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TOPIC HIGHLIGHT

2016 Gastric Cancer: Global view

Immunological battlefield in gastric cancer and role of immunotherapies

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Abstract

Like the wars predating the First World War where human foot soldiers were deemed tools in the battlefield against an enemy, so too are the host immune cells of a patient battling a malignant gastric cancer. Indeed, the tumour microenvironment resembles a battlefield, where the patient's immune cells are the defence against invading tumour cells. However, the relationship between different immune components of the host response to cancer is more complex than an "us against them" model. Components of the immune system inadvertently work against the interests of the host and become pro-tumourigenic while other components soldier on against the common enemy – the tumour cell.

Key words: Immune; Gastric cancer; Immune therapy; Immunology; Tumour microenvironment

Core tip: Many solid tumours are now being treated with immunotherapies and gastric cancer is no exception. Here we review the literature on molecular

subtypes of gastric cancer and how they each have different immunological responses and hence may be differentially responsive to these immunotherapies. We emphasise that while treatment of gastric cancer may be benefited by immunotherapy we should try to target this based on molecular and immunological signatures of the individual patient. This will match the ideal therapy to the specific patient and is a step forward on the pathos precision medicine.

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"Advances in medicine and agriculture have saved vastly more lives than have been lost in all the wars in history."

Carl Sagan

INTRODUCTION

Gastric cancer (GC) continues to be a significant cause of mortality globally, being the third leading cause of cancer-related death[1]. While there have been advances in the outcomes of many solid tumours[2-4], gastric adenocarcinoma, the predominant form of GC, has not shown the same degree of improvements in survival[5], despite aggressive multi-modality treatment[6]. Potent new immunotherapies induce host immune-mediated destruction of malignant cells and offer new hope in the battle against GC. Here we explore some of the positive and negative characteristics of the host immune response to the presence of a malignant cell.

It is incumbent on us to be aware that all cancers are not equal. The Cancer Genome Atlas (TCGA) Network has produced a landmark study using integrative genomics to molecularly phenotype four subtypes of GC[7] that are to some extent related to histological features of the disease. Previous studies suggest the histology of the tumour according to Lauren classification may explain some of the molecular heterogeneity of GC[8] but the host immune response to the cancer may also account for some of the differences. The TCGA study describes two particular subtypes both of which consist of predominantly intestinal type tumours that had a significant immunological association: the Epstein-Barr virus (EBV) subtype accounted for about 9%[7] of the GCs profiled and were characterised by a strong immune signature and; the MSI (Microsatellite Instability) subtype (22% of cancers in this study[7]), which had a high mutational load, also had a significant immune signature. While the other two GC subtypes [GS (Genomically Stable and predominantly diffuse) subtype and CIN (Chromosomal Instability subtype which are primarily intestinal in histology)] may have a host immunological response, this differed to the two immunogenic subtypes[7]. Here we explore some of the features of the immune response to GC to try and reconcile some of the clinical observations, such as differences in survival and also to explore the utility of immunotherapies for this particular cancer.

Currently, the immune context of GC comprises both anti- and pro-tumoural immune responses. The immune system includes inter-linked innate and adaptive arms, both have cellular and soluble effectors. The innate immune system cells respond to foreign antigens that are recognised *via* pathogen recognition receptors (PRR) for pathogen-associated molecular patterns (PAMPs) or danger associated molecular patterns (DAMPs)[9]. The PRR can recognise PAMPs or DAMPs derived from a diverse array of viruses, bacteria or tumour cells. The innate immune system is evolutionarily conserved and performs an immune surveillance role *via* cells [macrophages, dendritic cells (DCs), neutrophils and natural killer (NK) cells] and soluble factors such as, the complement system. There is considerable cross-talk between cells within the innate immune system as well as cross-talk with cells of the adaptive arm, for example, tissue resident DCs induce an adaptive immune response through antigen presentation[10]. The adaptive immune system recognizes and eliminates antigens; conventional T cells recognise antigen as peptide-major histocompatibility complex (MHC) on virus infected cells or tumour cells, whereas B cells recognise conformational antigen. Priming of naïve T and B cells to antigens occurs in the tissue draining lymph node of a particular organ. Effective antigen recognition and co-stimulation activates the antigen-specific T or B cell driving their proliferation and generation of effector and memory cells. Effector T cells traffic to the site of priming and participate in resolution of the threat/pathogen. Memory T cells reside in secondary lymphoid tissue (central memory), or the peripheral tissue (tissue resident memory cells) and can respond quickly to any future pathogen threat, termed "long term protective immunity". In healthy individuals the immune system is remarkably effective at responding to and eradicating a diverse array of pathogen threats; however the immune system can be a double-edged sword in cancer, which has the ability to shape the immune response to facilitate tumour cell growth and survival rather than eliminating the tumour[11].

