The role of immunogenic cell death in gastrointestinal cancer immunotherapy (Review)

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Abstract. Modern cancer immunotherapy techniques are aimed at enhancing the responses of the patients' immune systems to fight against the cancer. The main promising strategies include active vaccination of tumor antigens, passive vaccination with antibodies specific to cancer antigens, adoptive transfer of cancer-specific T cells and manipulation of the patient's immune response by inhibiting immune checkpoints. The application of immunogenic cell death (ICD) inducers has been proven to enhance the immunity of patients undergoing various types of immunotherapy. The dying, stressed or injured cells release or present molecules on the cell surface, which function as either adjuvants or danger signals for detection by the innate immune system. These molecules are now termed 'damage-associated molecular patterns'. The term 'ICD' indicates a type of cell death that triggers an immune response against dead-cell antigens, particularly those derived from cancer cells, and it was initially proposed with regards to the effects of anticancer chemotherapy with conventional cytotoxic drugs. The aim of the present study was to review and discuss the role and mechanisms of ICD as a promising combined immunotherapy for gastrointestinal tumors.

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1. Introduction

Modern cancer immunotherapy has been proposed to involve four primary promising strategies: i) Active vaccination of tumor antigens, ii) passive vaccination with antibodies specific to cancer antigens, iii) adoptive transfer of cancer-specific T cells, and iv) manipulation of the patient's immune response by inhibiting immune checkpoints. Additional emerging strategies include other antigen-non-specific interventions, including the applications of oncolytic or immune-enhancing viruses, innate immunity stimulators and immunogenic cell death (ICD) inducers (1). The focus of the present review was to describe the treatments that employ ICD to enhance the immunity of patients with gastrointestinal (GI) cancers.

The daily death of billions of ordinary cells from the human body goes essentially unrecognized by the immune system. This is crucial since the conservation of the entire bodies homeostasis includes the continuous turnover of various cell compartments. As such, the initiation of an immune response against dead-cell antigens would have detrimental outcomes. Conversely, the death of a few cells infected by a microorganism can trigger a potent antigen-specific immune reaction, which is associated with the clearance of the invading pathogen from the body and also enables the establishment of long-term

immunological memory (2). The first proposal of the 'danger theory' in 1994 by Matzinger (3) was that the immune system can distinguish between dangerous and innocuous endogenous signaling. In her famous essay, Matzinger suggested that 'unprogrammed cell death' could give rise to the unusual release of internal molecules from the cytoplasm, nucleus or membrane to activate dendritic cells (DCs). The dying, stressed or injured cells release or present molecules on the cell surface, which can function as either adjuvants or danger signals for detection by the innate immune system. These molecules are now termed 'damage-associated molecular patterns' (DAMPs) (4). The term 'ICD' was introduced to indicate a type of cell death that triggers an immune response against dead-cell antigens, particularly those derived from cancer cells, including DAMPs. This model was initially proposed with regard to anticancer chemotherapy, in view of clinical proof demonstrating that tumor-specific immune responses reflect the efficacy of anticancer treatments using conventional cytotoxic drugs (5).

Currently, various routinely employed anticancer agents include doxorubicin, epirubicin, idarubicin, mitoxantrone, bleomycin, bortezomib, cyclophosphamide (CY) and oxaliplatin. The list also includes certain anticancer agents that are currently under preclinical or clinical development, such as some microtubular inhibitors of the epothilone family. Certain drugs, including digoxin, digitoxin and zoledronic acid, act to convert otherwise non-immunogenic events of cell death into *bona fide* ICD inducers, and may thus be used as adjuvants in combinatorial immunotherapy regimens (6). The known clinically applied or experimental anticancer agents that induce ICD act via one or several of the following mechanisms: Inducing apoptosis, causing a severe focused stress of the endoplasmic reticulum, overcoming loss-of-function mutations that hide danger signals during tumorigenesis and downregulating the cancer-based induction of pro-inflammatory transcription factors (4). In addition, one important consideration is the complex interactions with DAMPs and their receptors, known as the pattern recognition receptors. Attempts have been made to identify and detect multiple DAMPs in order to facilitate the development of next-generation anticancer regimens, which, in addition to killing cancer cells, can simultaneously convert them into a cancer-specific therapeutic vaccine (7).

2. GI cancers

GI cancers are amongst the malignancies most frequently diagnosed in European patients. These include gastric cancer (GC), colorectal cancer (CRC), as well as cancers affecting the liver, particularly hepatocellular carcinoma (HCC), the biliary tract, such as cholangiocarcinoma (CCA), and the pancreas (pancreatic cancer; PC). The frequency with which these conditions are diagnosed presents a significant challenge for public health systems in Europe and worldwide (8).

Different cancers of the GI tract. Most commonly reported in patients in Asia, GC is notable for its particularly poor survival rates. The condition is associated with certain bacterial infections, such as *Helicobacter pylori* (*H. pylori*) infection, and the effects of other pathogens, including Epstein-Barr virus (9).

The annual number of new CRC diagnoses falls in the range of 1-2 million cases, placing CRC third in terms of the most frequently occurring cancers, and fourth amongst the most common causes of cancer-related mortality. The main CRC risk factors include age and history of chronic disease, as well as various aspects of patient lifestyles. There are three different pathogenic mechanisms that can lead to the onset of CRC: Microsatellite instability, chromosomal instability and a CpG island methylator phenotype (10,11).

