Gastric cancer and the epoch of immunotherapy approaches

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Abstract

The incidence of gastric cancer (GC) fell dramatically over the last 50 years, but according to IARC-Globocan 2008, it is the third most frequent cause of cancer-related deaths with a case fatality GC ratio higher than other common malignancies. Surgical resection is the primary curative treatment for GC though the overall 5-year survival rate remains poor (approximately 20%-25%). To improve the outcome of resectable gastric cancer, different treatment strategies have been evaluated such as adjuvant or perioperative chemotherapy. In resected gastric cancer, the addition of radiotherapy to chemotherapy does not appear to provide any additional benefit. Moreover, in metastatic patients, chemotherapy is the mainstay of palliative therapy with a median overall survival of 8-10 mo and objective response rates of merely 20%-40%. Therefore, the potential for making key beneficial progress is to investigate the GC molecular biology to realize innovative therapeutic strategies, such as specific immunotherapy. In this review, we provide a panoramic view of the different immune-based strategies used for gastric cancer treatment and the results obtained in the most significant clinical trials. In detail, firstly we describe the therapeutic approaches that utilize the monoclonal antibodies while in the second part we analyze the cell-based immunotherapies.

Key words: Gastric cancer; Immunotherapy; Monoclonal antibodies; T cells; Dendritic cells; NK cells

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Core tip: The overall 5-year survival rate of gastric cancer after surgery resection remains poor (approximately 20%-25%) also adopting different treatment strategies,
such as adjuvant chemotherapy, adjuvant chemoradiotherapy and perioperative chemotherapy. Several data support the idea that anti-gastric cancer (GC) specific immunotherapy could be an interesting therapeutic strategy. In this review, we provide a panoramic view of the various immune-based approaches adopted and the results obtained in the most significant clinical trials with GC patients.

INTRODUCTION

The incidence of gastric cancer (GC) fell dramatically over the last 50 years, but according to IARC-Globocan 2008, GC is still the third most frequent cause of cancer-related deaths after lung and liver cancer in male, and after breast and lung cancer in female[1,2]. Interestingly, there is a marked geographical variation in GC incidence, with the highest rate in Japan, China and Eastern Europe and the lowest in North America, India, Philippines and Australia. Histologically, there are three subtypes of gastric adenocarcinoma: intestinal, diffuse and indeterminate (mixed type). Of those, intestinal subtype influences the changes in the epidemiological incidence[3].

Worldwide, the well known epidemiological observation in gastric cancer includes: (1) If migrants from high risk areas move to low risk areas (China to North America), the incidence rate shows a remarkable reduction reaching to almost equal rates as in low risk countries[4]; and (2) preventable by lifestyle modification such as reduced salt intake and increased vegetable and fruit consumption, together with avoidance of smoking and countermeasures against Helicobacter pylori (H. pylori) infection, reduce the risk of gastric cancer[5].

Case fatality GC ratio is higher than other common malignancies, such as colon, breast and prostate cancer[6]. Cancer excision is the principal remedy for GC though the 5-year survival rate is not high (approximately 20%-25%). To improve the outcome of resectable gastric cancer, different treatment strategies have been evaluated such as adjuvant chemotherapy, adjuvant chemo-radiotherapy, and perioperative chemotherapy.

The US Intergroup 0116 trial reported the benefit of postoperative-chemo-radiotherapy using 5-fluorouracil (5-FU)/leucovorin in a US population. In this study, only 10 % of patients received D2 resection. In Korean patients after D2 resection, the ARTIST trial failed to show any benefit from adding radiotherapy to adjuvant chemotherapy in terms of 3-year disease-free survival.

The MAGIC trial compared perioperative chemotherapy with surgery alone and reported a prolonged 5-year overall survival in the perioperative chemotherapy arm. In resectable gastric cancer, the benefit of adjuvant chemotherapy compared with surgery alone has been clearly demonstrated. After D2 dissection, S-1 adjuvant chemotherapy improved the overall survival (ACTS-GC trial) and capecitabine/oxaliplatin combination chemotherapy improved 3-year disease-free survival (CLASSIC trial). To date, for resectable gastric cancer, the use of chemotherapy in addition to surgery has proved to be beneficial in decreasing the rate of recurrence and improving overall survival. The optimal sequence of chemotherapy and surgery, as well as the development of new optimal chemotherapeutic agents, are future goals for research. In D2-resected gastric cancer, the addition of radiotherapy to chemotherapy does not appear to provide any additional benefit[7,11].

First-line chemotherapy raises the overall survival (OS) of patients with advanced GC and the association of two drugs (generally fluorouracil and cisplatin) was more effective than monotherapy[12]. Moreover, the addition of a third drug (e.g., anthracycline or docetaxel) to a platinum-fluoropyrimidine association further increases the OS[13,14]. Lastly, both capecitabine and oxaliplatin had similar results to fluorouracil and cisplatin, respectively, about the OS and progression-free survival (PFS)[14,15].

In metastatic GC patients, chemotherapy is the column of palliative treatments with a median OS of 8-10 mo and objective response rates (ORRs) of merely 20%-40%[16].

Therefore, the potential for making key beneficial progress is to investigate the molecular biology of tumors to realize innovative therapeutic strategies, such as specific immunotherapy[17,18].

