Current status of immunotherapy against gastrointestinal cancers and its biomarkers: Perspective for precision immunotherapy

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Abstract
Immunotherapy has shown encouraging results for some types of tumor. Although enormous efforts have been made toward the development of specific immunotherapeutic strategies against gastrointestinal cancers, such as adoptive T-cell transfer, peptide vaccines, or dendritic cell vaccines, the efficacy of immunotherapies prior to the introduction of immune checkpoint inhibitors was not substantial. This article reviews immunotherapy for gastrointestinal malignancies, including cell therapy, peptide vaccine, and immune checkpoint inhibitors, and attempts to resolve the immunosuppressive conditions surrounding the tumor microenvironment, and to construct novel combination immunotherapies beyond immune checkpoint inhibitors.

KEYWORDS
gastrointestinal cancer, immune checkpoint inhibitor, immunity, microenvironment, precision immunotherapy
1 | INTRODUCTION

Gastrointestinal (GI) cancers are the most common human tumor worldwide, and the incidence and mortality are increasing every year. Several treatment strategies have been developed for GI cancers, including surgery, chemotherapy, radiotherapy, and molecularly targeted therapy. However, the overall survival (OS) of patients with GI cancer remains poor. Novel approaches to the treatment of GI cancer are thus needed.

Immunotherapy is a novel treatment strategy that is emerging as an effective and promising treatment option against several types of cancer. The first immunological treatments were carried out by Coley using a bacterial immunotoxin to patients with malignancy in 1891. The first notion of a role of immunity in cancer was postulated in 1909 by Smith, speculating that the immune system could repress the growth of carcinomas by recognizing tumor cells as foreign. In 1970, the concept of immunological surveillance was presented by Burnet, and an antigen recognized by cytolytic T lymphocytes on a human melanoma was finally reported by van der Bruggen et al.

Progress in this field is largely attributable to the identification of new immune-based targets, based on continued advances in the understanding of tumor immunology and the tumor microenvironment. Many types of immunomodulatory therapies have been demonstrated in the treatment of GI cancers, including non-specific biological response modifiers (OK432, lentian, PSK), interleukin (IL)-2-activated lymphocytes, tumor-specific reactive CD8+ T-lymphocyte transfer, dendritic cell (DC) vaccines, and tumor-associated antigen (TAA)-derived peptides. These immunotherapies have shown a certain degree of efficacy, but not durable objective responses. Confidence in the efficacy of immunotherapies was given a boost with the advent of immune checkpoint inhibitors, which was selected as a "Breakthrough of the Year 2013" by Science. Immunotherapy is now becoming mainstream as a treatment for GI cancer.

In the present study, we review immunotherapies for GI malignancies, including immune cell transfer therapy, peptide vaccine, immune checkpoint inhibitors, and combination immunotherapy beyond immune checkpoint inhibitors, by clarifying suppressive immune biomarkers surrounding the tumor microenvironment.

2 | IMMUNOTHERAPY AGAINST GI CANCERS

2.1 | Adoptive T-cell transfer

The concept of adoptive immunotherapy (AIT) for cancer treatment was presented by Mule et al in the form of IL-2 generated lymphokine-activated killer (LAK) cells combined with repeated injections of recombinant IL-2 (Table 1). Although LAK cells are non-specific killer cells that were considered effective against various types of tumor, the efficacy of LAK cells combined with high-dose IL-2 proved limited against metastatic GI cancer. The objective response rate (ORR) including complete response (CR) or partial response (PR) for colorectal cancer (CRC) was 11% (3 of 27 patients), and 0% (0/1) for esophageal cancer. Furthermore, severe toxicities were observed as a result of high-dose IL-2, which induces a vascular permeability leak that leads to fluid retention and interstitial edema, and results in circulatory failure, lung edema, and renal dysfunction. Hence, they made the shift to tumor-infiltrating lymphocytes (TIL), which are specific to tumor antigens and appear to offer far greater therapeutic potency than LAK cells.

Takayama et al conducted a randomized study to evaluate the efficacy of autologous lymphocytes activated in vitro with recombinant IL-2 and solid-phase antibody to CD3 as adjuvant therapy for curatively resected HCC. A total of 150 patients who had undergone curative resection for HCC were assigned to receive either AIT (n = 76) or no adjuvant treatment (n = 74). The immunotherapy group showed significantly longer DFS (P = .01) and disease-specific survival (P = .04) than the control group. No patients experienced grade 3 or 4 adverse events. These results suggested that transfer of non-specific-activated killer cells might be effective in preventing the intrahepatic recurrence of cancer.