The most common type of liver cancer is HCC, which originates from hepatocytes and accounts for ~4 in 5 liver cancer diagnoses, with an increased prevalence in China and Eastern Africa (12). The onset of HCC is often a consequence of the interaction between genetic characteristics and environmental factors. In particular, patients diagnosed with liver cirrhosis, or infection with the hepatitis B virus (HBV) or the hepatitis C virus (HCV), are more likely to develop HCC, while other risk factors include alcohol abuse, the ingestion of aflatoxin B1 or non-alcoholic steatohepatitis (13,14).

CCA is the second most frequently occurring primary hepatic cancer after HCC. CCA has been most commonly reported across Asia, although in recent years it has become increasingly more widespread in North America and Europe (15,16). CCA originates in the biliary tract and is subdivided by location into three different subtypes, namely perihilar, intrahepatic and distal CCA. Several risk factors, both common and rare, have been associated with CCA, including hepatobiliary parasites, Caroli disease, HBV and HCV infection, and exposure to toxins (17-19).

PC is the 14th most prevalent type of cancer and the seventh largest cause of annual cancer-associated deaths globally. PC risk factors include obesity, alcohol consumption and smoking, as well as *H. pylori* infection (20).

Latest approaches to GI cancer treatment. Several potential treatments are currently being developed for GI cancers. A number of these are targeted approaches that make use of biological properties to achieve their objectives, and can be employed alone or as components of combination or adjuvant treatments. The most widely applied treatments at present include surgery, as a means of resecting solid tumors; radiation therapy, as a means of managing localized solid tumors; chemotherapy, which involves the use of cytotoxic agents to eliminate cancerous cells; and hormonal therapy, which serves as a systematic approach with the aim of targeting all cancerous cells found in the body (21-23).

3. Immunotherapy in GI cancers

Patients diagnosed with GI cancers are typically subjected to a combination of treatments, including surgery, chemotherapy and/or radiation therapy; however, the survival rates remain poor, particularly when the cancer has reached an advanced stage, or in the case of metastatic disease (24,25). For this reason, it is imperative to develop more effective, novel techniques to address the problem, and immunotherapy appears to hold promise for this purpose. To date, a number of cytokines, including IFN-γ or IL-2, have been used to limit the activity of certain types of cancer, such as renal cell carcinoma and melanoma, and a moderate level of inhibitory activity has been reported (26). However,

the development of cancer vaccines has been less successful, with none generating statistically significant responses in test patients (23).

The progress in immunotherapy shows great potential in the context of GI cancers, whereas further therapies involving the administration of immunostimulatory monoclonal antibodies to treat GI malignancies are currently in their developmental phase. Immune checkpoint blockade is the primary type of immunotherapy currently used in GI cancer treatment. It can be anticipated that the vaccination approach will be streamlined with the lessons learned from initial successes, and the most suitable tumor-associated antigens will be identified for targeting. Adoptive cell therapies are now at an advanced stage of development and appear to hold significant promise for GI cancers. An improved understanding of the prevalent suppressive factors in patients with GI cancers may enable the development of superior strategies to limit immune suppression and promote endogenous immunity in patients. It is likely that deeper knowledge of the tumor microenvironment and the field of immunology will lead to the successful development of more efficacious treatments in the near future.

4. Vaccine therapy in GI cancers

Various cancer vaccines have been developed to date by using different technologies, including recombinant microorganisms, recombinant antigen cocktails, oncolytic viruses, DNA and gene therapy-based treatments, and anti-idiotypic antibodies. In addition, personalized vaccines have also been devised, including those based on adoptive cell transfer, or autologous cells and antigens. These may be more complex, and require specialized manufacturing approaches and expertise (27). However, although several trials have been conducted, approval has only been granted for one vaccine, which acts against metastatic castration-resistant prostate cancer. This vaccine is Dendreon's Provenge® (Sipuleucel-T), which was approved by the Food and Drug Administration in 2010 (28,29). Furthermore, two additional cancer vaccines, Vitespen® (30) and Melacine® (31), were approved in Russia and Canada, but not in the United States. These cancer vaccines rely upon the activation and strengthening of antitumor responses that target cancer. The dendritic cell-based cancer vaccine presents antigens and serves a critical role in formulating the immune response. They can also activate B cells, natural killer (NK) cells, as well as naïve and memory T cells, through the presentation of tumor antigens associated with major histocompatibility complex (MHC) molecules. When patients with cancer have higher numbers of DCs penetrating the tumors, this is a sign of reduced lymph node metastases and improved chances of survival (32). A number of approaches have been employed to create vaccines by loading DCs with tumor antigens. These include the use of synthetic peptides pulsed on DCs (33), DCs engineered with plasmid DNA (34), RNA (35) or viruses (36), tumor cell lysates combined with immature DCs (37) and, finally, DCs combined with whole tumor cells through polyethylene glycol or electroporation (38). The technique that uses DCs pulsed with MHC-restricted peptides, which are obtained from antigens known to be associated with tumors, is the most common type of vaccine approach (39-41). However, it can be challenging to use DCs for clinical

purposes, as these cells have a relatively short life span (42). To date, vaccines have shown little effectiveness in preventing GI cancers. The vaccines developed thus far have targeted melanoma-associated antigen (MAGE)-A3 (43), HER2 p369 peptide (44), gastrin-17 diphtheria toxoid (45), URLC10 or VEGFR1 epitopes (46) and heat shock protein (HSP) gp96 (47) in patients with GI cancers. Furthermore, chemotherapy has been tested alongside adjuvant Bacillus Calmette-Guérin (48). In cases of PC, a number of specific antigens serve as targets, including carcinoembryonic antigen (CEA), EGFR, Wilms' tumor 1 and VEGF, whereas GVAX® is a whole-cell vaccine that has been tested in trials in the metastatic, neoadjuvant and adjuvant settings. Positive outcomes have been reported in the metastatic setting when combined with ipilimumab, with a reported survival rate of 27% after 1 year (49).