THE IMMUNE SYSTEM AND CANCER

The immune system detects and eliminates tumour cells. This usually prevents cancer development through a process termed immune-surveillance[12,13]. Tumour-specific antigens (TSA) are antigens present only on tumour cells, while tumour-associated antigens (TAA) are antigens present on tumour cells as well as normal

cells. Expression of TSA and TAA generally results from tumour-associated genetic mutations. Tumour-resident DCs constantly sample the microenvironment *via* endocytosis, they process the TSA or TAA as peptides and assemble them on MHC, either in the endoplasmic reticulum for MHC class Ⅰ, or endosomes for MHC class Ⅱ. The DC requires an activation signal, such as a DAMP or PAMP, in order to mature and subsequently increase peptide MHC expression levels. Activated DCs change chemokine receptor and adhesion molecule expression making them responsive to chemokines emanating from the tumour draining lymph node (TDLN). Having migrated to the TDLN, the mature DC presents TSA/TAA on MHC class Ⅰ to $CD8^+$ T cell, or on MHC class Ⅱ to $CD4^+$ T cells, priming an antigen-specific T cell response[14]. For successful activation, Cytotoxic T cells (CTLs) require two signals from antigen processing cells (APCs); (1) antigen presentation, T-cell receptor (TCR) binding to peptide-MHC class Ⅰ molecules; and (2) co-stimulation, CD28 molecule on T cells binding to co-stimulatory molecules CD80 (B7-1) or CD86 (B7-2) on APCs. In the absence of signal 2, signal 1 induces immune tolerance to TAA/TSA. Signal 2 is only provided by mature DCs, as they express CD80/CD86 at higher levels. At this point, activated tumour-specific naïve T cells proliferate and form effector and memory T cells, as described for the pathogen response above. Tumour-specific $CD8^+$ effector T cells, also termed CTLs, traffic from the TDLN to the tumour and attack tumour cells presenting cognate antigen, with the help of $CD4^+$ helper T cells (Th cells), mainly Th1 cells. During the effector phase, T cells infiltrate the tumour (referred to as tumour infiltrating T lymphocytes or TILs) in response to chemokines, such as CX3CL1, CXCL9, CXCL10 and CCL5[15]. These TILs kill tumour cells by direct and indirect mechanisms. The direct mechanism utilises perforin and granzymes. Figure 1A outlines some of the aspects of antigen recognition, presentation and the effector immune cells (T cell and NK cell) killing of tumour cells. Tumour-specific CTL recognition of cognate antigen induces their activation and formation of an immune synapse (IS, a specialised molecular structure formed between a cytotoxic lymphocyte and a target cell) at the site of antigen recognition. Simultaneously, the CTL moves cytotoxic granules (containing perforin and granzymes) to the IS, these granules fuse with the CTL cell membrane and release their contents. Perforin polymerises and inserts into the tumour cell membrane forming a pore, this enables entry of granzyme B into the cytoplasm, which induces tumour cell apoptosis. Indirect mechanisms include secretion of cytokines including type Ⅰ IFN, IFN-γ and TNF[16,17]. After clearance, surviving $CD8^+$ T cells differentiate into T memory cells[18], which can retain anti-cancer properties and can enact faster and stronger anti-cancer immune response when they next encounter tumour cells.

Another cell type important in an early response to cancer is the NK cell. NK cells are part of the innate immune system that act non-specifically against tumour cells and can directly kill these cells. This type of anti-cancer immunity is reported in hematopoietic malignancies and solid tumours[19].

HOW CANCER ESCAPES FROM IMMUNE SYSTEM

The "immune-editing" paradigm was proposed to explain how tumour cells influence the behaviour of innate and adaptive immune cells through an immunosuppression process to finally present as a clinical tumour[20]. The immune-editing mechanism, which is the most important process during immunosuppression, consists of three sequential phases: elimination, equilibrium, and escape[20-22]. During the elimination phase, the immune system destroys developing tumour cells. In the equilibrium phase, sufficient tumour cells survive the immune attack to maintain tumour size, but there is no obvious tumour progression. During this phase, the immune system sculpts the immunogenicity of genetically unstable tumour clones. Finally, in the escape phase immune resistant tumour clones emerge, proliferate and spread either locally or to distant sites.

