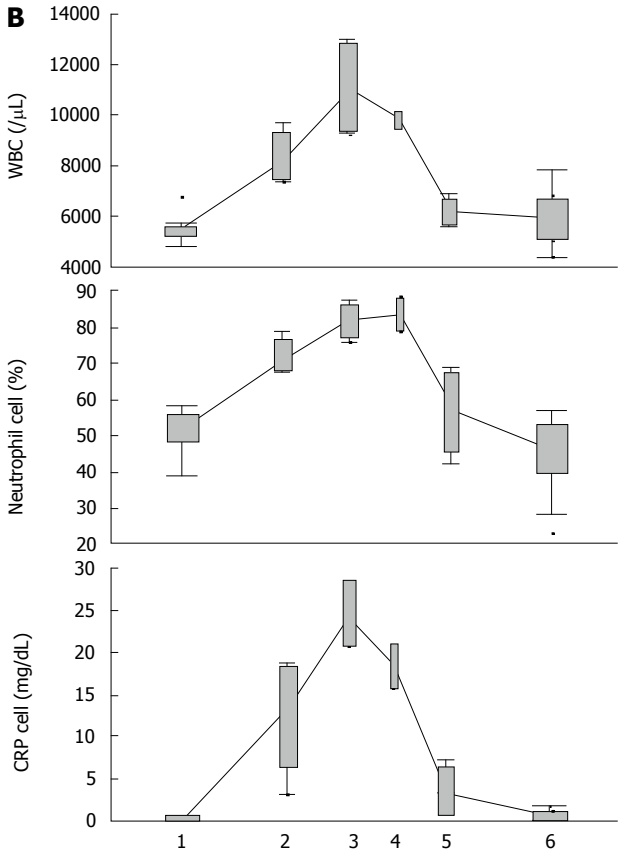


Figure 1 Imaging and macroscopic findings of granulocyte colony-stimulating factor producing hepatocellular carcinoma. A: CT scan one month before operation showed an irregular liver mass located in segment IV, approximately 60 mm in diameter with peripheral enhancement (white arrow head); B: T2-WI MRI one week before operation showed the rapidly growing liver mass with a 100 mm diameter (white arrow head); C: Macroscopic examination showed a large tumor (100 mm × 100 mm) that protruded through segment Ⅳ of the liver to the greater omentum; D: The irregular liver tumor in segment Ⅳ showed a central necrosis.





C

	2 d before operation	2 wk after operation	1 mo after operation	6 mo after operation
G-CSF (pg/mL)	42.0	26.9	25.2	24.0

Figure 2 Physiological and laboratory changes during the treatment. A: Changes in body temperature during the treatment; B: Laboratory changes during the treatment; 1: Steady state; 2: Admission; 3: Pre operation; 4: Post-operation; 5: Within 2 mo after operation; 6: More than 2 mo after operation; C: White blood cell count, neutrophil proportion and C-reactive protein were collected at various treatment points including "steady-state" (more than six months before admission), "before admission" (within six months of admission), "pre-operation" (from admission until operation), "post-operation" (from operation until discharge), "within two months of surgery" and "more than two months after operation".

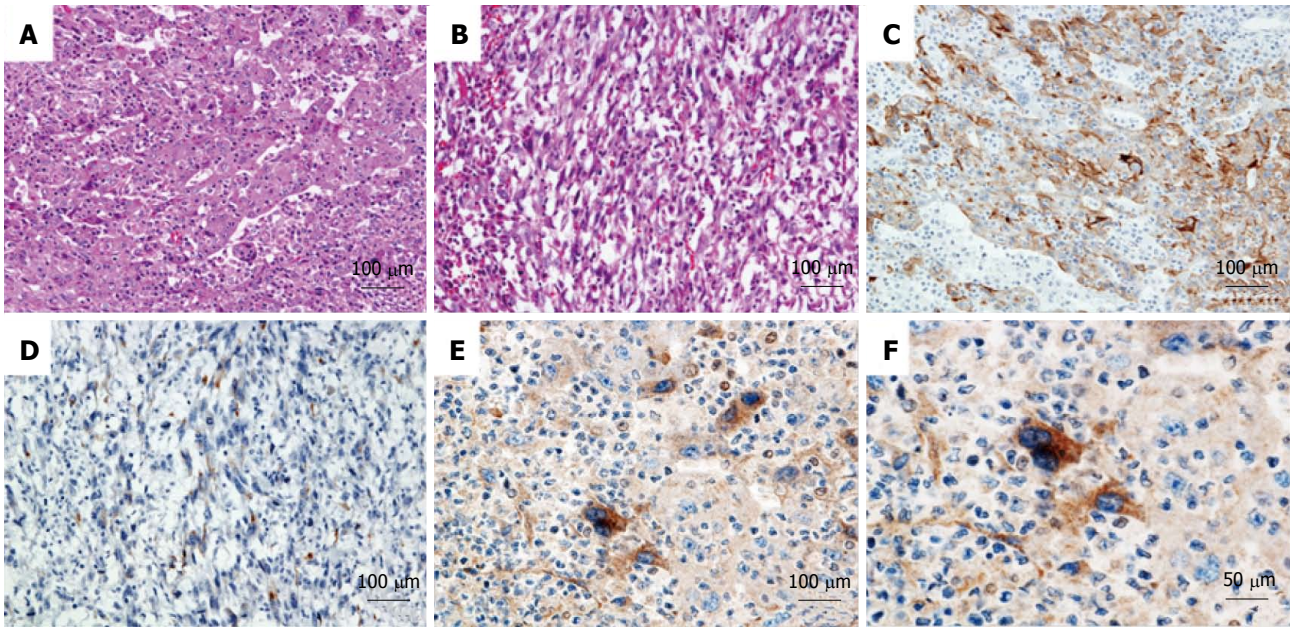


Figure 3 Histopathologic findings. Microscopic findings showed atypical poorly differentiated cells with a sheet structure (A); HCC tumor was also composed of sarcomatous spindle-shaped cells (B); in both samples, a drastic infiltration of the neutrophils was found (H and E, × 20). Immunohistochemical findings showed CAM5.2 positive in the moderately to poorly differentiated HCC lesion (C) and negative in the spindle-shaped cell lesion (D) (CAM5.2, × 20). Immunohistochemical examination showed that G-CSF was positive in the moderately to poorly differentiated HCC lesion (E, F) (G-CSF, × 20 and × 40). G-CSF: Granulocyte colony-stimulating factor; HCC: Hepatocellular carcinoma cell.

noted (Figure 3A and B). Immunohistochemistry showed the ordinary HCC cells to be positive for CAM5.2 (Figure 3C) and the sarcomatous area was positive for vimentin. The HCC cells were positive for G-CSF. These findings were supportive for the diagnosis of G-CSF producing HCC (Figure 3).

G-CSF

G-CSF is a glycoprotein (19.6 kDa) that stimulates cell proliferation and differentiation of precursor cells in the bone marrow. G-CSF is major extracellular regulator of hemopoiesis and the immune system, first named in the 1980s[12,13]. It not only changes mature precursor cells into fully differentiated neutrophils, but also enhances their functional activity[14]. These mechanisms have been exploited to produce a drug to increase neutrophils in patients with chemotherapy-induced neutropenia. Granulocyte colony-stimulating factor receptor (G-CSF R) is also member of the cytokine receptor family and functions in some cell surface adhesion or recognition process. This protein is essential for granulocytic maturation and plays a crucial role in the proliferation, differentiation and survival of cells along the neutrophilic lineage. Furthermore, there are reports on the relationship between G-CSF and cancers[15-17].

CLINICAL BEHAVIOR OF G-CSF PRODUCING TUMOR

Some cancers have been reported to produce certain humoral factors including cytokines, such as G-CSF, granulocyte macrophage colony-stimulating factor (GM-CSF), erythropoietin or parathyroid hormone, which cause paraneoplastic syndrome[18-21]. Paraneoplastic syndrome presents as various clinical disorders, such as anemia, hypercalcemia, erythrocytosis, granulocytosis and thrombocytosis, and is often reported in lung cancer[20,22]. Asano *et al*[1] first reported G-CSF producing lung cancer in 1997. After this report, various cases were reported with G-CSF producing tumors in lung, bladder, sarcoma, cervical and gallbladder cancers[3,6,7,20,22]. The G-CSF producing tumor has been described as having (1) a drastic WBC increase; (2) an elevation of G-CSF activity; (3) WBC decrease after tumor resection; and (4) evidence of G-CSF production in the tumor tissue[1]. In our case, high WBC counts and fever elevation were present without a bacterial infection preoperatively. Also, a contrast enhanced CT image revealed a non-typical and poorly differentiated HCC tumor. After radical hepatectomy, the serum WBC level and G-CSF activity were decreased to normal levels. Finally, immunohistochemical staining showed G-CSF production in the tissue inside the tumor. These findings fit the above definition and strongly suggested that our case was a G-CSF producing HCC[27-31].

PREVIOUS REPORTS OF G-CSF PRODUCING HEPATOCELLULAR CARCINOMA, INCLUDING OUR CASE

G-CSF producing HCC is extremely rare and only eight cases have been documented in the English literature

Table 1 Previous reported cases of Granulocyte colony-stimulating factor producing hepatocellular carcinoma

Case	Ref.	Year	Age	Sex	WBC[1] (/μL)	G-CSF[2] (pg/mL)	HCV	HBV	Pathology	Sarcomatous change	Treatment	IHC	Prognosis[3]	
1	Yamamoto *et al*[19]	1999	67	M	234000	251	+	-	Poorly dif. HCC	-	TAE + Chemotherapy	+	5 mo	Dead
2	Amano *et al*[20]	2005	70	M	26400	308	-	-	Poorly dif. HCC/CCC	+	Palliative surgery	+	1 mo	Dead
3	Aita *et al*[21]	2006	74	M	71700	286	-	-	Poorly carcinosarcoma	+	TAE	+	2 mo	Dead
4	Araki *et al*[22]	2007	66	M	45200	178	-	-	Poorly dif. HCC	+	Radical surgery + TAE	+	4 mo	Dead
5	Joshita *et al*[23]	2010	66	M	25450	62	-	-	Moderately dif. HCC	-	Radial surgery	+	4 yr	Dead
6	Kohno *et al*[24]	2012	46	M	51670	195	-	+	Moderately to poorly dif. HCC	+	Radical surgery + TAE + Chemotherapy	+	7 mo	Dead
7	Snyder *et al*[25]	2012	47	F	40000	58.2	-	-	Poorly dif. HCC	unknown	Radical surgery	unknown	1 mo	Dead
8	Ito *et al*[26]	2012	37	M	51600	342	-	+	Moderately to poorly dif. HCC	-	Radical surgery + Chemotherapy	+	2 yr	Alive
9	Our case	2016	79	M	13020	42	-	-	Poorly dif. HCC	+	Radical surgery	+	6 mo	Alive

[1]White blood cell count (normal value: 4000-8000/μL); [2]granulocyte-colony stimulating factor (normal value: < 39 pg/mL); [3]prognosis after diagnosis. HBV: Hepatitis B virus; HCV: Hepatitis C virus; WBC: White blood cell; G-CSF: Granulocyte-colony stimulating factor; HCC: Hepatocellular carcinoma; CCC: Cholangiocellular carcinoma; TAE: Transcatheter arterial embolization.

(Table 1). G-CSF producing tumors, including HCC, generally grow rapidly and have a poor prognosis. G-CSF is reported to be linked to tumor cell growth and progression[31,32]. Also, there might be a relationship between the secretion of G-CSF and the degree of cell differentiation[23]. Wang *et al*[33] compared the production of G-CSF between well- and poorly differentiated HCC using cell lines and concluded that only poorly differentiated HCC tends to produce G-CSF. As shown in Table 1, of nine reports including our case, six cases 76% (6/9) were pathologically diagnosed as poorly differentiated HCCs and two cases were moderate to poorly differentiated HCCs.

Tachibana *et al*[3] reported that the G-CSF production and G-CSF receptor expression exhibited by cancer cells both play crucial roles in mediating the malignant progression of nonhematopoietic cancer cells. Baba *et al*[34] and Segawa *et al*[35] both reported G-CSF as an autocrine growth factor considered to be necessary for tumor proliferation and metastasis[3,7,34,36]. As these reports demonstrate, the prognosis of patients with G-CSF producing HCCs is indeed very poor[26-28]. Specifically, in more than one-half of the cases, the patients died within approximately within six months after diagnosis. Therefore, some authors have suggested that surgical resection is not an effective strategy considering the poor outcome of this G-CSF producing HCC[26] because most patients were diagnosed in a far-advanced stage. Whereas, our case was diagnosed as a curatively resectable stage, such as a stage-Ⅱ (T2N0M0 UICC 7th), and the preoperative serum WBC counts and G-CSF levels were relatively lower than in previous reports (Table 1). Therefore, in our case, radical tumor resection was effective.

The serum levels of G-CSF are positively correlated with WBC counts[20,37]. Also, in our case, serum WBC counts, CRP and G-CSF levels were shifting in parallel during the treatment course and the trend seemed to be correlated with the growth of the liver tumor (Figure 2B and C). To date, these marker levels are being maintained at normal levels and will continue to be monitored, and the patient has had no recurrence in the six months following surgery.

G-CSF producing HCC as one of the differential diagnosis of fever of unknown origin

Fever of unknown origin (FUO) remains to be of considerable clinical importance. Classical FUO was defined by Petersdorf and Beeson[38] in 1961. In recent study about FUO, Bleeker-Rovers *et al*[39] showed that infection was the cause of FUO in 16% of the patients, cancer in 7% and non-infectious inflammatory diseases in 22%. Their report showed that in over 50% of the cases, the cause of fever was not found. Not only hematological malignancies, but also varieties of solid neoplastic diseases have been reported as occasionally associated with FUO without any associated infection[40]. Therefore, more physicians should include G-CSF producing tumors in the differential diagnosis of FUO.

CONCLUSION

Although G-CSF producing tumors are extremely rare, clinicians should consider this diagnosis for a patient with a continuous high fever of unknown origin and leukocytosis without evidence of infection. Early laboratory and imaging examinations should also be performed for an early diagnosis, effective treatment

and improved prognosis. Radical resection in the early stage of a G-CSF producing HCC might provide a more favorable outcome. Nevertheless, further studies and the accumulation of clinical cases are required to establish appropriate treatment strategies for patients with G-CSF producing HCCs.

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ORIGINAL ARTICLE

Basic Study

Tumor infiltrating lymphocytes in triple negative breast cancer receiving neoadjuvant chemotherapy

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Author contributions: Castaneda CA, Mittendorf E, Casavilca S and Wu Y contributed to the conception and design of the study and performed data analysis and interpretation; Castaneda CA, Casavilca S, Castillo M, Nunez T and Guerra H performed data acquisition, as well as providing administrative, technical, and material support; all authors drafted the article and made critical revisions related to the intellectual content of the manuscript, and approved the final version of the article to be published.

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Abstract

AIM

To determine influence of neoadjuvant-chemotherapy (NAC) over tumor-infiltrating-lymphocytes (TIL) in

triple-negative-breast-cancer (TNBC).

METHODS

TILs were evaluated in 98 TNBC cases who came to Instituto Nacional de Enfermedades Neoplasicas from 2005 to 2010. Immunohistochemistry staining for CD3, CD4, CD8 and FOXP3 was performed in tissue microarrays (TMA) sections. Evaluation of H/E in full-face and immunohistochemistry in TMA sections was performed in pre and post-NAC samples. STATA software was used and *P* value < 0.05 was considered statistically significant.

RESULTS

Higher TIL evaluated in full-face sections from pre-NAC tumors was associated to pathologic-complete-response (pCR) (*P* = 0.0251) and outcome (*P* = 0.0334). TIL evaluated in TMA sections showed low level of agreement with full-face sections (ICC = 0.017-0.20) and was not associated to pCR or outcome. TIL in post-NAC samples were not associated to response or outcome. Post-NAC lesions with pCR had similar TIL levels than those without pCR (*P* = 0.6331). NAC produced a TIL decrease in full-face sections (*P* < 0.0001). Percentage of TIL subpopulations was correlated with their absolute counts. Higher counts of CD3, CD4, CD8 and FOXP3 in pre-NAC samples had longer disease-free-survival (DFS). Higher counts of CD3 in pre-NAC samples had longer overall-survival. Higher ratio of CD8/CD4 counts in pre-NAC was associated with pCR. Higher ratio of CD4/FOXP3 counts in pre-NAC was associated with longer DFS. Higher counts of CD4 in post-NAC samples were associated with pCR.

CONCLUSION

TIL in pre-NAC full-face sections in TNBC are correlated to longer survival. TIL in full-face differ from TMA sections, absolute count and percentage analysis of TIL subpopulation closely related.

Key words: Triple-negative breast cancer; Survival; Tumor-infiltrating lymphocytes; Neoadjuvant therapy

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Core tip: We evaluated a series of 98 triple negative breast cancer cases who received neoadjuvant chemotherapy. Pre-neoadjuvant chemotherapy and post neoadjuvant chemotherapy samples were analyzed. We compared tumor-infiltrating lymphocytes evaluated in whole slides *vs* in tissue microarray slides. We also compared subsets of tumor-infiltrating-lymphocytes (TILs) evaluated through an absolute counting *vs* a percentage calculation. Our results confirm the predictive and prognostic role of tumor infiltrating lymphocytes, and evaluated the behavior of tumor infiltrating lymphocytes and lymphocyte subsets during chemotherapy in triple negative breast cancer. Our results increase the understanding of methodological issues for TIL evaluation as well as provide information about variation of the whole TIL population and TIL subpopulation during chemotherapy.