In the case of HCC, vaccines have shown no efficacy thus far. However, immune responses have been reported in phase I trials involving peptide vaccines (50), DC vaccines and tumor-associated antigens targeting oncolytic viruses, such as AFP (51), GPC-3 (52) and human telomerase reverse transcriptase (53). DC-based vaccines have been included in trials on CCA, whereas a phase II study was conducted in the adjuvant setting in order to target mucin 1 (MUC1) in the biliary tract and in PC via DC vaccination, and the tolerability was reported to be good, although it was not possible to draw definitive conclusions regarding the immune response (54). Further phase II clinical trials involving CRC-specific peptide vaccines were performed with patients diagnosed with HLA-A*2402-positive stage III CRC, with the findings suggesting that an immune response would be generated by the vaccine, leading to higher survival rates (55). For patients with PC, 13-mer mutant ras peptide vaccines were not only shown to be safe, but to also generate the appropriate immune response (56). Additionally, the p53 synthetic long peptide vaccine has been shown to be safe for patients with metastatic CRC in whom specific T-cell responses were induced (57). However, several issues must be addressed if a novel cancer vaccine is to be approved, including commercial, clinical, manufacturing, operational and regulatory concerns. Risk evaluation must also be conducted to make full use of the expertise available. Moreover, a number of clinical studies revealed that some patients may not benefit from the cancer vaccine treatment (primary resistance), and some responders may relapse after a period of response (acquired resistance). For example, PC is associated with the presence of a highly immunosuppressive microenvironment, which is characterized by a dense desmoplastic stroma that impedes blood flow to the area, inhibits drug delivery and suppresses antitumor immune response (58). In CRC and GC, it has been shown that only patients with the subset of mismatch-repair-deficient or microsatellite instability-high tumors are likely to respond to immunotherapy (59,60). Once these issues have been addressed, ICD may prove to be the answer for vaccine development.

5. Cancer vaccination is the target of ICD

DC maturation is a crucial step for immune activation. Once in the lymph nodes, DCs activate T cells via three canonical signals: Binding of T-cell receptors, co-stimulatory receptor

engagement, and the provision of cytokines and chemokines to facilitate T-cell polarization and differentiation. The ICD complementarily activates DCs. High mobility group box 1 (HMGB1) and HSP activate pro-inflammatory DCs through the Toll-like receptor (TLR)2/4-MyD88-NF-κB signaling pathway (61). Moreover, dying cancer cells also express calreticulin (CRT) on their plasma membrane, which signals to facilitate engulfment by DCs. The exposed CRT on the cell membrane can bind to CD91, the low-density lipoprotein-receptor-related protein 1, which promotes the engulfment of cellular compartments and debris by a mechanism that depends on the Rac family small GTPase 1 (61). Hence, the original concept for using the combination treatment of ICD stimulation and DC-based anticancer vaccines originated from significant evidence that cancer treatments, such as chemotherapy, radiation, phototherapy, phytotherapy and immunotherapy, could elicit danger signals from dying cancer cells. Notably, chemoradiotherapy is known to elevate serum HMGB1 in patients with esophageal squamous cell carcinoma, and the levels of HMGB1 were found to be positively correlated with patient survival (62). Moreover, oxaliplatin and mitoxantrone induced cancer cell death accompanied by an exposure of CRT and a release of HMGB1, HSP70 and ATP, thereby strongly inducing *in vitro* immune responses of DCs (63,64). Interestingly, it has been demonstrated that pretreatment with the oxaliplatin nanoparticle followed by a rechallenge by tumor inoculation in PC-bearing mice can enhance therapeutic efficacy by increasing the numbers of tumor-infiltrating activated cytotoxic T cells (64). In addition to the use of chemotherapy, botanical cancer therapy has also been introduced. Treatment with *Hemidesmus indicus* was shown to induce CRC cell apoptosis characterized by surface expression of CRT, increased HSP70 expression, and a release of ATP and HMGB1. This immunogenic agent is promising when combined with a DC-based anticancer vaccine (Table I) (65). Recently, our group demonstrated that a potent bioactive compound, honokiol, can induce CCA cell apoptosis, which is associated with the release of HMGB1 and HSP90. Incubation of DCs with CCA cells that were pretreated with honokiol induced DC maturation, and thus enhanced the priming of cytotoxic T cells to kill cancer cells (37). Therefore, the priming of DCs with immunogenic agents may maximize antitumor responses though DC stimulation.

Radiation as a cancer treatment also induces ICD, which may pave the way for anticancer vaccines in such patients. For example, in large orthotopic HCC, synergistic antitumor effects may be obtained when radiation is combined with the administration of IL-12. This has been shown to be associated with the activation of tumor-infiltrating DCs, CD8+ T cells and NK cells, as well as the suppression of tumor-infiltrating myeloid-derived suppressor cells (MDSCs) (66). Similar to radiation, phototherapy has been suggested to be a new platform for enhancing the immunogenicity of cancer. Photodynamic and photothermal therapies proficiently promote immunotherapy via the induction of ICD. In addition, phototherapy combined with oxygenation boosters can promote CRC cell apoptosis and induce ICD, thus facilitating DC maturation and inhibiting tumor growth and recurrence in animal models (67). Plasma-treated PBS, which is physical cold atmospheric plasma consisting of reactive oxygen and

nitrogen species, has demonstrated cytotoxic activity against PC cells with immunogenic features. This increases the potential of phagocytosing DCs and DC maturation, which may hold promise for combinations with DC cancer vaccines (68).

