Cooperation between chemotherapy and immunotherapy in gastroesophageal cancers

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ABSTRACT

Gastroesophageal cancers (GOCs) represent some of the most common cancers globally and are linked with poor survival rates. The current standard of care includes multimodal chemotherapy, radiotherapy and surgery. However, up to two-thirds of patients fail to derive benefit from these treatments, underscoring the urgent need to develop better, rationally-designed treatment strategies to enhance survival rates. Certain immunogenic chemotherapies can stimulate anti-tumour immune responses in GOC patients; therefore, combining immune checkpoint inhibitors (ICIs) with chemotherapy to prevent immune exhaustion is an attractive putative therapeutic strategy. Emerging studies demonstrate that immune checkpoint-intrinsic signalling in cancer cells supports several cancer hallmarks in addition to immune evasion, including proliferation, metastasis, glycolysis, DNA repair and chemo-resistance. Combining ICIs with chemotherapy may therefore potentially enhance chemosensitivity and suppress a range of immune-dependent and -independent tumourigenic processes in GOCs. This review summarises the current clinical trials investigating the efficacy of ICIs in GOCs. The immunogenic effects of chemotherapies and their effects on immune checkpoint expression is discussed, as is the important and emerging study of novel immune-independent functions of immune checkpoints in cancer.

1. Introduction

Gastroesophageal cancers (GOCs) are comprised of oesophageal cancer (OC) and gastric cancer (GC) and collectively have one of the highest incidence rates of all cancers, causing more than one million annual deaths globally [1]. Despite new therapies, OC has a dismal prognosis with a 5-year survival rate of 10–20% [2]. Five-year survival rates for GC vary from 70 to 95% in early stage patients and 20–30% in patients with advanced disease [3].

The two major histological subtypes of OC are oesophageal adenocarcinoma (OAC) and oesophageal squamous cell carcinoma (OSCC), which differ greatly in terms of risk factors, epidemiology, incidence and geographic distribution [2]. The main histological subtype of OC is OSCC, however OAC is the predominant subtype in Western countries [2]. The main histological subtype of GC is gastric adenocarcinoma and comprises 90–95% of GC cases [4].

The standard of care for patients with resectable advanced oesophagogastric junctional adenocarcinoma (OGJ) includes the peri-operative FLOT chemotherapy-based regimen [5]. The FLOT regimen includes 5-fluorouracil (5-FU), leucovorin, oxaliplatin and a taxane (such as the anti-microtubule agent docetaxel). Leucovorin enhances the binding of 5-FU to thymidylate synthetase and prolongs the half-life of 5-FU in vivo [6]. A multimodal approach involving combined chemotherapy is also an option for OSCC and OAC patients; the CROSS regimen (paclitaxel and carboplatin with a cumulative radiation dose of 41.4 Gy over 23 fractions) followed by surgery [7]. GC patients with HER2+ tumours (~20%) receive trastuzumab (anti-HER2 monoclonal antibody) in combination with a cisplatin and fluoropyrimidine-based chemotherapy regimen in the first-line setting [8].

Unfortunately, a significant proportion of GOC patients fail to benefit from the current standards of care, with only ~30% of OSCC and OAC patients achieving a complete pathological response [9]. Immune checkpoint inhibitors (ICIs) are a therapeutic option for treating GOCs and have already exhibited clinical efficacy in a wide range of cancer types. Immunotherapy is now considered by many as the fifth pillar of cancer therapy along with surgery, chemotherapy, radiotherapy and molecular targeted therapies [10].

ICIs block immune checkpoint (IC) pathways, reinvigorating anti-tumour immunity [10]. ICs control the magnitude and duration of the immune response, preventing overactivation of the immune system,

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which could lead to the development of autoimmune [11]. ICs include cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed death-1 (PD-1), lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin mucin domain-3 (TIM-3), T cell immunoglobulin and ITIM domain (TIGIT), V-domain immunoglobulin-containing domain suppressor of T cell activation (VISTA), HHLA2, butyrophilin-like 2, B and T lymphocyte attenuator, 2B4, B7–H3, B7–H4 and adenosine A2a receptor (A2aR) [10]. The receptors for ICs are predominantly expressed on activated T cells. IC ligands, such as PD-L1 and PD-L2 (ligands for PD-1), CD160 (ligand for herpes virus entry mediator), galectin-9 and carcinoembryonic antigen-related cell adhesion molecule 1 (ligands for TIM-3), and CD112 and CD155 (ligands for TIGIT), are found on the surface of antigen presenting cells (APCs) [10]. However, tumour cells exploit IC pathways to evade immune destruction through upregulation of IC ligands, such as PD-L1 and PD-L2 on their surfaces [12]. Tumour cells also secrete adenosine, which binds A2aR on T cells dampening anti-tumour T cell function [10].

Despite the vast array of ICs expressed on the surfaces of immune cells, to date the majority of related clinical trials in GOCs and other cancer types have largely focussed on testing the efficacy of blocking PD-1 and CTLA-4 IC pathways (Table 1). Anti-CTLA-4, anti-PD-1 and anti-PD-L1 ICIs have been FDA approved in first-, second- and third-line treatments for colorectal cancer [11]. ICIs have been FDA approved in first-, second- and third-line cancer types have largely focussed on testing the efficacy of blocking PD-1 and CTLA-4 IC pathways (Table 1). Anti-CTLA-4, anti-PD-1 and anti-PD-L1 ICIs have been FDA approved in first-, second- and third-line treatments for colorectal cancer [11].