Precisely how tumour cells evade the immune system (in the escape phase), as summarised in Figure 1B, is an area of active research, and can be broadly grouped into three main mechanisms including:

Immune recognition/ignorance: where tumour cells can control immune recognition *via* down-regulation of antigens and MHC molecules on the cell surface[23-25].

Immune suppression/tolerance: Where tumour-derived suppression mechanisms are driven *via* tumour-derived cytokines and influence the differentiation of immune effectors driving their functional polarization to suppressors. Immune suppressors include tumour associate macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory $CD4^+$ T cells. Macrophages within the tumour microenvironment have been described as pro-tumourigenic as they support cancer initiation and progression, or anti-tumourigenic based on differentiation patterns into M1 or M2 subtypes[26,27]. M1 macrophages have a tumouricidal activity by producing pro-inflammatory cytokines, such as IL-1, IL-6, IL-23 and TNF. M2 macrophages possess a tumour-promoting capacity by producing IL-10 and TGF-β. TAMs frequently have a spectrum of differentiation and, through the balance of M1 and M2 macrophage subtypes in the tumour microenvironment, may influence aggressiveness of the tumour and prognosis of patients. There is generally a poor outcome if M2 macrophages predominate in the tumour microenvironment[28,29]. Tumour cells may

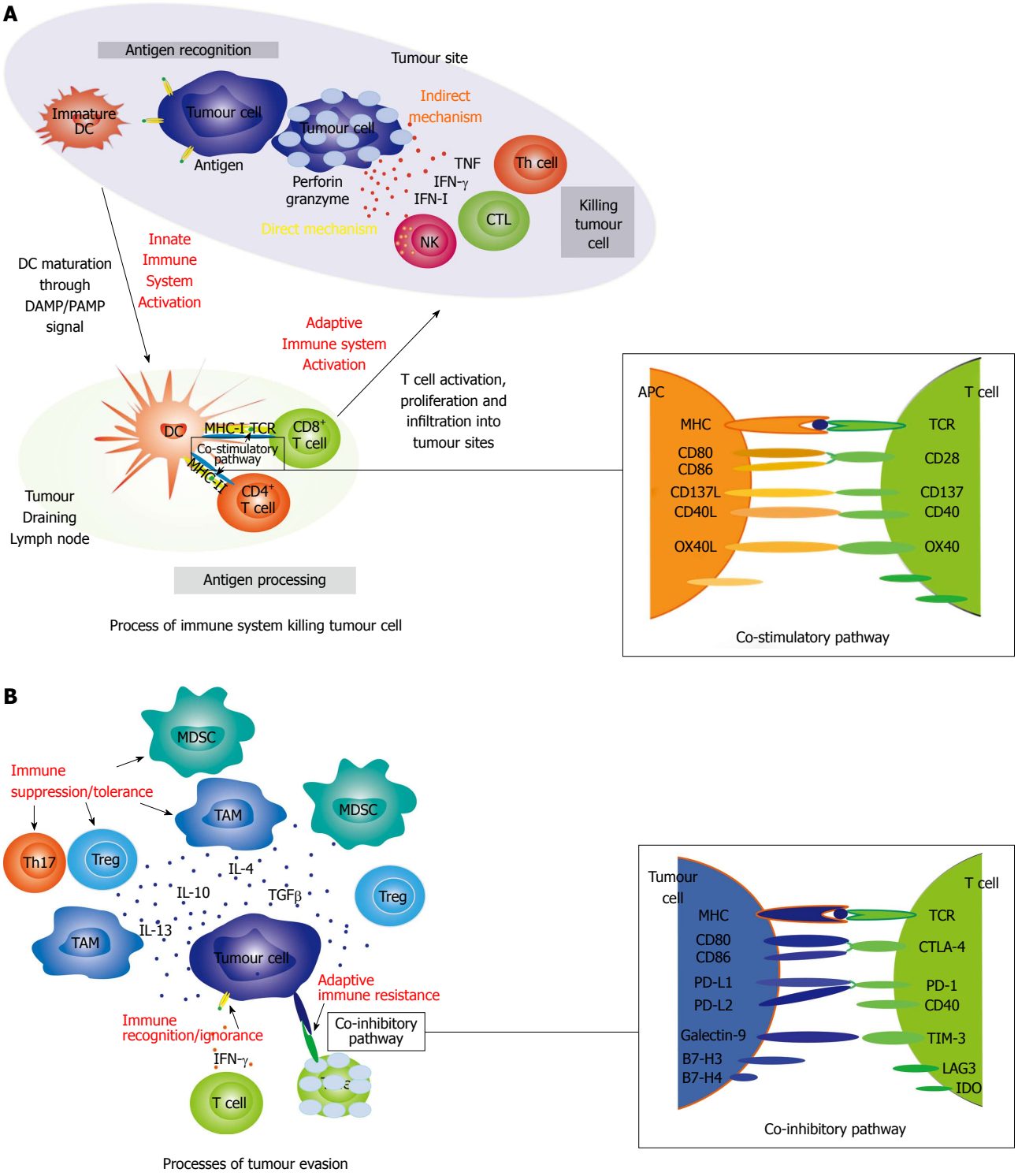

Figure 1 Process of immune system killing tumour and processes of tumour evasion. A: Outlines the process of immune system killing tumour cell. First, antigens (including neo-antigens) expressed by the tumour, are recognized by immature DC then the innate immune system is activated and the antigen is presented by APC to the T cell. The T cell becomes activated, then proliferates and infiltrates into tumour sites. The adaptive immune system is then responsible for activation of the effector immune cells (*e.g.*, CTL, Th cell or NK cell) that secrete cytokines to kill the tumour cell; B: Describes part of the processes of immune evasion. This is the mechanism by which the tumour cell evades the immune system (escape phase) through the processes of immune recognition/ignorance; immune suppression/tolerance and; adaptive immune resistance. DC: Dendritic cell; CTL: Cytotoxic T lymphocytes; Th cell: T helper cell; NK: Natural killer cell; APC: Antigen processing cell; MHC: Major histocompatibility complex; TCR: T-cell receptor.

