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REVIEW

Immunotherapy-based combination strategies for treatment of gastrointestinal cancers: current status and future prospects

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Abstract **Strategies in comprehensive therapy for gastrointestinal (GI) cancer have been optimized in the last decades to improve patients' outcomes. However, treatment options remain limited for late-stage or refractory diseases. The efficacy of immune checkpoint inhibitors (ICIs) for treatment of refractory GI cancer has been confirmed by randomized clinical trials. In 2017, pembrolizumab was approved by the US Food and Drug Administration as the first agent for treatment of metastatic solid tumors with mismatch repair deficiency, especially for colorectal cancer. Given the different mechanisms, oncologists have focused on determining whether ICIs-based combination strategies could achieve higher efficacy than conventional therapy alone in late-stage or even front-line treatment of GI cancer. This review discusses the current status of combining immune checkpoint inhibitors with molecular targeted therapy, chemotherapy, or radiotherapy in GI cancer in terms of mechanisms, safety, and efficacy to provide basis for future research.**

Keywords gastrointestinal cancer; immune checkpoint inhibitor; combination therapy

Introduction

In the last decade, the incidence and mortality rates of cancers have increased in China. The number of cancer-related deaths reached approximately 2 814 000 in China, making cancer as the leading cause of death [1]. Gastrointestinal (GI) cancers, including stomach, liver, colorectal, esophageal cancers, are the top five cancers with the highest incidence and mortality rates [2]. In China, a large proportion of newly diagnosed patients with GI cancer belong to cases with unresectable tumors or with distant metastasis [3–5]. Although multi-disciplinary treatment can improve treatment outcomes, management of patients with GI cancer and refractory diseases remains challenging [6,7].

The roles of host anti-tumor immunity against tumor development and progression have been widely investigated to understand the interaction between tumor cells and the host immune system. Immunotherapy, which aims to mobilize the host immune system against tumor cells, is a promising strategy for treatment of patients with cancer [8–11]. Since the emergence of immune checkpoint inhibitors (ICIs), immunotherapy has been widely used in clinical practice, particularly for treatment of solid tumors [12]. At present, ICIs, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, CD152) antibody (ipilimumab), programmed cell death 1 protein (PD-1, CD279) antibodies (nivolumab and pembrolizumab), and PD-1 ligand 1 (PD-L1, CD274) antibodies (atezolizumab, avelumab, and durvalumab) are approved by the US Food and Drug Administration. The efficacy of these inhibitors has been demonstrated in melanoma, lung cancer, renal cell carcinoma, bladder cancer, and colorectal cancer. ICIs have become one of the pillars of comprehensive cancer treatment [13–16].

Although predictive biomarkers, such as mismatch repair deficiency, tumor mutation burden, and PD-L1 expression, have been used to enrich patients who will probably benefit from ICIs, the fraction of patients with GI cancer who will acquire durable clinical response remains limited. The response rates to ICI monotherapy are approximately 5%–30% in gastroesophageal cancer, 10%–20% in hepatobiliary cancer, and 30%–50% in colorectal cancer with mismatch repair deficiency; meanwhile, the therapy has no clinical benefit in pancreatic cancer [16–21]. In this regard, strategies to optimize the

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clinical application of ICIs should be further investigated [22].

Conventional therapies, including molecular targeted therapy, chemotherapy, and radiation, can modulate the host anti-tumor immunity and exert possible synergistic effect with ICIs [23]. Scholars have attempted to combine ICIs with conventional therapies for GI cancer through early-stage clinical trials (Table 1). Numerous trials are underway to assess the efficacy of such combination (Table 2). In the present work, we aim to review current evidence of combining ICIs with molecular targeted therapy, chemotherapy, or radiotherapy for treatment of GI cancer.

Status of tumor immunity and immune checkpoint blockade

Tumor-immunity cycle derived by innate and adaptive immune systems should be completed to initiate anti-tumor immune responses and protect against tumor cells [24]. Immune cells, including antigen presenting cells (APC), natural killer (NK) cells, and cancer-specific cytotoxic T cells, in the tumor microenvironment participate in regulating anti-tumor immune responses. Tumor antigen-loaded dendritic cells migrate into lymphoid organs to prime naïve $CD8^+$ T cells and promote the activation of NK cells. The recognition of class I major histocompatibility complex (MHC) by T-cell receptor (TCR) and CD8 and the interaction of B7 and CD28, known as costimulatory signals, are necessary to prime naïve $CD8^+$ T cells into cytotoxic T cells. The activated cancer-specific T cells then destroy the tumor cells by releasing cytotoxins and expressing FAS ligands [25].