Castaneda CA, Mittendorf E, Casavilca S, Wu Y, Castillo M, Arboleda P, Nunez T, Guerra H, Barrionuevo C, Dolores-Cerna K, Belmar-Lopez C, Abugattas J, Calderon G, De La Cruz M, Cotrina M, Dunstan J, Gomez HL, Vidaurre T. Tumor infiltrating lymphocytes in triple negative breast cancer receiving neoadjuvant chemotherapy. *World J Clin Oncol* 2016; 7(5): 387-394 Available from: URL: http://www.wjgnet.com/2218-4333/full/v7/i5/387.htm DOI: http://dx.doi.org/10.5306/wjco.v7.i5.387

INTRODUCTION

Triple negative breast cancer (TNBC) represents 10% to 20% of breast cancer (BC) and is a biologically aggressive tumor with high response to Neoadjuvant chemotherapy (NAC) but poor outcome[1].

Prognostic and predictive role of tumor infiltrating lymphocytes (TILs) have been extensively studied in BC and different studies have found they are associated to better outcome in TNBC. Denkert *et al*[2] found that a high TIL percentage evaluated by Hematoxilin and Eosin (H and E) in full-face sections of pre-NAC samples can predict pathologic complete response (pCR) in a series of more than 1000 BC cases. Loi *et al*[3] evaluated the prognostic role of TIL percentage in more than 2000 node-positive BC and found that high TIL percentage are associated to better outcome only in the TNBC (*P* = 0.023). Adams *et al*[4] evaluated the role of TIL in two phase III adjuvant randomized BC trials and also found a better outcome only in the TNBC (*P* = 0.02)[5]. Finally, a recent meta-analysis and a retrospective analysis of 8897 TNBC confirm the prognostic value of TIL in TNBC[5,6]. Recent studies suggest that tumor infiltration by CD8 cytotoxic lymphocytes and absence of FOXP3 immunosuppressive regulatory cells could control tumor growth and carry a better prognosis[7-12].

Despite the international TILs working group defined harmonization criteria to evaluate TIL, there is some areas that still require a better understanding. One of these areas is the value of TILs in small pieces of tumor like tissue microarrays (TMA), as well as the value and the appropriate methodology to evaluate TIL subpopulations in TMA.

Finally, some recent studies also suggest a prognostic role of TIL in post-NAC samples, however, there is need for more information in this area because evaluation of these samples has special challenges to pathologists as neoadjuvant chemotherapy produces a spectrum of histopathologic changes including decrease in cancer cell number and changes in stroma composition that includes fibrosis, elastosis, collagenization, hyalinization, microcalcification, neovascularization, fibrinoid necrosis and mucinous changes[13].

This study aims to evaluate TIL variation during NAC through H and E in full-face and in TMA sections as well as the variation of TIL subpopulations through immunohistochemistry (IHC) staining in TMA sections.

MATERIALS AND METHODS

Patients and sample selection

We retrospectively reviewed the files of all new BC cases which came to the Instituto Nacional de Enfermedades Neoplasicas between 2005 and 2010, and we selected 98 TNBC cases with Clinical Stage Ⅱ-Ⅲ who went to surgery of breast tumor and axilla after receiving NAC. All core biopsy prior to NAC and breast tumor excision specimens were fixed in 10% neutral buffered formalin and embedded in paraffin and stored at Institutes Pathology Department Archive. Institutional review board approved the protocol of this study.

Staining and quantification of H and E, CD3, CD4, CD8 and forkhead box protein 3 IHC staining

Tumor areas were selected and a 0.6 cm punch from the formalin-fixed paraffin-embedded specimens were obtained and organized in 8-10 cylinder cards (TMA). H and E staining was performed in the full-face and in the TMA sections, and TIL was evaluated as the percentage of the stroma area of tumor that contained lymphocytic infiltrate through a 10% increment system under 200 × - 400 × magnification.

Process of IHC preparation included cutting 4 μm sections from the TMA, deparaffinating, rehydrating and processing sections using an automatized stainer (Autostainer Link 48, DAKO, Carpinteria, CA, United States) through standard methods. The following antibodies: CD3 (IS503, Dako), CD4 (IS649, Dako), CD8 (IS623, Dako) and FOXP3 (clone: 236A/E7) were used for staining of TMA section. Lymphocyte subsets were calculated through the percentage between lymphocytes/tumor cells in a 10% increment system, and through the absolute count of the lymphocytes in 5 high power fields under 200 × - 400 × magnification.

Clinical information and pathological response

We obtained clinical information from patient files archived at Instituto Nacional de Enfermedades Neoplasicas. The pathological therapeutic response of the surgically resected tumor was evaluated after NAC. The surgical specimens of breast lesions were cut into 5 mm slices and processed with H and E staining. A pCR was defined as the absence of all invasive cancer cells in breast and axillary lymph nodes, regardless of the presence of non-invasive cancer cells[11].

Statistical analysis

The mean percentage as well as the mean absolute number of immune cells was calculated, and those lesions above this mean were graded as increased. Analysis were also performed considering a high percentage of immune cells when were at least 50%. All statistical analyses were performed using STATA software version 12. Associations among variables were evaluated using Fisher's exact test or the χ^2 test. The Mann–Whitney *U* and Spearman's correlation tests were used to compare groups. The measurement of agreements between TIL evaluated in whole slide and TMA was used intraclass correlation coefficient (ICC). Kaplan-Meier estimation curves disease free and overall survival was applied. All tests were two sided, and a $P \leqslant 0.05$ was considered statistically significant.

RESULTS

Assessment of TILs by H and E

The characteristics of 91 pre-NAC and 80 post-NAC TN BC cases are reported in Table 1. Most cases were ductal infiltrating carcinoma (96.9%), inflammatory (29.6%), clinical stage Ⅲ (86.7%) and HGⅢ (75.5%). Most cases received neoadjuvant doxorubicin and paclitaxel (87.7%) and 29.6% obtained pCR. Sixty-six percent of the patients underwent mastectomy. After a median follow-up of 37.5 mo, there were 42% recurrences and 45% deaths. Pathologic complete response was associated to OS ($P = 0.0071$) but was not associated to DFS ($P = 0.1050$).

Median Pre-NAC TIL percentage in the full-face ($n = 91$) and in the TMA section ($n = 30$) was 40 ± 20 and 20 ± 15, respectively. Median post-NAC TIL percentage in the full-face ($n = 80$) and in the TMA section ($n = 58$) was 20 ± 15 and 10 ± 5, respectively (Table 2).

Pre-NAC TIL evaluated in full-face had low grade of agreement with TMA sections ($n = 30$) (ICC = 0.017). Post-NAC samples were larger and allowed to be divided in homogeneous ($n = 26$) and heterogeneous lesions ($n = 26$). Heterogeneous lesions had low level of agreement between full-face and TMA sections (ICC = 0.20), and homogeneous lesions had high level of agreement between full-face and TMA sections (ICC = 0.73).

Higher median pre-NAC TIL ($n = 91$) evaluated in full-face sections was associated to pCR (40% *vs* 30%, $P = 0.0251$), DFS (40% *vs* 20%, $P = 0.0076$) and OS (40% *vs* 30%, $P = 0.0334$); but not to age ($P = 0.1427$) nor inflammatory features ($P = 0.6401$), in the univariate analysis. Association between median TIL and pCR remained significant even with adjustment for age. Higher median pre-NAC TIL ($n = 30$) evaluated in TMA section was only associated to absence of inflammatory features (10% *vs* 30%, $P = 0.0387$).

Median post-NAC TIL evaluated in full-face ($n = 80$) or in TMA section ($n = 58$) was not associated to any of the previously mentioned features. Post-NAC median H and E TIL percentage evaluated in full-face section was similar in residual fibrous lesions ($n = 17$) (pCR) and in residual tumor lesions ($n = 63$) (no pCR) (20% *vs* 20%, $P = 0.6331$) (Table 3).

Classification of TIL with a cut-off of 50% did not identify a population associated to any of the previously mentioned features ($P = 0.16$ for pCR, $P = 0.14$ for DFS and $P = 0.64$ for OS).

NAC produced a statistically significant decrease in median TIL percentage when evaluated in full-face section ($n = 73$ $P < 0.0002$), but not when evaluated in TMA sections ($n = 16$ $P = 0.4321$) (Table 4).

Table 1 Clinical and pathological general features

Features	*n* = 98 (%)
Median age	49 ± 9
Clinical stage	
Ⅱ	13 (13.3)
Ⅲ	85 (86.73)
Histologic grade	
Ⅱ	23 (23.5)
Ⅲ	74 (75.5)
Subtype histologic	
Ductal	95 (96.9)
Lobular	2 (2.1)
Neoadjuvant chemotherapy	
AC	9 (9.2)
AC-Taxane	86 (87.7)
Taxane alone	3 (3.1)
Surgery	
Tumorectomy	29 (29.6)
Mastectomy	65 (66.3)
Unknown	4 (4.1)
Inflammatory	
No	69 (70.4)
Yes	29 (29.6)
pCR	
No	69 (70.4)
Yes	29 (29.6)
Recurrence	
No	56 (57.1)
Yes	42 (42.9)
Death	
No	53 (54.1)
Yes	45 (45.9)

AC: Adriamycin/cyclophosphamide; AC-Taxane: Adriamycin/cyclophosphamide, followed by treatment with taxane; pCR: Pathologic complete response.

Assessment of TIL subsets by IHC

Analysis of TIL subsets through IHC was calculated through percentage calculation and absolute counting methodology in TMA sections. Percentage calculation was significantly correlated with absolute counting for all markers in pre-NAC (CD3 $n = 27$ $r = 0.7182$, CD8 $n = 27$ $r = 0.6064$, FOXP3 $n = 26$ $r = 0.7192$) and in post-NAC (CD3 $n = 55$ $r = 0.7733$, CD4 $n = 30$ $r = 0.6129$, CD8 $n = 55$ $r = 0.7338$, FOXP3 $n = 47$ $r = 0.5387$) TMA sections that had enough material for both quantification methodologies (Table 5). The lymphocyte subset with highest absolute counts in the pre-NAC and post-NAC samples was CD8 (127 ± 193.5 and 156.5 ± 90.5) (Table 4).

Higher absolute counts of CD3, CD4, CD8 and FOXP3 in pre-NAC samples were associated with longer DFS ($n = 28$ $P = 0.003$, $n = 19$ $P = 0.0062$, $n = 28$ $P = 0.0096$ and $n = 29$ $P = 0.0019$; respectively). Higher absolute counts of CD3 in pre-NAC samples had longer OS ($n = 28$ $P = 0.0241$).

Higher absolute counts of CD4 in post-NAC samples was associated with age ($n = 54$ $P = 0.0393$) and pCR ($n = 54$ $P = 0.0095$).

Higher ratio of absolute counts of CD8/CD4 in pre-NAC and post-NAC samples was associated with pCR ($n = 17$ $P = 0.0343$ and $n = 43$ $P = 0.0086$ respectively).

Table 2 Tumor infiltrating lymphocytes in pre-neoadjuvant-chemotherapy and post-neoadjuvant-chemotherapy samples (median ± interquartile range)

TIL population	(Me ± IQR)
Pre-NAC TIL	
Median percentage TIL H and E full-face ($n = 91$)	40 ± 20
Median percentage TIL H and E TMA ($n = 30$)	20 ± 15
Median counting TIL CD3 ($n = 28$)	244.5 ± 253.8
Median counting TIL CD4 ($n = 19$)	48 ± 107.5
Median counting TIL CD8 ($n = 28$)	102 ± 98.8
Median counting TIL FOXP3 ($n = 29$)	22 ± 35
Post-NAC TIL	
Median percentage TIL H and E ($n = 80$)	20 ± 15
Median percentage TIL H and E TMA ($n = 58$)	10 ± 5
Median counting TIL CD3 ($n = 68$)	156.5 ± 200.8
Median counting TIL CD4 ($n = 54$)	12 ± 27.5
Median counting TIL CD8 ($n = 70$)	75.5 ± 93.5
Median counting TIL FOXP3 ($n = 70$)	7.5 ± 14.5

NAC: Neoadjuvant-chemotherapy; TIL: Tumor-infiltrating-lymphocyte; TMA: Tissue microarrays; Me ± IQR: Median ± interquartile range.

Higher ratio of absolute counts of CD4/FOXP3 in pre-NAC sample was associated with longer DFS ($n = 16$ $P = 0.0389$). Higher ratio of absolute counts of CD4/FOXP3 in post-NAC sample was associated with pCR ($n = 30$ $P = 0.003$).

Higher ratio of absolute counts of CD4/CD3 in post-NAC samples was associated with pCR ($n = 48$ $P = 0.0095$).

DISCUSSION

We have evaluated pre and post-NAC samples in an effort to produce a comprehensive analysis of the role of TIL variation during NAC in TNBC samples. Evaluation of pre-NAC H and E staining of full-face sections found that those tumors with higher TILs are associated to both pCR and better outcome. These results are similar to those found by Denkert *et al*[2] in the neoadjuvant setting and by Loi *et al*[3] and Adams *et al*[4] in the adjuvant setting, and confirm accuracy of our methodology.

We did not find an association between prognosis and TILs in post-NAC samples evaluated in full-face or in TMA sections. García-Martínez *et al*[14] evaluated 121 BC cases and found that high TIL level in pre-NAC samples was associated to pCR. TIL in pre-NAC and post-NAC were not associated to outcome. By other side, Dieci *et al*[15] evaluated 278 TNBC with residual disease after NAC and found that those residual lesions with high level of TIL had better prognosis.

We found that TIL percentage evaluated in full-sections were higher in pre-NAC than post-NAC samples. No association between TIL variation (pre- *vs* post-NAC) and response to NAC was found. Post-NAC samples of those cases who obtained pCR were similar to TIL levels in those cases who did not obtain pCR (residual cancer). By other side, Dieci evaluated 19 selected cases with

Table 3 Comparison between tumor-infiltrating-lymphocytes evaluated in full-face and tissue microarrays sections

Features	H and E in the full-face section			H and E in TMA section		
	n	Me ± IQD	*P*-value[2]	*n*	Me ± IQD	*P*-value[3]
Pre-NAC	91			30		
Age			0.1427			0.6313
≤ 49	50	40 ± 20		19	20 ± 15	
> 49	41	20 ± 15		11	20 ± 25	
pCR			0.0251[1]			0.2227
No	63	30 ± 20		15	30 ± 25	
Yes	28	40 ± 17.5		15	20 ± 10	
Inflammatory			0.6401			0.0387[1]
Yes	27	40 ± 15		8	10 ± 6.5	
No	64	35 ± 20		22	30 ± 15	
DFS			0.0076[1]			0.1601
< 32 mo	46	20 ± 20		20	15 ± 13.8	
≥ 32 mo	45	40 ± 20		10	25 ± 25	
OS			0.0334[1]			0.7214
< 41 mo	51	30 ± 20		22	20 ± 15	
≥ 41 mo	40	40 ± 20		8	20 ± 15	
Post-NAC	80			58		
Age			0.8547			0.5684
≤ 49	43	20 ± 15		31	10 ± 15	
> 49	37	20 ± 15		27	10 ± 5	
Inflammatory			0.4582			0.1299
Yes	26	15 ± 10		16	10 ± 0	
No	54	20 ± 15		42	10 ± 15	
pCR			0.6331			0.1299
No	63	20 ± 15		44	10 ± 7.5	
Yes	17	20 ± 10		14	10 ± 7.5	
DFS[3]			0.2450			0.2573
< 32 mo	35	20 ± 15		22	10 ± 0	
≥ 32 mo	28	20 ± 15		22	10 ± 15	
OS[3]			0.5973			0.6948
< 41 mo	40	20 ± 15		27	10 ± 10	
≥ 41 mo	23	20 ± 15		17	10 ± 5	

[1]$P < 0.05$; [2]U Mann Whitney test; [3]Only cases with incomplete pathological response in post NAC. DFS: Disease free survival; Me ± IQD: Median ± interquartile deviation; OS: Overall survival; pCR: Pathologic complete response; NAC: Neoadjuvant-chemotherapy.

Table 4 Comparison of tumor-infiltrating-lymphocyte evaluated in pre- *vs* post-neoadjuvant-chemotherapy samples

Population of lymphocytes	*n*	Pre-NAC Me ± IQR	Post-NAC Me ± IQR	*P*-value[2]	*P* corrected value[3]
Percentage					
H and E whole slide	73	40 ± 15	20 ± 15	< 0.001	< 0.002[1]
H and E TMA	16	30 ± 17.5	15 ± 15	0.4321	
Absolute counting					
CD3	22	244.5 ± 315.5	255.5 ± 267	0.8583	
CD4	7	14 ± 94	32 ± 43	0.6721	
CD8	21	127 ± 193.5	156 ± 90.5	0.7544	
FOXP3	21	18 ± 31	12 ± 19.5	0.0917	

[1]$P < 0.05$; [2]Wilcoxon signed rank test; [3]Bonferroni correction. NAC: Neoadjuvant-chemotherapy; TMA: Tissue microarrays; Me ± IQR: Median ± interquartile range.

high TIL level in post-NAC samples and found that lower TIL increased during NAC[15], and Demaria *et al*[16] found that those cases with higher response have an increase of TIL during NAC in a series of 25 BC.

Although lymphocytic infiltration has demonstrated to behave as a prognostic and predictive marker in breast cancer, there are some aspects without standardization. We evaluated TIL in the 0.6 cm TMA, and we found that the TIL percentages differ from those found in the full-face sections. TIL percentage in TMA was not associated to pCR nor prognosis. Breast tumors and especially TNBC are heterogeneous lesions and our findings indicate that TILs concentration is also heterogeneous inside the different tumor areas. The evaluation of only one region of the tumor through a TMA cylinder appears not to produce confident information about immune reaction against the whole tumor.