The next advance was antigen-specific cytotoxic T lymphocytes (CTL) for the management of effector cells as treatment.

In Japan, Aruga et al reported autologous tumor-specific CTL, induced from peripheral blood mononuclear cells (PBMC) cultured with autologous tumor cells. These CTL were injected through the hepatic artery into patients with unresectable liver tumors. Among 15 treated patients (13 with hepatocellular carcinoma [HCC] and 2 with metastatic liver cancer), two CR, three PR, and four minor responses were observed without any severe treatment-associated systemic adverse events.

We have assessed the efficacy of CTL against pancreatic ductal adenocarcinoma (PDAC). Patients with curatively resected PDAC received AIT with CTL stimulated using MUC1-expressing human cell lines (MUC1-CTL), and the results indicated that MUC1-CTL might prevent liver metastasis. For the next step, combination therapy using MUC1-CTL and gemcitabine was carried out. A total of 43 patients who underwent radical pancreatectomy received treatment with MUC1-CTL and gemcitabine after surgery. MUC1-CTL were induced and given i.v. three times, and gemcitabine was given according to the standard regimen for 6 months. No severe treatment-associated systemic adverse events were encountered in the 43 treated patients. In the adequate treatment group (n = 21) in which the relative dose intensity of gemcitabine was ≥50% and ≥2 MUC1-CTL treatments were provided, disease-free survival (DFS) was 15.8 months, and OS was 24.7 months. Liver metastasis was found in seven patients only (33%), and local recurrence occurred in four patients (19%). Combination therapy with AIT and GEM might prevent liver metastasis and local recurrence.

As described above, adoptive immunotherapies have shown a certain degree of efficacy (Table 1). To obtain more effective arms, revolutions in technologies are needed; these include expanding...
| Tumor type | Target | Key drug and study design | Treatment line | Phase | Allocation | Sample size | Clinical efficacy | irAE | Reference |
|------------|--------|---------------------------|----------------|-------|------------|-------------|-----------------|------|-----------|
| CRC        | Non-specific | LAK with IL-2 | Late | Pilot | Review in an institute | 27 | 3 PR of 27 (11%) | SAE as a result of high-dose IL-2: fluid retention, circulatory failure, lung edema, and renal dysfunction | 22 |
| HCC        | Non-specific | Activated killer cells | Adjuvant | II | Randomized | 150 | Longer DFS ($P = .01$) and DSS ($P = .04$) than control | No severe irAE | 23 |
| Liver tumor$^a$ | Autologous tumor | CTL, HAI | Late | Pilot | Retrospective | 15 | 2 CR and 3 PR of 15 (33%) | No severe irAE | 24 |
| PDAC       | MUC1    | MUC1-CTL and gemcitabine | Adjuvant | Pilot | Retrospective | 21 | DFS, 15.8 M, OS 24.7 M (median) | No severe irAE | 25 |
| PDAC       | MUC1    | MUC1-CTL, MUC1-DC, and gemcitabine | 1st | Pilot | Retrospective | 42 | MST, 13.9 M; 1 CR (2.4%); 3 PR (7.1%) and 22 SD (52.4%) | No severe irAE | 15 |
| HCC        | HSP70   | HSP70-DC | Late | I | Dose escalation | 12 | 2 CR (17%), 5 SD | Grade 3 liver abscess | 14 |
| CRC        | Oncoantigens | Peptide cocktail with IFA | Late | I | Dose escalation | 18 | MST, 13.5 M; 1 CR (6%); 6 SD (33%) | No severe irAE | 26 |
| CRC        | Oncoantigens | FOLFOX + peptide cocktail with IFA | 1st | II | One arm, HLA-blind | 96 | ORR and OS did not differ from control group | Grade 5 IP, 2 in study group, 1 in control group | 17 |
| PDAC       | Oncoantigens | Gemcitabine + peptide cocktail with IFA | Adjuvant | II | One arm | 30 | Median DFS, 15.8 M | No severe irAE | 18 |
| HCC        | GPC3    | GPC3 peptide with IFA | Adjuvant | II | One arm | 41 | Recurrence rate, 28.6% (1 year), 39.4% (2 years) | No severe irAE | 27 |
| CRC        | Tumor specific | TSA with various adjuvants | Late | I/II | Review | 527 | 1 CR and 4 PR (ORR, 0.9%) | Not evaluated | 20 |

Liver tumor$^a$: HCC 13 patients, CRC 2 patients.