The behavior of immune response is centered on a task partition involving the innate (especially macrophages, dendritic and NK cells) and specific immune response (T and B lymphocytes), that often cooperate to obtain an efficacious anti-cancer response. Up to now, various strategies (vaccines, T cells infusion or cytokines) have been proved to be able to stimulate the immune system, exploiting essentially two principal mechanisms: (1) strengthening the anti-tumor response (by raising the amount of effective cells and/or cytokines/chemokines); or (2) increasing the immunogenicity and/or susceptibility of cancer cells.

However, the neoplastic cells are able to develop various strategies to evade immune surveillance: decrease of tumor antigen or MHC expression, modulation of Fas-L, secretion of inhibitory cytokines [interleukin (IL)-10 and/or TGF-β] and generation of regulatory cells such as Tregs (regulatory cells) and myeloid-derived suppressor cells (MDSC)[19,20].

In other words, the prerequisite for an effective anti-tumor immune-based treatment is the stimulation of a successful cancer-specific immune response, able

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to crack the immunological tolerance of tumor cells. The aim of this review is to provide a panoramic view of the different immune-based strategies used for GC management (Figure 1) and discuss the data of the most significant clinical trials. In detail, firstly we describe the therapeutic approaches that utilize the monoclonal antibodies while in the second part we analyze the cell-based anti-GC treatments.

**MONOCLONAL ANTIBODIES DIRECT TO MOLECULAR AND CELLULAR GASTRIC CANCER TARGETS**

The typical paradigm of drug development, especially for targeted therapies, has primarily been in the metastatic setting, followed by introduction into chemoradiation, and finally, subsequent evaluation in a randomized trial.

In this paragraph, we will focus on three of the most widely studied therapies with monoclonal antibodies (moAb) in gastric cancer: anti-epidermal growth factor receptor (anti-EGFR), anti-HER2 and anti-vascular endothelial growth factor (anti-VEGF); as they have been evaluated by this paradigm. The second part will focus on pathways and drug targets currently under evaluation for gastric cancer.

**Anti-epidermal growth factor receptor (cetuximab/panitumumab/matuzumab)**

EGFR (ERBB1) is an element of the ERBB transmembrane growth factor receptor family, that promotes and modulates, via a receptor-associated tyrosine kinase (TK), various cell processes such as apoptosis or proliferation[21].

The EGFR hyperexpression shows a relationship with augmented invasion and more unfavorable prognosis of patients with esophageo-gastric cancers (EGC)[22-25]. In addition, the anti-EGFR MoAb therapy is ineffective in colorectal cancer (CRC) patients that have K-ras mutations[26-28].

Lately, Janmaat et al.[29] showed mutated K-ras in 8.7% patients with EGC; but the prognostic role of K-ras status in the anti-EGFR therapy is practically indefinite.

Existing anti-EGFR treatments in EGC patients consist of oral TK inhibitors (TKIs; erlotinib, gefitinib) and moAb (cetuximab, panitumimab and matuzumab).

Cetuximab obstructs the ligand junction with the EGFR[30], promotes EGFR internalization[31] and also, can start the immune-mediated cytotoxicity[32,33].

Due to the better ORR and time-to-progression (TTP) for the cetuximab/irinotecan association compared with the irinotecan monotherapy[34], the FDA (Food and Drug Administration) has been approved the cetuximab use in irinotecan-refractory CRC.

In addition, the FDA has been authorized the Panitumumab therapy of chemo-refractory EGFR-positive CRC, because a recent study showed an amelioration in ORR and PFS over best current treatment[35]. Besides, a phase I study reported a stable disease (SD), for 7 mo, in one refractory EGC patient, treated with panitumumab[36].

Lastly, a recent study showed that one patient, with esophageal cancer (EC), cured with Matuzumab (the last anti-EGFR moAb) had a durable six-month PR[37]. Also, the combination of matuzumab with the ECX regimen (epirubicin/cisplatin/capecitabine) registered encouraging results as first-line therapy in patients with EGFR+ gastric cancer. The ORR in 20 evaluable patients was 65% with a median TTP of 5.2 mo[38].

**Metastatic results:** Numerous phase II studies have been performed with cetuximab in combination with chemotherapy in advanced EGC. One of the first trials[39] evaluated cetuximab with FOLFIRI in thirty-eight patients with untreated advanced gastric or GE junction adenocarcinoma. Cetuximab was given with an initial loading dose of 400 mg/m² followed by weekly doses of 250 mg/m². The overall response rate was 44.1%, with a median survival of 16 mo. In another randomized phase II study, cetuximab was added to 3 chemotherapy regimens: ECF (epirubicin, cisplatin, 5-FU), IC (irinotecan/cisplatin), and FOLFOX[40]. The response rates were 58%, 38%, and 51% in the 3 arms, respectively.

The role of anti-EGFR therapy in advanced EGC was tested in a phase III study evaluating the efficacy of panitumumab with combination chemotherapy in the REAL 3 study[41]. Patients with inoperable/metastatic esophageal, gastric, or GE junction cancer were randomized to receive EOC (epirubicin, oxaliplatin, capecitabine) with or without panitumumab. An early planned interim analysis showed that the panitumumab arm was statistically inferior after 553 (76%) patients were enrolled. Median survival was 11.3 mo in the chemotherapy-alone arm vs 8.8 mo for chemotherapy plus panitumumab (hazard ratio (HR) = 1.37, P = 0.013). Although patients with rash in the panitumumab arm did better than those without rash, the subgroup of patients with rash still had a numerically worse median survival than the entire chemotherapy-alone group.