also induce an M1 to M2 switch, mediated by TGF-β[30]. Several studies have identified an association with the density of TAMs in the microenvironment of GC and a poor outcome[31,32]. MDSCs are a group of activated but immature myeloid cells with strong immunosuppressive capacity that have been shown to support tumour cell growth, differentiation, and metastasis[33,34]. $CD4^+$ T cell response to tumour-derived antigen in the context

of TGF-β induces up-regulation of the key transcription factor (FoxP3) and regulatory T cells (Tregs) functional polarization. Tregs are powerful suppressors of the tumour-specific T cell responses (both CTL and effector $CD4^+$ T cells) and can be found at increased numbers in patient TILs, both at the tumour margin and inside the tumour itself[35].

Adaptive immune resistance: where tumour cells can induce T cell inactivation through a process described as "adaptive immune resistance"[36]. When CTL's recognise cognate antigens on tumour cells their effector mechanisms include secretion of IFN-γ, IFN-γ binding to tumour cell IFN-γR induces JAK-STAT signalling and up-regulation of tumour cell programmed death ligand-1 (PD-L1) expression. CTL recognition of antigen induces programmed death-1 (PD-1) expression. Binding of PD-L1 to its receptor PD-1 on T cells, delivers an inhibitory signal to the T cell IS and results in T cell paralysis. The over-arching result is tumour cell resistance to killing by T cells[36,37]. The molecules involved in the immune co-inhibitory pathways are called immune checkpoints. These molecules have an important normal physiological role, and are important in turning off the immune system once effective T cell effector function has been achieved (*i.e.*, once antigen has been cleared). The checkpoint inhibitors include: PD-1 (also known as CD279) and its ligands PD-L1 (B7-H1; also known as CD274) and PD-L2 (B7-DC; also known as CD273); Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152) and its ligands CD80 and CD86; T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) and its ligand galectin-9; lymphocyte-activation gene 3 (LAG3, also known as CD223) and Indoleamine-pyrrole 2,3-dioxygenase (IDO). These pathways are discussed later as they have been transformed into successful immunotherapies in the war against cancer. Other components of the adaptive immune response are two subcategories of T cells, the Treg and Th17 cell. Tumour cells can induce Tregs, which in turn promote tumour progression by secreting TGF-β, as well as Th17 cells, which accelerate tumour progression by producing IL-17[38].