Several clinical studies have corroborated the concept that ICD-inducing pretreatment may act as an immunomodulator (Table I). There are ~50 trials currently investigating the benefit of ICD in vaccines against cancer, mostly PC and CRC. A phase I/II trial study, in which 22 patients with HCC have been enrolled, is investigating treatment interventions comprising pre-infusion of CY and vaccination using a multi-peptide-based HCC vaccine (IMA970A) plus CV8102 adjuvant (RNAdjuvant®). This trial is investigating whether IMA970A and CV8102 are safe and whether they can trigger an immune response against the tumor under pre-conditioning by induction of ICD (NCT03203005). Moreover, mFOLFOX6, which is a formulation of 5-fluorouracil (5-FU), leucovorin and oxaliplatin, has been used in combination with nivolumab and vaccination with a CV301 peptide vaccine in patients with advanced CRC (NCT03547999). Another clinical trial including patients with PC used CY followed by vaccination with a GVAX peptide cancer vaccine. However, pretreatment with CY did not improve overall survival or disease-free survival when compared with the uncombined intervention (NCT00727441). Collectively, the majority of preclinical and clinical data have demonstrated that combining the immunogenic potential of ICD with cancer vaccination is a promising approach that could achieve future translational success.

6. Checkpoint inhibitor therapies in GI cancers

The field of immuno-oncology has witnessed significant advances with regard to immune checkpoint inhibitors (ICIs). Immune checkpoints are the mechanisms through which T-cell immune responses are regulated (69). Tumor cells are considered to be able to avoid host immune clearance when T-cell immune responses are downregulated. If immune checkpoints can be targeted, the endogenous response of the immune system to tumors may be used as a means of addressing the risk of disease. A number of antibodies have been shown to be effective against immune checkpoints, and more are under examination (69). These antibodies most commonly serve to target cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1). CTLA-4 acts as a receptor capable of inhibiting the activation of T cells (70). Accordingly, when CTLA-4 is blocked, the T cells will proliferate and become activated. PD-1 is expressed on T cells and other immune cells, and PD-L1 serves as one of its ligands. PD-1 and PD-L1 binding creates an inhibitory signal affecting T cells; by contrast, if the binding is inhibited, the T cells will become activated, leading to a heightened response from cold tumor (non-inflammatory T-cell) to hot tumor (inflammatory T-cell) immune response (71-73). Another targeted checkpoint receptor protein is the lymphocyte activation gene-3, which may control the activity of T cells through its binding with MHC class II-molecules (74). However, earlier studies examining such checkpoint inhibitors in CRC were not very successful, phase II trials involving the CTLA-4 inhibitor, tremelimumab, in patients diagnosed with metastatic CRC have shown no significant levels of efficacy (75,76).

Table I. List of studies on immunotherapies associated with ICD in gastrointestinal cancers.

Type of therapy	Type of cancer	ICD inducer	Treatment condition	Summary of results	Reference/NCT
DC-based anticancer vaccine	PC	Encapsulated oxaliplatin induced exposure of CRT and release of HMGB1 HSP70 and ATP	Vaccination with oxaliplatin-treated cancer cells and subsequent rechallenge with tumor inoculation	Enhanced the therapeutic efficacy by increasing tumor-infiltrating cytotoxic T cell numbers	(64)
	HCC	Radiation	Combination treatment of radiation and IL-12 in an orthotopic tumor model	Inhibited tumor growth by activation of DCs, tumor-infiltrating CD8^{+} T cells and NK cells, and suppression of tumor-infiltrating MDSCs	(66)
	CCA	Honokiol induced release of HMGB1 and HSP90	Culture of DCs with honokiol-treated CCA cells, and subsequently priming with T lymphocytes for cancer cell killing in vitro	Honokiol-treated CCA cells induced DC maturation and type I IFN production and enhanced cancer cell killing by effector T lymphocytes	(37)
	CRC	Phototherapy induced CRT exposure and release of ATP	Combination treatment of phototherapy and oxygenation booster in tumor-bearing mice	Facilitated DC maturation and inhibited tumor growth and recurrence	(67)
	CRC	S-1/CPT-11 chemotherapy	Phase I trial to evaluate the safety and immune response of RNF43-721 peptide vaccine combined with chemotherapy	No results yet	NCT00641615
	HCC	CY	Phase I/II vaccination of multi-peptide-based HCC vaccine plus adjuvant by a single pre-vaccination of low-dose (CY) in patients with very early, early, intermediate stage HCC	No results yet	NCT03203005
	CRC	mFOLFOX6	Phase II trial of perioperative CV301 vaccination in combination with nivolumab and systemic chemotherapy for metastatic CRC	No results yet	NCT03547999
	GC or gastroesophageal cancer	Cisplatin and 5-FU	Phase III trial of G17T immunogen in combination with chemotherapy in patients with metastatic or locally recurrent cancer	No results yet	NCT00020787
	PC	CY	Phase II study by pretreatment with CY before injecting with GVAX peptide cancer vaccine	Overall survival of 34.2, 15.4 and 16.5 months, and disease-free survival of 18.92, 8.54 and 5.56 months for GVAX without CY, GVAX with intravenous CY and GVAX with p.o. CY, respectively	NCT00727441

Table I. Continued.