Different articles have evaluated the role of TIL

Table 5 Relationship between percentage and absolute counting methodologies of tumor-infiltrating-lymphocyte subpopulations

Population of lymphocytes	*n*	rho[2]	*P*-value	*P* corrected value[3]
Pre-NAC				
CD3	27	0.7182	< 0.001	< 0.004[1]
CD4	17	0.5071	0.0378	0.1512
CD8	27	0.6064	0.0008	0.0032[1]
FOXP3	26	0.7192	< 0.001	< 0.004[1]
Post-NAC				
CD3	55	0.7733	< 0.001	< 0.004[1]
CD4	30	0.6129	0.0003	0.0012[1]
CD8	55	0.7338	< 0.001	< 0.004[1]
FOXP3	47	0.5387	0.0001	0.0004[1]

[1]$P < 0.05$; [2]Spearman correlation coefficient test; [3]Bonferroni correction. NAC: Neoadjuvant-chemotherapy.

subpopulations (IHC staining), however some of them have measured them by percentage (resembling methodology used for TIL evaluation with H and E)[2,3] and other have measured by an absolute counting[7,8,17-21].

We compared both methodologies in 0.6 cm TMA tumor samples for CD3, CD4, CD8 and FOXP3 lymphocyte subpopulations and we found a significant correlation between both methodologies.

We also evaluated the association between levels of CD3, CD4, CD8 and FOXP3 lymphocyte subpopulations (absolute counting) and clinical features. Although our sample size for evaluating lymphocyte subpopulations is small, we found that higher absolute counts of CD4 lymphocytes in post-NAC samples were associated with pCR. Higher absolute counts of CD3, CD4, CD8 and FOXP3 lymphocytes in pre-NAC samples were associated with DFS, and higher absolute counts of CD3 lymphocytes in pre-NAC samples had longer OS.

Finally, we also found an association of pCR with higher ratio of absolute counts of all CD8/CD4 in pre-NAC, and CD8/CD4, CD4/CD3, CD4/FOXP3 in post-NAC samples. Higher ratio of absolute counts of CD4/FOXP3 in pre-NAC sample was associated with longer DFS.

The role of one TIL subpopulation and the role of the relationship between two TIL subpopulations over tumor behavior have been previously described and some of authors confirm our findings. Rathore *et al*[9] found that higher levels of $CD3^+$, $CD4^+$ and $CD8^+$ TILs was significantly associated with good prognosis in a series of 123 early BC cases. Kim *et al*[22] reported that lower number of $CD8^+$ TILs in breast tumors were significantly associated with lymph node metastasis, higher stage and high proliferative index in a series of 72 early BC cases. Increased number of FOXP3 lymphocytes was associated to lymph node metastasis, high proliferative index and shorter DFS. Ladoire *et al*[10] evaluated 56 BC cases who went to NAC and found that high CD8 and absence of FOXP3 infiltration was associated to pCR. Miyashita *et al*[11] evaluated 131 TNBC patients treated with NAC and found that high CD8 TIL levels and high CD8/FOXP3 ratio in residual tumor had better outcome. García-Martínez *et al*[14] described a decrease of CD4, an increase of CD8 and an absence of changes of FOXP3 during NAC. High levels of CD3 and CD4 in pre-NAC were associated to pCR. A decrease of CD3 and CD4 during chemotherapy was associated to pCR. A decrease of CD3 during NAC was also associated to better DFS and OS. They also found that higher ratio of CD4/CD8 in pre-NAC was associated to a pCR. They also evaluated six public genomic datasets with around 1000 BC patients treated with NAC and found that higher CD4 count in post-NAC samples was associated to pCR. Finally, they found that high levels of CD3 in post NAC was associated to better DFS[14]. Other authors have also evaluated the role of TIL ratios, such as CD8/CD4[23] or FOXP3/CD3[12], as an alternative approach to better integrate the information provided by each TIL subpopulation[14].

Differences among mentioned authors and our findings could be explained by our small population size, analysis of a not representative area in the TMA samples or changes in $CD4^+$ TILs phenotype from effectors to suppressors. Therefore, our results need to be validated in larger series. Remarkably, our work is the first to our knowledge to evaluate TIL in BC tumors from Latinoamerican women. And, it is the first to compare the evaluation of TIL percentage in full-sections and in TMA sections, as well as to compare the evaluation of TIL levels through percentage analysis and through absolute counting.

Identification of biomarkers and evaluation of therapy in the neoadjuvant setting has become a major challenge in BC since they could speed-up the development and approval of new drugs[24]. pCR is a validated surrogate for drug efficacy in the neoadjuvant setting but its specificity needs still to be improved. The finding of a biomarker related to host immunity in the pre or post-NAC samples could have the benefit to predict response not only to chemotherapy but also to immune checkpoint modulators.

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COMMENTS

Background

Triple-negative-breast-cancer (TNBC) is associated to poor outcome and is highly prevalent among Latinoamerican women. Tumor-infiltrating-lymphocytes (TILs) have been associated to higher response to chemotherapy and better outcome in TNBC when evaluated in retrospective and prospective series as well as meta-analysis. An international TILs working group defined harmonization criteria to evaluate them in 2015, however, there is still some areas requiring a better understanding. There is not information describing TILs in small pieces of tumor, and the value and the appropriate methodology to evaluate TIL subpopulations in tissue microarrays (TMA). There is small information describing variation of TIL during chemotherapy.

Research frontiers

TNBC has higher prevalence in Latinoamerican women and is a poor prognostic malignancy without target therapy. Chemotherapy is the only available treatment for TNBC. TIL appears to identify prognosis in TNBC and some recent studies are evaluating if it predicts response to chemotherapy or immunotherapy. Therefore, information about TIL variation during chemotherapy is an important issue as it is the scenario we need to improve treatment efficacy.

Innovations and breakthroughs

As TNBC malignancy and role of TIL as biomarker are important issues, more research about relevance of TIL in TNBC is needed. Therefore, the authors revealed that TIL evaluated in a small area of tumor differs to those evaluated in full-face samples, and loss their prognostic and predictive value. The authors also found that evaluation of lymphocyte subsets can be equally performed through absolute counting or percentage calculating, and can provide prognostic information.

Applications

Based on the present study, the authors can suggest that full-face samples (and not core samples) are used for TIL evaluation in H and E. Absolute counting and percentage calculating could be considered appropriate for evaluation of TIL subsets.

Terminology

TIL is a biomarker with current strong evaluation in different malignancies including TNBC. TIL is accepted as those stromal mononuclear cells inside the tumor but not in contact with cancer cells nor inside tumor niches. TMA is a technique allowing immunohistochemistry staining of many tumor samples at the same time.

Peer-review

The paper is very interesting and well written.

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ORIGINAL ARTICLE

Case Control Study

Cigarette smoking, dietary habits and genetic polymorphisms in *GSTT1*, *GSTM1* and *CYP1A1* metabolic genes: A case-control study in oncohematological diseases

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Abstract

AIM

To analyze the association between oncohematological diseases and *GSTT1*/*GSTM1*/*CYP1A1* polymorphisms, dietary habits and smoking, in an argentine hospital-based case-control study.

METHODS

This hospital-based case-control study involved 125 patients with oncohematological diseases and 310 control subjects. A questionnaire was used to obtain sociodemographic data and information about habits. Blood samples were collected, and DNA was extracted using salting out methods. Deletions in *GSTT1* and *GSTM1*

(null genotypes) were addressed by PCR. *CYP1A1 MspI* polymorphism was detected by PCR-RFLP. Odds ratio (OR) and 95%CI were calculated to estimate the association between each variable studied and oncohematological disease.

RESULTS

Women showed lower risk of disease compared to men (OR 0.52, 95%CI: 0.34-0.82, P = 0.003). Higher levels of education (> 12 years) were significantly associated with an increased risk, compared to complete primary school or less (OR 3.68, 95%CI: 1.82-7.40, P < 0.001 adjusted for age and sex). With respect to tobacco, none of the smoking categories showed association with oncohematological diseases. Regarding dietary habits, consumption of grilled/barbecued meat 3 or more times per month showed significant association with an increased risk of disease (OR 1.72, 95%CI: 1.08-2.75, P = 0.02). Daily consumption of coffee also was associated with an increased risk (OR 1.77, 95%CI: 1.03-3.03, P = 0.03). Results for *GSTT1*, *GSTM1* and *CYP1A1* polymorphisms showed no significant association with oncohematological diseases. When analyzing the interaction between polymorphisms and tobacco smoking or dietary habits, no statistically significant associations that modify disease risk were found.

CONCLUSION

We reported an increased risk of oncohematological diseases associated with meat and coffee intake. We did not find significant associations between genetic polymorphisms and blood cancer.

Key words: Cancer; Oncohematological disease; Case-control study; Lifestyle; Diet; Tobacco; Xenobiotic metabolizing genes; *GSTT1*; *GSTM1*; *CYP1A1*

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Core tip: Cancer is considered as a multi-factorial disease. Except certain genetic abnormalities, viruses, environmental exposures and chemotherapeutic agents, it is not well defined which are the risk factors for these diseases (leukemia, lymphoma, multiple myeloma, among others). Here, we analyzed lifestyle and genetic polymorphisms as risk factors for blood cancer. We reported an increased risk of disease associated with meat and coffee intake. No significant associations were found between metabolic gene polymorphisms and disease. Our study offers relevant insights into diverse aspects of oncohematological diseases etiology, particularly genes and environmental factors, in an Argentinean population.

Cerliani MB, Pavicic W, Gili JA, Klein G, Saba S, Richard S. Cigarette smoking, dietary habits and genetic polymorphisms in *GSTT1*, *GSTM1* and *CYP1A1* metabolic genes: A case-control study in oncohematological diseases. *World J Clin Oncol* 2016; 7(5): 395-405 Available from: URL: http://www.wjgnet.com/2218-4333/full/v7/i5/395.htm DOI: http://dx.doi.org/10.5306/wjco.v7.i5.395

INTRODUCTION

Xenobiotic metabolizing enzymes (XME), coded by a family of xenobiotic metabolizing genes (XMG), transform endo and exogenous compounds in hydrophilic by-products, which are more easily excreted from the tissues[1]. It is well known since decades that genetic differences in the metabolism of drugs and environmental chemicals exist[2]. These differences are due to pharmacogenetic polymorphisms, which are allele variants that occur with a relatively high frequency in the population, and are generally associated with anomalies in gene expression or enzymatic function. Moreover, pharmacogenetic polymorphisms have been associated with an increased risk of some types of cancer, due to: (1) an impaired ability to inactivate endogenous or exogenous mutagenic molecules; or (2) the conversion of metabolites into highly reactive and toxic compounds. Both polymorphisms and the levels of exposure to their substrates, may impact on cancer susceptibility.

The cytochrome P450 family of enzymes is responsible for catalyzing phase Ⅰ metabolism reactions. CYP1A1 is a member of the CYP family and plays an important role in the metabolism of estrogen and polycyclic aromatic hydrocarbons (PAHs), catalyzing the activation of pro-carcinogenic PAHs[3]. Dysfunction of CYPs enzymes can cause damage to DNA, lipids and proteins, leading to carcinogenesis[4,5]. A commonly studied single nucleotide polymorphism (SNP) in *CYP1A1* gene is the T3801C (also named *MspI* polymorphism, *2A or m1), a T to C mutation in the 3′ flanking region of the gene. The C variant becomes more highly inducible than the T variant[6], which may cause enhanced enzymatic activity, thus modifying susceptibility to adduct formation and cancer risk[7]. In fact, T3801C polymorphism was associated with leukemia and cervical, hepatocellular, lung, prostate, and head and neck cancer[8].

Glutathione S-transferases (GSTs) constitute a superfamily of phase II detoxification enzymes which play a key role in cellular protection against environmental carcinogens, drugs, toxins and by-products of oxidative stress. GSTs catalyze the conjugation of reduced glutathione (GSH) to a wide variety of electrophilic compounds to facilitate their cellular excretion. In addition, as non-enzymatic proteins, GSTs can modulate signaling pathways that control cell proliferation, cell differentiation, apoptosis, anti- and pro-inflammatory functions and DNA damage processing, among other processes[9]. Genetic polymorphisms in *GST* genes are common in the human population. GSTM1 and GSTT1 exhibit variations in copy number due to complete gene deletion, resulting in the loss of enzymatic activity. The absence of enzyme has been associated with lung, breast and gastrointestinal cancer, among others[10], and also with adverse side effects and toxicity in chemotherapies[11].

Lifestyle and dietary habits are additional risk factors for cancer. Diet is known to modulate the immune system, and it may also influence cancer susceptibility through changes in the energy balance and in the levels of carcinogens and anticarcinogens[12]. Cigarette smoke contains more than 7000 chemicals and compounds, from which more than 70 are associated with cancer[13]. Benzene, present in tobacco smoke, is a strong carcinogen associated with leukemia and lymphoma development[14], and has long been recognized as hematotoxic[15].

It should not be forgotten that cancer susceptibility results from genetic and environmental factors, individually or in combination. According to this, it is expected that genetic, dietary and lifestyle factors interact with each others.

Several studies have inquired the epidemiologic risk factors associated with leukemia, lymphoma and/or myeloma. Except certain genetic abnormalities, viruses, environmental exposures and chemotherapeutic agents, little is known about risk factors that develop these onco-hematological diseases.

Argentina is within the range of countries with medium to high incidence of cancer, according to the International Agency for Research on Cancer (IARC) data for 2012. They estimated an incidence of 14.2 new cases/year/100000 persons for Hodgkin lymphoma (HL), leukemia, non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) all together[16]. During 2012, nearly 3830 patients have died because of these diseases according to the Statistics and Health Information Office[17]. Between 2007 and 2011, oncohematological diseases account for the 6.5% of all cancer deaths[18].

The aim of this study was to analyze the association between oncohematological diseases and genetic polymorphisms in *GSTT1*, *GSTM1* and *CYP1A1*, dietary habits and cigarette smoking, in an argentine hospital-based case-control study.

MATERIALS AND METHODS

Subjects

A hospital-based case-control study was performed, involving 125 patients with oncohematological diseases and 310 control subjects. Participants were recruited between June 2013 and March 2015 at the Unit of Diagnosis, Treatment and Support for Hematological Diseases of the Acute Care General Hospital "Prof. Dr. Rodolfo Rossi" (La Plata, Buenos Aires, Argentina). The study was approved by the hospital's Ethics Committee. The International Classification of Diseases for Oncology information was not available.

Cases were patients diagnosed with acute lympho-blastic leukemia (ALL, n = 10), acute myeloblastic leukemia (AML, n = 18), chronic lymphoblastic leukemia (CLL, n = 10), chronic myeloblastic leukemia (CML, n = 20), MM (n = 29), HL (n = 18) and NHL (n = 20). Controls included patients frequently visiting the Unit for routine checks of disorders unrelated to cancer or preoperative blood analyses (*i.e*., for ophthalmic surgeries, pre-employment medical examinations, anemia, among others). All participants reside in Argentina. Cases and controls with previous history of cancer or pathologies closely related to oncohematological diseases were excluded from the study.

Patients were invited to participate in the study and signed an informed consent. A questionnaire was used to obtain sociodemographic data and information about habits, such as cigarette smoking (never, former and current smoker), and consumption of grilled/barbecued meat (times per month), canned food (times per week), alcohol (times per week) and coffee (cups per day). No other food or beverage items were asked. The survey also included weight, height, medication, working conditions, family history of cancer, history of disease (only in cases), and functionality data (quality of sleep, fatigue, changes in appetite and mood, *etc*.). The overall case and control response rate was higher than 90% for both groups.

All surveys were addressed by the same person. Blood samples were collected and kept in Vacutainer tubes with K2-EDTA (3.6 mg), and DNA was extracted from whole blood using salting out methods.

GSTT1 and GSTM1 genotype assay

GSTT1 and *GSTM1* gene deficiency resulted from the deletion of the loci (null alleles). The detection was performed by a multiplex PCR, using GSTT1 forward primer 5'-TTC CTT ACT ggT CCT CAC ATC TC-3' and GSTT1 reverse primer 5'-TCA CCg gAT CAT ggC CAg CA-3' (459 bp product), GSTM1 forward primer 5'-CTg CCC TAC TTg ATT gAT ggg-3' and GSTM1 reverse primer 5'-CTg gAT TgT AgC AgA TCA TgC-3' (273 bp product), and a third pair of primers, forward 5'-TCC AgC AgT TTC ATg AgA TgC-3' and reverse 5'-gAg gTC ATT TCA Tag CTg AgC-3' for a 221 bp product of the gene CLOCK, as an internal control of the reaction (Figure 1). PCR conditions were as follows, in a final volume of 15 μL: 1 × buffer, 50 ng DNA, 0.25 μmol/L each primer, 200 μmol/L dNTPs, 2 mmol/L $MgCl_2$, 0.45 U Taq Platinum Polymerase (Invitrogen, Life Technologies) and H_2O up to 15 μL. PCR cycling consisted in an initial denaturation at 94 ℃ for 5 min, followed by 35 cycles of 1 min at 94 ℃, 1 min at 59 ℃ and 1 min at 72 ℃, with a final extension at 72 ℃ for 5 min. Verification of PCR products, and subsequent identification of genotype, were performed using 2% agarose gels stained with GelRed (Biotium Inc., CA, United States). The absence of PCR product defines the null allele.

CYP1A1 genotype assay

MspI polymorphism was detected by PCR-RFLP. A product of 420 bp was amplified by PCR in a final volume of 15 μL, containing buffer 1 ×, 2 mmol/L $MgCl_2$, 250 nmol/L each primer, 200 μmol/L dNTPs, 0.4 U Taq DNA Polymerase Recombinant (Invitrogen), 30 ng DNA and H_2O up to 15 μL. Primers were: forward

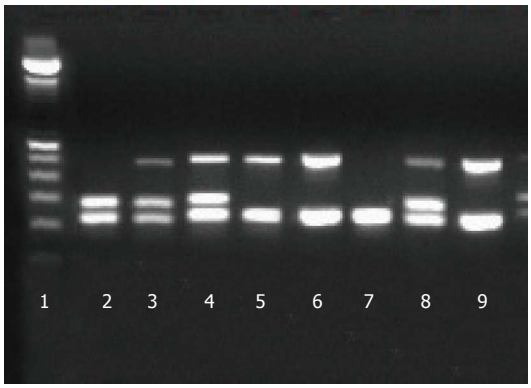

Figure 1 Gel electrophoresis showing PCR products of GSTT1 (459 bp), GSTM1 (273 bp) and CLOCK (221 bp). Numbered lanes represents: 1: 100 bp molecular weight marker; 3, 4, 8: T^+/M^+; DNA from samples with positive GSTT1 and GSTM1 alleles, plus control CLOCK gene band; 7: T^-/M^-; Double null genotype of GSTM1 and GSTT1 (in the presence of CLOCK PCR product); 5, 6, 9: T^+/M^-; GSTT1 positive/GSTM1 null; 2: T^-/M^+; GSTT1 null/GSTM1 positive.