The tumor cells will finally acquire immune escape and grow into macroscopic neoplasms due to immunoediting [26]. Genetic and epigenetic changes in the tumor cells with the immunosuppressive microenvironment

Table 1 Current data on combining ICIs with other therapies for GI cancer

Trial	Phase	Line	Treatment	No.	Efficacy	Adverse events
Anti-angiogenesis agents						
Colorectal cancer						
Abstract 2651 [55]	2	1st	B + A + FOLFOX	23	ORR 52% PFS 14.1 m, DOR 11.4 m	NR
NCT01633970 [54]	1b	⩾3rd, dMMR	B + A	10	ORR 30%	G3/4 40%; all-grade 80%
Gastric cancer						
NCT02999295	1/2	2nd	Ram + N	46	PR 22%, DCR 59%	G3/4 13%; all-grade 87%
NCT02572687 [58]	1a/b	⩾2nd	Ram + D	29	ORR 17% PFS 2.6 m, OS 6.4 m	G3/4 72%; all-grade 100%
Hepatocellular cancer						
NCT03006926 [59]	1b	1st	L + P	18	PR 46%, SD 46%	All-grade 94%
NCT02715531 [60]	1b	1st	B + A	26	PR 62%	G3/4 35%; all-grade 81%
Other targeted agents						
Colorectal cancer						
NCT02437136 [72]	2	⩾2nd	Entinostat + P	16	1PR, 5SD	G3/4 50%; all-grade 100%
NCT01988896 [73]	1	⩾3rd	C + A	23	ORR 17%	G3/4 34.8%;
NCT02788279 [74]	3	⩾3rd	C + A /A/ Reg	363	OS 8.9 m /7.1 m/8.5 m ORR 2.7%/2.2%/2.2%	G3/4 45%/10%/49%
Gastric cancer						
NCT02689284 [69]	2	2nd, HER2 (+)	M + P	60	ORR 16%, DCR 54%	G3/4 16%
Chemotherapy or radiotherapy						
Colorectal cancer						
Abstract 3541 [11]	2	1st	FOLFOX6 + P	30	ORR 53% 8 w DCR 100%	G3/4 36.7%
NCT02437071 [85]	2	⩾3rd	Radiation/ablation + P	19	ORR 9%	All-grade 73%
Gastric cancer						
NCT02335411 KEYNOTE-059 Cohort 2 [83]	2	1st, HER2 (−)	CF + P	25	ORR 60% PFS 6.6 m OS 13.8 m	G3/4 76%

Abbreviations: A, atezolizumab; B, bevacizumab; C, cobimetinib; D, durvalumab; DCR, disease control rate; DOR, duration of response; G, grade; L, lenvatinib; M, margetuximab; m, month; N, nivolumab; NR, not reported; ORR, objective response rate; OS, overall survival; PR, partial response; P, pembrolizumab; PFS, progression free survival; Ram, ramucirumab; Reg, regorafenib; SD, stable disease; w, week.

Table 2 Ongoing clinical trials on combining ICIs with other therapies for GI cancer

Trial	Phase	Patients	Treatment	End point
Esophageal cancer				
NCT03044613	2	Neoadjuvant cStage II/III	Nivolumab±ipilimumab followed by carboplatin + paclitaxel + RT + nivolumab	Safety
NCT03377400	2	1st-line, SCC	Durvalumab or tremelimumab + CCRT	PFS
NCT03437200	2	1st-line	Arm A: chemoradiation + nivolumab Arm B: chemoradiation + nivolumab + ipilimumab	12 m PFS
Gastric cancer				
NCT03006705	3	Adjuvant pStage III (D2)	Arm A: nivolumab + S-1 or CapeOX Arm B: placebo + S-1 or CapeOX	RFS
NCT03221426 KEYNOTE-585	3	Perioperative	Arm A: pembrolizumab + XP; Arm B: placebo + XP Arm C: pembrolizumab + FLOT; Arm D: placebo + FLOT	OS, pCR
NCT03382600 KEYNOTE-659	2b	1st-line	Arm A: pembrolizumab + oxaliplatin + S-1 Arm B: pembrolizumab + cisplatin + S-1	ORR
NCT03488667	2	Perioperative	Pembrolizumab + mFOLFOX6 before and after surgery	ypRR
NCT02918162	2	Perioperative	Chemotherapy + pembrolizumab before and after surgery Pembrolizumab maintenance	24 m DFS
NCT03257163	2	Perioperative	Pembrolizumab before surgery Pembrolizumab + capecitabine after surgery	RFS
NCT03409848	2	1st-line, HER2 (+)	Arm A: trastuzumab + nivolumab + ipilimumab Arm B: trastuzumab + nivolumab + mFOLFOX6	OS
NCT02872116		1st-line	Arm A: nivolumab + ipilimumab followed by nivolumab Arm B: XELOX Arm C: FOLFOX Arm D: nivolumab + XELOX Arm E: nivolumab + FOLFOX	OS: A vs. B + C; D + E vs. B + C
NCT03342937	2	1st-line	Pembrolizumab + CapeOx	PFS
NCT03413397	2	⩾2nd-line	Pembrolizumab + lenvatinib	ORR
NCT03453164	1/2	⩾2nd-line	Nivolumab + radiotherapy	DCR
NCT02999295	1/2	⩾2nd-line	Nivolumab + ramucirumab	6 m PFS
Colorectal cancer				
NCT02563002 KEYNOTE-177	3	1st-line, dMMR	Arm A: pembrolizumab Arm B: chemotherapy	PFS, OS
NCT02788279	3	⩾3rd-line	Arm A: atezolizumab Arm B: cobimetinib + atezolizumab Arm C: regorafenib	OS
NCT03414983	2/3	1st-line	Arm A: nivolumab + FOLFOX + bevacizumab Arm B: FOLFOX + bevacizumab	PFS
NCT03174405	2	1st-line	Avelumab + cetuximab + FOLFOX	12 m PFS
NCT03202758	1/2	1st-line, Kras MT	Durvalumab + tremelimumab + FOLFOX	Safety
NCT03475004	1/2	3rd-line	Pembrolizumab + bevacizumab + binimetinib	ORR
NCT03332498	1/2	>3rd-line	Pembrolizumab + ibrutinib	4 m DCR
Hepatocellular cancer				
NCT03434379	3	1st-line	Atezolizumab + bevacizumab Sorafenib	ORR/OS
NCT03439891	2	1st-line	Nivolumab + sorafenib	Safety/ORR
NCT01658878	1/2	1st-line	Nivolumab + cabozantinib	Safety/ORR
NCT03382886	1	⩾2nd-line	Nivolumab + bevacizumab	Safety
Biliary tract cancer				
NCT03101566	2	1st-line	Nivolumab + gemcitabine + cisplatin Nivolumab + ipilimumab	6 m PFS
NCT03111732	2	⩾2nd-line	Pembrolizumab + oxaliplatin + capecitabine	5 m PFS