Type of therapy	Type of cancer	ICD inducer	Treatment condition	Summary of results	Reference/NCT
Immune checkpoint blockade Immune	CRC	Cisplatin and CBP501 induced cell surface exposure of CRT and release of HMGB1	Combination treatment with cisplatin, CBP and anti PD-1 or PD-L1	CBP501 enhanced cisplatin-induced ICD and increased the efficacy of immune checkpoint inhibitors against tumors in mice	(80)
	CRC	PARP and PI3K inhibitors induced exposure of CRT on cell membrane	Nano-formulation of PARP and PI3K inhibitors combine with X-ray irradiation and anti-CTLA-4 immunotherapy	PARP and PI3K inhibitors sensitized efficacy of anti-CTLA-4 by improved tumor control and increased tumor infiltrating lymphocytes	(81)
	CRC	Lurbinectedin induced exposure of CRT, release of ATP, release of HMGB1 and type I IFN responses	Combination treatment of lurbinectedin and immune checkpoint blockade	Strong antitumor response as both prophylaxis and therapy in a cancer-bearing mouse model	(123)
	CRC	NIR-PIT induced release of cellular ATP and HMGB1	CD44-targeted NIR-PIT combined with CTLA4 or PD-1 blockade	Combination of NIR-PIT with antibody blockade showed greater tumor inhibition with prolong survival	(83)
	CRC	Trifluridine/tipiracil plus oxaliplatin induced surface CRT exposure, eIF2a-dependent endoplasmic reticulum stress, HMGB1 and ATP release	Trifluridine/tipiracil plus Oxaliplatin combined anti PD-1 antibody	Combination treatment enhanced the antitumor efficacy via elimination of type-2 tumor-associated macrophages	(124)
	HCC	Oxaliplatin elicited cell surface exposure of CRT and release of HMGB1	Combination treatment with oxaliplatin and anti-PD-1	Combination therapy with oxaliplatin and anti-PD-1 inhibited tumor growth, activated CD8+ T-cell expansion and DC maturation	(82)
	CRC	FOLFOXIRI	Phase II, FOLFOXIRI plus bevacizumab with atezolizumab treatment for patients with unresectable metastatic CRC	No results yet	NCT03721653
	CRC	FOLFOX	Phase I/II, FOLFOX combination treatment with durvalumab and tremelimumab in patients with metastatic CRC	No results yet	NCT03202758
	GC	5-FU and cisplatin	Phase II, 5-FU and cisplatin combination treatment with pembrolizumab in patients with advanced GC or gastroesophageal junction cancer	Pembrolizumab + 5-FU + cisplatin showed manageable safety and promising antitumor activity	NCT02335411

Table I. Continued.

Type of therapy	Type of cancer	ICD inducer	Treatment condition	Summary of results	Reference/NCT
T-cell adoptive transfer	CRC	Mitochondria-targeted small molecule, IR-780 induced cell surface exposure of CRT and release of HMGB1 and ATP	Combination treatment of cytotoxic T-cell adoptive transfer with IR-780	Improves the inhibitory effect on tumor growth due to DC maturation and synergistic effector T-cell priming and tumor infiltration	(119)

PC, pancreatic cancer; HCC, hepatocellular carcinoma; CRC, colorectal cancer; GC, gastric cancer; ICD, immunogenic cell death; HMGB1, high mobility group box 1; CRT, calreticulin; MDSC, myeloid-derived suppressor cell; DC, dendritic cell; NK cells, natural killer cells; Treg, regulatory T cell; TLR, Toll-like receptor; PD1, programmed death 1; PD-L1, programmed death ligand 1; CY, cyclophosphamide; FOLFOX, oxaliplatin, 5-FU and leucovorin; FOLFOXIRI, FOLFOX and irinotecan; 5-FU, 5-fluorouracil; HSP, heat shock protein; CBP, calmodulin-binding peptide; NIR-PIT, near-infrared photoimmunotherapy; CTLA-4, cytotoxic T lymphocyte-associated protein 4; eIF2a, eukaryotic translation initiation factor 2A; PARP, poly adenosine diphosphate-ribose polymerase.

As a consequence of tumor heterogeneity, along with the complexity of immunosuppression, treatment of GC, as has been the case with CRC, has shown little success in the area of immunotherapy, for reasons possibly related to mechanisms that are incompletely understood. Previous findings have revealed that a certain level of checkpoint inhibition can be achieved, often in correlation with enhanced classification and characterization of GC and an improved understanding of its histopathology (48). PC presents the greatest challenge amongst all types of GI cancer in the context of immunotherapy, most likely as a result of inadequate immunogenicity, along with a low mutational burden and a unique vascular and stromal microenvironment. These conditions make it very difficult for the immune cells and molecules to penetrate into the tumors, particularly compared with the conditions in other types of cancer (77). If the microenvironment exhibits immunosuppressive qualities, checkpoint inhibition may represent a suitable goal for HCC immunotherapy, although success has been limited to date in the case of single-agent checkpoint blockade (78).