| Trial     | Treatment regimen                                                                 | IC(s) tested       | Cancer-type       | Clinical outcomes              |
|-----------|-----------------------------------------------------------------------------------|--------------------|-------------------|-------------------------------|
| NCT02730546 | pembrolizumab + neoadjuvant chemotherapy, and radiation therapy                   | anti-PD-1          | resectable OC     | recruiting, data pending.     |
| NCT02735239 | durvalumab alone followed by capcitabine and oxaliplatin or combination durvalumab + tremelimumab + oxaliplatin and capcitabine or durvalumab + paclitaxel + carboplatin + radiation or durvalumab + FLOT (5-FU, leucovorin, oxaliplatin and docetaxel) | anti-PD-L1 and anti-CTLA-4 | OC                        | active, data pending.          |
| NCT02559687 | pembrolizumab                                                                  | anti-PD-1          | OC, OJG           | mPFS: 2 months and mOS: 5.8 months [13]. |
| NCT02564263 | pembrolizumab versus paclitaxel + docetaxel + irinotecan                          | anti-PD-1          | OAC and OSCC      | mOS for OAC cohort: 7.1 months (pembrolizumab) versus 7.1 months (chemotherapy), OSCC cohort: 8.2 months (pembrolizumab) versus 7.1 months (chemotherapy) versus 6.7 months (chemotherapy). 12-month OS rates for OAC cohort: 32% (pembrolizumab) versus 24% (chemotherapy), OSCC cohort: 39% (pembrolizumab) versus 25% (chemotherapy) and for OAC/OSCC with PD-L1 expression CPS ≥10: 42% (pembrolizumab) versus 20% (chemotherapy), (n = 628) [14]. |
| NCT02349975 | tremlumabumub versus durvalumab versus tremelimumab + durvalumab                 | anti-CTLA-4, anti-PD-L1 | OJG, GC           | data pending.                  |
| NCT02370498 | pembrolizumab                                                                   | anti-PD-1          | OJG, GC           | mOS: 9.1 months (pembrolizumab) versus 8.3 months (paclitaxel) and mPFS: 1.5 months (pembrolizumab) versus 4.1 months (paclitaxel) [15]. |
| NCT02589496 | pembrolizumab                                                                   | anti-PD-1          | OJG, GC           | overall response rate: 24.6%, 85.7% of patients with MSI-H responded and 100% patients with Epstein-Barr virus’ responded [16]. |
| NCT02901301 | triplet pembrolizumab + trastuzumab + chemotherapy (capcitabine + cisplatin)    | anti-PD-1          | HER2+ GC          | mPFS: 8.6 months, mOS: 18.4 months and objective response rate of 76.7%, n = 43 [17]. |
| NCT02494583 | pembrolizumab versus pembrolizumab + cisplatin + 5-FU/or/pacetaxel + 5-FU/or/capcitabine | anti-PD-1          | OJG, GC           | mOS: 10.6 months (pembrolizumab) versus 11.1 months (chemotherapy) and 12.5 months (combined pembrolizumab and chemotherapy). mPFS: 2 months (pembrolizumab) versus 6.4 months (chemotherapy) and 6.9 months (pembrolizumab and chemotherapy) [18]. |
| NCT02625610 | avelumab versus oxaliplatin + capcitabine/5-FU                                   | anti-PD-L1         | OJG, GC           | mOS: 10.4 months (avelumab) versus 10.9 months (chemotherapy), objective response rate: 13.3% (avelumab) versus 14.4% (chemotherapy). mPFS: similar between arms (HR 1.04 [95% CI 0.85–1.28]) for n = 49.9 [19]. |
| NCT02625623 | avelumab versus irinotecan + paclitaxel                                         | anti-PD-L1         | OJG, GC           | mOS: 4.6 months (avelumab) versus 5.0 months (chemotherapy) and mPFS: 1.4 months (avelumab) versus 2.7 months (chemotherapy) (n = 371) [20]. |
| NCT02872116 (checkMate 649) | nivolumab + ipilimumab versus nivolumab + oxaliplatin + FOLFOX/XELOX versus FOLFOX/XELOX | anti-PD-1, anti-CTLA-4 | OJG, GC           | data unavailable [21]. Trial ended due to toxicities and death. |
| NCT02864381 | nivolumab versus nivolumab + GS-5745                                           | anti-PD-1, MMB-9 inhibitor | OJG, GC           | data pending [22].          |
| NCT02340975 | second-line patients received durvalumab + tremelimumab (arm A), or durvalumab (arm B) or tremelimumab monotherapy (arm C), and third-line patients received durvalumab + tremelimumab (arm D). | anti-PD-1, anti-CTLA-4 | OJG, GC           | overall response rates were 7.4%, 0%, 8.3% and 4.0% in the four arms, respectively. PFS rates at 6 months were 6.1%, 0%, 20% and 15% and 12-month OS rates were 37.0%, 4.6%, 22.9%, 38.8%, respectively [23], n = 6 in phase Ib and n = 107 in phase II (arm A: 27; arm B: 24; arm C: 12; arm D: 25) in phase II. |

**BSC**, best supportive care; **CI**, confidence interval; **CPS**, combined positive score; **HR**, hazard ratio; **MSI-H**, microsatellite instability-high; **mOS**, median overall survival; **mPFS**, median progression free survival; **OS**, overall survival.
settings, spanning a wide range of malignancies, including melanoma, lymphoma, renal cell carcinoma, lung cancer and microsatellite instability-high (MSI-H) cancers [24–27]. They have been approved as monotherapies, as a dual anti-PD-1/anti-CTLA-4 cocktail or in combination with chemotherapy or anti-angiogenic drugs (receptor tyrosine kinase inhibitors against vascular endothelial growth factor receptor 1, 2 and 3) [24–27].

Two ICIs have been FDA approved as part of second and third-line settings for treating GOCs. In 2017, single agent pembrolizumab (Keytuda), an anti-PD-1 monoclonal antibody, was FDA-approved for the treatment of advanced or recurrent GC or OGJ cancers in the third-line setting for tumours expressing PD-L1 (combined positive score (CPS) ≥1) [28]. In 2019, single agent pembrolizumab was also granted FDA approval in the second-line setting for the treatment of GSCC patients with recurrent, locally-advanced, or metastatic disease, whose tumours express PD-L1 (CPS ≥10%) [29].