(*Continued*)

Trial	Phase	Patients	Treatment	End point
Pancreatic cancer				
NCT02620423	1	2nd-line	Pembrolizumab + chemotherapy	Safety
NCT03250273	2	⩾2nd-line	Nivolumab + entinostat	ORR
NCT02879318	2	1st-line	Tremelimumab + durvalumab + G + nab-paclitaxel Gemcitabine + nab-paclitaxel	OS

modulated by the tumor cell itself result in immune escape. The low expression of class I MHC and B7 on tumor cells is the most common mechanism, and the high expression of PD-L1 causes the exhaustion of effector T cells. In tumor stroma, immunosuppressive molecules, such as transforming growth factor-β (TGFβ), indoleamine 2,3-dioxygenase, arginase, and nitric oxide synthase, released by tumor cells can form the immunosuppressive microenvironment [27,28]. TGFβ can induce immune escape by promoting the exclusion of T cells and blocking the acquisition of the Th1 effector phenotype [29,30]. Regulatory T cells (Tregs), which can suppress the activation of effector T cells, are usually recruited into the tumor stroma. Chemokines produced by tumor cells, such as CCL22, can promote Tregs infiltration by activating CCR4 [31]. Intratumoral hypoxia can also suppress the activation of effector T cells [32].

Immune checkpoints, namely, CTLA-4, PD-1, and PD-L1, have been identified as favorable targets to reverse tumor immune escape. CTLA-4, which is upregulated on activated T cells, competes with CD28 to bind to B7 and then transduces the inhibitory signal into T cells. Therefore, the persistent activation of effector T cells can be maintained by targeting CTLA-4. CTLA-4 inhibition can also enhance naïve T cell priming in lymphoid organs and deplete Tregs in the tumor microenvironment [33,34]. The PD-1/PD-L1 pathway is another inhibitory signal of effector T cell and inhibits TCR-mediated T cell activation and proliferation, resulting in T cell exhaustion and apoptosis. Upregulation of PD-L1 is commonly detected on tumor cells and Tregs in the tumor microenvironment. Therefore, inhibiting the PD-1/PD-L1 pathway mainly overcomes immune escape in the tumor microenvironment [35].

Combination with anti-angiogenesis targeted therapy

The vascular endothelial growth factor (VEGF) pathway promotes tumor angiogenesis and plays a critical role in tumor development and progression [36]. The suppressive effect of VEGF on host anti-tumor immune response has been recognized [37,38]. (1) VEGF can interfere with the maturation of DCs and effector T cells and inhibit the function of cytotoxic T cells [39]. (2) Overexpression of VEGF can promote the intratumoral accumulation, differentiation, and proliferation of Tregs [40,41]. (3) Accumulation of myeloid-derived suppressor cells, which inhibit tumor-directed T cell response, is regulated by the VEGF pathway [42]. Immature myeloid cells, which impede the maturation of DCs, are also recruited by VEGF into the tumor microenvironment [43,44]. (4) VEGF suppresses the expression of endothelial intercellular adhesion molecule-1 on endothelium cells and interferes with the trafficking of antitumor effector T cells by inhibiting cell adhesion [39]. (5) The abnormal anatomy and function of intratumoral vessels induced by VEGF can also suppress the infiltration of effector T cells [45,46] (Fig. 1).