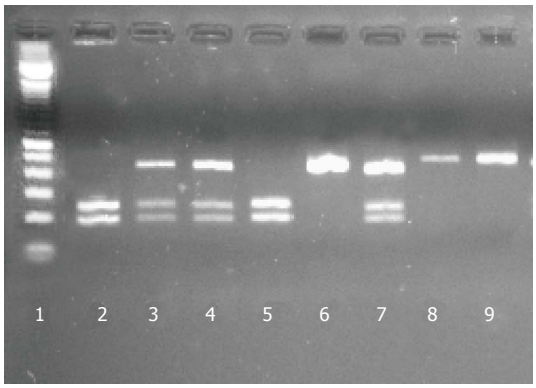

Figure 2 Gel electrophoresis showing PCR-RFLP product of CYP1A1 gene digested with MspI restriction enzyme. *CYP1A1* gene T3801C variant gives the following digestion band patterns: TT, 420 bp; TC, 420 bp and 237 bp + 183 bp; and CC, 237 bp + 183 bp. Numbered lanes represents: 1: 100 bp molecular weight marker; 2, 5: CC homozygous type; 3, 4, 7: TC heterozygous type; 6, 8, 9: TT homozygous type.

5′-ACC CCA TTC TgT gTT ggg TT-3′ and reverse 5′-TAg AgA ggg CgT AAg TCA gCA-3′. Cycling conditions were as follows: An initial denaturation at 94 ℃ for 5 min, followed by 35 cycles of 30 s at 94 ℃, 30 s at 58 ℃ and 40 s at 72 ℃, with a final extension at 72 ℃ for 5 min.

After checking amplification in 2% agarose gel stained with Gel Red, 7 μL of PCR product were digested with 5U of *MspI* enzyme (Thermo Scientific), buffer and H_2O up to 15 μL. Incubation time was 5 h at 37 ℃. Verification of digested products was carried out in 2% agarose gels stained with Gel Red. The C variant has the restriction site, generating 237 and 183 bp products (Figure 2).

Statistical analysis

Odds ratio (OR) and 95%CI were calculated to estimate the association between each variable studied and oncohematological disease. χ^2 test was applied to obtain the statistical significance of the association. Analyses were performed with the softwares STATA 11.1[19] and Epidat 4.0[20]. Genotype and allele frequencies were calculated and tested for Hardy-Weinberg Equilibrium using the software GenAlEx 6.5[21]. The sample size of this survey achieved 80% power to detect an OR = 2. *P*-values ⩽ 0.05 were considered statistically significant. The statistical review of the study was performed by a biomedical statistician (Gili JA, one of the authors).

RESULTS

Study population

In this association study, a total of 125 cases were compared to 310 controls, all of them patients from the Acute Care General Hospital "Prof. Dr. Rodolfo Rossi". Demographic characteristics are listed in Table 1, and were already published by our group in Cerliani *et al*[22]. Missing data for each variable were not included in the analysis, nor are detailed in the tables. The maximum number of missing data in a variable was 18, representing 4.14% of the samples. Of all variables in the questionnaire, only height and weight were excluded due to > 10% missing data. There was no significant difference in the mean age of cases and controls. Women showed lower risk of disease compared to men (OR 0.52, 95%CI: 0.34-0.82, *P* = 0.003). Higher levels of education (> 12 years) were significantly associated with an increased risk, compared to complete primary school or less (OR 3.68, 95%CI: 1.82-7.40, *P* < 0.001 adjusted for age and sex). Marital status did not show association with the disease.

Tobacco, dietary habits and XMG polymorphisms

With respect to tobacco, none of the smoking categories showed association with oncohematological diseases. Regarding dietary habits, consumption of grilled/barbecued meat 3 or more times per month showed significant association with an increased risk of disease (OR 1.72, 95%CI: 1.08-2.75, *P* = 0.02). Daily consumption of coffee also was associated with an increased risk (OR 1.77, 95%CI: 1.03-3.03, *P* = 0.03). Since control patients with gastrointestinal problems could create a spurious OR (given that they may be abstaining from coffee), patients from the Gastroenterology Unit of the hospital were removed from the analysis, as well as control patients under treatment with gastric protectors or medication prescribed for other gastrointestinal issues. Therefore 19 controls were excluded from the analysis; nevertheless, coffee consumption still remains statistically correlated with disease, showing the same OR range (OR 1.01-3.02). No association was observed with consumption of canned food or alcohol. When assessing the risk associated with consumption of coffee and grilled/barbecued meat, within each of the pathologies, results showed no differences between groups for coffee consumption, but CML and MM cases exhibited a significant association with consumption of grilled/barbecued meat 3 or more times per month, adjusted for age, sex and educational

Table 1 Demographic characteristics of the population under study *n* (%)

	Cases *n* = 125	Controls *n* = 310	OR (95%CI)	*P*
Age mean (SD)	48.5 (16.6)	51 (18.5)	-	0.507
Sex				
Male	74 (59.2)	134 (43.2)	Ref.	0.003
Female	51 (40.8)	176 (56.8)	0.52 (0.34-0.82)	
Education				
⩽ 7 yr (complete primary school or less)	174 (56.31)	55 (44)	Ref.	
12 yr (complete secondary school)	115 (37.22)	48 (38.4)	1.2 (0.73-1.95)	< 0.001[1]
> 12 yr (complete college)	20 (6.47)	22 (17.6)	3.68 (1.82-7.4)	
Marital status				
Single	79 (25.48)	27 (21.6)	Ref.	
In couple/married	162 (52.26)	86 (68.8)	1.7 (0.99-2.95)	0.05[1]
Divorced/separated	27 (8.71)	5 (4)	0.64 (0.21-1.9)	0.42[1]
Widowed	42 (13.55)	7 (5.6)	0.75 (0.27-2.13)	0.59[1]
Type of malignancy				
ALL	10 (8)			
AML	18 (14.4)			
CLL	10 (8)			
CML	20 (16)			
MM	29 (23.2)			
HL	18 (14.4)			
NHL	20 (16)			

[1]Adjusted for age and sex. $P < 0.05$ considered statistically significant. ALL: Acute lymphoblastic leukemia; AML: Acute myeloblastic leukemia; CLL: Chronic lymphoblastic leukemia; CML: Chronic myeloblastic leukemia; MM: Multiple mieloma; HL: Hodgkin lymphoma; NHL: Non-Hodgkin lymphoma; Ref: Reference category; SD: Standard deviation.

Table 2 Allele and genotype frequencies for *CYP1A1* *2A polymorphism, and *GSTT1/GSTM1* null polymorphisms

	Cases *n* = 125	Controls *n* = 310
Allele frequencies		
CYP1A1		
T	0.7	0.65
C	0.3	0.35
Genotype frequencies (*n*)		
GSTT1*null	0.18 (22)	0.18 (55)
GSTM1*null	0.5 (61)	0.47 (140)
CYP1A1		
TT	0.53 (64)	0.47 (140)
TC	0.34 (42)	0.37 (113)
CC	0.13 (16)	0.16 (47)

level (data not shown). With regard to *GSTT1*, *GSTM1* and *CYP1A1* polymorphisms, results showed no significant association with oncohematological diseases. *CYP1A1 MspI* polymorphism was not in Hardy-Weinberg equilibrium. Allele and genotype frequencies are detailed in Table 2. Results from the association analysis between tobacco, dietary habits, XMG polymorphisms and oncohematological diseases are described in Table 3. When analyzing the interaction between XMG polymorphisms and tobacco smoking or dietary habits, no statistically significant associations that modify disease risk were found (data not shown).

DISCUSSION

Carcinogenesis is considered as a multi-step and multifactorial process that implied different genetic alterations and several biological pathways. Thus, it is expected that cancer risk factors interact with each others. Genetic polymorphisms may play different roles in cancer susceptibility according to the genetic background, environmental exposures and lifestyles[8]. Despite this, we did not find evidence of interaction between genetic and lifestyle factors, probably because the sample size is not big enough for this type of analysis.

Regarding tobacco use and dietary habits, we reported an increased risk of oncohematological diseases associated with grilled/barbecued meat intake 3 or more times per month, and with daily consumption of coffee, after adjustment for age, sex and educational level. Moreover, for both factors that showed an increased risk when analyzing all malignancies combined, we have also assessed the risk associated with each of the major groups within the case category. While for coffee consumption results showed no differences between groups, grilled/barbecued meat intake (3 times or more per month) has shown a significant correlation with CML and MM cases. However, it has to be considered that the sample size becomes small when divided by each pathology. Therefore, the real effect of each risk factor for a specific type of leukemia, lymphoma or myeloma might be determined in future studies by analyzing a larger set of samples.

Although cigarette smoke has been associated with mutagenic/carcinogenic effects, inflammation and immune suppression in animals and humans[23,24], previous studies on cigarette smoking and its association with blood cancers have generated inconsistent findings. Regarding NHL, studies reported little or no association

Table 3 Association between tobacco, dietary habits, xenobiotic metabolizing genes polymorphisms, and oncohematological diseases *n* (%)

	Cases *n* = 125	Controls *n* = 310	OR (95%CI)	*P*
Tobacco smoking status				
Never	49 (39.8)	142 (46.4)	Ref.	
Former	47 (38.2)	100 (32.7)	1.36 (0.84-2.19)	0.202
Current	27 (22)	64 (20.9)	1.22 (0.70-2.13)	0.478
Consumption of canned food				
0-2 times/wk	121 (96.8)	292 (94.8)	Ref.	0.370
3 or more times/wk	4 (3.2)	16 (5.2)	0.60 (0.14-1.92)	
Consumption of grilled/barbecued meat				
0-2 times/mo	69 (55.2)	217 (70.7)	Ref.	0.021[1]
3 or more times/mo	56 (44.8)	90 (29.3)	1.72 (1.08-2.75)	
Alcohol drinking				
0-3 times/wk	110 (88)	268 (86.5)	Ref.	0.665
4 or more times/wk	15 (12)	42 (13.5)	0.87 (0.43-1.68)	
Consumption of coffee				
< 1 cup/d	90 (72)	259 (83.5)	Ref.	0.037[1]
1 or more cups/d	35 (28)	51 (16.5)	1.77 (1.03-3.03)	
GSTT1				
Presence	100 (82)	245 (81.7)	Ref.	0.942
Null	22 (18)	55 (18.3)	0.98 (0.54-1.74)	
GSTM1				
Presence	61 (50)	160 (53.3)	Ref.	0.534
Null	61 (50)	140 (46.7)	1.14 (0.73-1.78)	
CYP1A1				
TT	64 (52.5)	140 (46.7)	Ref.	0.280
TC + CC	58 (47.5)	160 (53.3)	0.79 (0.51-1.24)	

[1]Adjusted for age, sex, and educational level. $P < 0.05$ considered statistically significant. Ref.: Reference category.

with use of cigarettes/tobacco, or patterns related with duration or intensity of exposure (detailed in[25-27]). In a case-control study from Sweden, the effects of smoking on the risk of AML were weak and no significant[28]. However, a cohort study from the same country showed a 50% increased risk of AML for current smokers[29]; this study also indicated no association between current and former smokers with CML, ALL, CLL or MM. An Indian case-control study reported an increase of 2.1 fold in the risk of leukemia in the cigarette smokers, compared to non-smokers[30]. A US cohort study that evaluated risk factors for AML, showed hazard ratios of 1.79, 2.42 and 2.29 for former smokers of > 1 pack/d, current smokers of ⩽ 1 pack/d, and current smokers of > 1 pack/d, respectively[31]. A meta-analysis done by Fircanis *et al*[32] including over 7500 cases of AML, found an increased risk of disease associated with smoking, regardless of sex, geographical region, study design and quality of studies. They also reported a higher risk with higher intensity and longer duration of smoking.

It is possible that the exposure to agents present in the smoke varies given the different usage patterns, exposure pathways and manufacturing processes.

A recent study done by Rubinstein *et al*[33] showed that 29.7% of the general population of Argentina, Chile and Uruguay (n = 7524) smoke cigarettes. The CARMELA study (Cardiovascular Risk Factor Multiple Evaluation in Latin America), performed between 2003 and 2005, reported that Buenos Aires (Argentina, n = 1482) and Santiago (Chile, n = 1655) have the highest smoking prevalence among the seven Latin American cities studied, with no gender differences (38.6% and 45.4% respectively)[34]. Our study reports that 20.9% of the controls and 22% of the cases are current smokers, with higher percentages for former smokers (32.7% for controls and 38.2% for cases). These results are probably due to that this study is based on hospital population, which strongly encourages quitting. Considering the high proportion of people exposed to tobacco in these countries, it would be interesting to continue assessing its impact on the development of blood cancer, given the limited information available.

Diet, probably among the most modifiable environmental factors, contributes to the development of 30%-35% of cancers[35]. Association studies in the field may be inconsistent due to differences in the frequency of food intake, varieties of available food, and different methods of preparation among populations.

According to a recent publication of the IARC Monograph Working Group, consumption of processed meat was classified as "carcinogenic to humans" (group 1), and consumption of red meat as "probably carcinogenic to humans" (group 2A)[36]. Meat processing can result in formation of N-nitroso compounds and PAH, while cooking it can produce heterocyclic aromatic amines and PAH. High-temperature cooking, such as grilling and barbecuing, produces the highest amounts of these carcinogens. Barbecued red meat is a frequent dish

among the Argentinean population, where there is a high consumption of animal protein and fats obtained mainly from red meat[37,38]. Several epidemiologic studies from Córdoba, an Argentinean province, report significant associations between consumption of red meat, or dietary patterns that contains it, and breast, colorectal, prostate, and urinary tract cancers[37-42]. According to Navarro *et al*[38], all meats were associated with an increased risk for colorectal cancer when barbecued, a similar result to that observed in our study. In two other surveys, the Southern Cone dietary pattern (red meat, starchy vegetables and wine consumptions) was associated with higher risk of urinary tract tumors[39] and colorectal cancer[37]. In a case-control study from Uruguay, red meat, lamb, and boiled meat were associated with the risk of squamous cell carcinoma of the esophagus[43].

In relation to oncohematological diseases, there are no studies in Argentina concerning diet and lifestyle as risk factors. Several studies from other countries evaluated this possible association, with inconsistent results. Most of them reported no association between meat consumption and increased risk of NHL or CML[44-46]. A case-control study from US done by Li *et al*[47] reported a positive association between an increased risk of AML and beef intake among women. Our results are the first to report a significant association between oncohematological diseases and consumption of barbecued/grilled meat in our population. However, some bias could exist since portion size was not assessed, and the questionnaire does not differentiate between red and white meat. Despite this, our results are relevant given the fact of a high and frequent consumption of these foods in Argentina.

It has been suggested that light to moderate alcohol consumption has beneficial effects due to advantageous host cellular and humoral immune responses[48]. On the other hand, ethanol was classified as carcinogenic to humans by the IARC. Regarding hematologic malignancies, a pooled analysis from the International Lymphoma Epidemiology Consortium reported that ever or current drinking were associated with a lower risk of NHL, compared with non-drinkers[49]. However, they did not find a dose-response relation or a stronger trend with longer duration. A meta-analysis performed with 18 studies, including 5694 cases with MM and 7142 with leukemia, did not find any association between alcohol drinking and MM or leukemia risks[50,51]. As with beef consumption, Li *et al*[47] reported a positive association between beer and wine intake and AML, only among women. An Italian case-control study showed no clear association between leukemia or NHL and alcohol consumption[27]. In line with some of these reports, we found no association between alcohol consumption and oncohematological diseases in the Argentinean population under study.

Regarding coffee consumption, there is no consistent evidence suggesting protective or deleterious effects. On the one hand, coffee may decrease the risk of cancer through antioxidant, antihormonal, and anti-inflammatory mechanisms[52]. On the other hand, caffeine and Topo Ⅱ inhibitors may elevate cancer risk. A United States cohort study reported no association between coffee intake and risk of all cancers combined, but they observed a decrease in the risk of endometrial cancer for women drinking 1 or more cups per day[53]. Although some studies on solid tumors have reported a protective effect (*i.e*., on liver, colorectal, breast and endometrial cancer[54-57]), studies on hematopoietic malignancies in adults are rare. In our study, daily consumption of coffee was associated with an increased risk of disease. An Indian case-control study showed a 40% reduction in the risk of leukemia for coffee drinkers[30], while an Italian one reported an increase in the risk of NHL[58]. Other studies found no significant associations between coffee consumption and hematologic cancer[44,59,60]. In 1991, the IARC Working Group classified coffee as possibly carcinogenic to humans (group 2B). Given the large number of studies published on the subject since that IARC publication, the IARC Advisory Group recommends a review of the evidence, giving to this exposure high priority for its inclusion in the monographs to be published between 2015 and 2019[61].

Genetic variations in XMG may be important factors in the etiology of onco-hematological diseases. Although they have low penetrance, they are highly prevalent in most populations, giving the chance to identify potential carcinogens and populations at higher risk of cancer[62]. These polymorphisms also interact with other polymorphisms and/or particular environmental factors, which vary between and within ethnic groups[8]. The increased activity/inducibility of CYP1A1*2A may contribute to the accumulation of genetic changes due to an increased production of mutagenic agents. In a similar way, the decreased activity of GSTs due to gene deletions may lead to a more intense cellular oxidative stress, increasing the level of DNA damage.