Chen et al., performed a meta-analysis for clinical trials testing the efficacy of anti-PD1, anti-PD-L1 and anti-CTLA-4 ICIs in advanced GCs and OGJs, which demonstrated that the addition of ICIs to the second- and third-line setting for treating GOCs improves some, but not all survival endpoints [30]. The objective response rates were 9.9%, 12.0% and 2.1%, respectively and the disease control ratios were 33.3%, 34.7% and 30.1%, respectively [30]. The median progression-free survival (mPFS) was 1.6, 1.6 and 2.9 months, respectively and the median overall survival (mOS) of the three groups was 6.0, 5.4 and 7.7 months, respectively [30]. ICIs targeting the PD-1 pathway were more effective in GC patients who were PD-L1+ [30], MSI-H, Epstein-Barr virus positive or had a high tumour mutational burden (TMB) [30]. These features have demonstrated the greatest success for distinguishing responders from non-responders, acting as surrogate markers of pre-existing anti-tumour immunity [30].

Immunogenic ‘hot’ tumours are characterized by T cell infiltration, the presence of effector mediators, such as granzyme B, in addition to interferon-gamma (IFN-γ)-induced PD-L1 expression [31]. However, ICIs are thought to be largely ineffective in non-immunogenic ‘cold’ tumours, where there is an absence of pre-existing anti-tumour immunity and therefore no immune response to reinvigorate [32]. Thus, it is crucial to elucidate the underlying cellular and molecular mechanisms that contribute to the generation of non-immunogenic tumours in order to enable the design of rational therapeutic approaches to convert ‘cold’ tumours to ‘hot’ tumours, or alternatively be able to stratify these patients to receive treatments other than circumstance ineffective ICIs. Immunogenic cytotoxic chemotherapies are emerging as a valuable tool to convert ‘cold’ tumours to ‘hot’ tumours, widening the therapeutic window and benefits of ICIs to a greater spectrum of patients [33]. Furthermore, resistance to PD-1 ICIs can be mediated through upregulation of other ICIs including TIM-3 in lung cancer [34]. Therefore, co-blockade of multiple ICs has been suggested as a method to overcome/prevent IC blockade resistance and enhance the efficacy of ICIs targeting the PD-1 pathway.

This review will outline the clinical rationale for blocking ICs in GOCs. An up-to-date summary of clinical trials testing the efficacy of ICI monotherapies, co-blockade of multiple ICs and chemotherapy-ICI combinations in GOC patients is provided. The molecular and clinical rationale for combining chemotherapy with ICs to enhance treatment outcomes for GOC patients is also highlighted. Additionally, recent studies uncovering novel functions of ICs in promoting the hallmarks of cancer other than immune evasion will be discussed.

2. Overcoming ICI resistance using conventional chemotherapy regimens

Emerging studies demonstrate that the presence of tumour-associated antigens and neoantigens is a superior biomarker of response to ICIs compared with the presence of tumoral PD-L1 expression [35]. Studies from The Cancer Genome Atlas reveal that only 22% of GCs are MSI-H, suggesting that a significant proportion of GC patients are unlikely to respond to ICIs, thus requiring additional therapeutics to sensitize their tumours to ICIs [36,37]. As such, chemotherapies that induce DNA damage in cancer cells, consequently increasing their immunogenicity through the generation of neoantigen-yielding nonsynonymous mutations, are emerging as an attractive tool to sensitize TMB-low tumours to ICIs [38]. However, chemotherapy also exerts a range of effects on the immune system depending on the agent and dose given, either leading to an augmentation of anti-tumour immunity or the induction of immunosuppression [39]. The ability of chemotherapy to induce immunogenic tumour cell death (ICD) determines how the dying tumour cell interacts with the immune system and whether an anti-tumour immune response will be triggered [40]. Chemotherapy-induced DNA-damage is associated with increased antigen presentation and the recruitment of APCs to the tumour microenvironment (TME) [41–43]. Therefore, chemotherapy is a potentially useful strategy to overcome low TMB and enhance anti-tumour immunity. Conventional chemotherapy regimens are administered using a maximal tolerated dosing schedule, typically resulting in lymphodepletion and destruction of both anti-tumour and immunosuppressive immune cells [44]. In response to chemotherapy-induced lymphodepletion, homeostatic T cell reconstitution occurs, generating new populations of T cells, which are subsequently educated in the thymus [44]. This temporal therapeutic window offers an opportunity to shape the T cell repertoire towards tumour antigens released from tumour cells that have died via chemotherapy-induced ICD. Several supporting studies have demonstrated that the clinical efficacy of chemotherapy is not solely due to the direct killing of tumour cells but also results from the restoration of immunosurveillance [45]. Individual chemotherapies have a range of effects on the immune system; the immunomodulatory mechanisms of each chemotherapy used in the treatment of GOCs is summarized in Table 2.

Immunostimulatory chemotherapies induce ICD via the release of damage-associated molecular patterns (DAMPs). ICD is characterized by the induction of tumour cell apoptosis and concurrent appearance of DAMPs on the cell surface or by the release of DAMPs into the extracellular TME [54]. DAMPs indirectly trigger anti-tumour immunity via binding to pattern recognition receptors, such as CD91 and toll-like receptor-4 on APCs, inducing maturation and activation of DCs and subsequent activation and mobilisation of anti-tumour T cells to the tumour site [55]. Studies highlighting the different mechanisms of ICD induced by specific chemotherapies used for treating GOCs are outlined below.

| Chemotherapy | Chemotherapeutic class | Mechanism of immunomodulation |
|--------------|-----------------------|-------------------------------|
| 5-FU | anti-metabolite | increases the frequency of tumour-infiltrating CTLs [46], depletes tumour-associated MDSCs [47], release of HSPs [48], enhances DC maturation and cross-presentation of tumour antigens to T cells [49] |
| oxaliplatin | alkylating agent | increases T cell recognition, induces CRT expression, HMGB1 and ATP release, increases the CTLs/regulatory T cell ratio, depletes MDSCs, improves the activity of neutrophils and macrophages [49] |
| docetaxel | taxane | regulatory T cells and MDSC depletion [50], DC maturation [51], single agent effects unknown. |
| carboplatin | alkylating agent | depletes regulatory T cells [52] and MDSCs [53], induces DC maturation [51] |

ATP, adenosine triphosphate; CRT, calreticulin; CTLs, cytotoxic T lymphocytes; DC, dendritic cell; 5-FU, 5-fluorouracil; HMGB1, high mobility group box protein 1; HSP, heat shock protein; MDSCs, myeloid-derived suppressor cells.
Calreticulin (CRT) is a pre-apoptotic marker which translocates from the endoplasmic reticulum to the cell surface as a result of endoplasmic reticulum stress [56]. Membrane exposure of CRT acts as a phagocytic ‘eat me’ signal and attracts APCs to the tumour site. Binding of CRT to CD91 on the surface of DCs and macrophages mediates phagocytosis of the dying tumour cell and subsequent antigen processing and presentation to T cells [56]. Binding and activation of CD91 also induces the production of pro-inflammatory tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6 [57]. Oxaliplatin has been shown to induce cell surface CRT expression in colorectal cancer [49] and murine lung carcinoma [58], while docetaxel induced CRT cell surface expression in breast, prostate and colorectal cancer cell lines in vitro [59].