Molecular agents (such as monoclonal antibodies and tyrosine kinase inhibitors), which target the VEGF pathway, have been administered in clinical practices for treatment of GI cancer. The efficacy of bevacizumab, an anti-VEGF antibody, has been demonstrated in metastatic colorectal cancer (mCRC) [47]. The multi-targeted tyrosine kinase inhibitor (TKI) regorafenib targeting VEGFR2 has been approved for treatment of refractory colorectal cancer [48]. Sorafenib is used as the standard first-line therapy for advanced or metastatic hepatocellular cancer (HCC) [49]. Other TKIs targeting VEGFRs, including lenvatinib and cabozantinib, can also be used to treat patients with HCC [50]. Ramucirumab, a fully human monoclonal antibody targeting VEGFR2, has been approved as second-line regime, monotherapy or combination with paclitaxel for treatment of advanced gastric cancer [51].

In pre-clinical investigation, the combination of bevacizumab with atezolizumab, an anti-PD-L1 antibody, increased the infiltration of intra-tumoral $CD8^+$ T cells into the xenografts of renal cell carcinoma. Increased CX3CR1 was observed on peripheral $CD8^+$ T cell, which suggested that the combined therapy could improve the migration of T cells [37]. Bevacizumab could also enhance the presentation of tumor antigens in breast, lung, and colorectal cancer. VEGFR2 inhibitors showed synergic effect with anti-PD-1 antibodies on inhibiting the tumor growth of colon cancer cell lines *in vivo*, but they did not