**Chemoradiation results:** Chemoradiation with cetuximab has been extensively studied in the phase II setting. One clinical study evaluated 60 patients treated with cetuximab, paclitaxel, and cisplatin in combination with radiation therapy. A pathologic complete response rate of 27% was seen with this regimen[42]. In the Swiss Group for Clinical Cancer Research phase I b/II trial (SAKK 75/06), 28 patients with adenocarcinoma or squamous cell carcinoma were treated with induction cisplatin, docetaxel, and cetuximab followed by radiation therapy to 45 Gy along with concurrent cisplatin and cetuximab. A pathologic
complete response (pCR) rate of 32% was seen with this regimen. Neither of these studies demonstrated excess risk with the addition of cetuximab. In contrast, ECOG 2205 evaluated a neoadjuvant regimen of cetuximab in combination with infusional 5-FU, oxaliplatin, and radiation therapy. The study was closed after an excessive number of early deaths. Four of 18 patients died postoperatively of the acute respiratory death syndrome (ARDS) despite compliance with strict radiation lung dosimetry guidelines. This high rate of ARDS, not seen in other studies of 5-FU with oxaliplatin and radiation, raised the possibility that cetuximab may have added to the risk of postoperative pulmonary complications.

**Evolution of chemoradiation:** Radiation Therapy Oncology Group (RTOG) 0436 is a randomized phase III trial of cisplatin, paclitaxel, and radiation therapy to 50.4 Gy with or without cetuximab in inoperable esophageal cancer. In the spring of 2012, the study underwent a planned interim analysis to document superiority of the cetuximab arm as measured by clinical complete response rate. The study failed to meet this end point and closed to further patient enrollment.

The SCOPE1 study from the United Kingdom is a similarly designed 2-arm randomized phase II/III study comparing cisplatin/capecitabine/radiation with or without cetuximab. This study will also undergo a planned analysis after the phase II portion to document a freedom from treatment failure rate exceeding 75% at 24 wk in the cetuximab containing arm.

Given the negative results of the REAL 3 trial, and RTOG 0436 closing enrollment to adenocarcinoma due to a lack of efficacy with cetuximab, it is unlikely that anti-EGFR strategies will be further developed in the United States in unselected patients.

**Anti-HER2/ERBB2 (trastuzumab)**

HER-2/neu (ERBB2) is part of the ERBB TK receptor family. The ligand of these receptors leads to homo/hetero-dimerization of the receptors and with their formation displaying a distinct hierarchy. In this system, HER-2/neu has a key role because each receptor with a specific ligand promotes the association with Her-2/neu. This predilection is more influenced by Her-2/neu hyperexpression, as seen in numerous types of human tumor cells.

About EGCGs, HER-2/New hyperexpression has been shown in esophageal cancer and GE Junction carcinoma. HER-2/neu hyperexpression has been connected with increased invasion and poor response to neo-adjuvant chemotherapy or overall reduced survival.

The anti-HER2/neu mAb treatment that has been tested in EGC patients is Trastuzumab, that exercises its role by different ways: blocking HER-2 receptor dimerization, favoring the receptor demolition and promoting the cytotoxicity. Currently, it has been used in association with chemotherapy for HER-2/neu+ and node+ breast cancer.

**Metastatic results:** The proof of the therapeutic benefit of HER2-directed therapy in gastric and GE cancer comes from the trastuzumab for gastric cancer trial, a large randomized trial of trastuzumab added to standard chemotherapy in HER2+ advanced gastric cancer. In this study, patients with HER2+ gastric or GE cancer were randomized to either trastuzumab and chemotherapy or chemotherapy alone.

Chemotherapy consisted of (5-FU or capecitabine in combination with cisplatin given every 3 wk for 6 cycles. Trastuzumab was continued until disease progression. HER2 positivity was defined as 3+ staining by immunohistochemistry (IHC). Tumors with IHC 2+ staining had to be confirmed by the evidence of amplification by fluorescence in situ hybridization.

Tumors from 3807 patients were centrally tested for HER2 status, of which 22.1% were HER2+. These 594 patients were randomized to 1 of the 2 treatment groups, with well-balanced clinical characteristics. A planned interim analysis was performed after 75% of the events had occurred, and the independent data monitoring committee recommended release of the data because the prespecified boundary had been exceeded, with a median follow-up of 17.1 mo. Median survival was improved with the addition of trastuzumab to chemotherapy from 11.1 to 13.5 mo (P = 0.0048; HR = 0.74; 95%CI: 0.60-0.91). The overall response rate was also improved from 34.5% to 47.3% with the addition of trastuzumab (P = 0.0017). The toxicity was similar in both arms.

Specifically, there was no difference in congestive heart failure. Asymptomatic decreases in left ventricular ejection fraction were similar in both arms (4.6% with trastuzumab, 1.1% without). Based on this study, trastuzumab was approved in the setting of HER2+ advanced gastric and GE cancer.

**Chemoradiation results:** Recently it has been performed a pilot study of trastuzumab added to chemoradiation in patients with locally advanced esophageal carcinoma.