2) Following, exposure of pre-apoptotic CRT on the cell surface, adenosine triphosphate (ATP) is released from lysosomes during the blebbing phase of apoptosis into the extracellular TME [60]. ATP acts through the ATP-purinergic P2Y2 ligand-receptor axis, functioning as a chemotactic signal attracting DCs and macrophages, leading to their maturation [60]. Oxaliplatin has been shown to induce ATP secretion in colorectal cancer [49] and murine lung carcinoma [58].

3) The release of high mobility group box protein 1 (HMGB1) from dying cancer cells binds toll-like receptor (TLR)-4 on the surface of APCs, stimulating their activation and maturation [61]. Studies have demonstrated that HMGB1 is important for ensuring optimal processing and phagocytosis of tumour peptides [61]. HMGB1 also binds the receptor for advanced glycation end products resulting in downstream activation of NF-κB and MAPK, promoting DC maturation and subsequent migration to the lymph node [61]. Docetaxel (lung adenocarcinoma [62]), oxaliplatin (colorectal cancer [49] and lung carcinoma [59]) and 5-FU (colon carcinoma cells [63]) all induce tumour cell secretion of HMGB1. Paclitaxel, but not carboplatin, was found to induce ICD through the release of HMGB1 and activation of TLR-4-dependent and -independent pathways in ovarian cancer [64].

4) ICD results in the increased production and release of inducible heat shock proteins (HSPs), part of the adaptive stress response, namely HSP70 and HSP90, which enhance DC maturation via binding to CD91 on their surface [65]. HSPs are also known as chaperone proteins due to their regular function in mediating the refolding of misfolded proteins or in the degradation of damaged proteins [65]. 5-FU induces production of HSP70 in GC [48].

5) Additionally, chemotherapy can also activate the stimulator of IFN genes (STING) pathway, which is critical in generating an effective anti-tumour immune response [67]. Cyclic GMP-AMP synthase detects cytosolic double-stranded DNA, another DAMP, resulting in the production of cyclic GMP-AMP, which activates STING and induces the expression of type I interferons [67]. Type I interferons induce

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**Fig. 1.** Immunogenic chemotherapies stimulate anti-tumour immunity via induction of ICD. Mechanisms of ICD induced by chemotherapies used to treat gastrointestinal malignancies. Different chemotherapies induce ICD through diverse pathways and include the release of tumour antigens from dying cancer cells, translocation of CRT to the tumour cell surface and the secretion of DAMPs, such as HMGB1 and ATP. CRT, HMGB1 and ATP bind their respective receptors calreticulin receptor (CRTR), toll-like receptor-4 (TLR-4), and the P2RX7 receptor on immature DCs, respectively. The binding of DAMPs to their receptors on immature DCs results in their maturation and subsequent migration to lymph nodes. In the lymph nodes, mature DCs activate antigen-specific cytotoxic CD8+ T cells (CTLs). Activated CTLs then migrate to the tumour and kill tumour cells expressing their cognate tumour antigens. Certain chemotherapies result in the activation of STING and subsequent production of type I interferons further enhancing anti-tumour immunity.
the release of CXCL10, an effector T cell chemoattractant that binds CXCR3 on effector T cells [68]. Paclitaxel has been shown to induce the activation of STING in breast cancer cells in \textit{vivo} [69], while oxaliplatin induces the production of type I interferons in ovarian cancer in \textit{vivo} [70].

GOCs are highly heterogeneous cancers and emerging evidence suggests that they can be stratified into unique immune-based subtypes [71,72]. In a study of 1524 GC patients, distinct TME phenotypes were identified and associated with prognosis. The high TME score subtype was characterized by immune activation and was an independent prognostic biomarker in predicting response to immunotherapy. Immune activation was defined by enrichment for genes involved in response to viruses (IFNG, TRIM22, CXCL10, CXCL9, and CD8A), response to IFN-γ (HLA-DPβ1, CCL4, CCL5, and IFNG) and T-cell activation (TRBC1, IDO1, CD2, NLRP3, and CD8A). The low TME score subtype was enriched for genes involved in extracellular matrix remodelling (DCN, TIMP2, POXF2, and MYH11), epithelial mesenchymal transition (EMT) (ACTA2, TGFBI11, and SFRP1), cell adhesion and angiogenesis (PDGFR, GREM1, and TMEEM100), which are considered suppressive factors for T cells and may be responsible for significantly worsening prognosis in GC patients [71].

Relative to other tumour types, OAC has a relatively high TMB and ranked 5th of 30 tumour types in terms of TMB [73,74]. A study of 129 OAC patients identified 3 subgroups based on mutational signatures. The “mutagentic” subgroup displayed the highest TMB, neoantigen burden and CD8+ tumour infiltrating lymphocyte density, which may lead to an increased response to ICIs [75].

An additional study of 551 OAC patients demonstrated a three-way association between hypermutation, activation of the Wnt pathway and loss of immune signalling genes, such as β2 microglobulin, a component of the major histocompatibility complex-I, which is associated with T cell exclusion from the tumour [76]. Hypermutation is associated with higher immune activity, while Wnt dysregulation and loss of β2 microglobulin is associated with immune escape [77]. This provides an acquired mechanism through which OAC may prevent immune surveillance induced by a high TMB, potentially contributing to the lack of response to ICIs in OAC.