7. Induction of ICD sensitizes blockade inhibitor therapies

In this decade, antibodies directed against immune checkpoints have been intensely investigated in patients with cancer following the discovery by the Nobel prize winners James P. Allison and Tasuku Honjo, who demonstrated that cancer therapy can be enhanced by the inhibition of negative immune regulation (79). CTLA-4 and PD-1 are the receptors that are commonly found on the surface of activated T cells. The interaction of CTLA-4 and PD-1 with their ligands inhibits activated T cells and converts them into exhausted T cells, which abrogates the antitumor response. The targeting of CTLA-4 and PD-1 molecules has demonstrated durable response rates, increased survival time of responders and a manageable safety profile. Recently, checkpoint inhibition plus chemotherapy has been considered for use in the first-line setting for the treatment of CRC (11). The potential clinical responses may be associated with the induction therapy with ICD inducers and cancer immunotherapy.

It has been reported that calmodulin-binding peptide (CBP)501, a CBP that can induce ICD when combined with cisplatin treatment, can induce cell membrane exposure of CRT and the release of HMGB1. Treatment of CRC-bearing mice with CBP501 and cisplatin, and subsequently with anti PD-1 or PD-L1 antibodies, significantly enhanced the antitumor activity of immune blockade via upregulating the percentage of tumor-infiltrating CD8^{+} T cells (80). Similarly, in CRC-bearing mouse models, the combination treatment of PARP and PI3K inhibitors induced radio-sensitization and the induction of ICD. Thus, subsequent anti-CTLA-4 treatment strongly inhibited tumor growth and increased the numbers of tumor-infiltrating lymphocytes (TILs) (81). The induction treatment of not only CRC, but also HCC, has been investigated using immunogenic chemotherapy, through oxaliplatin promoting the exposure of CRT and the release of HMGB1. In HCC, oxaliplatin combined with anti PD-1 antibodies achieved marked tumor suppression, activation of CD8^{+} T cells and stimulation of DCs (82). Moreover, radiation and phototherapy are additional examples of immunogenic cancer

therapies that sensitize the immune checkpoint blockade. CD44-targeted near-infrared photoimmunotherapy combined with anti CTLA-4 and PD-1 antibodies was shown to inhibit tumor growth and prolong the survival of CRC-bearing mice via a mechanism associated with the induction of ATP and the release of HMGB1 (83).

The ICD inducer-enhancing immune blockade has been proven in several clinical reports (Table I), but has not been well established in GI cancers. Current phase II trials have been investigating the ICD-inducing effect of 5-FU, leucovorin and oxaliplatin (FOLFOX) and FOLFOX plus irinotecan (FOLFOXIRI) to enhance the efficacy of ICIs. Patients with unresectable metastatic CRC have been treated with either FOLFOX/FOLFOXIRI or anti-PD-L1/CTLA-4 antibodies. After each treatment cycle, the safety, disease progression, death and intolerable toxicity will be continuously recorded (84,85). Furthermore, the use of 5-FU and cisplatin is encouraged owing to their safety and strong enhancement of the antitumor response in advanced GC when combined with pembrolizumab (86).

The insight into the mechanism of ICD-sensitized immune blockade inhibitors is under investigation. Two possible effects have been proposed, depending on the type of ICD inducer. The first effect is the direct consequence of the chemotherapeutic agents proficiently upregulating PD-L1 expression. Those that exert this effect include 5-FU, gemcitabine, cisplatin, oxaliplatin, doxorubicin and paclitaxel (87). This is an important method for increasing the sensitivity to blockade inhibitors. Treatment with FOLFOX can activate the secretion of IFN-γ from PD-1$^+$ CD8$^+$ T cells, which is associated with the overexpression of PD-L1 on tumor cells. Hence, the combination treatment of FOLFOX with anti PD-1/PD-L1 antibodies has achieved complete cure in CRC-bearing mice, while monotherapy was unsuccessful (88). Similarly, the anthracycline drug, epirubicin, could upregulate PD-L1 expression in HCC, which causes sensitization to immune blockade therapy (89). The other effect would be due to the indirect function of ICD inducer agents via modulating the tumor microenvironment. This may be associated with the depletion of regulatory T cells and MDSCs that potentiates stronger antitumor responses (66). Taken together, these results from preclinical and clinical studies have indicated that the concept of priming treatment with ICD inducer agents prior to checkpoint blockade treatment could elicit a stronger antitumor response.

8. Adoptive therapies in GI cancer

A potential approach to cancer treatment is harnessing the properties of T cells and NK cells to attack tumor cells. NK cells and TILs are of both predictive and prognostic value in GI cancers (90). These properties can be applied through the various modalities of adoptive cell therapies, typically involving the isolation of immune cells obtained from patients diagnosed with cancer, whereupon they can be genetically modified in order to strengthen their capacity to identify and kill cancerous cells. By expanding these isolated cells *ex vivo*, they can then be re-introduced to the patient, in a form of treatment which can theoretically be effective for all patients with cancer who do not demonstrate adequate cancer immunity without assistance, and would therefore be incapable of