Regarding *CYP1A1 MspI* polymorphism, allele frequencies vary between ethnic groups, being 0.058, 0.149 and 0.218 for the C allele (*2A) in Caucasians, Asians and Africans, respectively[63]. Roco *et al*[64] reported a *2A allele frequency of 0.37 for a Chilean population. In this study, *2A allele frequencies were 0.34 for controls and 0.30 for cases, similar to that described for the Chilean population and away from that reported for Caucasians. Our genotypic data about this polymorphism deviate from Hardy-Weinberg equilibrium; this may be because the sample does not represent the entire population variability, or due to genotyping errors that create a bias towards increased homozygosity.

Our association analysis between *MspI* polymorphism and blood cancer showed an OR = 0.79 (95%CI: 0.51-1.24), with no statistical significance. Association studies between CYP1A1 *MspI* polymorphism and cancer have been inconsistent. A meta-analysis from 268 studies performed by He *et al*[8] showed that the variant *2A was associated with an increased risk of leukemia, cervical, hepatocellular, head and neck, lung and prostate cancer, but not with other cancer types. Another meta-analysis done by Han *et al*[65] reported a higher risk of leukemia associated with this variant, which remains significant for Caucasians when stratified by ethnicity.

They also reported an increased risk for ALL and AML, especially in childhood ALL in Caucasians. Among Asians, Lu *et al*[66] showed that the presence of the *MspI* polymorphism increased the risk of AML. On the other hand, a meta-analysis performed by Zhuo *et al*[67] did not find significant associations between CYP1A1 variant and AML risk.

Many studies have analyzed the possible association between hematological cancer and the deletions of *GSTT1* and *GSTM1*, with disparate results. In this study, frequencies for null genotypes were 0.18 in cases and controls for GSTT1, and 0.5 and 0.47 for GSTM1, in cases and controls respectively. Reported frequencies for GSTT1*null in controls were 0.13-0.26 for Caucasians[63], 0.10-0.12 for Chilean and Argentinean populations[64,68-70], and 0.09-0.24 for Native American Argentineans[71]. GSTM1*null has higher frequencies, with values of 0.42-0.60 for Caucasians[63], 0.36-0.46 for Chilean and Argentinean populations[64,68-70], and 0.29-0.49 for Native American Argentineans[71]. Our study showed an OR = 0.98 (95%CI: 0.54-1.74) for the GSTT1 deletion, and an OR = 1.14 (95%CI: 0.73-1.78) for GSTM1 deletion. He *et al*[62] carried out a meta-analysis, showing that GSTM1*null genotype significantly increased the risk of AML in East Asians, while GSTT1*null increased it in Caucasians. Double-null genotypes were associated with AML in both ethnic groups. Several case-control studies reported significant associations between GSTT1*null genotype and AML, CML, CLL and acute leukemia[72-78]. Conversely, other studies did not find such associations with all leukemia, acute leukemia, CML, AML or MM[79-82]. A similar scenario occurs with GSTM1*null genotype: many studies showed a significant increased risk of NHL, CLL, AML, MM, CML, and acute leukemia associated with the null genotype[72,73,76,78,83,84], while other ones reported no differences in cancer risk between controls and CML, AML, MM, acute leukemia and all leukemia cases[74,75,77,79-82]. In general, studies reported moderate OR, with values from 1 to 3; however, values up to 7 were reached in some assays, with OR values even greater when analyzing double null genotypes. It should be noted that these case-control studies are from different countries with different ethnic backgrounds, and with a variable number of participants.

Our study has strengths that are worth mentioning. Face-to-face interviews were performed by the same person, with all cases and controls, thus addressing reliable data about personal information, habits and lifestyle. Furthermore, although sample size is small, it has the power to detect an OR = 2, a value close to that reported in similar case-control studies. Even though cases and controls were not age- and gender-matched, statistical adjustment were used to minimize potential biases. It should be recall that hospital-based studies may have some bias, due to controls that might have benign diseases which are prone to turn malignant. This study design could reduce the generalization of the results to the general population. Additionally, hematological malignancies are heterogeneous illnesses, with potentially different causes. A larger number of samples will allow us to conduct studies of risk factors for each pathology independently.

There is a lack of studies for oncohematological diseases etiology in an argentinean population, particularly for genes and environmental factors. Taking that into account, our goal through the study was to address and offers relevant insight into diverse aspects for these pathologies in our population.

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COMMENTS

Background

Risk factors for oncohematological diseases are not completely defined. As in other cancer pathologies, blood cancer susceptibility is related to both lifestyle and genetic factors. In regards to the Argentinean population, these pathologies are barely studied, even in relation to highly frequent risk factors for this population.

Research frontiers

In the latest years, although specific diet patterns for Argentina have been evaluated as risk factors related to cancer development by many studies, hematological cancer was not included. Moreover, studies about tobacco, alcohol and coffee as risk factors for oncohematological diseases are still not conclusive; furthermore, the results can vary between different populations and study designs. On the other side, genetic variants in coding genes for enzymes associated to carcinogenic compounds metabolization are known genetics risk factors linked to cancer. Allelic frequencies for these enzymatic variants are different between populations; therefore, it becomes difficult to extrapolate an estimated risk from one population to another. For Argentina, genetic frequencies for metabolizing enzymes are already known; however, available data are limited to just a few cities and, mostly, for healthy population.

Innovations and breakthroughs

In that regards, in this work the authors reported an association between oncohematological diseases with coffee and meat intake. With respect to the latter risk factor, the result becomes extremely relevant given the fact of high meat consumption in Argentina. The results are the first one showing a possible association in the authors' population. Moreover, Argentina has a medium-high cancer incidence; every research implying an evaluation of different risk factors frequently found in the population, could help in contributing with new information, in order to dilucidate which specific factors are really involved in the actual incidence of blood related cancer diseases.

Applications

The result could be joined with the whole set of studies looking to evaluate the impact of genetics and lifestyle factors in cancer development. The results reported for coffee and barbecued/grilled meat intake, could be taken into account to go deeply in future studies by evaluating specific kinds of meat, portion size, other cooking methods, *etc*. This could allow researchers to classify, more specifically, which particular characteristics related to nutrition patterns are associated with cancer development in a population. Additionally, each oncohematological pathology could be also analyzed individually from the main group, due to the fact that each specific genetic or lifestyle factor could be involved in different pathways and/or specific disease stages, independently.

Terminology

Odds ratio (OR), in statistics, the OR is a way to quantify how strongly the presence or absence of property A is associated with the presence or absence

of property B in a given population. Oncohematological diseases or hematology-oncology, the diagnosis, treatment and prevention of cancer developed in blood cells, and the research associated to them. Hematology-oncology includes diseases such as leukemias and lymphomas, as well as other blood disorders (*i.e.*, iron deficiency anemia, hemophilia, sickle cell disease, and thalassemias).

Peer-review

The study indicates an increased risk of oncohematological diseases associated with meat and coffee intake. Overall the manuscript was written in a clear and concise manner.

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ORIGINAL ARTICLE

Observational Study

Factors associated with cervical cancer screening in a safety net population

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Abstract

AIM

To identify factors associated with Papanicolaou-smear (Pap-smear) cervical cancer screening rates in a safety net population.

METHODS

From January 2012 to May 2013, the use of Pap-smear was determined for all patients seen at the breast clinic in a safety net hospital. Health literacy assessment was performed using the validated Newest Vital Sign. The records of patients were reviewed to determine if they had undergone Pap-smears for cervical cancer screening. Sociodemographic information was collected included age, education, monthly income, race/ethnicity, employment, insurance status, and primary care provider of the

patient. Logistic regression analysis was then performed to determine factors associated with utilization of Pap-smears. Crude and adjusted odds ratios derived from multivariate logistic regression models were calculated as well as the associated 95%CIs and *P*-values.

RESULTS

Overall, 39% had Pap-smears in the prior 15 mo, 1377 consecutive women were seen during the study period and their records were reviewed. Significantly more patients with adequate health literacy underwent Pap-smears as compared to those with limited health literacy (59% *vs* 34%, $P < 0.0001$). In multivariate analysis, patients with adequate health literacy, younger patients, and those with later age of first live birth were more likely to undergo Pap-smears. Patients whose primary care providers were gynecologists were also significantly more likely to have Pap-smears compared to other specialties ($P < 0.0001$). Patients younger than 21 years or older than 65 years underwent screening less frequently (11% and 11%, respectively) than those 21-64 years (41%, $P < 0.0001$). Race, ethnicity, language, and insurance status were not associated with Pap-smear screening rates.

CONCLUSION

Patient health literacy and primary care physician were associated with Pap-smear utilization. Development of interventions to target low health literacy populations could improve cervical cancer screening.

Key words: Cervical cancer; Health literacy; Prevention; Screening; Pap-smear

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Core tip: Patient health literacy and type of primary care physician were associated with Papanicolaou-smear utilization. Development of interventions to target low health literacy populations could improve cervical cancer screening and therefore improve screening in populations most at risk for cervical cancer.

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INTRODUCTION

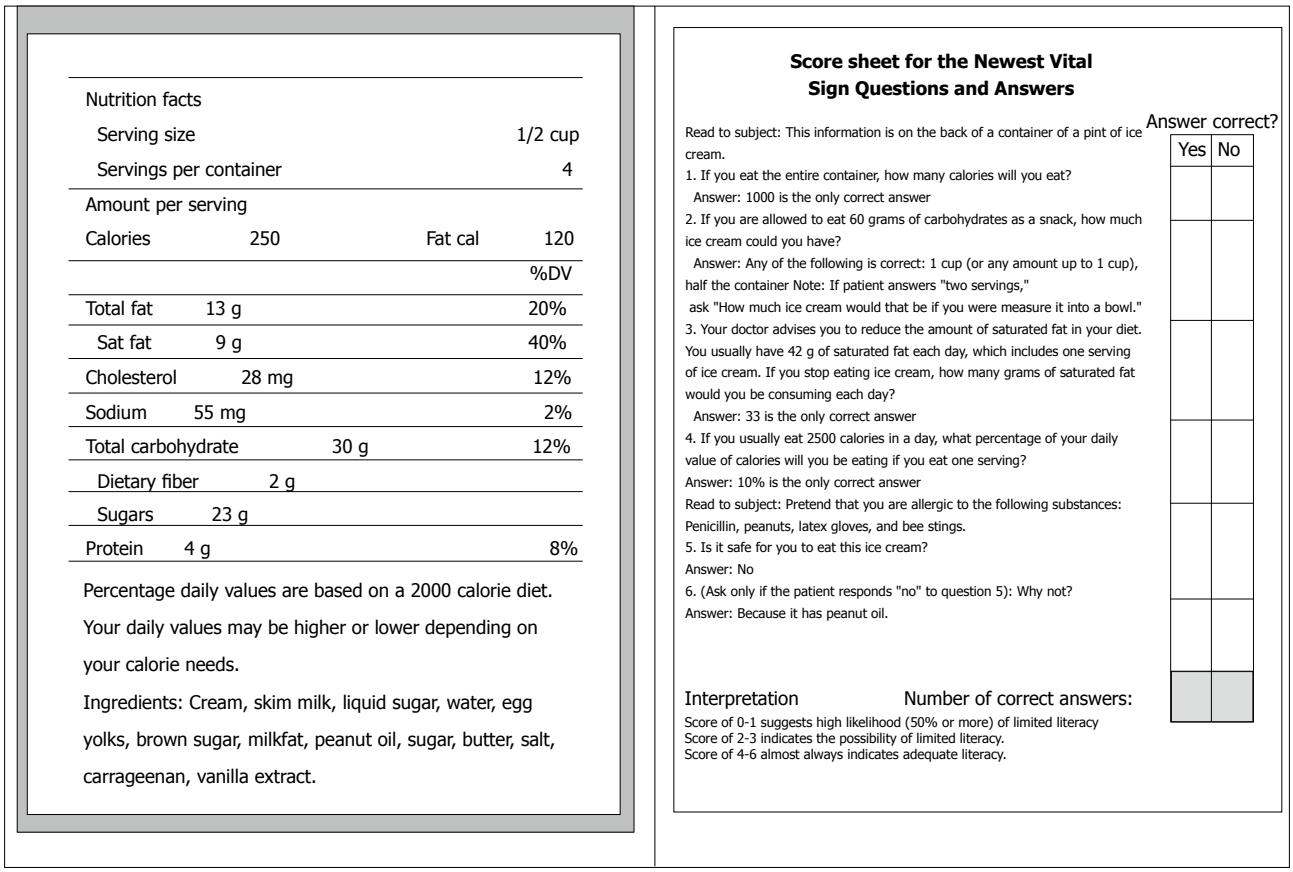
Cervical cancer is one of the most preventable and treatable female cancers. While cervical cancer death rates have decreased, it remains a significant burden in all countries. Cervical cancer screening rates are most suboptimal among recent immigrants to developed countries, in countries without screening programs, among racial and ethnic minorities, women from low socioeconomic backgrounds, and underinsured populations[1-3]. Most studies show higher rates of cervical cancer and lower compliance with cervical cancer screening in these populations[1-3].

Previous studies have found that patients do not understand the concept of screening or did not realize that Pap-smears are a test for cervical cancer. One population studied was less likely to undergo screening because they "didn't have problems/symptoms"[4]. A second study found that patients thought Papanicolaou-smear (Pap-smears) were for infections, gonorrhea, or HIV[5]. Health literacy is the degree to which individuals have the capacity to obtain, process, and understand basic health information needed to make appropriate health decisions[6]. In 2003, the National Assessment of Adult Literacy (NAAL) reported that over 89 million American adults have limited health literacy skills and that individuals with limited health literacy come from all parts of society[7,8]. In fact, the NAAL survey, which rated health literacy skills in four levels ranging from "below-basic" to "proficient", showed that more than 40% of high school graduates and 13% of college graduates have health literacy skills at the lowest two levels and are thus considered to have limited health literacy[8,9]. Similarly, the 2011 Programme for the International Assessment of Adult Competencies which evaluated adults in 23 industrialized countries indicated continuing issues: A significant proportion of adults scored below average for literacy, numeracy, and problem solving in technology-rich environments[10]. The number of patients with low health literacy is increasing and is more common among those with low educational attainment, immigrants, elderly, and racial/ethnic minorities[7,11]. These individuals are less able to navigate the health care system and less likely to participate in preventive health care[12-16]. The current study was performed to identify factors that influence use of Papanicolaou cytology smears for cervical cancer screening in a safety net population.

MATERIALS AND METHODS

This study was conducted at Maricopa Medical Center in Phoenix, Arizona, and reviewed and approved by the medical center's institutional review board. The institutional review board also granted a waiver of informed consent for this study. Maricopa Medical Center is the safety net hospital for Maricopa County, which includes the city of Phoenix and the surrounding metropolitan area. Maricopa County is the state's most populous area with nearly four million of Arizona's 6.5 million inhabitants. Maricopa Medical Center serves a patient population of which 78% of patients are from racial/ethnic minority groups and 79% are underinsured, uninsured, or insured by Medicaid.

Beginning on January 1, 2012 and continuing until May 31, 2013, every patient seen in the Breast Clinic underwent a health literacy assessment as part of their routine history and physical examination. Health literacy



Nutrition facts

Serving size	1/2 cup
Servings per container	4

Amount per serving

Calories	250	Fat cal	120

		%DV
Total fat	13 g	20%
Sat fat	9 g	40%
Cholesterol	28 mg	12%
Sodium	55 mg	2%
Total carbohydrate	30 g	12%
Dietary fiber	2 g	
Sugars	23 g	
Protein	4 g	8%

Percentage daily values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

Ingredients: Cream, skim milk, liquid sugar, water, egg yolks, brown sugar, milkfat, peanut oil, sugar, butter, salt, carrageenan, vanilla extract.

Score sheet for the Newest Vital Sign Questions and Answers

Read to subject: This information is on the back of a container of a pint of ice cream.

1. If you eat the entire container, how many calories will you eat?
Answer: 1000 is the only correct answer
2. If you are allowed to eat 60 grams of carbohydrates as a snack, how much ice cream could you have?
Answer: Any of the following is correct: 1 cup (or any amount up to 1 cup), half the container Note: If patient answers "two servings," ask "How much ice cream would that be if you were measure it into a bowl."
3. Your doctor advises you to reduce the amount of saturated fat in your diet. You usually have 42 g of saturated fat each day, which includes one serving of ice cream. If you stop eating ice cream, how many grams of saturated fat would you be consuming each day?
Answer: 33 is the only correct answer
4. If you usually eat 2500 calories in a day, what percentage of your daily value of calories will you be eating if you eat one serving?
Answer: 10% is the only correct answer

Read to subject: Pretend that you are allergic to the following substances: Penicillin, peanuts, latex gloves, and bee stings.

5. Is it safe for you to eat this ice cream?
Answer: No
6. (Ask only if the patient responds "no" to question 5): Why not?
Answer: Because it has peanut oil.

Answer correct?	Yes	No
Number of correct answers:		

Interpretation

Score of 0-1 suggests high likelihood (50% or more) of limited literacy
Score of 2-3 indicates the possibility of limited literacy.
Score of 4-6 almost always indicates adequate literacy.

Figure 1 The newest vital sign instrument for health literacy assessment. Available from: URL: http// www.pfizerhealthliteracy.com/.

assessment involves the use of validated instruments. The Newest Vital Sign was chosen because it can be administered in about 2 min, and therefore is feasible for use in clinical practice rather than strictly a research setting. In addition, it has the advantage of having been validated in both English and Spanish[17-19].