Contextually, a “one size fits all” approach will unlikely be suitable for treating GOCs. The available evidence to date supports the premise that certain tumours will likely respond better to ICIs, whereas tailored combination approaches will be required to sensitise other tumours.

3. The double-edged sword of chemotherapy provides a therapeutic niche for ICIs

3.1. Chemotherapy upregulates immune checkpoints on the surface of cancer cells

Chemotherapy may be a double-edged sword in cancer, tumour-promoting or tumour-inhibiting, depending upon the circumstances. Several studies have highlighted a role for the immune system in the development of chemoresistance. In particular, chemotherapy-induced PD-L1 upregulation on the surface of breast cancer cells results in immune evasion via ligation of PD-L1 to PD-1 on T cells, thereby inducing T cell apoptosis [78]. Additionally, in \textit{vivo} and in \textit{vivo} murine model studies [79] demonstrate that cisplatin upregulates PD-L1 on head and neck squamous cell carcinoma cells [79] and ovarian cancer cells [80].

It has been demonstrated that 5-FU increases PD-L1 expression on the surface of colorectal and OAC cancer cells in \textit{vivo} [81]. However, the post-treatment expression levels of PD-L1 on OAC cells from \textit{ex vivo} tumour tissue that received 5-FU, cisplatin and radiation, was not significantly different compared with matched treatment-naïve tumour tissue (n = 10) [81]. Several studies have demonstrated that DNA damage signalling upregulates PD-L1 expression on the surface of cancer cells [82,83] and that PD-L1 cancer cell-intrinsic signalling mediates DNA repair, including base excision repair in osteosarcoma, breast and colorectal cancer [83]. This suggests that the 5-FU-induced PD-L1 upregulation on cancer cells in \textit{vivo} may be as a result of the 5-FU-induced DNA damage, which would be repaired by the time the biopsy is taken post-treatment [81].

In a study by Ng et al., PD-L1 was expressed in 21% of OSCC tumours, as determined by immunohistochemistry (n = 84) [84]. PD-L1 staining positively correlated with advanced stage III and IV disease and lymph node metastasis [84]. Combination cisplatin and 5-FU and combination carboplatin and paclitaxel significantly upregulated PD-L1 on OSCC cells in \textit{vivo} via the EGFR/ERK and MAPK/MEK pathways. The chemotherapy-induced PD-L1 upregulation may be a pro-survival strategy to protect against chemotherapy-induced cell death and/or the chemotherapy may be selecting for a more aggressive and resistant clone, highlighting the double-edged sword of chemotherapy [84].

A study by Fournel et al., reported significantly increased PD-L1 expression on both lung cancer cells and tumour-infiltrating immune cells following cisplatin-based chemotherapy (n = 22) [85]. Cisplatin treatment also significantly increased PD-L1 expression on tumour cells in nude and immunocompetent mice bearing lung carcinoma tumours [85]. Combined anti-PD-L1 antibody and cisplatin significantly reduced tumour growth compared to single agent treatment and controls in Lewis lung murine models [85].

Overall, the chemotherapy-induced upregulation of PD-L1 on the surface of tumour cells is presenting a therapeutic target. It is unclear what mechanisms are regulating the chemotherapy-induced upregulation of PD-L1; be it in response to chemotherapy-induced cancer cell secretion of cytokines known to upregulate PD-L1, such as IFN-γ [81] and transforming growth factor-β [86], or DNA damage signalling [82,83]. Emerging studies have demonstrated that cancer cell-intrinsic signalling of PD-L1 and other ICs promote various immune-independent hallmarks of cancer and that blockade of these pathways may have additional benefits in terms of reducing overall tumour burden [87–90].

3.2. Immune checkpoint signalling promotes a range of cancer hallmarks in addition to immune evasion

Several studies have identified PD-1 [89–93], TIM-3 [94,95], VISTA [96,97] and TIGIT [98] IC receptors on the surface of cancer cells across a range of malignancies. Tumour-expressed IC receptors have novel immune-independent functions associated with various hallmarks of cancer via cell-intrinsic signalling including glycolysis [87], DNA repair [88], proliferation [89], invasion and migration (Fig. 2) [94,95].

Kollmann et al., recently reported that PD-1 is expressed on 77% of OAC patient tumours (n = 168) at levels greater than 5%, as determined by immunohistochemical analysis [91]. Additionally, PD-1 has been found to be preferentially expressed on melanoma cancer stem-like cells (CSCs) in melanoma xenografts and melanoma patient biopsies, as characterized by the expression of tumour-initiating cell determinant ABCB5 [99]. CSCs exist as a small sub-population of cells within a tumour, which are more resistant to therapies and persist following conventional chemoradiotherapy [100]. CSCs are thought to be primarily responsible for tumour initiation, treatment resistance and disease recurrence [100] and destruction of CSCs is essential for complete tumour eradication [101]. It remains to be determined if blocking PD-1 on the surface of CSCs can render them more sensitive to chemotherapy-induced cell death, or whether this may contribute to the striking clinical efficacy of PD-1 ICIs in melanoma - a question that certainly warrants further investigation.

A study by Hsu et al., demonstrated that the process of EMT upregulates PD-L1 on CSCs via the EMT/β-catenin/STT3/PD-1 signalling axis [102]. Induction of EMT promotes N-glycosyltransferase STT3 transcription through β-catenin and subsequent STT3-dependent N-glycosylation inducing PD-L1 upregulation [102]. The enrichment of PD-L1 on CSCs may enable this aggressive subpopulation of cancer cells, considered largely responsible for treatment resistance and disease
Fig. 2. Cancer cell-intrinsic signalling of IC ligands and cognate receptors promotes a range of immune-independent hallmarks of cancer. Several studies in a range of cancer types, including lung, melanoma, breast, colorectal, gastric and oesophageal cancers, have demonstrated that cancer cell-intrinsic signalling of various IC ligands and receptors promotes a range of hallmarks of cancer other than immune evasion. This figure summarises the results from a range of studies demonstrating that activation of these pathways promotes proliferation, invasion, migration, metabolism, DNA repair and confers chemoresistance.