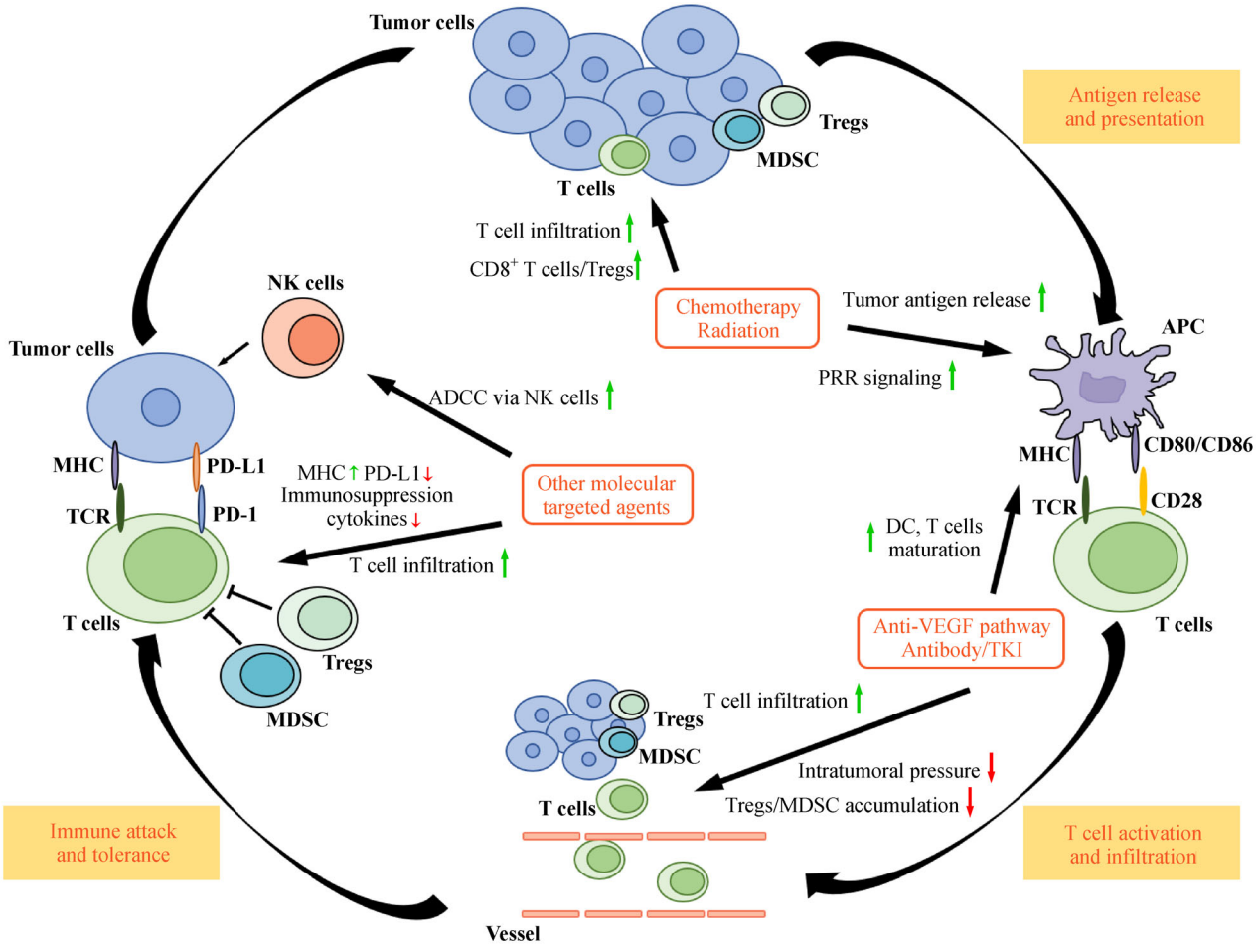

Fig. 1 Mechanisms of ICI interaction with conventional therapies against tumor cells.

interfere with T cell infiltration and activation [52]. Moreover, normalization of the function and anatomy of tumor vessels after anti-angiogenesis therapy could facilitate the infiltration of immune cells into tumor lesion, thereby enhancing the effect of immune checkpoint inhibition [46,53].

A phase Ib study (NCT01633970) evaluated the combination of bevacizumab and atezolizumab in MSI-high mCRC patients as rescue therapy. Ten patients were enrolled. The confirmed overall response rate (ORR) was 30% with a median follow-up of 11.1 month, and the overall survival (OS) was not reached. Grade 3 proteinuria was observed in 1 patient (10%) [54]. In another phase Ib study, atezolizumab was combined with FOLFOX plus bevacizumab as first-line treatment for mCRC patients. A total of 23 patients were enrolled, and ORR was 52% with a median progression-free survival (PFS) of 14.1 months and median duration of response (DOR) of 11.4 months. Biomarkers analysis showed that patients with increased $CD8^+$ T cell infiltration and expression of cytotoxic T cell signatures had prolonged disease control duration [55]. In comparison with the results of NO16966 trial (FOLFOX plus bevacizumab: ORR 38%, PFS 9.4 months) [56], the addition of atezolizumab might improve the efficacy of FOLFOX plus bevacizumab.

For gastric cancer, a phase Ia/b study (JVDF trial, NCT02572687) reported the combination of ramucirumab (8 mg/kg) with durvalumab (750 mg) in treating 29 advanced gastric or gastro-esophageal junction adenocarcinoma patients who previously received at least one line of chemotherapy. Confirmed partial response (PR) was 17% (5/29). Median PFS was 2.6 months, and OS was 6.4 months [57,58]. Another phase 1/2 study (NCT02999295) evaluated the combination of ramucirumab (8 mg/kg) with nivolumab (3 mg/kg, every 2 weeks) as second-line therapy. A total of 46 patients were enrolled. PR was 22% (10/46), and disease control rate (DCR) was 59%. For ramucirumab monotherapy as second-line treatment for gastric cancer, the ORR was only 2.9%. Median PFS and OS were 2.1 and 5.2 months, respectively [51]. Results indicated that the efficacy of the combination of ramucirumab with ICI was better than monotherapy. More data

should be obtained from future randomized controlled clinical trials.

For hepatocellular cancer, anti-VEGFR TKI is one of the major choices for patients in advanced stage, but the efficacy of its combination with ICIs is still unclear. Clinical trials evaluating the combination of sorafenib, lenvatinib, axitinib, cabozantinib, or regorafenib with nivolumab, pembrolizumab, or avelumab are now ongoing. The preliminary result from a phase Ib trial (NCT03006926) evaluating lenvatinib (8–12 mg daily) plus pembrolizumab (200 mg, ever 3 weeks) as first-line therapy for unresectable HCC patients (BCLC stage B/C, Child-Pugh class A) showed that both PR and stable disease (SD) were 46% (6/13) in 13 evaluable patients. Most common adverse events were decreased appetite and hypertension (56% each, all grade) [59]. Bevacizumab (15 mg/kg) plus atezolizumab (1200 mg) was administrated every three weeks as first-line treatment for the chemotherapy naïve unresectable or metastatic HCC patients in a phase Ib study (NCT02715531). A total of 26 patients were enrolled. For 21 efficacy-evaluable patients, the PR was 62% (13/22). Hypertension was observed in 19% patients (all grade) [60]. According to the promising result, a multicenter, open-label, and randomized phase III trial of bevacizumab plus atezolizumab versus sorafenib in advanced HCC is now ongoing (NCT03434379).