The Newest Vital Sign uses a nutrition label, similar to the nutrition label present on all packaged food in the United States. The nutritional label was tested as one of many scenarios developed by a panel of health literacy experts based on concepts and types of scenarios used in health literacy research and in general literacy assessments. The candidate scenarios tested involved both reading and numeracy skills as this was driven by research indicated that these skills are highly correlated with one another, plus an intuitive understanding that patients must be able to use and understand both text and numbers if they are to successfully deal with today's health care system. Of the candidate scenarios tested, the nutritional label performed the best and has been validated and correlates well with more complicated health literacy instruments[17].

The assessment involves asking the patient five questions (and a sixth qualifier question, if needed) about the nutrition label (Figure 1). One point is awarded for each correct answer. Instructions accompanying the nutrition label specify what the interviewer should ask and they list the only acceptable answer(s). Scoring instructions for the Newest Vital Sign place patients into 3 groups, depending on how many of the 6 questions are answered correctly. A score of 0-1 indicates a high likelihood of limited health literacy. A score of 2-3 indicates the possibility of limited health literacy. Score of 4-6 indicate adequate health literacy. The instrument validation, including its sensitivity and specificity among Spanish speakers has been previously described[17].

The details of incorporating routine health literacy assessment as part of the standard history and physical examination have been described previously[19]. In short, the assessment was performed by the clinician during the history and physical examination. However, if the patient was Spanish speaking, the assessment was performed by a hospital certified Spanish translator or a clinician who was a native-Spanish speaker. No special personnel or other resources were used for performance of the health literacy assessment. Patients were excluded from health literacy assessment if they did not speak English or Spanish, had visual impairment that prevented them from viewing the nutritional label, or had cognitive limitations due to developmental delay, dementia, psychiatric illness, or head injury.

The records of patients receiving care during the study period were reviewed to determine if they had undergone Papanicolaou cytology smears (Pap-smears) for cervical cancer screening. If there was no record in the medical system of a Pap-smear, the patient was

Table 1 Patient sociodemographics

	All patients (n = 1318)
Mean age, years (SD)	45.0 (13.1)
Race/ethnicity	
Non-Hispanic White	230 (17%)
African American	131 (10%)
Hispanic	888 (67%)
Other	69 (5%)
Language, English	591 (45%)
Mean age of menarche (SD)	12.8 (1.88)
Mean pregnancies (SD)	3.3 (2.32)
Mean live births (SD)	2.7 (1.97)
Mean age of first live birth (SD)	21.0 (4.43)
Education, years	
6 or less	286 (22%)
7-11	340 (26%)
High school/equivalent	324 (25%)
Some college	368 (28%)
Adequate health literacy	229 (17%)
Body mass index in kg/m^2 (SD)	29.3 (7.57)
Marital status - married	555 (42%)
Employment, employed	456 (35%)
Insurance status	
Commercial	60 (5%)
Medicare	45 (3%)
Medicaid	312 (24%)
None	900 (68%)
Monthly income $US (SD)	1099 (878.46)
Screening mammography (age ≥ 40 yr)	253/899 (28%)
Pap smear in last 15 mo	514 (39%)

SD: Standard deviation; $US: United States dollars.

asked about their last screening and documentation was obtained *via* contact with prior health facilities or providers to confirm the date and results.

Patients were excluded and no attempt was made to obtain Pap-smear results on patients if they had undergone hysterectomy for reasons other than cervical cancer or had not yet initiated sexual activity. All other patients were considered to have undergone screening if they had done so in the year based on the more guidelines and recommendations for annual Pap-smears as of January 2012. Many insurance payors, however, will not pay for repeat screenings in durations even one day less than one calendar year. Therefore patients will often be scheduled for subsequent screenings in 13 or 14 mo. To account for this issue, a 15 mo time period was chosen to give some room for error on the "annual screening".

For all patients, sociodemographic information was collected and included age, education, self-reported monthly income, race/ethnicity, employment status, and insurance status. Patients were also queried about their reproductive status, height and weight, current smoking status, and use of alcohol. Finally, data were collected on whether or not the patient had a primary care provider and the type of provider.

Statistical analysis

Age, education, body mass index (height/weight^2), number of live births, and estimated monthly income were analyzed as continuous variables. Race/ethnicity (non-Hispanic white *vs* other, Hispanic *vs* non-Hispanic), employment status, insurance status (uninsured *vs* insured), and primary care provider were analyzed as categorical variables. Adequacy of health literacy was analyzed as a categorical variable. Patients with Newest Vital Sign 4-6 were categorized as having "adequate health literacy". Patients with Newest Vital Sign scores of 0-1 and 2-3 had similar use of Pap-smears and were therefore combined into a single group labeled "low health literacy". Analysis of the data did not demonstrate differences when the patients were categorized in three groups or in the two groups as presented. A two-sample *t*-test was used to determine if there were significant differences in continuous variables between women who did and did not undergo Pap-smears. A Fisher's exact test was used to assess differences in the categorical variables. All statistical tests were two sided and significance levels were set at 0.05.

Logistic regression analysis was then performed with the dependent variable being whether or not a patient had undergone Pap-smears. Independent variables included health literacy (adequate *vs* low literacy) and the sociodemographic variables listed in Table 1. Factors shown to have $P < 0.1$ in univariate analysis were included in the multivariate model. Crude and adjusted odds ratios (ORs) derived from multivariate logistic regression models were calculated as well as the associated 95%CIs and *P* values.

The statistical methods of this study were performed and reviewed by a biomedical statistician (CHH).

RESULTS

A total of 1377 consecutive patients were seen from January 1, 2012 and continuing until May 31, 2013. Fifty-nine patients were excluded because they were not yet sexually active or had hysterectomy for an indication other than cervical cancer. The remaining 1318 patients made up the study population (Table 2). The average age of the women was 45 years and the minority was non-Hispanic White (17%), while 10% were African American and 67% were Hispanic. Only 45% spoke English as their primary language. The vast majority (92%) were underinsured (24% Medicaid and 68% uninsured). The mean monthly income was $1099. Patients completed an average of 10 years of education.

Overall, 39% underwent a Pap-smear in past year. Table 1 shows the rates of Pap-smear use according to various sociodemographic variables, health literacy, and other factors. Several factors were significantly associated with Pap-smear uptake, including age, education, employment status, number of pregnancies, age at first live birth, and menopausal status. When evaluated by health literacy, significantly more patients with adequate health literacy underwent Pap-smears as compared to those with low health literacy (59% *vs*

Table 2 Patient factors associated with use of Pap-smear for cervical cancer screening

Variables	Unadjusted			Adjusted[2]	
	Rate	OR	*P*-value[1]	OR	*P*-value
Age (per year)	45.17 ± 12.64	0.97 (0.96, 0.98)	< 0.0001	0.97 (0.96, 0.99)	< 0.0001
Non-Hispanic White race		0.92 (0.68, 1.23)	0.6029	-	
No	429/1088				
Yes	86/230				
Hispanic ethnicity		1.16 (0.91, 1.47)	0.2294	-	
No	158/430				
Yes	357/888				
Pregnancies (per pregnancy)	3.31 ± 2.28	0.95 (0.90, 0.99)	0.0277	1.07 (0.95, 1.20)	0.2537
Live births (per birth)	2.70 ± 1.95	0.91 (0.85, 0.96)	0.0011	0.90 (0.77, 1.04)	0.1556
Age of 1st live birth (per year)	20.96 ± 4.42	1.05 (1.02, 1.07)	0.0013	1.04 (1.01, 1.08)	0.0121
Body mass index, kg/m^2 (per unit)	29.29 ± 7.57	0.988 (0.972, 1.003)	0.1224	-	
Language		1.09 (0.87, 1.36)	0.4622	-	
English	235/619				
Other	280/699				
Education (per year)	10.40 ± 3.80	1.05 (1.02, 1.08)	0.0012	1.00 (0.96, 1.04)	0.9472
Low HL	368/1056	2.73 (2.04, 3.66)	< 0.0001	2.05 (1.38, 3.03)	0.0003
Adequate HL	136/229				
Married		1.19 (0.95, 1.48)	0.1375		
No	285/763			-	
Yes	230/555				
Menopausal		0.42 (0.32, 0.54)	< 0.0001	0.88 (0.58, 1.31)	0.5192
No	410/909				
Post	105/409				
Employed		1.39 (1.11, 1.76)	0.0053	1.04 (0.78, 1.37)	0.8023
No	313/862				
Yes	202/456				
Income (per $1000US/mo)	1.10 ± 8.78	1.44 (1.26, 1.64)	< 0.0001	1.29 (1.09, 1.51)	0.0024
Insured	141/418	1.40 (1.10, 1.78)	0.0076	1.22 (0.90, 1.65)	0.1977
Uninsured	374/900				
Current smoker		0.80 (0.60, 1.06)	0.1211		
No	423/1054			-	
Yes	92/264				
Screening mammography		1.46 (1.11, 1.92)	0.0081	1.69 (1.22, 2.35)	0.0018
No	397/1064				
Yes	118/254				
Primary care provider	427/1182	3.24 (2.24, 4.70)	< 0.0001	2.81 (1.84, 4.29)	< 0.0001
Others gynecologist	88/136				

[1]Derived from a Fisher's exact test for categorical variables and a logistic regression model for continuous variables; [2]Adjusted for age, pregnancies, live births, age of 1st live birth, years of education, HL, menopause, employment, income, insurance, use of screening mammography, and type of primary care provider. HL: Health literacy as measured by Newest Vital Sign.

34%, $P < 0.0001$). Although patients without a primary care provider had Pap-smear rates similar to those with such a provider, the type of provider made a difference. Women who had a gynecologist for their primary care provider were significantly more likely to have Pap-smears (65%) as compared to those who had an internist (23%) or family practitioner (42%, $P < 0.0001$). Patients less than 21 years of age and those older than 65 years underwent screening less frequently (11% and 11%, respectively) than those age 21-54 years (41%, $P < 0.0001$).

In multivariate analysis, older age was significantly associated with lower likelihood of undergoing Pap-smear use ($P < 0.001$), whereas older age of first live birth ($P < 0.01$) and higher income were associated with higher use ($P < 0.0096$). Patients who underwent screening mammography were more likely to undergo Pap-smears (OR = 1.69; 95%CI: 1.22-2.35, $P = 0.0018$). The type of primary care provider also had a significant effect as patients whose primary care provider was a gynecologist were significantly more likely (OR = 2.81; 95%CI: 1.84-4.29, $P < 0.0001$) to undergo screening than those with other types of providers. Level of health literacy also affected use of Pap-smears as those with adequate health literacy were twice as likely to participate as those with low health literacy (OR = 2.05; 95%CI: 1.38-3.03, $P = 0.0003$).

DISCUSSION

Results of this study show that the use Pap-smears in this underinsured population was suboptimal, at 39%. We identified several factors that were independently associated with Pap-smear utilization. Younger patients, patients with later first live birth, and those who participated in screening mammography were more

likely to utilize Pap-smears. Women with a gynecologist as their primary care provider and those with adequate health literacy were most likely to undergo Pap-smears.

The finding that Pap-smear screening is underutilized in an underinsured population is not new[20]. However, the factors associated with low screening rates within these populations can provide insight into factors that may improve compliance or to provide targeted screening. In particular, women with adequate health literacy underwent Pap-smear screening twice as much as those with low health literacy. On the other hand, many sociodemographic factors which are thought to influence use of preventative services, such as race/ethnicity, education, employment, and insurance status were not found to be associated with Pap-smear use in the current study[21-23]. We previously reported that limited health literacy was the strongest predictor of non-use of breast cancer screening[14]. In that study, 57% of patients did not realize they should undergo screening mammography or did not understand the concept of screening[14]. In the current study, we found that participation in screening mammography was associated with higher rates of Pap-smear screening, suggesting that understanding the concept of preventive care and the ability to navigate the health care system - both key components of health literacy - are critical to Pap-smear screening. Since it is unclear whether an individual's level of health literacy can be modified, development of strategies to increase awareness of the importance of prevention in populations with health disparities and limited literacy are critical to improve compliance[24].

In our population comprised of a significant proportion Hispanic women, ethnicity was not a predictor of use of Pap-smears. It is possible that level of acculturation, which we did not assess, may influence screening uptake in these women. However, health literacy may be an adequate proxy for acculturation, as it has been associated with factors related to acculturation (*i.e.,* language, education, employment)[19]. In the current population, patients with adequate health literacy, had reproductive behavior consistent with higher levels of acculturation (fewer pregnancies 2.7 *vs* 3.6; fewer live births 1.8 *vs* 3.1; and later age of first live birth 22 years *vs* 20 years; $P < 0.01$ for all three factors). Patients with characteristics associated with higher levels of acculturation, were more likely to utilize Pap-smears suggesting that acculturation might play a role[25].

Although having a primary care provider did not influence Pap-smear use, the type of provider was significant. Patients whose primary provider was a gynecologist were three times as likely to undergo screening with Pap-smears compared to those with other providers. It also appears that Pap-smears were not over-utilized as patients under the age of 21 years or over the age of 65 years were less likely (both 11%) to undergo Pap-smears compared to the rest of the population (41%). The time frame of this study was 3 years after the 2009 American College of Obstetrics and Gynecology (ACOG) Practice Bulletin, which was when these age recommendations were changed[26]. This study indicates reasonable assimilation of the 2009 recommendations.

There are limitations to the current study. First, because we asked patients about their use of Pap-smears prior to verifying use, it is possible that some patients were unable to recall this information, and this could underestimate the use of Pap-smears. Not being able to recall that the test was completed or the results, however, somewhat defeats the purpose of screening, since not knowing the results would make patients unaware of necessary follow-up. A second limitation is that in November 2012, there was a change in recommendations for screening to every 3 years[20]. It is possible that clinicians and practitioners immediately instituted the recommendations and could have contributed to the low use. Implementation of new guidelines into clinical practice, however, is frequently inefficient and often requires several years to be implemented[27,28]. Further, there were no differences in the use of Pap-smears before (38%) or after (41%) the change in recommendations, indicating this did not affect the results. A third limitation is that Human papilloma virus testing was not included as part of the study and results may have been different had we included this evaluation. Human papilloma virus testing alone, however, is not recommended for cervical cancer screening in any age group. Pap-smear cytology alone remains the recommended (age 21-29 years) or acceptable (age 30-65 years) method for screening[20].

Most clinicians feel that patients with limited health literacy do not exist in their practice. Surveys in the United States and internationally, however, demonstrate that significant proportions of adults in all countries have limited health literacy skills[7-11]. Patients with limited health literacy are found in all clinical practices and the number of patients with limited health literacy is increasing, particularly with the aging population, increasing number of immigrants, and patients with low educational attainment[7,8,10,11]. No strategies have yet been proven to improve or increase health literacy. Therefore increased awareness of patients with limited health literacy is important in all clinical settings.

The current study found that specialty of the primary care physician, health literacy, along with the patient's age and older age at first childbirth were associated with Pap-smear utilization. Patients with low health literacy exist in all countries and clinical practices and their numbers are increasing. Development of interventions to target low health literacy populations could improve cervical cancer screening.

COMMENTS

Background

Cervical cancer is one of the most preventable and treatable female cancers. Cervical cancer screening rates are most suboptimal among recent immigrants to developed countries, in countries without screening programs, among racial

and ethnic minorities, women from low socioeconomic backgrounds, and underinsured populations. Most studies show higher rates of cervical cancer and lower compliance with cervical cancer screening in these populations.

Innovations and breakthroughs

Previous studies have found that patients do not understand the concept of screening or did not realize that Papanicolaou-smears (Pap-smears) are a test for cervical cancer. The number of patients with low health literacy is increasing worldwide and these individuals are less able to navigate the health care system and less likely to participate in preventive health care. The current study is the largest series of consecutive patients at a single institution to evaluate the association of Pap-smear utilization with health literacy and other sociodemographic factors.

Applications

This study found that specialty of the primary care physician, health literacy, along with the patient's age and older age at first childbirth were associated with Pap-smear utilization. Patients with low health literacy exist in all countries and clinical practices and their numbers are increasing. Development of interventions to target low health literacy populations could improve cervical cancer screening.

Terminology

Health literacy is the degree to which individuals have the capacity to obtain, process, and understand basic health information needed to make appropriate health decisions. In 2003, the National Assessment of Adult Literacy reported that over 89 million American adults have limited health literacy skills and that individuals with limited health literacy come from all parts of society. Similarly, the 2011 Programme for the International Assessment of Adult Competencies which evaluated adults in 23 industrialized countries indicated similar issues: A significant proportion of adults scored below average for literacy, numeracy, and problem solving in technology-rich environments.

Peer-review

Few papers have examined the association of health literacy and use of Pap-smears for cervical cancer screening. The authors of the current study evaluated sociodemographic factors which affected use of Pap-smears. This study found that patients with adequate health literacy and specialty of their primary care provider were more likely to use Pap-smears than other patients.

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ORIGINAL ARTICLE

Observational Study

Bone and soft tissue tumors presenting as sciatic notch dumbbell masses: A critical differential diagnosis of sciatica

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Abstract

AIM

To study the clinical findings and characteristic features in sciatic notch dumbbell tumors (SNDTs).

METHODS

We retrospectively reviewed the clinical outcomes and characteristic features of consecutive cases of SNDTs (n = 8).

RESULTS

Buttock masses occurred in three patients with SNDT (37.5%). Severe buttock tenderness and pain at rest were observed in seven patients with SNDTs (87.5%). Remarkably, none of the patients with SNDTs experienced back pain. Mean tumor size was 8.4 ± 2.0 cm (range, 3.9 to 10.6 cm) and part of the tumor mass was detected in 2 patients in the sagittal view of lumbar magnetic resonance imaging (MRI).

CONCLUSION

The clinical information regarding to SNDTs is scarce. The authors consider that above mentioned characteristic findings may facilitate the suspicion of pelvic pathology and a search for SNDT by MRI or computed tomography should be considered in patients presenting with sciatica

without evidence of spinal diseases.