CR, complete response; CRC, colorectal cancer; CTL, cytotoxic T cell; DFS, disease-free survival; DSS, disease-specific survival; GPC3, glypican-3; HAI, hepatic arterial infusion; HCC, hepatocellular carcinoma; HLA, human leukocyte antigen; HSP, heat-shock protein; HSP70-DC, dendritic cells transfected with HSP70 mRNA; IFA, incomplete Freund's adjuvant; IL-2, interleukin-2; IP, interstitial pneumonia; irAE, immune-related adverse effects; LAK, lymphokine-activated killer cells; M, month; MST, median survival time; MUC1-DC, dendritic cells transfected with MUC1 mRNA; ORR, objective response rate; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PR, partial response; SAE, severe adverse event; SD, stable disease; TSA, tumor-specific antigen.
neoantigen recognized TIL or genetically engineered T cells such as T-cell receptor (TCR) T cells and chimeric antigen receptor (CAR) T cells.\textsuperscript{31}

So-called CAR-T therapy was also selected as a “Breakthrough of the Year 2013” by Science.\textsuperscript{21} CAR-modified T cells (CAR-T) targeting CD19 showed durable effects against leukemia, achieving complete remission.\textsuperscript{22} For gastrointestinal tumor, CAR-T therapies remain experimental.\textsuperscript{23} In clinical studies of CRC\textsuperscript{34} and biliary tract and pancreatic cancers,\textsuperscript{35} some promising results have been reported in the form of PR and long-term stable disease without uncontrollable toxicities. The novel next-generation CAR-T therapy was reported by Adachi et al.\textsuperscript{36} They engineered CAR-T cells to express IL-7 and chemokine (C-C motif) ligand 19 (CCL19) (7 × 19 CAR-T cells), as these factors are essential for the maintenance of T-cell zones in lymphoid organs. In mice, 7 × 19 CAR-T cells achieved complete regression of pre-established solid tumors with anti-tumor activity superior to that of conventional CAR-T cells. Histopathological analyses showed increased infiltration of DC and T cells into tumor tissues following 7 × 19 CAR-T-cell therapy,\textsuperscript{26} which might be adapted against gastrointestinal cancers in the near future.

2.2 | Dendritic cell vaccines

Dendritic cells are antigen-presenting cells specialized for the induction of a primary T-cell response. A clinical pilot study reported generation of DC in the presence of granulocyte-macrophage colony-stimulating factor and IL-4. These cells were pulsed with tumor lysate or a cocktail of TAA-derived peptides. This method to induce DC has been used as a standard worldwide.\textsuperscript{37} The study analyzed 16 patients with advanced melanoma, and objective responses were evident in five of these 16 evaluated patients.

For patients with PDAC, the clinical efficacy of immunotherapy using both DC transfected with MUC1 mRNA (MUC1-DC) and MUC1-CTL was evaluated in a pilot study with gemcitabine. Forty-two patients with unresectable or recurrent PDAC were enrolled, and median survival time was 13.9 months, with a 1-year survival rate of 51.1%. Of the 42 patients, one patient achieved CR (2.4%), three patients had PR (7.1%), and 22 patients had stable disease (SD) (52.4%). The disease control rate (DCR) was thus 61.9%. No significant differences in DFS were apparent between patients with and without any recurrence was achieved in two patients (for at least 44 and 33 months) and SD in five patients. That study indicated that HSP70-DC therapy is both safe and effective in patients with HCC.\textsuperscript{14}

DC vaccines might gain a place in novel combination immunotherapy.

2.3 | Peptide vaccines

Since the first clinical trial of a melanoma antigen gene-1-derived peptide-based vaccine was reported in 1995,\textsuperscript{39} various types of next-generation peptide vaccine are currently under development.\textsuperscript{16} Here, we present some successful reports from among these numerous studies.

We conducted phase I and phase II trials using HLA-A*24:02-restricted peptides, three derived from oncoantigens and two from vascular endothelial growth factor receptors (VEGFR) against CRC. In the phase I study, 18 HLA-A*2402-positive CRC patients for whom standard therapy had failed were enrolled, and 0.5 mg, 1.0 mg, or 3.0 mg each of the peptides was mixed with incomplete Freund’s adjuvant (IFA) and then s.c. injected. Vaccine treatment was well tolerated without any severe treatment-associated systemic adverse events.\textsuperscript{26} One patient who achieved CR remains alive without recurrence more than 10 years after the initial vaccinations, and six patients showed stable disease for 4-7 months. Median overall survival time (MST) was 13.5 months.