Combination with other targeted agents

Anti-ERBB family antibodies comprise another important type of targeted agents, which have been widely used in clinical practices for GI cancer. Aside from directly inhibiting important signal pathways, which control proliferation, anti-apoptosis, and invasion of tumor cells, recombinant antibodies can also induce host anti-tumor immune response through antibody-dependent cell-cytotoxicity (ADCC), antibody-dependent cell-phagocytosis and complement-dependent cytotoxicity [61]. The identification of the immunoglobulin G1 (IgG1) backbone of antibodies, such as cetuximab and trastuzumab, is necessary to mobilize immune responses [62].

The immune modulation of cetuximab has been widely investigated (Fig. 1). In comparison with chemotherapy alone, immune cell infiltration ($CD3^+$, $CD8^+$, and $CD56^+$) into liver metastatic sites was increased after treatment with cetuximab plus chemotherapy in colorectal cancer [63]. Cetuximab can also induce ADCC via NK cells and increase the HLA class I molecule expression on tumor cells [64]. Activated NK cells can kill tumor cells to release tumor antigens, which are presented by DCs to prime effector T cell. NK cells can also directly interact and improve the function of other immune cells, including DCs and macrophages [62]. Therefore, cetuximab can modulate both innate immune response and adoptive immune system. However, a bidirectional effect of cetuximab on immune response was also noticed. The activation of the immunosuppressive pathway as a feedback mechanism was observed after cetuximab administration. Cetuximab could enhance Tregs infiltration and trigger PD-L1 expression on tumor cells, which exhausted the effector T cells [65,66]. Some of these phenomena could also be observed after treatment with trastuzumab. In breast cancer, increased tumor-infiltrating lymphocytes was associated with the efficacy of trastuzumab-based treatment, while PD-L1 was upregulated in tumor cells, which resisted trastuzumab [67,68]. Considering their bidirectional effect on anti-tumor immunity, immune checkpoint inhibitors may reverse the immunosuppressive effect of anti-ERBB antibodies by unleashing the function of effector T cells and impeding Tregs activation.

The efficacy of anti-ERBB antibodies plus ICIs is still under investigation in GI cancer. A phase II clinical trial (NCT03409848) for gastric cancer is now ongoing to assess the efficacy of trastuzumab combined with nivolumab plus mFOLFOX6 (Table 2). The combination of margetuximab, a novel anti-HER2 monoclonal antibody, with pembrolizumab was administrated in HER2-amplified and PD-L1 positive gastroesophageal adenocarcinoma patients as second-line therapy after trastuzumab progression in a phase II trial (NCT02689284). A total of 60 patients were enrolled. ORR was 16%, and DCR was 54% in 57 evaluable patients. Notably, for patients with HER2 amplification and PD-L1 positivity by circulating tumor DNA (ctDNA) detection, ORR was 57% and DCR was 86%. Treatment-related adverse events (TRAEs, $\geqslant$ grade 3) occurred in 13% of the patients, and serious adverse events (SAEs) included autoimmune hepatitis (two cases) and pneumonitis (one case) [69] (Table 1).

For other molecular targeted agents, including small-molecule kinase inhibitors and epigenetic modulators, immune sensitization is mainly modulated by the regulating expression of immune molecules [70] (Fig. 1). BRAF inhibitors increased expression of MHC class I molecules. EGFR kinase and mTOR inhibitors can decrease the transcription of PD-L1 and inhibit the function of $FOXP3^+$ Tregs. Epigenetic modulators such as histone deacetylase inhibitors and methylation inhibitors can increase the expression of NK-cell-activating ligands, MHC class I and II molecules, and proinflammatory cytokines. MEK inhibitors decrease the immunosuppressive cytokine expression (IL-1, IL-6, IL-8, IL-10, and VEGF) in tumor microenvironment [71]. Oral class I-selective histone deacetylase inhibitor entinostat (5 mg, daily) plus pembrolizumab (200 mg, every 3 weeks) was used to treat mCRC patients with pMMR (NCT02437136). In total 16 patients, a patient acquired PR, and five patients had SD. Common TRAEs include fatigue (37.5%), arthralgia (18.8%), and increased alkaline phosphatase (18.8%) [72]. The efficacy of MEK inhibitor cobimetinib plus

atezolizumab was evaluated in a phase I trial. A total of 23 mCRC patients were enrolled and cobimetinib was provided with incremental dosage. The ORR was 17% (4PR, 5SD). Notably, three out of four patients with PR had mismatched repair proficiency (pMMR) [73]. However, the efficacy of cobimetinib plus atezolizumab was not validated by phase III trial. In IMblaze370 trial (NCT02788279), mCRC patients with pMMR were randomized at 2:1:1 ratio to receive atezolizumab plus cobimetinib, atezolizumab monotherapy, or regorafenib. Results showed that the median OS among the three groups did not significantly differ (8.9 months vs. 7.1 months vs. 8.5 months), as well as PFS and ORR. Therefore, IMblaze370 trial did not meet its primary endpoint. Grade 3/4 TRAEs rate was 45% in the atezolizumab plus cobimetinib group, and common TRAEs included diarrhea (56%), rash (42%), and nausea (32%) [74].