Patients were required to have HER2 positivity (HER2 2+/3+ expression). Chemoradiation was delivered with a dose of 50.4 Gy along with concurrent weekly cisplatin (25 mg/m2) and paclitaxel (50 mg/m2). In cohort 1, 3 patients received a 2-mg/kg bolus dose in week 1 followed by a weekly dose of 1 mg/kg. In cohort 2, 3 patients received a 3-mg/kg bolus dose in week 1 followed by a weekly dose of 1.5 mg/kg. In the third cohort, 13 patients received a 4-mg/kg bolus dose in week 1 followed by a weekly dose of 2 mg/kg. Maintenance trastuzumab was given for 1 year at a dose of 6 mg/kg every 3 wk. Despite the advanced treatments, the overall survival for HER2+ patients was inferior to HER2- patients (P = 0.0007).
disease in many patients, such as celiac adenopathy (37%) or retroperitoneal (37%), a striking 3-year overall survival of 47% was observed, although a lot of patients did not undergo surgery owing to extensive adenopathy or medical morbidities. Additionally, there were no observed increases in adverse events from the addition of concurrent or maintenance trastuzumab. Because surgery was not required, there was no meaningful pCR data.

Trastuzumab emtansine, or T-DM1, is an antibody-drug conjugate linking trastuzumab to a highly potent anti-microtubule agent. Preclinical data on human GC cells and xenografted tumors suggested that T-DM1 is more effective than trastuzumab. Recently, a phase III study evaluating T-DM1 vs lapatinib and capecitabine in HER22+ trastuzumab-refractory breast cancer demonstrated an improvement in median survival favoring T-DM1 (not reached vs 23.3 mo; HR = 0.621; 95%CI: 0.475–0.813; P = 0.0005)59. Additionally, T-DM1 had a higher response rate (43.6% vs 30.8%) and duration of response (12.6 mo vs 6.5 mo). Furthermore, T-DM1 showed antitumor effects even in xenografted tumors that had developed resistance to trastuzumab. Based on this evidence, an international phase II/III trial in second-line advanced EGC will open randomizing between T-DM1 vs a taxane (weekly paclitaxel or q3w docetaxel).

**Evolution of chemoradiation:** Based on the positive results observed in the metastatic setting with the addition of trastuzumab as well as the encouraging safety and efficacy data from the Brown group, the RTOG has initiated a randomized trial, RTOG 1010, studying the addition of trastuzumab to chemoradiation. In this study, patients with operable locally advanced adenocarcinoma of the esophagus and GE junction are centrally screened for HER2 positivity. If the tumor is found to be HER2+, patients are randomized to concurrent and maintenance trastuzumab in addition to chemoradiation. Chemoradiation consists of a dose of 50.4 Gy along with weekly carboplatin (AUC 2) and paclitaxel (50 mg/m²). The results of this ongoing trial will inform the future use of trastuzumab in localized HER2-overexpressing esophageal and GE junction cancer60.

**Anti-vascular endothelial growth factor (bevacizumab):**

The action of most powerful angiogenic factor, VEGF, is started by linking to various high-affinity transmembrane receptors, most remarkably VEGFR types 1 and 261.

VEGF is over-expressed in different cancers62 and besides, in esophageal and gastric cancer the hyperexpression correlates with cancer stage, bad prognosis and reduced survival63–70. Also, the moAb bevacizumab is an anti-VEGF monoclonal antibody, that associated with the chemotherapy increases the ORR and TTP in patients with CRC71 NSCLC72 and breast cancer73. It seems that bevacizumab have a double anti-cancer effect: as anti-angiogenic factor and also increasing chemotherapy drug delivery, favoring the decrease of interstitial fluid pressures74,75.

**Metastatic results:** Multiple phase II studies evaluated bevacizumab in combination with a variety of chemotherapy regimens in esophagogastric cancer. In a phase II study76, the addition of bevacizumab to cisplatin and irinotecan showed a response rate of 65% and a median survival of 12.3 mo. In another phase II study, Shah et al77 evaluated bevacizumab in combination with docetaxel, cisplatin, and 5-FU. This regimen yielded a response rate of 67% and an impressive median survival of 16.8 mo. Similarly, a high response rate of 68% was observed when bevacizumab was combined with docetaxel, cisplatin, and irinotecan78.

With these higher obtained response rates, a randomized phase III trial was performed evaluating the efficacy and safety of bevacizumab in combination with chemotherapy. The Avastin in Gastric Cancer (AVAGAST) trial randomized patients with inoperable locally advanced or metastatic gastric or GE junction adenocarcinoma with no previous therapy to bevacizumab or placebo in combination with capecitabine (or 5-FU) and cisplatin. 774 patients were randomized, with 95% of patients having metastatic disease79. The median survival was 10.1 mo for chemotherapy alone vs 12.1 mo for chemotherapy plus bevacizumab (HR = 0.87; P = 0.1002). Although this result did not reach statistical significance, there was an improvement in progression-free survival from 5.3 to 6.7 mo (HR = 0.80; P = 0.0037), and the overall response rate increased from 29.5% to 38% (P = 0.0121). However, despite this negative trial, some of the trends in the secondary endpoints have led to further evaluation of bevacizumab in the metastatic setting.