Key words: Sciatic notch; Dumbbell masses; Sciatica; Differential diagnosis; Bone and soft tissue tumor

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Core tip: The author retrospectively studied the clinical outcomes and characteristic findings of consecutive cases of sciatic notch dumbbell tumors (SNDTs) and found that buttock mass, severe buttock pain at rest and lack of back pain may facilitate the suspicion of pelvic pathology and a search for SNDT by magnetic resonance imaging or computed tomography should be considered in patients presenting with sciatica without evidence of spinal diseases.

Matsumoto Y, Matsunobu T, Harimaya K, Kawaguchi K, Hayashida M, Okada S, Doi T, Iwamoto Y. Bone and soft tissue tumors presenting as sciatic notch dumbbell masses: A critical differential diagnosis of sciatica. *World J Clin Oncol* 2016; 7(5): 414-419 Available from: URL: http://www.wjgnet.com/2218-4333/full/v7/i5/414.htm DOI: http://dx.doi.org/10.5306/wjco.v7.i5.414

INTRODUCTION

Sciatica is a very common disorder. Accurate diagnosis of sciatica is problematic, since it may be caused by various pathologies, including lumbar disc herniation (LDH), spinal degeneration, inflammatory diseases, trauma, metabolic or circulatory events, and tumors[1].

In particular, intra- and extra-pelvic sciatic notch dumbbell-shaped tumors [sciatic notch dumbbell tumors (SNDTs)] may cause classic sciatica by invading the sacrum, sacral plexus, and sciatic nerve. The lifetime incidence of LDH was considered to be 2%-3% and that of spinal tumor was 1 per 100000. Meanwhile, the occurrence of SNDTs is considered to be rare and there were no reports of case series with large number and the accurate incidence rate of SNDT remains unknown[2-4], and most spine and orthopedic surgeons therefore have little or no experience with sciatica caused by undiagnosed SNDTs.

Retroperitoneal bone and soft tissue sarcomas may present as SNDTs. These have a poor prognosis because of high rates of local recurrence, mortality, and surgical morbidity[5]. Early recognition of malignant SNDTs should increase the chance of survival, and thus it is essential to promptly and accurately diagnose sciatica caused by such tumors. However, very little clinical information regarding SNDTs has been published thus far[6].

In this study, we studied retrospectively the clinical outcome of 8 patients suffered from sciatica due to SNDTs. We also evaluated the characteristic features of these tumors on physical examination and imaging analysis, with the aim of improving the differential diagnosis of sciatica.

MATERIALS AND METHODS

Clinical findings

This study was approved by institutional review board in our hospital. The clinical findings and surgical records of consecutive eight cases of SNDTs were retrospectively reviewed. The following information was retrieved from medical records: Demographic details, disease history, imaging manifestations, tumor pathology, details of surgical treatment, and postoperative survival and tumor recurrence. SNDT specimens were used for histopathological analysis and final diagnosis. To establish the tissue diagnosis, either computed tomography (CT)-guided needle biopsy or open biopsy were carried out. All patients underwent a clinical examination in which the chief complaint and mode of illness onset were noted in a standardized manner. Physical examination was performed at the first medical visit. Patient delay was defined as the duration between the onset of each patient's initial symptoms and their first physician consultation, while physician delay was defined as the period from the patient's first medical visit for their symptoms until the date of accurate diagnosis.

Review of radiographic images

X-ray, magnetic resonance imaging (MRI), and CT images of the pelvis were taken in all cases. Lumbar MRI was performed in 7 patients. Two orthopedic surgeons with more than 5 years' experience independently investigated all imaging results: For X-rays, bone destruction and matrix mineralization; for MRIs, tumor size, tumor boundaries, and relation between the sciatic nerve and mass on lumbar MRI sagittal imaging; and for CT, osteolytic bone destruction, tumor calcification, and enlargement of the sciatic foramen.

RESULTS

Clinical features of SNDTs

There were 8 cases of SNDTs (5 males and 3 females). Patients' ages at their first medical visits ranged from 12 to 81 years, with a mean age of 35.6 ± 21.2 years (mean ± SD). With regard to the McCormick scale[7], one case was grade Ⅰ, grade Ⅱ in 3, grade Ⅲ in 2, and grade Ⅳ in 2. Histologically, 2 SNDTs were undifferentiated pleomorphic sarcomas, and there was one case each of osteosarcoma, Ewing sarcoma, malignant peripheral nerve sheath tumor, neurinoma, carcinosarcoma, and solitary fibrous tumor. The patient delay averaged 11.8 mo (2 to 26 mo), while the physician delay averaged 2.6 mo (1 to 7 mo). Table 1 presents a summary of the patients' clinical demographics.

Chief complaints and physical examination findings at the first medical visit

All 8 cases presented with sciatica. A palpable mass in one buttock was detected in 3 of the 8 cases. Of the 8

Table 1 Clinical features of 8 patients with sciatic notch dumbbell tumors

Case	Age/sex	McCormick scale	Histology	Patient delay (mo)	Physician delay (mo)
1	41/F	Ⅱ	Solitary fibrous tumor	26	7
2	12/F	Ⅰ	UPS	12	4
3	27/F	Ⅲ	Neurinoma	20	3
4	36/F	Ⅱ	Carcinosarcoma	2	1
5	81/M	Ⅳ	Osteosarcoma	5	2
6	15/F	Ⅳ	UPS	2	1
7	35/F	Ⅱ	Ewing's sarcoma	15	2
8	38/M	Ⅲ	MPNST	12	1

UPS: Undifferentiated pleomorphic sarcoma; MPNST: Malignant peripheral nerve sheath tumor; M: Male; F: Female.

Table 2 Summary of chief complaints and physical examination findings in patients with sciatic notch dumbbell tumors

	SNDT (n = 8)
Sciatica	8
Pain at rest	7
Back pain	0
Palpable mass in the buttock	3
Buttock tenderness	7
Motor weakness	4
Sensory loss testing	6
Positive SLRT	6

SNDT: Sciatic notch dumbbell tumor; SLRT: Straight leg rising test.

cases, 7 reported severe buttock tenderness, 4 reported muscle weakness, 7 reported pain at rest, and 6 reported a positive straight leg raise test (SLRT) (Table 2). Six cases experienced unilateral sensory disturbances of the lower limb. The dermatomal distribution of symptoms was as follows: L5 in 2 cases, L5 to S1 in 2 cases, S1 in one case, and S2 in one case. A positive SLRT was noted in 6 of the 8 cases. Remarkably, no patients experienced back pain. Taken together, the clinical features suggestive of SNDTs were as follows: A chief complaint of pain at rest, lack of back pain, a unilateral buttock mass, and severe buttock tenderness.

Imaging features of SNDTs

On initial plain radiographs, osteolytic bone destruction was detectable in 2 of the 8 cases, while matrix mineralization was observed in one case. In each of the 8 cases, MRI of the pelvis showed a large intrapelvic and extrapelvic tumor adjacent to the sciatic notch. Maximal diameters of the mass ranged from 3.9 to 10.6 cm (mean 8.4 ± 2.0 cm). Tumor borders were clearly defined in 3 cases and poorly defined from the adjacent organ in 5 patients. The sciatic nerves were clearly connected to the tumors in 3 cases. Lumbar MRI was performed in 7 patients; importantly, in 2 of these, part of the tumor mass was detected in the sagittal view but not in axial views. On CT, destructive invasion to the bone by the tumor progression was found in 5 cases. Ectopic calcification was observed in one case and enlargement of the sciatic foramen was found in 4 cases. Imaging features of SNDTs are summarized in Table 3.

Table 3 Radiological features of 8 patients with sciatic notch dumbbell tumors

Modalities	Descriptions	Value
X-ray	Bone destruction	2
	Matrix mineralization	1
MRI	Tumor size (cm)	8.4 ± 2.0 (range, 3.9 to 10.6)
	Indistinguishable tumor boundary	5
	Connection of the sciatic nerve	3
	Mass on lumbar MRI sagittal image	2
CT	Osteolytic bone destruction	5
	Tumor calcification	1
	Enlargement of sciatic foramen	4

MRI: Magnetic resonance imaging; CT: Computed tomography.

Treatment and clinical outcomes of SNDTs

Two of the 8 cases were managed with surgery, one using a wide surgical margin and the other *via* intralesional resection. Chemotherapy was administered to the 5 patients who did not undergo surgical tumor resection. Combination of adriamycin, ifosfamide, cisplatin, and etoposide were used for chemotherapy. Chemotherapy resulted in a complete response in one case, a partial response in 2 cases, stable disease in one case, and progressive disease in one case. Five patients received radiotherapy, 3 patients underwent conventional radiotherapy, and 2 patients were treated with carbon-ion curative radiotherapy. Local recurrence occurred in 2 cases and distant metastases to the lung were observed in one case. At final follow-up, one of the 8 patients had died of disease, one was continuously disease-free, one showed no evidence of disease, and 5 were alive with disease.

Case presentations

Case 1: An SNDT in a 41-year-old woman. She suffered from neurogenic claudication and sciatica for 9 mo. Examination showed moderate sensory loss in the right S1 dermatome and a nerve stretch test was positive.

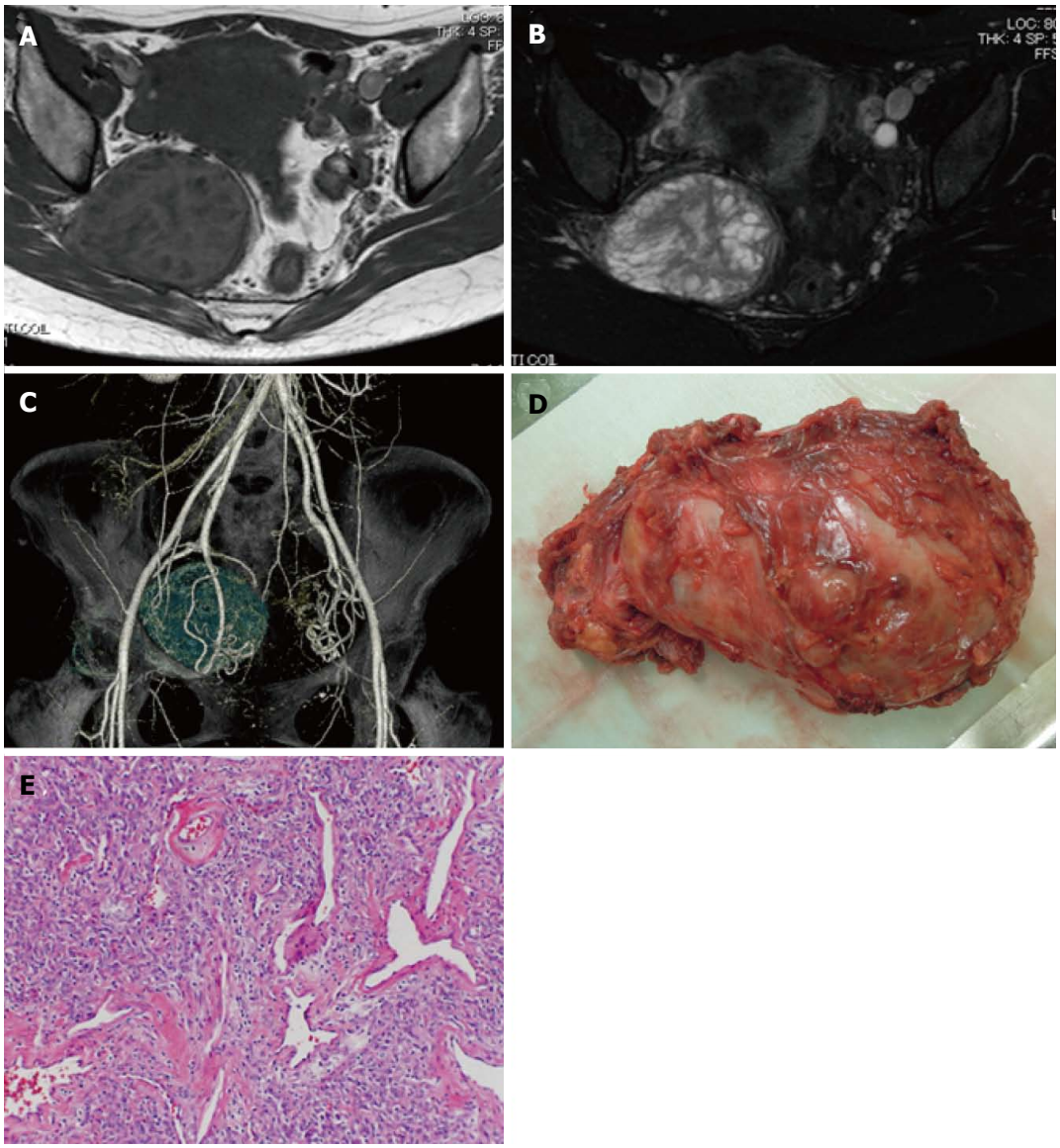

Figure 1 Case 1: An sciatic notch dumbbell tumor in a 41-year-old female. A and B: Axial MRI revealed an SNDT with a 7.7-cm diameter. The tumor showed a mixed intensity signal on T1- (A) and T2-weighted (B) images; C: 3D-CT angiography clearly demonstrated the relationship between the tumor and major vessels; D: Macroscopic appearance of the resected tumor showing a gray-white, dumbbell-shaped mass with surrounding soft tissue; E: Postoperative pathology confirmed the diagnosis of solitary fibrous tumor. The specimen showed cellular proliferation of mildly atypical spindle or oval cells arranged in short fascicles that were associated with dilated sclerotic blood vessels displaying a hemangiopericytoma-like appearance. Hematoxylin and eosin, original magnification 100 ×. MRI: Magnetic resonance imaging; SNDT: Sciatic notch dumbbell tumor; CT: Computed tomography.

Lumbar MRI revealed no pathological findings; however, an intrapelvic dumbbell-shaped mass compressed lumbosacral plexus (Figure 1A and B). The maximal diameter of the mass was 7.7 cm, and the lesion showed mixed signal intensity both on T1- and T2-weighted images. 3D-CT angiography clearly demonstrated the relationship between the tumor and major vessels (Figure 1C). A diagnosis of solitary fibrous tumor was made by CT-guided biopsy. The tumor was resected by a one-stage combined transabdominal and transgluteal (extrapelvic) approach (Figure 1D). A postoperative surgical specimen showed cellular proliferation of mildly atypical spindle or oval cells arranged in short fascicles that were associated with dilated sclerotic blood vessels displaying a hemangiopericytoma-like appearance (Figure 1E). The surgical margin was negative and the postoperative course was uneventful. The patient noted a significant improvement in pain. There was no local recurrence or distant metastasis at 25 mo after surgery.

DISCUSSION

Common symptoms of unilateral sciatica include pain radiating down the lower extremity, pain with motion of the hip joint, and pain in the buttock, groin, and low back. LDH is by far the most common and well-known cause of sciatica[8]. Other frequent causes include hip diseases[9], degenerative lumbar spinal disease, spinal infection, spinal and spine tumors[10], and vascular diseases[11]. Local compression of sciatic nerve by tumors

and/or trauma may cause sciatica. Importantly, several reports have demonstrated that benign, malignant, and metastatic bone and soft tissue tumors in the pelvis also cause symptoms that are typical and suggestive of sciatica[12]. In particular, pelvic tumors arising adjacent to the sciatic notch have the ability to form huge dumbbell-shaped tumors[5], and compress the lumbosacral plexus, thus causing sciatica.

However, SNDTs are quite rare and there are few previous studies on these tumors. Thomas *et al*[13] presented 35 cases of neurogenic tumors around the sciatic nerve, 11 of which occurred at the sciatic notch. There have been few reports of SNDTs producing sciatica; these include lipoma[14] and a range of cancers[15]. Due to this rarity, SNDTs are rarely considered in the potential diagnosis of sciatica.

Physical examinations may be insufficient to distinguish SNDTs from other cause of sciatica. From the finding in this study, the authors suggest that the diagnosis of SNDT should be considered in sciatica with the following characteristics: (1) a palpable mass in one or both buttocks; (2) severe buttock tenderness; and (3) chronic pain at rest. Importantly, lack of back pain is another characteristic feature of sciatica caused by SNDTs and this should alert the clinician to consider for alternative diagnosis for sciatica. Overall, the combination of the aforementioned features may warrant the consideration of sciatic nerve compression by an SNDT, a diagnosis that may be clarified preoperatively by MRI or CT of the pelvis.

This case series demonstrated the detailed imaging features of SNDTs, and we found that in certain cases SNDTs could be identified by careful inspection of pelvic X-ray or lumbar MR images. Specifically, 2 patients showed abnormal X-ray findings and 2 demonstrated tumor masses on sagittal sections of lumbar MR images. These findings may be helpful for physicians who suspect the existence of SNDTs in patients with sciatica. SNDTs often form extraordinarily large, asymptomatic soft-tissue masses before the lesions become evident on clinical examination, even in cases of benign neurogenic tumors.

The presence of inadequate operative margins has been proven to be an independent and adverse prognostic factor in local recurrence of sarcoma of the pelvis[16]. Tumor location is a critical factor for tumor resectability. In cases of SNDTs, *en bloc* resection of SNDTs is not feasible because of the complex anatomic features of the surrounding organs, including the pelvic bone, lumbosacral nerve plexus, and large blood vessels. A recent report described a safe resection of certain cases of SNDTs by combination of one-stage transabdominal and transgluteal approach[5]. Accordingly, we applied this method in one case and achieved complete tumor resection with no impaired neural function. Thus, we believe that SNDTs can be resected safely and completely if the tumor displaces rather than directly involves the lumbosacral plexus (including the sciatic nerve).

In conclusion, early recognition and treatment is important in bone and soft tissue tumors. Thus in patients presenting with sciatica without evidence of spinal diseases such as LDH, prompt and accurate diagnostic strategies should include the suspicion of pelvic pathology and a search for SNDT by MRI or CT.

COMMENTS

Background

Sciatica, defined as pain radiating from the back into the buttocks and lower extremities, is a very common disorder. The differential diagnosis of sciatica is difficult, since it may be caused by various pathologies including sciatic notch dumbbell tumors (SNDTs).