Combination with chemotherapy or radiotherapy

Chemotherapy and radiotherapy both directly execute non-specific anti-tumor activity on tumor cells by inhibiting cell mitosis and inducing cell apoptosis. Tumor antigens from killed tumor cells can be presented by APCs and can create a polyvalent tumor cell vaccine *in situ*. Then, a cascade of promoting adaptive antigen-specific immune response will be set off. Therefore, chemotherapy and radiotherapy can mobilize host anti-tumor immune response [75]. Chemotherapy and radiotherapy also regulate the immune system directly. The expression of class I MHC molecule and PD-L1 on tumor cells can be increased during genotoxic treatment [76,77]. The immunosuppressive architecture of tumor microenvironment is also distorted by chemotherapy and radiation, thereby facilitating infiltration of immune cell and transfection of drugs. Enhanced $CD45^+CD8^+$ T cells, reduced $CD11b^+Gr1^+$ myeloid cells infiltration, and increased ratio of $CD8^+$ T cells to Tregs can be observed after chemotherapy and radiotherapy [78] (Fig.1).

The mechanisms of genotoxic therapies, which modulate immunogenic effects, are mainly mediated by enhancing the pattern recognition receptor (PRR) signaling upon recognizing tumor antigens, which is known as immune adjuvanticity [71]. The principle assault of chemotherapy and radiotherapy is on cellular DNA, which causes genomic integrity disruption, DNA double strand break, DNA cross-links, and other chromosomal abnormalities. Damage-associated molecular pattern molecules (DAMPs) including adenosine triphosphate (ATP) and high mobility group box 1 (HMGB1) protein are released by dying and stressed cells. Toll-like receptors, the major type of PRR on innate immune cells, can be activated by binding with DAMPs. The activation of innate immunity then stimulates DC maturation, antigen presentation, and type I IFN production [79].

The clinical efficacy of combining ICI with chemotherapy has been demonstrated in non-small cell lung cancer (NSCLC). A meta-analysis reported that the ORR and DCR for combination of ICI with chemotherapy were 47.0% and 80.9%, respectively [80,81]. In KEYNOTE-189 trial, a phase 3, double-blind, placebo-controlled randomized trial assessed the pemetrexed and platinum-based chemotherapy plus either pembrolizumab or placebo as first-line therapy for non-squamous NSCLC patients without sensitizing EGFR or ALK mutations. Results showed that both PFS (8.8 months vs. 4.9 months, HR 0.52, 95%CI 0.43–0.64, $P < 0.001$) and OS rate at 12 months (69.2% vs. 49.4%, HR 0.49, 95%CI 0.38 – 0.64, $P < 0.001$) were improved by combination with pembrolizumab [82].

In gastric cancer, KEYNOTE-059 trial cohort 2 (NCT02335411) evaluated the efficacy of pembrolizumab plus cisplatin and fluoropyrimidine regime as first-line treatment for HER2-negative patients [83]. Results showed that in 25 patients, the ORR was 60% (PD-L1 positive 68.8%, PD-L1 negative 37.5%) with PFS of 6.6 months and OS of 13.8 months. Grade 3/4 TRAEs occurred in 76% of the patients. In comparison with the results of KEYNOTE-059 cohort 3 (pembrolizumab as first-line therapy for PD-L1-positive gastric cancer patients), the ORR of pembrolizumab monotherapy was 26.0% (CR 7%), while the median OS was 20.7 months [57]. Results indicated that a relative increased ORR of the combined therapy did not achieve a survival benefit of advanced gastric cancer patients. For colorectal cancer, pembrolizumab plus FOLFOX6 was employed as first-line treatment in 30 patients. The ORR was 53%, and the 8-week DCR was 100%. Adverse events could be tolerated with grade 3/4 AE of 36.7% [11] (Table 2).

Preoperative or adjuvant radiotherapy is now administrated to treat gastroesophageal cancer and rectal cancer, whereas palliative radiation for advanced or metastatic GI cancer is often used as part of the comprehensive treatment to control cancer-related symptoms and improve the quality of life. Based on several studies with small sample size, local radiation could cause tumor shrinking outside the scope, which was known as the abscopal effect [84]. Mechanisms of abscopal effect might be mediated by mobilizing anti-tumor immune response. The host adaptive immunity can be activated by radiation, including NK cell mobilization, $CD8^+$ T cell infiltration, antigen presentation of DCs, and upregulation of immunostimulatory cytokines, which can kill tumor cells outside the target lesion of radiation. Therefore, immune checkpoint inhibition can play a synergistic role with radiotherapy by promoting systemic immune response in host environment [85]. In NSCLC, a retrospective study compared the efficacy of hypo-fractionated radiation plus nivolumab and nivolumab

monotherapy. For a total 35 pretreated patients, 15 patients received combination therapy. The one-year OS (57.8% vs 27.4%, $P = 0.043$) and PFS rates (57.8% vs. 20.6%, $P = 0.040$) were both relative higher in the combination group [86].