**Chemoradiation results:** An interesting study80 demonstrated that bevacizumab could change tumor physiology of rectal cancer and theoretically potentiate the effects of radiation therapy. In localized esophageal cancer, a similar approach was used in a phase II trial evaluating bevacizumab with erlotinib in a neoadjuvant chemoradiation study81. Patients with stage I–III esophageal or GE junction cancer were enrolled. Ninety-five percent of patients had adenocarcinoma, and 93% of patients had stage II or III disease. Bevacizumab was added to a regimen consisting of carboplatin (AUC 5, days 1 and 22), paclitaxel (200 mg/m², days 1 and 22), and continuous infusion of 5-FU (225 mg/m² per day, from day 1 to 35) in combination with radiation therapy to 45 Gy. Of sixty patients enrolled, a pathologic complete response rate of 30% was observed.

Another phase II study, reported results of pre-operative chemoradiation with cisplatin, irinotecan, and bevacizumab. Patients with Siewert I/II adenocarcinoma
of the esophagus received induction chemotherapy with cisplatin, irinotecan, and bevacizumab. This was followed by concurrent chemotherapy with cisplatin, irinotecan, and bevacizumab in combination with radiation therapy to 50.4 Gy. Surgery was followed by adjuvant bevacizumab. A pathologic complete response was seen in 4 of 33 patients (12%). Progression-free survival and overall survival were 14 and 30 mo, respectively\(^{60}\).

**Evolution of chemoradiation:** The negative primary result of the AVAGAST study has mitigated some of the enthusiasm for bevacizumab in the context of chemoradiation for esophageal cancer. Given the lack of improvement in the pathologic complete response rate in the phase II study discussed earlier in the text compared with historical control groups, further development of bevacizumab with chemoradiation for esophageal cancer is currently not being pursued in a phase III study\(^{60}\).

**Anti-hepatocyte growth factor/mesenchymal-epithelial transition factor (rilotumumab)**

The cell surface receptor c-MET [mesenchymal-epithelial transition factor (MET)] and its ligand hepatocyte growth factor (HGF) are potential therapeutic targets in esophagogastrectomy cancer. Physiologic MET tyrosine kinase activation is mediated by binding of HGF, leading to signal transduction down multiple downstream pathways, including those involving Ras, PI3K, mTOR, STAT3, and NF-κB\(^{85,86}\). Additionally, the HGF/MET axis can stimulate tumor endothelial cells, thereby altering the tumor microenvironment and promoting angiogenesis\(^{85,86}\). Physiologic MET signaling can be altered by ligand/receptor overexpression or gene amplification as well as MET gene mutations\(^{85}\). Specifically, MET gene amplification is a driver in some esophagogastrectomy cancers\(^{87-90}\).

Additionally, MET gene mutations have been documented in hereditary and sporadic renal carcinoma, esophagogastrectomy cancer, hepatocellular cancer, head and neck cancer, ovarian carcinoma, small-cell lung cancer, and glioma\(^{85,91,92}\). Strategies to inhibit the HGF/MET axis include blocking both the ligand and the receptor.

Rilotumumab is a human moAb to HGF. In a randomized phase II study, patients with newly diagnosed GC were randomized to receive 1 of 2 doses of rilotumumab (15 mg/kg or 7.5 mg/kg) in combination with ECX (epirubicin, cisplatin, capecitabine) chemotherapy or chemotherapy alone\(^{93}\). Tumors that were IHC\(^+\) in > 50% of cells were defined as MET high. In the MET-high subgroup, representing approximately half of the patients, the 2 rilotumumab arms had a median survival superior in the chemotherapy-alone arm (11.1 mo vs 5.7 mo; HR = 0.29; 95%CI: 0.11-0.76; \(P = 0.012\)). In contrast, the MET-low patients in the 2 rilotumumab-containing arms had a trend toward a worse survival than the MET-low patients in the chemotherapy-alone arm (HR = 1.84; 95%CI: 0.78-4.34). In the chemotherapy-alone arm, patients with MET-high tumors had a worse overall survival (HR = 3.22; 95%CI: 1.08-9.63) than those with MET-low tumors. This study suggested that expression, as opposed to amplification, may be a reasonable biomarker. In this study, MET expression was both predictive (good) for anti-HGF antibody therapy and prognostic (poor). Based on these data, a phase III study has been planned in the first-line setting for EGC patients with MET-high tumors.

**CELL-BASED IMMUNOTHERAPY APPROACHES**

**Therapies with T cell**

The central anti-cancer role of T cells has been highlighted by the documented cancer incidence in immunodeficient disorders\(^{94}\) and by evidence that the intra-tumoral T cell infiltration is associated with better patient survival\(^{95}\).

Currently, there isn't FDA-approved adoptive T-cell therapy, but the growing new acquisitions on the cancer nature and lymphocyte role do hope that, shortly, the adoptive T-cell therapy can become a clinical cancer practice. Topical information obtained from adoptive transfer in lymphodepleted hosts\(^{96}\), the immunosuppressive capacity of Tregs\(^{97}\) and the utilize of better culture systems\(^{98}\) have not yet been tested in clinical studies.

Essentially exist two different therapeutic protocols of T cell-based anti-cancer treatment: (1) cytotoxic T lymphocytes (CTL); (2) tumor infiltrating lymphocytes (TIL) (Figure 1).