responding to ICIs. Adoptive cell therapy as a cancer treatment can involve various strategies, which have either been previously examined in clinical settings, or are undergoing trials to assess their suitability against GI cancers (91). One particular approach of adoptive cell therapy makes use of the immunotherapeutic properties of cytokine-induced killer cells (CIK), which can be obtained through the treatment of peripheral blood lymphocytes using IFN-γ. This is a type of monoclonal antibody that counteracts the CD3 molecule, along with IL-2. CIK cells are predominantly expansions of CD3$^+$/CD8$^+$/CD56$^-$ cells to become terminally differentiated CD56-positive NK cells. These are uniquely able to identify the tumor cells regardless of the presence or absence of antibodies and MHC molecules and, accordingly, have the ability to identify any tumor cells lacking MHC molecules on their surface (92). In addition to T-cell adoptive transfer, NK cell-based immunotherapy has shown promising antitumor effects in a number of studies (93-95). Adoptive transfer is being used to increase the infiltration of NK cells in the tumor site by using NK cells from different origins, such as autologous cells, allogeneic peripheral blood mononuclear cells, umbilical cord blood, human embryonic stem cells and induced pluripotent stem cells (90). However, although preclinical data indicate high efficacy of NK cell adoptive transfer in *in vitro* and *in vivo* studies, there is little information on the clinical efficiency of this method. A phase I clinical trial demonstrated that HSP70-induced activation of autologous NK cells was achieved in patients without any treatment-related negative side effects, but no significant clinical response was observed due to the high tumor burden and limited sample size (96). Moreover, adoptive macrophage transfer has recently become a hot research field (97,98). Due to their innate immune function and more prominent penetrating ability, macrophages may kill tumor cells when T-cell therapy fails (99). However, sufficient clinical evidence is urgently needed to support pre-clinical data, particularly in the field of GI cancer treatment.

In order to make adoptive cell therapy more readily applicable and more effective in destroying tumor cells, relatively more recent approaches have sought to introduce antitumor antigen receptors to regular T cells, which may have promising therapeutic applications. It is possible to eliminate the requirement for MHC interaction and co-stimulatory molecules when T cells are engineered to include chimeric antigen receptors (CAR), in which the B-cell receptor-derived and T-cell receptor domains are combined (100,101). To date, the antitumor qualities from the adoptive transfer of CAR-T cells has been evident in the case of advanced hematological malignancies, but in the case of solid tumors the results have been less promising (102,103). It may be possible to explain this as a consequence of the expression of heterogeneous tumor antigens, the immunosuppressive network activity within the microenvironment of the tumor, the T-cell trafficking into solid tumors, which is less than optimal, and the absence of the necessary co-stimulatory signals to achieve CAR-T cell persistence following infusion (100,101). The affinity of HER2-directed CAR-T cells for cancer cells of the GI tract is high, even for cells exhibiting low levels of HER2 expression. Moreover, CAR-T-HER2 cells may offer the potential to inhibit disease recurrence of metastasis. Specific CAR-T-HER2 cells
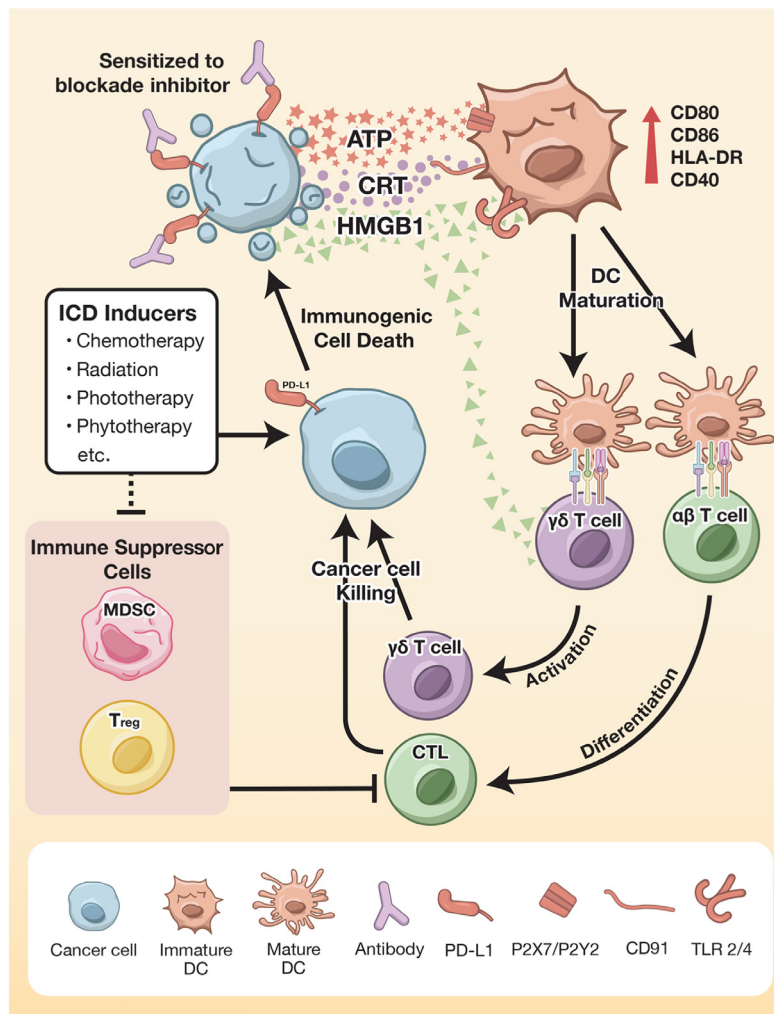
Figure 1. ICD enhances the antitumor immune response. Anticancer therapies, such as chemotherapeutic agents, radiation and phytotherapy, elicit the exposure of CRT on cell membranes and the extracellular release of ATP, HMGB1 and HSP. Interestingly, some types of ICD inducers increase PD-L1 expression, which increases the sensitivity to blockade inhibitor treatment. ICD potentiates DCs though upregulation of costimulatory signals, thereby strengthening adaptive immune system activation. Activation of αβ T cells results in differentiation to cytotoxic T cells, whereas activation of γδ T cells is facilitated by either mature antigen-presenting cells or as a direct effect of ICD. These modulate antitumor activity by cytotoxic granules and proinflammatory cytokine production. Moreover, the ICD inducer plays a role in the inhibition of immune suppressor cells. Therefore, ICD may be considered as priming therapy that may be suitably combined with cancer immunotherapy. ICD, immunogenic cell death; HMGB1, high mobility group box 1; CRT, calreticulin; MDSC, myeloid-derived suppressor cell; DC, dendritic cell; Treg, regulatory T cell; CTL, cytotoxic T lymphocyte; TLR, Toll-like receptor; PD-L1, programmed death ligand 1; HSP, heat shock protein.