These are some of the physiological mechanisms that a tumour cell can exploit to survive, perpetuate and invade a host organism resulting in poor outcomes seen in many malignancies.

IMMUNOGENIC SUBTYPES OF GC

Integrated genomic analysis of GC showed that molecular subtypes have distinct signatures. The EBV and MSI subtypes have significant immune signatures (Figure 2A) compared to the CIN and GS subtypes (Figure 2B). It is recognised that MSI cancers result in increased tumour cell mutational load[39] and presentation of neo-antigens[40] resulting in an augmented host immune response with increased TILs[41-43], DCs and macrophages[44]. EBV-associated GC also has an increased density of TILs[41,45-47]. Despite a significant host immune response these tumours persist, likely due to immune escape mediated by over-expression of immune checkpoints, such as PD-L1 and PD-L2[7]. Llosa *et al*[48] found similar changes to the tumour microenvironment of MSI colorectal cancer. These MSI cancers showed up-regulation of immune checkpoints, such as PD-1, PD-L1, LAG-3, CTLA-4 and IDO. In colorectal cancer MSI-high specimens are often associated with high infiltration of $CD3^+$, $CD4^+$ and $CD8^+$ cells and show more frequent infiltration by PD-1 positive intraepithelial lymphocytes compared to microsatellite stability samples[49]. Indeed, colorectal cancer is a good example of how the immunological reaction to the tumour has been used as a prognostic marker and may identify a group of cancers that can be targeted with specific immunotherapies[50,51]. The mechanisms for the robust immune response in the EBV subtype remain unclear, but are likely due to long term inflammation induced by the infection in the stomach[52].

An association between lymphocytic infiltration and survival in GC was first proposed over 100 years ago[53]. Since then numerous studies have shown an increased density of TILs is associated with favourable clinical outcomes in a variety of solid tumours[54-57], including GC[58]. This holds true for intratumoural B cells[42] and NK cells[59] in GC. However, GC expression of the checkpoint inhibitor PD-L1 is associated with poor clinical outcomes[60-66]. Tumour expression of PD-L1 is not universally a negative predictor as patients with ovarian cancer expressing high PD-1 (generally thought to be expressed by the immune cells) and PD-L1 levels in tumour cells as well as TILs having favourable prognosis[67]. These contrasting results are partially explained by methodological differences in the studies where most investigators report on the intra-tumoural immune component only and ignore the peri-tumoural context of the cancer or focus on particular components of the immune response in isolation and ignore the dynamic environment that is the tumour microenvironment. Importantly, another variable that is not factored in the GC literature is the molecular characteristics of the tumour cell itself. As described in the Asian Cancer Research Group study from Cristescu *et al*[68], tumours of the MSI subtype (similar to TCGA) have the best prognosis of the subtypes described in their study. Marrelli *et al*[69] confirm the favourable prognosis of MSI in non-cardia intestinal gastric tumours. Kim *et al*[70] investigated the type and density of TILs and macrophages in the MSI-high subgroup of GC and found that increased density of intra-tumoural $CD8^+$ and $FoxP3^+$ TILs was associated with a good prognosis. It was further shown that the balance of TILs and TAMs (M2-polarized macrophages) also showed favourable prognostic significance[71].