**Cytotoxic T lymphocytes:** Improved CTL cell culture technology has permitted the first clinical tests for adoptive transfer of CTLs and this technique\(^{99,100}\) seems to result in substantial activity in melanoma patients: 40% of patients showed an anti-tumor immune responses\(^{101}\). Similar results were obtained by Yee et al\(^{102}\) in an independent trial in which engraftment of the CTLs was detectable up to two weeks after T-cell transfer in all patients.

Survivin has been demonstrated to be an excellent target for immunotherapy in various cancer types and recent data suggest a role also in gastric cancer\(^{103}\). In this study, elevated efficiency was obtained upon inducing survivin-derived peptide-specific CTL from mononuclear cells isolated from blood of healthy donors. The induced CTLs showed specific lysis against tumor cells in vitro, and vs primary cell cultures isolated from GC patients. These data suggest that survivin epitope peptide could be a promising vaccine candidate for GC immunotherapy.

Instead, another recent study\(^{104}\) examined the possibility of using cancer-specific immunotherapy based upon mitotic centromere-associated kinesin (MCAK), a new cancer antigen. To evaluate the
feasibility of developing cancer immunotherapy using MCAK peptides, Kawamoto et al.\[104\] studied HLA-A*0201 and 12402 as targets for CTLs. The CTLs killed HLA-A*0201/12402 colon and gastric cancer MCAK\+ cells, as well as the peptide-pulsed target cells, in an HLA-l restricted manner. These results prospect the opportunity of designing peptide-based immunotherapeutic treatments for patients with MCAK\+ gastric cancer.

Of late, Kim et al.\[105\] demonstrated the anti-gastric cancer power of cytokine-induced killer (CIK) cells (essentially T CD80+ cells), that were isolated from the human peripheral blood mononuclear cells (PBMC), cultured in medium with IL-2 and anti-CD3 antibody. The CIK cells were able to destroy, in vitro, the MKN74 cells (a human gastric cancer cell line) and to inhibit the MKN74 tumor growth in nude mouse model. These results suggest the potential use of CIK cells as adoptive GC immunotherapy patients, as described in different studies\[106,107\]. In fact, the CTLs from GC patients are capable to attack the autologous cancer cells, recognizing specific tumor-associated antigens\[107,108\], such as MG7-antigen, that shows a big potential for starting immune responses to gastric cancer\[109,110\]. In addition, the use of HLA-A-restricted allogeneic GC cells to stimulate tumor-specific CTLs could be a different immunotherapeutic approach for GC patients\[111\].

Of note, different studies suggest that the association of CIK cells with chemotherapy can be functional in advanced GC patients\[112,113\]. In fact, the patients cured with the combined therapy showed a significant decrease of serum levels of the cancer markers and a marked improvement of life quality, in comparison to patients treated only with chemotherapy.

In summary, preclinical/clinical evidence supports the idea that CIK cell immunotherapy can be a successful anti-GC treatment, but it is still unclear what is the injection via which guarantees the best distribution of effector cells.

In a mouse model of gastric cancer Du et al.\[114\] observed the distribution of CIK cells injected by three different via of infusion: peritumoral (pt), intravenous (iv) and intraperitoneal (ip).

They demonstrated that the pt injection produced a considerable tumor infiltration of CIK cells for 48 h and induced the most tumor inhibition in comparison to the ip or iv infusion, that caused a very small CIK intratumoral accumulation and a short in vivo inhibition of tumor growth only following injection. In conclusion, the pt injection of CIK can be an effective and minimally invasive approach of adoptive cellular immunotherapy for GC patients.

Adoptive transfer therapy with TILs: The use of TILs as adoptive transfer therapy is a “not immediate” therapeutic approach because it requires about six weeks before the T cells would be ready for infusion. In fact, the protocol necessitates firstly the T cell isolation from neoplastic tissue, after the in vitro
expansion and finally the selection of tumor-specific T cells. In addition, only 30%-40% of the biopsies yield acceptable T-cell populations and the whole process\textsuperscript{115}. So, the adoptive transfer of TILs has been promising in preclinical models\textsuperscript{116} but not in clinical trials\textsuperscript{117,118}, except for the melanoma patients for easy surgical availability of the tumor tissue. However, should technical limitations of current tissue culture approaches are overcome; new data indicate that the presence of TILs positively correlates with patient survival in ovarian and colorectal cancer\textsuperscript{95,119} and have a important role in pancreatic cancer\textsuperscript{120}, thus prompting the enforcement of this protocol for other usually encountered epithelial cancers.

In the past, we have demonstrated, in GC patients, the functional role of TILs reactive vs different peptides of GC-associated antigens\textsuperscript{121}. We have documented a peptide-specific T-cell response in 17 out of 20 enrolled patients and the majority of specific TILs had an effective role showing a T helper 1 (Th1) cytokine profile with high cytokoty activity. In other words, in most of GC patients, a specific type-1 T-cell response to GC antigens was detectable and would have the potential of killing the cancer cells. But, in order to get 	extit{“in vivo”} tumor cell destroying, the the quantity and quality of tumor-specific T cells almost certainly need to be enhanced by vaccination with the appropriate cancer antigens/peptides or by injection of the autologous cancer-specific T cells, previously expanded 	extit{in vitro}.