In a phase II trial, mCRC patients who had progressed after two or more standard regimes were treated with pembrolizumab plus radiotherapy or ablation. Primary results showed that ORR was 9% in 19 cases, while the rate of adverse events with any grade was 73% (NCT02437071) [85]. Currently, the efficacy of radiotherapy combined with ICIs has not been demonstrated by perspective clinical trials in GI cancer. For esophageal cancer, several clinical trials, which evaluate the combination of chemoradiotherapy with ICIs as neoadjuvant or first-line treatment are now ongoing (Table 2).

ICI monotherapy activity was not observed in advanced pancreatic cancer patients due to the low immunogenicity and lymphocyte infiltration of tumor lesions [87]. To reverse the immunosuppression of pancreatic cancer, the combination of ICIs with cytotoxic agents, including gemcitabine, irinotecan, 5-FU, and albumin-bound paclitaxel is being evaluated by clinical trials. A randomized phase II trial (NCT02879318) is now ongoing to assess the efficacy of gemcitabine + nab-paclitaxel + durvalumab + tremelimumab versus gemcitabine plus nab-paclitaxel in pancreatic cancer patients. The primary outcome is OS. Although the response of ICIs monotherapy in biliary tract cancer (BTC) is higher than that in pancreatic cancer, the result is also preliminary. A phase Ib trail enrolled 24 advanced BTC patients with PD-L1 expression over 1%. Four patients obtained PR, and four had SD [88]. Clinical trials for NCT03101566 (nivolumab plus gemcitabine/cisplatin) or NCT03111732 (ipilimumab and pembrolizumab plus CapOX) are now ongoing. Before the publication of these results, chemotherapy is still the major strategy for advanced pancreatic and biliary tract cancer patients.

Future perspectives

The advantage of ICI combined with conventional treatment, including targeted therapy, chemotherapy, and radiotherapy, has been observed in preclinical research and preliminary clinical trials in GI cancer based on current evidence. However, due to the limited sample size, the treatment efficacy of the combined strategy should be validated by randomized controlled clinical trials before administration in clinical practices.

The toxicities of combined therapy have not been fully assessed. Adverse events of immune checkpoint inhibitors are unique to conventional therapy. Hypothyroidism, hyperthyroidism, increased ALT, pneumonitis, colitis, and hypophysis are the most common grade 3/4 adverse events [35]. In NSCLC, the results of phase III randomized clinical trials showed that the combination of pembrolizumab with chemotherapy did not significantly increase the frequency of grade 3/4 adverse events [82]. In GI cancer, the toxicities of combination strategy should also be evaluated in a large sample size.

To further optimize the benefits of combined therapy of ICIs, some essential factors should be concerned. For ICIs, the endeavor to identify predictive biomarkers had been made. The predictive values of genomic (microsatellite instability, tumor mutation burden, PD-L1 amplification), transcriptomic (RNA signature of immune response), and proteomic (PD-L1 expression) biomarkers have been assessed by perspective clinical trials [89]. In combination therapy, the possible use of present biomarkers to enrich patients who will benefit from combination therapy remains unclear. The optimal dosage of ICIs, sequence of ICIs and conventional therapy, and optimal compatibility of chemotherapy agents or radiotherapy technologies should also be considered in future investigations [85].

With the success of immune checkpoint blockade in the late stage of cancer, its possible application as front-line or even adjuvant therapy to mobilize host anti-tumor immunity at the early stage of disease in immunosuppressive microenvironment has not been induced by multiple therapies or tumor evolution and is preferred to be investigated [90]. Neoadjuvant immunotherapy was assessed in NSCLC. In a pilot study, two preoperative doses of nivolumab were administered in resectable NSCLC patients (stage I, II or IIIA). Twenty out of 22 patients underwent resection. Major pathological response occurred in 9 patients (45%). The recurrence-free survival rate after 12 months was 80% [91]. Clinical trials of ICIs as neoadjuvant or adjuvant treatment for gastro-esophageal cancer are also ongoing (Table 2).

Conclusions

Immune checkpoint therapy mainly mobilizes host anti-tumor immune response, which is different from the mechanisms of conventional therapy. With the development of oncologic research, treating cancer patients in their entirety, including tumor cells and the host environment, is critical. Therefore, the combination of immune checkpoint therapy with conventional therapies is promising, and its potential benefit has been observed in several preliminary trials. For GI cancer, investigations of combination therapy are now ongoing, which will provide more valuable clinical evidence to improve patient outcome.

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Compliance with ethics guidelines

Chenfei Zhou and Jun Zhang declare that they have no conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval by a relevant institutional review board or ethics committee.

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