3.3. Immune checkpoint signalling can confer chemoresistance via immune-independent mechanisms

Several studies demonstrated that IC cancer cell-intrinsic signalling offers protection to cancer cells against chemotherapy-induced cell death [106]. One study showed that a 5-FU resistant GC cell line SGC7901/5-FU expressed significantly higher levels of cell surface PD-1 than 5-FU sensitive SGC7901 cells [107]. Upregulation of PD-1 in SGC7901 cells protected against 5-FU chemotherapy-induced cell death, increased proliferation and upregulated the expression of the Bcl-2 anti-apoptotic protein and chemotherapy efflux pump, ATP binding cassette subfamily C member 1 [107]. Downregulating PD-1 expression in SGC7901/5-FU cells reduced 5-FU resistance demonstrated by a decrease in proliferation and increase in apoptosis [107].

Other studies have reported a higher expression of PD-1 and PD-L1 on tumour cells that are more resistant to cisplatin in small cell lung cancer [93] and GC [107] in vitro and ex vivo. PD-1 and PD-L1 were expressed at significantly higher levels on cisplatin resistant (H69R and H82R) compared to cisplatin sensitive small cell lung cancer cells (H69P and H82P) in vitro [93]. Additionally, small cell lung cancer tumour tissue in the treatment-naive setting had lower expression of PD-1 and PD-L1 than resistant tumours [93]. Of significant clinical relevance, blockade of PD-1 and PD-L1 has been shown to reduce cisplatin resistant small cell lung cancer cell sensitivity to cisplatin [93]. Several studies have demonstrated that DNA damage signalling upregulates PD-L1 expression on the surface of cancer cells [82,83,108] and that PD-L1 cancer cell-intrinsic signalling mediates DNA repair [108], including base excision repair in osteosarcoma, breast and colorectal cancer [83]. Therefore, blockade of PD-L1 on the surface of GOC cells could prevent repair of cancer cell metabolism, proliferation, metastasis and invasion. It is possible that chemotherapeutic drugs may indirectly activate these immune-independent pathways via upregulation of IC ligands and receptors on cancer cells and immune cells. Therefore, chemotherapy may predispose patients to respond to ICIs as a result of higher IC expression and ultimately enhance response rates. However, recent studies have also shown that IC cancer cell-intrinsic signalling can confer chemoresistance via immune-independent mechanisms [87-90].

Recurrence, to evade immune destruction.

Studies in other cancers, including pancreatic ductal adenocarcinoma, have demonstrated that activation of PD-1 signalling in vitro using recombinant PD-L1 promotes proliferation via MAPK signalling [92]. Similarly, PD-1 overexpressing murine melanoma tumours demonstrate increased tumourigenicity compared with PD-1 knockout murine melanoma tumours in NOD SCID gamma mice (IL-2Rγ-chain−/−), identifying an immune-independent role for cancer cell-intrinsic PD-1 signalling in tumourigenesis [104].

Interestingly, it has been shown that the levels of TIM-3 in GC tissue are higher in adjacent normal gastric tissue [95]. Decreased galectin-9 and increased TIM-3 correlated with poor prognosis in GC [95]. Similarly, TIM-3 was also identified on the surface of HeLa cervical cancer cells in vitro and high expression of TIM-3 on cervical tumour cells correlated with advanced cancer grade and decreased OS compared with patients who had lower tumour cell TIM-3 expression [94]. Downregulating TIM-3 using adenoviral mutants encoding anti-sense TIM-3 significantly decreased the in vitro invasive capacity of HeLa cells, highlighting an immune-independent role for this IC [94].

It has been shown that the A2aR IC receptor is also expressed on the surface of GC cells. Activation of A2aR by adenosine promotes GC cell metastasis by enhancing PI3K-AKT-mTOR signalling [105]. The expression of A2aR positively correlates with TNM stage and is associated with poor outcomes [105]. Binding of adenosine to A2aR induces migration and invasion, increases pseudofoot and ciliary growth and ultimately enhances cell survival [105]. Human GC tumour xenograft mice injected with A2aR knockout GC cells had significantly reduced numbers and size of micrometastatic lung lesions compared to mice injected with GC cells expressing A2aR [105].

Collectively, these studies highlight the inhibitory effects of ICs on the various hallmarks of cancer (other than immune evasion), including chemotherapy resistance, DNA repair, proliferation, migration and invasion. This figure summarises the results from a range of studies demonstrating that activation of these pathways promotes proliferation, invasion, migration, metabolism, DNA repair and confers chemoresistance.
chemotherapy-induced DNA damage, thereby enhancing chemotherapy toxicity.

4. Clinical trials assessing ICI-chemotherapy combinations

There is minimal clinical data available to determine if the addition of ICIs to chemotherapy regimens will enhance the efficacy of chemotherapy in GOCs. However, a range of clinical trials are ongoing to answer this clinically relevant question.

Results from the phase III keyote-062 trial with 763 OGI/GC patients show the objective response rate is higher in the combined pembrolizumab and chemotherapy arm (cisplatin + 5-FU/capecitabine) versus the chemotherapy alone arm. The mOS was 10.6 months (pembrolizumab) versus 11.1 months (chemotherapy) and 12.5 months (combined pembrolizumab and chemotherapy). The mPFS was 2 months (pembrolizumab) versus 6.4 months (chemotherapy) and 6.9 months (pembrolizumab-chemotherapy) [18].

The addition of ICIs to chemotherapy for the treatment of lung cancer patients substantially enhanced the efficacy of chemotherapy-inducing synergistic effects with improved and durable clinical responses [109]. A phase II study in non-squamous non-small cell lung cancer (n = 123) demonstrated that the addition of pembrolizumab significantly enhanced response rates to carboplatin and pemetrexed, which led to the accelerated FDA approval (overall response rate: 55% for pembrolizumab-chemotherapy arm versus 29% for chemotherapy only arm) [110]. The PFS was 19 months for the pembrolizumab-chemotherapy arm versus 8.9 months for the chemotherapy only arm (median follow-up of 18.7 months) [110]. This is encouraging data for the use of ICIs to enhance response rates to chemotherapy regimens.