It is remarkable to note that not always the lymphocytic infiltrate has an anti-cancer role and often the TILs can promote the expansion of tumor cells. Recently, we have investigated the functional profile of HP0175-specific TILs in GC patients, infected with 	extit{H. pylori}. The TILs cells were able to produce IL-17 and IL-21 in response to HP0175 and showed poor cytolytic activity and high helper activity for monocyte MMP-2, MMP-9 and VEGF production. In a nutshell, these data suggest that HP0175 drives gastric Th17 response and promoting pro-inflammatory low cytotoxic TIL response, so providing a link between 	extit{H. pylori} and gastric cancer\textsuperscript{122}.

In addition, different studies highlight that most of GC TILs show a Treg profile. Recently, Shen et al\textsuperscript{123} demonstrated that that CD4\textsuperscript{+} and CD8\textsuperscript{+} TILs were not associated with the OS of GC patients and that in the tumor sites, higher Tregs/CD8\textsuperscript{+} ratio was an independent factor for worse OS (\(P = 0.037\)). The 1-year, 2-year and 3-year OS rates were 90%, 77.5% and 70% for the group with intratumoral high Tregs/CD8\textsuperscript{+} ratio, compared with 100%, 94.3% and 90.5% for the group with intratumoral low ratio. So, intratumoral high Tregs/CTLs ratio was a prognostic factor for GC patients. Accordingly, an independent study showed that a higher Tregs/Th cell ratio is associated with an unfavorable prognosis and locoregional recurrence pattern in gastric cancer\textsuperscript{124}.

It can be inferred that a combination of deletion of Tregs and stimulation of effector T cells may be a successful immunotherapy to prolong survival of GC patients.

**Dendritic cell-based vaccination**

Antigen presentation by dendritic cells (DCs) is essential to start the cellular immune responses required for tumor immunotherapy\textsuperscript{125,126} (Figure 1). In addition, in mouse models ex vivo generated DCs can provoke antigen-specific T-cell responses\textsuperscript{127}, supporting the use of DC-based anticancer vaccines in clinical studies\textsuperscript{128}.

In GC patients the number of DCs correlates with the clinical stage and prognosis: patients with abundant DCs infiltration showed a better 5-year survival rates than patients with smaller amount of DCs\textsuperscript{129,130}. Moreover, it has been documented that the use of adjuvant immunotherapy enhances the survival in resected GC patients with small tumor DCs infiltration\textsuperscript{131}.

Of the 325 trials reported in ClinicalTrials. Gov on DC therapy, six studies involve GC patients (Table 1)\textsuperscript{132-134} but only three have been terminated (Table 1) and only two have published their results.

Kono et al\textsuperscript{132} reported a phase-I clinical study of GC patients treated with DCs pulsed with HER-2/ neuro-peptides. After the vaccination, one (out of 9 patients HER-2/neu\textsuperscript{+}) showed decreased levels of CEA and CA19-9 while two registered a significative cancer regression (> 50%). Of note, the vaccine protocol did not register considerable side effects.

Recently Sadanaga et al\textsuperscript{133} published the results of a phase-I trial, where twelve patients, with advanced gastrointestinal carcinoma, were immunized with DC pulsed with MAGE-3 peptides without significant side effects. After vaccination, in four patients was registered the presence of peptide-specific CTL while in seven was observed the serum decrease of cancer markers. In addition, small cancer regressions were highlighted in three patients.

Finally, Galetto et al\textsuperscript{135} described that memory T cell specific to GC antigens could be activated by cancer-loaded autologous DCs, isolated from blood mononuclear cells and activated by stimulation with apoptopic autologous tumor cells.

Nevertheless, the clinical application of DC vaccines has been limited for the the short lifespan of DCs, and one of the factors threatening DC survival is antigen-specific CD8\textsuperscript{+} that acquire cytolytic activities after activation by DCs\textsuperscript{136}.

In recent times, Kim et al\textsuperscript{138} ameliorated the efficiency of a DC vaccine with a small interfering RNA (siRNA) targeting phosphatase and tensin homolog (PTEN), that has a key role as a negative regulator in the signal transduction of the PI3K/AKT pathway\textsuperscript{137}.

The PTEN downregulation in DCs resulted in AKT-dependent maturation, which generated a considerable
surface hyperexpression of costimulatory molecules and the chemokine receptor, CCR7, leading to an increased T cell activation in vitro and a migration to a draining lymph node in vivo, respectively. In addition, the PTEN siRNA transfected DCs (DC/siPTEN) showed an augmented survival and most remarkably, DC/siPTEN generated a major number of cancer-specific Tc cells and a stronger anticancer response in vaccinated mice compared to the controls.

In short, these data suggest that manipulation of the PI3K/AKT pathway with the siRNA system could improve the efficacy of a DC-based tumor vaccine, such as in GC treatment.

Immunosuppressive factors, such as IL-10, secreted by DCs (or other regulatory cells) downregulates the Dcs functionality by specific surface receptors (e.g., IL-10R). Recent data showed that the targeting IL-10 receptor with siRNA, can increase the effectiveness of DC-based vaccine, suggesting a potential clinical use of siRNA.[138]

In addition, very interesting are the results of He et al.[139] concerning the opportunity of increasing the anticancer immunity through GM-CSF gene-modified DCs. After GM-CSF gene modification, DCs are able to secrete elevated levels of GM-CSF and have a major propensity to be maturated. In this way, the DCs increase their ability of activating the proliferation of T cells. In addition, in vitro the dendritic cells with GM-CSF gene modified can stimulate specific CTL to kill the cancer cells.