Research frontiers

There is little clinical information relating to SNDTs.

Innovations and breakthroughs

This study clearly describes clinical outcomes and characteristic features of SNDTs and this may improve the differential diagnosis of sciatica.

Applications

Practical clinical tips for differential diagnosis of sciatica.

Terminology

SNDTs: Sciatic notch dumbbell tumors.

Peer-review

This is a very good article.

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CASE REPORT

Male papillary breast cancer treated by wide resection and latissimus dorsi flap reconstruction: A case report and review of the literature

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Abstract

Breast cancer (BC) in men represents between 0.5% and 1% of all BC diagnosed each year. We report a case of advanced BC in a 62-year-old male treated at our interdisciplinary Breast Cancer Center. The patient presented with a newly diagnosed large, symptomatic mass in his left breast. Clinical examination showed a not movable mass of 16 cm diameter, deforming the whole breast; the overlying skin was livid and hypervascularized. Enlarged lymph nodes were palpable in the axillary pit. He had no concomitant diseases at time of presentation. He denied any first- or second degree family medical history of cancer of any type and he never received radiotherapy. Ultrasound guided minimal-invasive 14-gauge core biopsy revealed a moderately differentiated encapsulated papillary carcinoma with high expression of estrogen and progesterone receptors (both > 80%, IRS 12) and HER2-negative. Because of the tumor size a mastectomy with axillary dissection and chest wall reconstruction using a latissimus dorsi flap was performed. Histological analysis showed invasive growth besides typical (non-invasive) papillary carcinoma and was classified as invasive solid papillary carcinoma; pT3 (10 cm), pN0 (0/15), M0,

R0; OncotypeDX Recurrence Score indicated low risk (RS: 2). After discussion in the interdisciplinary tumor board meeting, radiation therapy and tamoxifen were recommended. The patient had an uneventful recovery and is disease-free after two years of follow-up. Male BC is typically diagnosed at an advanced stage, most likely due to a lack of awareness that men can develop BC. Therefore, in case of a large tumor, a flap-based thoracic reconstruction may be required.

Key words: Male breast cancer; Papillary carcinoma; Reconstruction; Latissimus dorsi flap; Rare tumors

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Core tip: Male breast cancer (BC) is typically diagnosed at an advanced stage, most likely due to a lack of awareness that men can develop BC. Therefore, in case of a large tumor, a flap-based thoracic reconstruction may be required.

Banys-Paluchowski M, Burandt E, Banys J, Geist S, Sauter G, Krawczyk N, Paluchowski P. Male papillary breast cancer treated by wide resection and latissimus dorsi flap reconstruction: A case report and review of the literature. *World J Clin Oncol* 2016; 7(5): 420-424 Available from: URL: http://www.wjgnet.com/2218-4333/full/v7/i5/420.htm DOI: http://dx.doi.org/10.5306/wjco.v7.i5.420

INTRODUCTION

Breast cancer (BC) in men represents between 0.5% and 1% of all breast cancers diagnosed each year[1]. Few epidemiological or clinical trial data on male BC are available. The disease appears to share similar risk factors and characteristics with postmenopausal BC in women[2]. Favorable parameters, such as low nuclear grade and positive hormone receptor status, are more common for men and postmenopausal women than for premenopausal women. As in women, the most common subtype is invasive ductal carcinoma, while lobular tumor types are rarely diagnosed in men. In contrast to female BC, men are significantly more likely to present with a more advanced stage at diagnosis and with lymph node involvement[3]. We report an interesting case of a large papillary invasive breast cancer in a 62-year-old man.

CASE REPORT

A 62-year-old Caucasian male presented at the certified Breast Cancer Center, Klinikum Pinneberg, Germany, with a newly diagnosed large, symptomatic mass in the left breast. Clinical examination showed a not movable mass of 16 cm diameter, deforming the whole breast (Figure 1); the overlying skin was livid and hypervascularized.

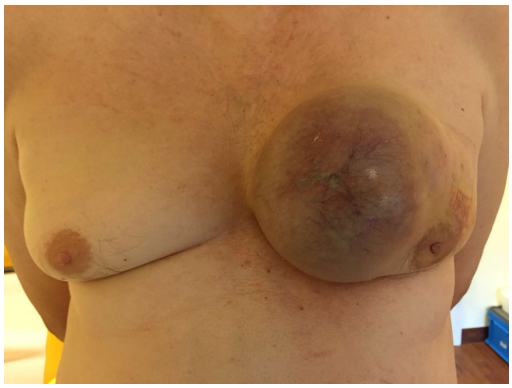

Figure 1 Photodocumentation at time of presentation.

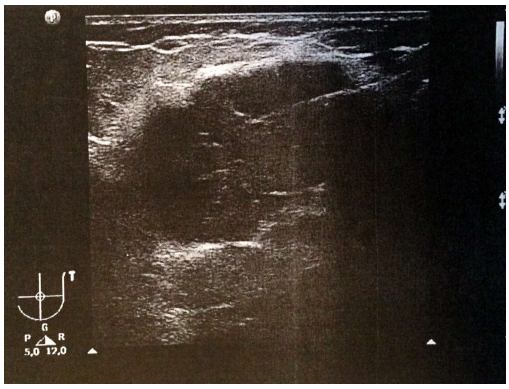

Figure 2 Breast ultrasound shows a large irregular structure of low echogenicity with spiculae measuring > 15 cm × 15 cm (BI-RADS 5).

Enlarged lymph nodes were palpable in the axillary pit. He had no concomitant diseases at time of presentation; his previous surgeries included circumcision as a child and he was a nonsmoker. He denied any first- or second degree family medical history of cancer of any type and he never received radiotherapy. His serum estradiol levels were normal. The patient reported that he had noticed the tumor one year prior to presentation; the reason for eventually seeing a doctor was an increasing pain, most likely due to the pressure on the skin by the growing tumor. There was no nipple discharge. At ultrasound, the lesion was scored BI-RADS 5 (Figure 2). Axillary lymph nodes were suspicious on sonography. Mammography was not possible due to tenderness on palpation.

Ultrasound guided minimal-invasive 14-gauge core biopsy revealed a moderately differentiated non-invasive encapsulated papillary carcinoma with high expression of estrogen and progesterone receptors (both > 80%, IRS 12) and HER2-negative (Figure 3). Because of the tumor size a mastectomy with axillary dissection and chest wall reconstruction using a latissimus dorsi (LADO) flap was performed. Histological analysis showed invasive growth besides typical non-invasive papillary carcinoma and was classified as invasive solid papillary carcinoma (Figures 4 and 5). The TNM stage was pT3 (10 cm), pN0 (0/15), M0 and the resection margins were at least 5 mm in all directions. After discussion in our interdisciplinary

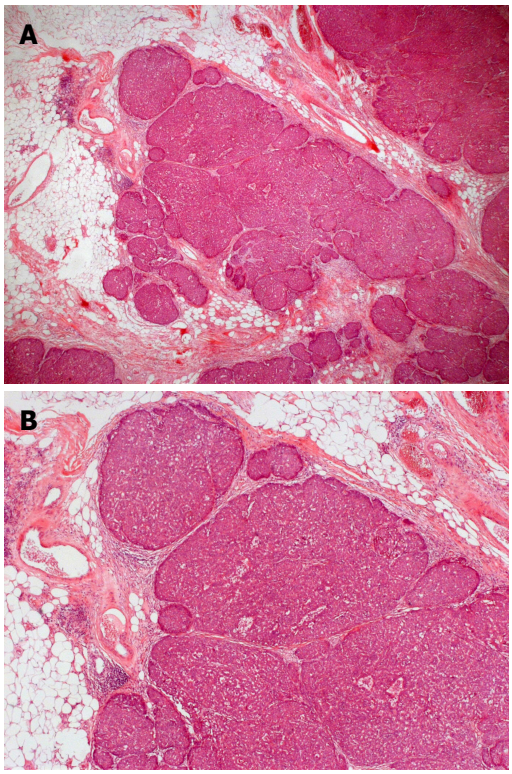

Figure 3 Solid papillary carcinoma (*in situ*), composed of expansile rounded nodular epithelial masses. A: Low magnification 12.5 ×; B: Low magnification 25 ×.

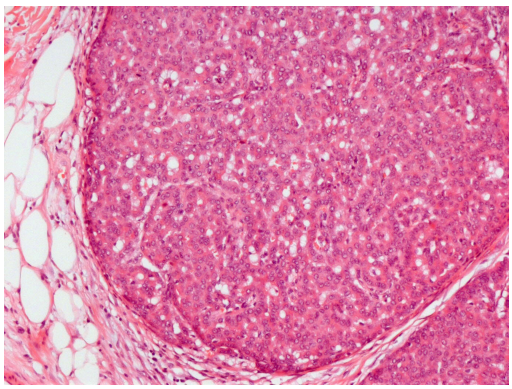

Figure 4 Relatively bland tumor cells with ovoid nuclei and indistinct nucleoli. Fine fibrovascular septa are seen within the epithelial islands (medium magnification, 100 ×).

tumor board we conducted the OncotypeDX test which indicated low risk (Recurrence Score: 2). Radiation therapy and tamoxifen were recommended. The patient was referred for genetic counseling but decided against genetic testing. He had an uneventful recovery and is disease-free after two years of follow-up (Figure 6).

DISCUSSION

To our knowledge, this is the first case report on a giant invasive papillary carcinoma in a man treated by LADO flap-based reconstruction of the thoracic wall. Papillary

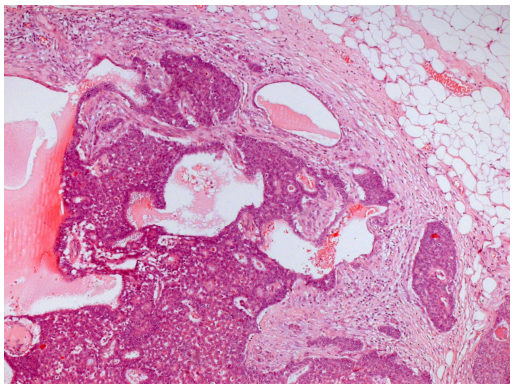

Figure 5 Solid papillary carcinoma (invasive) - tumor cell islands with irregular jagged contours within a desmoplastic stroma (medium magnification, 50 ×).

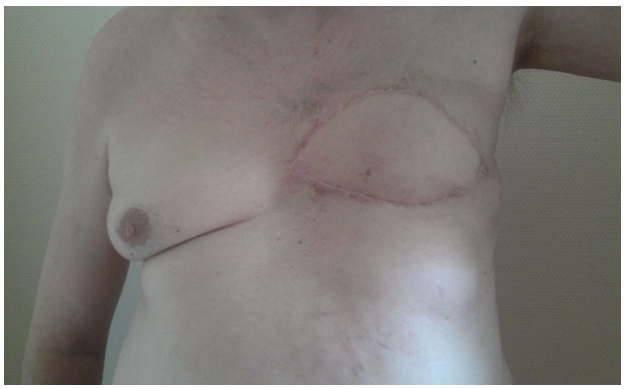

Figure 6 Postoperative clinical presentation.

breast cancer is a very rare type of BC with an estimated incidence of approximately 1% of all breast cancer cases[4]. In terms of histopathology, papillary carcinoma refers to a morphologically heterogeneous group of lesions, all of which are characterized by arborescent fibrovascular stalks lined by epithelial cells. In most cases of invasive papillary carcinoma, ductal carcinoma *in situ* is also present. Available epidemiological data suggest an improved survival of patients with papillary carcinoma in comparison to the more common invasive ductal cancer[4]. In men, papillary tumor type is more common than in women: In a large series of 778 men with invasive BC, 34 (4.4%) were diagnosed with this tumor subtype[5]. In case of our patient, the minimally invasive core biopsy showed in situ papillary cancer, while the examination of the whole surgical specimen revealed invasive growth as well.

According to the epidemiological data from the Surveillance, Epidemiology, and End Results Program of the American National Cancer Institute, men tend to be older than women at the time of diagnosis, with a median age of 67 years compared with 62 years for women[3]. Further, men are more likely to be diagnosed with advanced disease: At time of diagnosis, 20% of women had tumors smaller than 1 cm compared with only 9.8% of men, 38% of men had regional lymph node involvement compared with 29% of women, and more

Table 1 The comparison of male and female breast cancer with respect to diagnostics and therapy

	Female breast cancer	Male breast cancer
Epidemiology	Very common (125 new cases per 100000 women per year)	Very rare (1.2 new cases per 100000 men per year)
Average age at diagnosis	62 yr	67 yr
Diagnostics	Mammography, sonography; in selected cases MRI	Sonography; mammography if possible; in selected cases MRI
Association with BRCA mutation	5%-10% of all cases are BRCA-positive	10%-20% of all cases are BRCA-positive
Surgery	Breast-conserving surgery (70%-80% patients) or mastectomy; sentinel node biopsy in cN0	Mastectomy; sentinel node biopsy in cN0
Reconstruction techniques	Implant or flap-based reconstruction after mastectomy	Flap-based reconstruction of thoracic wall in case of a large tumor
Chemotherapy	Recommendation depends on tumor biology and tumor load; adjuvant or neoadjuvant use; usually anthracycline- and taxane-based	
Endocrine therapy	Recommended in hormone receptor positive tumors; tamoxifen or aromatase inhibitor	Recommended in hormone receptor positive tumors; tamoxifen
HER2-targeted treatment	Trastuzumab recommended in HER2-positive tumors	
Radiation therapy	Recommended after breast-conserving surgery; thoracic wall or lymph node radiation in case of higher tumor load	Thoracic wall or lymph node radiation in case of higher tumor load

MRI: Magnetic resonance imaging; HER2: Human epidermal growth factor receptor 2.

men had distant metastasis at time of diagnosis than women. On the other hand, tumor biology appeared to be more favourable in men than in women: Men have a significantly higher proportion of hormone receptor positive tumors than women (91% of men and 76% of women present with ER-positive disease)[3]. With regard to molecular gene expression assays, there is limited evidence available related specifically to men, although tumors in men display very similar gene signatures to those in women[6] and OncotypeDX-based clinical trials such as the Ontario trial include male BC patients as well[7]. With respect to risk factors, genetic factors including BRCA mutations, family history, age, androgen/estrogen imbalance, radiation therapy and environmental exposures seem to predispose to male breast cancer[8]. In context of genetic counseling, BRCA2 germline mutation leads to a 100-fold increase in breast cancer risk in male carriers while this association is less established for the BRCA1 mutation. The cumulative risk of BC for male BRCA1 mutation carriers at age 70 years is 1.2% compared to 6.8% for BRCA2 mutation carriers[9]. Another risk factor, the excessive estrogen stimulation, may be due to exogenous hormonal exposure (*i.e.*, hormonal treatment or estrogen-containing compounds/testosterone), overweight, chronic liver diseases and thyroid disease[10]. Men with Klinefelter syndrome are more at risk for developing breast cancer as well[11].

The approach to men with a suspicious breast mass is similar to that of women and includes mammography, ultrasound and biopsy (Table 1). As in women, male breast cancer is classified according to the TNM staging system and tumor biology (estrogen receptor, progesterone receptor and HER2 status) is crucial for choosing adequate systemic therapy. Most men with early stage disease undergo a simple mastectomy. Flap-based reconstruction of the thoracic wall may be necessary in case of a very large tumor[12]. Both the LADO flap and the abdominal tissue transfer, such as transverse rectus abdominis muscle flap or deep inferior epigastric perforator flap, are reliable techniques. The use of LADO flap in a variety of reconstructive settings has been described since early 20th century[13]. In this surgical technique, the pedicled musculocutaneous flap is dissected along with the fat and skin overlying the vascularized muscle and tunneled subcutaneously under the axilla to be transposed into the wound in the thoracic wall. Beyond breast or thoracic wall reconstruction, the LADO flap may be used in men for phalloplasty after penile trauma or in case of congenital anomalies of the penis; in this setting, the flap is not pedicled but is transferred as a "free flap" to another body site and the circulation is re-established *via* microsurgical anastomosis between the flap and the femoral vessels[14].

Most experts agree that sentinel node biopsy should be performed in early male breast cancer in absence of clinically or sonographically suspicious nodes; this approach is in accordance with the ASCO clinical guidelines[15]. Although large clinical trials on sentinel node biopsy in men have not been carried out, smaller studies confirm this technique to be as feasible as in women[16,17].

As in women, adjuvant therapy of male breast cancer may include radiation therapy, endocrine therapy, chemotherapy, and HER2-targeted treatment. In the absence of large clinical trials focusing on male BC, therapy recommendations radiation therapy, chemo- and HER2-therapy mirror those for women. In the context of endocrine therapy, the use of tamoxifen rather than an aromatase inhibitor is recommended[18].

COMMENTS

Case characteristics

A 62-year-old male with a newly diagnosed large, symptomatic mass in the left

breast.

Clinical diagnosis
A not movable mass of 16 cm diameter, deforming the whole breast; the overlying skin livid and hypervascularized; enlarged lymph nodes in the axillary pit.

Differential diagnosis
Benign breast tumor.

Imaging diagnosis
Ultrasound: Large irregular structure of low echogenicity with spiculae (BI-RADS 5), suspicious axillary lymph nodes.

Pathological diagnosis
Core biopsy: Moderately differentiated non-invasive encapsulated papillary carcinoma with high expression of estrogen and progesterone receptors (both > 80%, IRS 12) and HER2-negative. Surgical specimen: Invasive solid papillary carcinoma, pT3, pN0 (0/15), M0, OncotypeDX low risk (Recurrence Score: 2).

Treatment
Mastectomy with axillary dissection and chest wall reconstruction using a latissimus dorsi flap.

Experiences and lessons
This case report describes a rare case of male papillary breast cancer and emphasizes the importance of flap-based reconstruction in case of a large tumor.

Peer-review
This is a case report of rare men breast cancer and then the author gave a short literature review. The review provides some useful information.

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