An ongoing phase II trial demonstrated that 39% of OSCC patients (9/31) achieved a pathological complete response to combination atezolizumab and CROSS treatment [111]. However, no CROSS only arm was included in the trial design so it is not possible to determine if adding atezolizumab to the CROSS regimen enhanced efficacy [111]. However, the authors suggest that despite the small cohort, this was a promising result as the CROSS trial demonstrated that 23% of OC patients (47/161) receiving CROSS achieved a pathological complete response rate compared with 39% of OSCC patients in this phase II trial (9/31) [9].

An ongoing phase II study (KEYNOTE-059, NCT02335411) in GC and OGI patients is investigating pembrolizumab in three different arms, which include: (1) pembrolizumab, 5-FU and cisplatin in treatment-naive patients, (2) single agent pembrolizumab in previously treated patients and (3) treatment-naive patients. Preliminary safety data from the pembrolizumab, 5-FU and cisplatin arm reported that grade 3–4 treatment related adverse effects were documented in 37% of patients (n = 18, median follow-up was 5.5 months). This trial is forecast to reach completion in May 2022 [112].

5. Co-blockade of multiple immune checkpoints in GOCs to enhance response rates

5.1. Co-blockade of ICs boosts anti-tumour immunity

The vast array of IC receptors and ligands expressed on immune cells and cancer cells suggests non-redundant functions with unique roles in immunological tolerance in cancer but ultimately synergise to inhibit T cell function. Co-expression of PD-1 with other ICs characterizes a more dysfunctional population of tumour-infiltrating lymphocytes (TILs) compared with TILs expressing PD-1 alone in GC patients [113]. Dual blockade of CTLA-4 and PD-1 can achieve a more effective anti-tumour immune response as both the CTLA-4 and PD-1 pathways inhibit T cell activation and function using non-redundant mechanisms [114]. Combined use of nivolumab and ipilimumab has been FDA-approved in melanoma, MSI-H and DNA mismatch repair-deficient metastatic colorectal cancer and kidney cancer [115]. Unfortunately, the majority of patients still fail to benefit from CTLA-4, PD-1 and PD-L1 ICIs. Therefore, co-blockade of novel ICs, such as TIM-3, TIGIT and LAG-3 are being investigated in clinical trials. These novel IC receptors exhibit unique functions in tissue sites where they each regulate distinct aspects of immunity [116]. It remains to be determined if specific ICs may be co-opted by specific cancer types to evade anti-tumour immunity depending on the tissue site. A greater fundamental understanding of the specialized functions of the array of ICs that exist will inform the rational design of therapeutic strategies for GOC patients.

A study by Zong et al., demonstrated that TILs in GC patients have increased expression of CTLA-4, TIGIT, TIM-3 and PD-1 and the percentage of peripheral blood PD-1 ‘TIM-3’ and PD-1 ‘TIM-3’ T cells was significantly higher than circulating levels in healthy donors [113]. Furthermore, the predominant fraction of TILs was comprised of PD-1 ‘TIM-3’ double positive cells [113]. PD-1 inhibition enhanced cytokine production in vitro and co-blockade of PD-1 and TIM-3 synergistically enhanced cytokine production (IFN-γ, TNF-α and IL-2) in vitro [113]. Additionally, the percentage of CD3+ TIM-3+ TILs expressing PD-1 positively correlated with tumour size and lymph node metastasis in GC patients [113]. The presence of lymph node metastasis and larger tumour sizes in patients with a higher percentage of CD3+ PD-1+ TILs may indicate immune escape due to immune exhaustion.

Wilms’ tumour 1 (WT1) is often overexpressed in cancer cells and knockdown of WT1 induces mitochondrial damage and inhibits cancer cell growth [117]. WT1 antigen is a promising target for DC-based vaccines in several malignancies, including GC [117]. The expression of TIGIT, PD-1 and TIM-3 was upregulated in GC patients with limited WT1-specific CD8+ T cell proliferation and function following administration of a WT1-targeted DC-based vaccine [118]. TIGIT-expressing PD1 ‘Tim3’ CD8+ T cells comprised the predominant subset of TILs, however, TIGIT ‘PD1 ‘Tim3’ represented the most dysfunctional subset of WT1-specific CD8+ T cells. Co-blockade of PD-1, TIGIT and TIM-3 enhanced the growth, proliferation and cytokine production (IFN-γ, TNF-α and IL-2) of WT1-specific CD8+ T cells in vitro [118]. This may suggest that the co-expression of TIGIT, TIM-3 and PD-1 may identify exhausted tumour-antigen specific T cells. Reinivigoration using TIM-3, TIGIT and PD-1 co-blockade may represent an attractive and tailored therapeutic approach to reinvigorate tumour-antigen specific T cells in this cohort of GC patients.

An additional study demonstrated that the level of PD-1+ , TIM-3+ and PD-1 ‘TIM-3’ peripheral blood CD4+ and CD8+ T cells was significantly higher in OC patients compared with healthy donors, whereas the expression of TIGIT was significantly lower [119]. While the expression of PD-1+ , TIM-3+ and TIGIT+ and PD-1 ‘TIM-3’ CD4+ and CD8+ T cells was significantly higher in OC tumour tissue compared with ‘normal’ adjacent oesophageal mucosa [119]. This suggests that single agent blockade of PD-1 alone may not be sufficient to reinvigorate exhausted T cells and perhaps co-blockade of TIM-3 and PD-1 may be required in these patients.

Studies in other cancers, such as colon carcinoma and fibrosarcoma tumour models have demonstrated that dual blockade of PD-1 and LAG-3 synergistically promotes anti-tumour immunity and reduces tumour growth [120]. Preliminary data from a phase I/IIa clinical trial testing the efficacy of combined PD-1 and LAG-3 blockade in advanced melanoma patients demonstrated an objective response rate of 12.5% (n = 48) [121]. This patient cohort was heavily pre-treated and either refractory or had relapsed on anti-PD-1/PD-L1 therapy [121]. Anti-PD-1 and LAG-3 expression in at least 1% of immune cells within the tumour margin achieved a higher objective response rate of nearly three-fold compared to those with less than 1% expression (20% (n = 25) versus 7.1% (n = 14), respectively) [121]. This clinical data suggests that upregulation of other ICs may play a role in immune escape and treatment resistance to other ICs and that combined blockade of LAG-3 and PD-1 in PD-1/PD-L1 refractory patients may help overcome resistance.