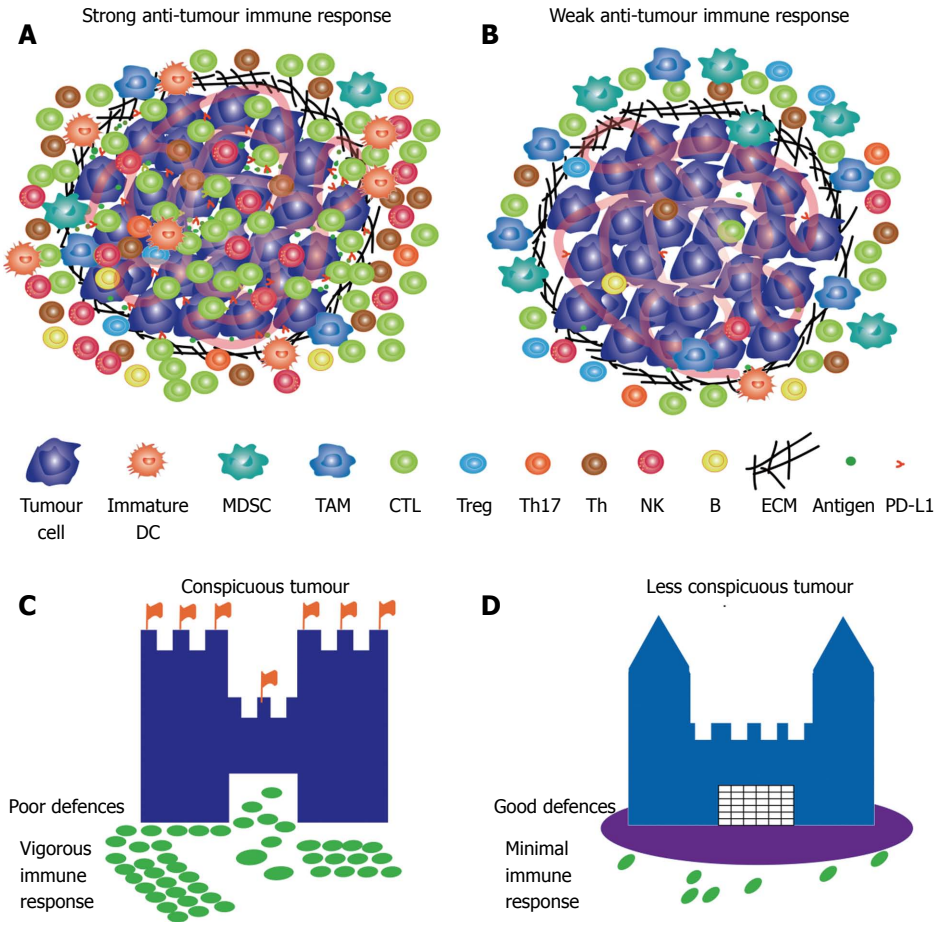

Figure 2 Two different tumour microenvironments of different molecular subgroups of gastric cancer. A: Example of a strong immunogenic tumour with increased antigen presentation and immune checkpoints expression, more TILs and other effector immune cells infiltration into the tumour; B: Example of a weak anti-tumour immune response with less antigen expression, less TILs and other effector immune cell infiltration into tumour and increased numbers of immunosuppressive cells; C: Schematic diagram representing poor defences from a conspicuous tumour with vigorous immune response; D: Schematic diagram representing good defences from a less conspicuous tumour with minimal immune response. MDSC: Myeloid-derived suppressor cell; TAM: Tumour associate macrophages; Treg: T regulatory cell; Th17: T helper cell-17; ECM: Extracellular matrix; MHC: Major histocompatibility complex; TCR: T-cell receptor.

IMMUNOTHERAPY OF CANCER

The immune system is an integral part of the tumour microenvironment and immune cell evasion by tumour cells has recently been highlighted as one of the hallmarks of cancer[11]. Promotion, or activation, of the immune system, referred to as "immunotherapy", has also been proposed as an option for targeted treatment. Unlike chemotherapy, which uses potent drugs to eliminate tumour cells or control their growth, cancer immunotherapy involves boosting the immune system of a patient to eliminate or control a malignancy. Using the immune checkpoint inhibitors (ICIs), T-cells can be re-activated or maintained in an active state allowing them to recognize and eliminate tumour cells. Documented clinical responses of ICIs in a number of cancer types, especially in solid tumours, including melanoma, non-small cell lung cancer, renal cell carcinoma[2-4] have been reported and provide us with new anti-cancer strategies. The use of immunotherapy, especially the ICIs, for treating GC is still in its infancy with several clinical trials underway.

CTLA-4, one of the immune checkpoints, is expressed on the surface of T cells following recognition of antigen. T cell CTLA-4 has a higher affinity than CD28 for APC CD80/CD86. This transduces an inhibitory signal to T cells serving as a "brake" on T cell activation. Ipilimumab, an IgG1 antibody which blocks CTLA-4 activity, allows ongoing APC priming of antigen-specific T cells in the TDLN (*i.e.*, brake removed). An additional proposed mechanism of ipilimumab action includes targeting of CTLA-4^{hi} intra-tumoural Tregs tipping the balance of effector T cells: Treg in favour of an anti-tumour response. Ipilimumab was the first approved immune checkpoint therapy and has shown a survival benefit in advanced stage melanoma patients[72,73]. The repercussions of meddling with the physiologic processes governing immunity is an increase in a variety of immune related side effects, including skin lesions (rash, pruritus, and vitiligo), colitis, thyroiditis, hypophysitis, and hepatitis[74]. A clinical trial in GC patients with unresectable, locally advanced or metastatic cancer following first line standard chemotherapy with a fluoropyrimidine/platinum combination (NCT01585987)