Finally, in comparison with the vaccination alone, DCs vaccination and the preventive removal of Tregs substantially enhances the activation of tumor-specific T-cell responses[140].

**Treatments by using NK cells**
The NK cells are able to arrest the metastatic spreading of human cancers[141] and also, the intra-cancer infiltration of NK cells is associated with a better prognosis of GC patients[142]. The key function of NK cells in anti-tumor response gives us the possibility to counteract the cancer progression by manipulating the NK cell "arms". However, some obstacles make it difficult to the therapeutic use of NK cell-based treatments: (1) unfinished characterization of the specific function of the different NK cell subpopulations;
About gastric cancer, a recent study evaluated the NK number in 72 patients with gastric adenocarcinoma and its correlation with patient survival. The conclusions are that patients with high concentration of NK cells showed a higher survival rate compared to the low concentration, especially in the advanced stage.

Moreover, very interesting data have been obtained by Saito et al. which demonstrated that the frequency of apoptotic NK cells in GC patients was significantly higher than in normal controls. Moreover, their frequency was related to the GC progression. Fas+ NK cells were significantly more common in GC patients compared with normal controls and Fas expression was closely related to the frequency of NK cell apoptosis. Also, the frequency of tumor-infiltrating NK cell apoptosis was significantly higher than that of circulating NK cell apoptosis. Furthermore, apoptotic circulating NK cells significantly decreased after surgery compared to before surgery.

Finally, Voskens et al. showed that numerous cytotoxic NK cells can be obtained from cancer patients co-culturing autologous PBMC with K562 cells. Of note, the ex vivo development increased the cytotoxic activity of NK cells vs the autologous derived, suggesting a future clinical application, as cell-based immunotherapy, for autologous expanded NK cells (as alone as and in association with specific monoclonal antibodies).

Very interesting are the recent results about lupeol, a triterpene that has curative action vs various diseases. Recently, Wu et al. showed that lupeol is able to favor the proliferation of NK cells, increasing also their killing action vs the GC cells. Moreover, lupeol inhibit the proliferation of different GC cell lines. These data suggest that lupeol could serve as a potential agent against gastric cancer alone or with adoptive transfer of NK cells.

**FUTURE DIRECTIONS**

We have used this review to provide a panoramic view about the current immunotherapeutic anti-GC approaches, some of which have been used in clinical trials with fairly good results about the tumor regression and patient survival. But, the role of immunotherapy for gastric cancer continues to evolve. As the current development suggests, gastric cancer therapy has suffered from a relative lack of gastric-specific biological exploration. The most common developmental path to date has been limited to the study of immune-based therapies that have demonstrated efficacy in other somewhat similar diseases, and then have been tested in gastric cancer.

Moreover, an additional great challenge in the field is to develop randomized clinical trials validating the medical benefits to justify the logistics and especially the costs of these personalized cell treatments.

Usually the clinical trials enroll patients with advanced GC, this factor could determine an unfavorable result, because the anti-cancer battle of current immune response is already a lot compromised. For this purpose, it would be strategic to recruit early-stage GC patients, that being in the early stages of tumor development, may better react to the immunotherapy strategies.

Finally, to set up successful anti-GC immunotherapy approaches, it is necessary to understand the “fine” immune escape mechanisms, adopted by the cells of gastric cancer.

Past and recent studies have supplied new insights about the thick crosstalk between tumor and immune cells. Comprehending this operative dialogue and the hierarchic grade of the various cancer-immune evasion mechanisms at distinct steps of neoplastic evolution, will guide the development of innovative curative strategies aiming to demolish the “tumor fortress”.

So, it will be remarkable to evaluate the pathways of the various components that modulate the growth and mobility of Tregs, MDSCs and tolerogenic DCs within cancer-draining lymph nodes and the cancer surroundings.

Another important target for the future anti-GC immunotherapy treatment could be the immune checkpoints that are inhibitory pathways hardwired into the immune system. The immune checkpoints play critical roles for physiological homeostasis because they are essential for maintaining self-tolerance and modulating the duration and amplitude of physiological immune responses in order to minimize collateral tissue damage. But, these checkpoints may also allow immune escape in cancer.

Checkpoint pathways are regulated by ligand/receptor interactions. For example, programmed death-1 receptor (PD-1) and CTL-associated antigen 4 (CTLA-4) are inhibitory molecules whose presence on lymphocytes signifies a blunted immune response. PD-1 negatively regulates T cell responses and downregulation and eventually apoptosis is initiated following binding of a PD-1 ligand with PD-1. PD-1 ligands, PD-L1 or PD-L2, are frequently expressed on tumor cells and can thus thwart the immune response. One approach to overcome this inhibition of the immune response has been to target immune checkpoints with blocking moAb. For example, PD-1 moAb binds to the PD-1 receptors on T cells and inhibits their binding to the ligands on tumor cells thus preventing the tumors from down regulating the cytotoxic lymphocyte response. This approach has been successful clinically in advanced melanoma and phase I clinical trials of anti-PD-L1 moAb are under investigation for gastric cancer.

It is realistic to declare that, in the future effective anti-GC immunotherapy strategies must include combined approaches, which should use both systemic radio/chemotherapy and transplantation, to diminish
the burden or to remove immune suppressive cells, and tailored immunotherapies customized to each single patient.

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