Collectively, these studies suggest that co-blockade of multiple ICs,
such as TIGIT, PD-1, LAG-3 and TIM-3, could achieve better reinvigoration of anti-tumour immunity than monotherapy, which warrants further investigation through clinical trials in GOC patients. However, the level of immune-related adverse events (irAEs) that accompanies co-blockade of multiple ICs is an important consideration.

5.2. Chemotherapy alters immune checkpoint expression on T cells; how will this inform the administration and timing of chemotherapy-ICI combinations for GOC patients?

The effect of chemotherapy on IC expression on T cells is an important factor that requires consideration. This information will enable rational design of chemotherapy-ICI combinations and help guide the appropriate timing of these treatments for trial design in GOC patients. Following neoadjuvant chemotherapy in GC patients the majority demonstrated a significant increase in the expression levels of CD4, CD8, PD-1, PD-L1 and TIM-3, as determined by immunohisto-chemical analysis (n = 60 paired) [122]. This increase in TILs further supports the immunogenic properties of chemotherapies in GC patients [122]. Additionally, the increase in ICs suggests potential exhaustion of the induced anti-tumour immune response and highlights a role for ICs in combination therapy in the neoadjuvant and the adjuvant setting for GC patients [122]. Interestingly, a small percentage of patients demonstrated a significant decrease in CD4, CD8, PD-1, PD-L1 and TIM-3 expression following neoadjuvant chemotherapy treatment, suggesting a subset of patients with a distinct tumour biology resistant to the immunomodulatory effects of chemotherapies and unlikely to achieve benefit from ICIs [122]. Changes in the expression of PD-1, PD-L1 and TIM-3 revealed strong pairwise correlation, further supporting the rationale for co-blockade of multiple ICs [122]. Furthermore, high expression levels of CD8, PD-1 and PD-L1 following neoadjuvant chemotherapy were positive prognostic factors of OS [122]. The increase in cytotoxic T lymphocytes and ICs may likely be surrogate markers of ongoing anti-tumour immunity and may stratify patients who could benefit from ICIs.

IC blockade is a promising therapeutic strategy in GOCs, however, a substantial proportion of tumours lack TILs or a pre-existing anti-tumour immune response [32]. Therefore, the timing of delivery of ICIs with other treatment modalities is critical. The addition of adjunct therapies, such as conventional chemotherapy regimens, offers an opportunity to stimulate anti-tumour immunity, whereby ICIs can prevent exhaustion of the subsequent induced anti-tumour immunity. Administering ICIs prior to chemotherapy would therefore be less effective as concurrent or potentially subsequent administration of immunogenic chemotherapy with IC blockade. Additionally, studies outlined in this review have shown that chemotherapy directly upregulates ICs on cancer cells in vitro, further supporting a rationale for administering ICIs concurrently with conventional chemotherapies.

6. Combination regimens increase the frequency of irAEs

Emerging evidence suggests that ICI-induced irAEs are associated with improved responses [123]. However, enhancing the efficacy of ICIs by the addition of chemotherapy may subsequently escalate irAE profiles and may ultimately represent a barrier to advancing dual ICI combinations and ICI-chemotherapy combinations in the clinic. It is recognised that the frequency of irAEs increases with the duration and dose of ICI administered [124]. Additionally, agents targeting CTLA-4 are associated with more frequent irAEs compared to agents targeting PD-1 and PD-L1, and combinations of both anti-CTLA-4 and anti-PD-1/PD-L1 agents results in higher incidences of irAEs [125]. Thus, the choice of ICI has a considerable impact on irAE frequency and intensity. To date, trials in GOCs combining chemotherapy with ICIs indicate a manageable safety profile, although higher incidences of irAEs in the ICI-chemotherapy arm compared with ICI alone arm is often reported. In a phase I/IIb trial of GC patients testing the efficacy of anti-PD-1 alone versus combination anti-PD-1-oxaliplatin 77.6% experienced at least one irAE and 22.4% experienced a grade 3 or higher irAE in the anti-PD-1 arm. However, in the anti-PD-1-oxaliplatin arm 94.4% of patients experienced at least one irAE and 38.9% experienced at least one grade 3 or higher irAE. In the phase III keynote-062 trial with 763 OJG and GC patients, the incidence of grade 3–5 drug-related adverse events was 17% for the pembrolizumab arm, 69% for the chemotherapy arm and 73% for the combination pembrolizumab-chemotherapy arm [18]. However, although an increase in irAEs was observed with the addition of chemotherapy, the safety profile reported was clinically manageable [37].

7. Concluding remarks

The studies outlined in this review highlight both an immune- and non-immune-based rationale for blocking ICs on cancer cells, either alone or as part of a multimodal chemotherapies regimen. ICIs could potentially limit immune resistance, but also block the various tumour-promoting functions of IC cancer cell-intrinsic signalling. Further studies are required to investigate which ICs are expressed by cancer cells in GOCs and to elucidate the immune-dependent and -independent functions of these ICs. This will inform the rational design of clinical trials testing ICI combinations with conventional cytotoxic regimens to achieve better response rates for GOC patients.

Development of clinically applicable prognostic and predictive tools will be crucial in stratifying patients into suitable treatment arms, such as those likely to benefit from ICI monotherapy and those who are unlikely to respond. For non-responders, the development of clinically applicable methods to identify what specific therapeutic combinations, if any, are required and capable of sensitising immunologically ‘cold’ tumours to ICIs are needed. Combination chemotherapy and ICI regimens offer a potential strategy to alter the TME sufficiently to convert ‘cold’ tumours to ‘hot’ tumours with the ultimate outcome of converting non-responders to responders.

Author contributions

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Declaration of competing interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Abbreviations

A2aR Adenosine A2a receptor
ATP Adenosine tri-phosphate
BSC Best supportive care
CI Confidence interval
CPS Combined positive score
CRT Calreticulin
CRTR Calreticulin receptor
CSCs Cancer stem-like cells
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