have been developed, which are consistently active within the cardiovascular system, and which can accumulate within and inhibit tumors (104). In the context of PC, CAR-T-cell therapy has been the subject of a number of studies. The tumor-specific antigens of PC can be targeted by CAR-T-cell therapy. In particular, antigens that are overexpressed, such as CEA (NCT02349724), mesothelin (NCT02159716), HER2 (NCT02713984) and MUC1 (NCT02587689), are promising targets. In HCC, there have been no complete CAR-T-cell tests, but there is some evidence that the antigens CEA, MUC1 and glypican-3 could be effectively targeted (105,106). For GC, only a few studies have examined CAR-T-cell therapy to date (107,108), but the results are cautiously optimistic when developing CAR-T cells to target 3H11 (109), despite an inability to overcome the biological obstacles presented by solid tumors. A further study based on the overexpression of the folate 1 receptor (FOLR1) in GC compared with healthy tissue showed that FOLR1 CAR-T cells display antitumor properties

by recognizing FOLR1-positive GC cells (110). In the case of hepatobiliary cancer, small-scale trials have reported positive outcomes of immunotherapy, and recent work has assessed the potential of CAR-T cells when treating cancers of the biliary tract (111). The suggested targets include EGFR, mesothelin and HER2, due to their propensity to be overexpressed in the aforementioned malignancies (112-114).

Although adoptive transfer therapy is promising and intensely under review, the major obstacle of this approach in GI cancer treatment is the heterogeneity of solid tumor, particularly in colon and GC. The heterogenicity is caused by genomic instability, which contributes to clonal evolution and immune evasion resulting in immune-resistance and tumor recurrence. Interestingly, combination treatment of cellular adoptive transfer and chemotherapy can improve clinical outcomes and may prevent recurrence in patients with advance GC that may be attributed to the synergistic effect of ICD (115). Moreover, multiclonal elimination of tumor cells could be

improved by using multi-peptide vaccine or broad-array tumor antigen (116,117). Overall, these would be the solution for immune exhaustion in clinical settings of adoptive therapies.

9. Implications of ICD and adoptive cell transfer

The implications of adoptive transfer of cytotoxic T cells or CAR-T cells with ICD has not been extensively studied in the context of GI cancer treatment. However, it has been shown that the antitumor immune response of cytotoxic T cells may lead to immunogenic tumor cell death, improving their own tumor cell-killing capacity (118). Interestingly, treatment with mitochondria-targeted small molecules, including ATP, CRT and HMGB1, can induce upregulation of ICD in both *in vitro* and *in vivo* models. This is surprising, as priming by an ICD inducer effectively suppressed tumor growth and lung metastasis by enhancing the adoptive T-cell therapy against colon cancer in a mouse model (119). Moreover, it has been demonstrated that the mitochondrial DAMPs could play a role as immunomodulators through activation of γδ-T cells. Mitochondrial DAMPs induced expression of TLR2 and TLR4, which may positively regulate the antitumor response (120,121). TLR/type I IFN/CXCL10 has been proposed as the signaling pathway implicated in the recruitment of CXCR3⁺ T cells and the activation of γδ-T cells (122). Hence, not only the direct impact from activated antigen-presenting cells, but also T-cell-based anticancer therapy may be targeted when considering ICD (Table I). However, although the implications of T-cell therapy and ICD appear to be very promising, sufficient evidence is currently lacking.

10. Conclusions

ICD plays a key role in enhancing the efficacy of immunotherapy. DC-based anticancer vaccines directly activate and strengthen the co-stimulatory signal through DAMPs to further stimulate the immune response. The treatment outcomes from both the immune checkpoint blockade and adoptive transfer cell therapy may be enhanced by using ICD inducer agents. The mechanisms involving ICD-enhanced immunotherapy in GI cancer treatment are demonstrated in Fig. 1. These findings confirm the effectiveness of induction therapies combined with immunotherapy. Intervention may be implemented at only one or a few cycles at effectively low doses to avoid adverse effects and to obtain optimal ICD induction. Neoadjuvant chemotherapy can induce either cancer cell apoptosis or necrosis, which effectively promotes ICD induction, particularly HMGB1 release. In addition to the HSP family, CRT and S100 protein expression is elicited by chemotherapy and may be the primary DAMP molecules strengthening the efficacy of immunotherapy. However, the use of ICD inducers in immunotherapy for GI cancers has been limited due to a lack of research evidence, accounting for only 5-10% of all clinical trials. Further investigation should yield more positive outcomes regarding the induction of ICD in immunotherapy for GI cancer.

Availability of data and materials

Not applicable.

Authors' contributions

WS wrote the paper. CN designed and created the graphic figure. SP and AJ wrote and edited the paper. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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