has recently been completed however initial results are not promising with poorer PFS (secondary endpoint) in the ipilimumab treated group[75]. Tremelimumab, another anti-CTLA-4 monoclonal antibody, was investigated as a second-line treatment for patients with unselected metastatic gastric and oesophageal adenocarcinomas. The results were disappointing and among 18 recruited patients only one patient achieved a partial response[76].

A second immune checkpoint target is the PD-1/PD-L1 axis. PD-1 is present on the surface of activated T-cells, B-cells and monocytes whilst PD-L1 is found on the surface of tumour cells and antigen presenting cells (macrophages and DCs)[2]. Similarly PD-L2, expressed exclusively on DCs, is also a ligand for PD-1 and has been shown to inhibit T-cell activation, proliferation and cytokine production[77]. Checkpoint inhibitor antibodies directed to the PD-1/PD-L1 pathway are thought to largely rescue function of pre-existing tumour-specific TILs.

Anti-PD-1 (nivolumab, pembrolizumab) or anti-PD-L1 (MSB0010718C, BMS936559, MPDL3280A, Medi4736) agents are humanized monoclonal antibodies, which inhibit binding of PD-1 to PD-L1 and restore T cell activity. Due to promising results from initial clinical trials utilising these antibodies in melanoma and in other cancer types, they are currently being explored in GC. A phase I clinical trial (NCT01928394) using nivolumab in GC patients has been completed and initial results showed objective responses occurred in patients irrespective of PD-L1 status[78].

Pembrolizumab has been tested in GC patients selected based on immunohistochemical staining of PD-L1 (NCT01848834)[79,80] and initial results reported at ESMO 2014[79] with updated results presented at ASCO 2015[80]. Eligible patients had PD-L1 positive staining in stromal or $\geqslant$ 1% tumour nest cells. Based on these criteria this study observed a 40% rate of PD-L1 positive cancers and demonstrated manageable toxicity and promising antitumour activity in advanced GC[80]. When used in melanoma patients, a positive response was associated with expression of four specific immune signatures (presented as an abstract)[81]. These findings were recapitulated in the GC[82] patients suggesting that screening for expression of these signatures could be used as a method to best select patients who might benefit from this treatment. A large number of clinical trials testing these drugs in combination with standard chemotherapies are currently underway and have been reviewed in detail elsewhere[83].

Several anti-PD-L1 monoclonal antibodies, including Avelumab (MSB0010718C), Durvalumab (Medi4736) and Atezolizumab (MPDL3280A) and BMS936559, are under evaluation in digestive cancers, including GC[84]. GCs comprise only a small minority of the patients recruited to the early phase clinical trials currently underway and as such only limited data on their efficacy is currently available[85]. It is worth noting that therapeutic strategies should be carefully considered. Whilst targeting the PD-1/PD-L1 + PD-L2 checkpoint pathways should increase anti-tumour efficacy, this may come at the cost of increased "off tumour target" toxicity. Therapies targeting only PD-L1 whilst maintaining PD-L2 activity may result in decreased anti-tumour effects coupled with decreased toxicity. There are currently no PD-L2 specific inhibitors available.

Therapeutic strategies targeting both CTLA-4 and PD-1 in combination are currently being tested in GC in the hope of identifying synergistic effects. The CheckMate032 (NCT01928394) trial testing the effects of nivolumab as a sole agent, or in combination with ipilimumab in a variety of solid cancers, including GC, and in a refractory setting is currently recruiting. This combination has previously showed successful tumour regression in the setting of melanoma[86].

The molecular, genetic and immunological heterogeneity described by the TCGA highlights a need to stratify patients based on their likelihood of responding to different treatment options including immunotherapy. Despite this, many of the clinical trials described above recruited GC patients of all subtypes which, unfortunately may dilute out the potential positive effects of these therapies. EBV and MSI subtypes of GC are associated with a vigorous immunological reaction, as well as over-expression of immune checkpoints, highlighting these two subtypes of GC as particularly attractive candidates for immune checkpoint blockade, and indeed trials in these particular GC subtypes are underway.

The EBV subtype described by the TCGA[7] is characterised by a high prevalence of mutations in the *PIK3CA* suggesting a possible therapeutic role for PI3K inhibitors. This subtype is also associated with a high prevalence of DNA hypermethylation and amplifications in the genes *CD274* and *PDCD1LG2* which encode the immunosuppressive proteins PD-L1 and PDL-2, which highlights this subtype as an ideal candidate for immunotherapy[7,83]. A clinical phase Ⅱ/Ⅲ trial (NCT02488759, CheckMate358) plans to test the efficacy of nivolumab in subjects with virus- associated tumours including EBV-positive GC. This trial is currently in the recruitment phase. Given that most of these patients have concurrent immune infiltrate and harbour mutations in targetable genes, an adjuvant approach including a targeted therapy in conjunction with a PD-1 inhibitor such as pembrolizumab may be warranted. Such a treatment combination would need to be evaluated to ensure that the targeted therapy doesn't directly inhibit immune effector cell signalling pathways.

The MSI TCGA GC subtype was characterised by high levels of microsatellite instability and elevated mutation rates[7]. Unsurprisingly gastrointestinal tumours that are MSI-H or mismatch repair deficiency, when compared to microsatellite stable tumours, have

shown promising immune-related objective response rates (ORR; 40% *vs* 0%) and progression-free survival (PFS; 78% *vs* 11%) when treated with PD-1 inhibitor, pembrolizumab[87]. This emphasizes this subtype as a potential candidate for immunotherapy.

Genomic amplifications in receptor tyrosine kinases were a distinguishing feature of the CIN subtype as defined by TCGA[7]. Many of these are candidates for treatment with molecular targeted therapies. A phase I clinical trial testing the effects of Pembrolizumab in combination with ramucirumab (NCT02443324) is currently recruiting and may be particularly effective in this subgroup. This group was also enriched for *TP53* mutations.

The GS TCGA subtype[7] (20% of all cases) comprised predominantly of tumours classified as diffuse GC, with poorer survival compared to the intestinal type GC, by the Lauren classification and was associated with mutations in *CDH1* and *RHOA* genes as well as aneuploidy. At this stage it is unclear whether this subtype would benefit from existing immunotherapies and warrants specific investigation.

With the significant clinical benefits from immune checkpoint blockade drugs, novel opportunities are emerging for GC treatment. To improve effectiveness of GC immunotherapy, novel criteria based on different molecular and immunological subtypes to predict potential response and prognosis are needed. Galon *et al*[88,89] have established an "immunoscore" in colorectal cancer based on the number and location of $CD3^+$ and $CD8^+$ cells[90]. This type of classification could be useful in GC. While we have focused on the immune component of the tumour microenvironment we must not lose sight that GC remains heterogeneous and while we may co-opt the immune system in destroying some cancers others may have mechanisms of resistance to avoid this form of killing. Therefore combination therapies may be the way of the future and we will need to be cognizant of the ensuing toxicities these therapies may invoke. It is important to also recognize the microenvironmental and immunological impact of the more traditional chemotherapeutics[91]. Examples include oxaliplatin, a platinum drug used often in GC chemotherapy which induces immunogenic cell death and provides a release of tumour antigens[92].

CONCLUSION

In most communities GC is diagnosed late and subsequently has poor prognosis. There are now exciting new therapies that utilise the host's immune system to fight back. However, data to date suggests we need to use these therapies judiciously to derive maximum benefit. GC is molecularly and immunologically heterogeneous, and this heterogeneity influences the tumour microenvironment in different ways. Returning to the battlefield analogy, the immunogenic or immune activating GC subtypes, EBV and MSI, are likely to be more conspicuous to the immune system by the expression of larger numbers of neo-antigens and other foreign epitopes that stimulate a vigorous immunological response that can be augmented by current therapies (Figure 2C), whereas the less immunogenic GCs, the CIN and GS subtypes, are more stealthy, with less antigen presentation providing a stronger defensive system against the host immune attack (Figure 2D). Like the battles in the wars of old, you need to choose your battlefield carefully and one of the key strategies, as enunciated by Sun Tzu, is to "know thy enemy", which translates to understanding the molecular nature of the cancer you are treating.

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