The phase II study was conducted to evaluate the efficacy of this approach in combination with oxaliplatin-based chemotherapy as a first-line therapy. Ninety-six chemotherapy-naive CRC patients with measurable metastatic or resectable lesions were enrolled under masking of HLA-A status. Although ORR and OS did not differ between the HLA-A*2402-matched and unmatched groups, a significantly delayed response was observed in the subgroup with a neutrophil-to-lymphocyte ratio (NLR) <3.0 according to the Harrington-Fleming method. Although the incidences of serious adverse events (SAE) were broadly similar between groups, that of neutropenia was relatively higher in the HLA-A*2402-matched group than in the unmatched group. Interstitial pneumonia that led to death was observed in two cases in the HLA-matched group and in one case in the HLA-unmatched group.\textsuperscript{17}

In the adjuvant setting, Miyazawa et al reported that 30 patients with resected PDAC were treated using a peptide cocktail vaccine containing epitope peptides derived from KIF20A, VEGFR1, and VEGFR2 combined with gemcitabine as a single-arm multicenter phase II study. No serious (more than grade 3) immune-related adverse events (irAE) were encountered. Median DFS was 15.8 months. This study also conducted comparisons with 15 patients treated using gemcitabine alone as a prospective control group that did not meet eligibility criteria as a result of HLA-A type only, for whom the median DFS was 12.0 months. No significant difference was seen between the two groups (P = .504). Significant differences in DFS were apparent between patients with and without KIF20A-specific CTL responses (P = .027), and between patients
with and without KIF20A expression \( (P = .014) \). In addition, all four patients who underwent R0 resection with KIF20A expression showed no recurrence with KIF20A-specific CTL responses.\(^{18}\)

Sawada et al identified in glypican-3 (GPC-3) an HLA-A*24, HLA-A*02 restriction peptide with extreme cancer specificity. In a phase I study, they reported safety, and immunological and clinical responses.\(^{40}\) A subsequent trial showed a durable effect against giant HCC, although the patients died from circulatory failure as a result of tumor thrombus, which occupied most of the right atrium.\(^{41}\)

In the next phase II study of GPC3 peptide vaccine as an adjuvant therapy for HCC, no significant difference in recurrence rate was found between 35 patients treated with surgery plus vaccination and 33 patients who underwent surgery alone (28.6% vs 54.3% at 1 year and 39.4% vs 54.5% at 2 years, respectively; \( P = .346, .983 \)). In a subgroup analysis, 25 patients treated with vaccination showed GPC3-positive tumors and a significantly lower recurrence rate compared to that in the 21 GPC3-positive patients who received surgery alone (24% vs 48% at 1 year and 52.4% vs 61.9% at 2 years, respectively; \( P = .047, .387 \)). GPC3 peptide vaccine improved the 1-year recurrence rate in patients with GPC3-positive tumors.\(^{27}\)

Although tumor-associated antigen-derived peptide vaccines have been shown to provide effective induction of antigen-specific immunity, the clinical efficacy has not proven durable (Table 1). As a result, no peptides are covered by the National Health Insurance. Pooled results of clinical trials show a very weak clinical response rate of \(<1\%\) for the active specific immunization procedures currently available for advanced CRC.\(^{20}\) Rosenberg et al reported that the objective response rate was low (2.6%) in their cancer vaccine trials of 440 patients, even though the main target was melanoma, which is highly immunogenic.\(^{42}\)

Combination immunotherapy appears needed for peptide vaccinations such as immune checkpoint inhibitors,\(^{43}\) novel immune adjuvants,\(^{44}\) COX-2 inhibitors,\(^{45}\) and anti-epidermal growth factor receptor (EGFR) antibodies.\(^{46}\) Such novel approaches are described in detail in later sections.

Neoantigens, a class of HLA-bound peptides that arise from tumor-specific mutations, are highly immunogenic because they are not present in normal tissues and hence bypass central thymic tolerance. Ott et al demonstrated the feasibility, safety, and immunogenicity of a vaccine targeting up to 20 predicted personal tumor neoantigens using machine learning approaches to reliably predict those mutated peptides with high-affinity binding of autologous HLA molecules. Of six vaccinated patients, four showed no recurrence at 25 months after vaccination, whereas two with recurrent disease were subsequently treated with anti-programmed cell death protein 1 (PD-1) therapy and achieved CR, with expansion of the repertoire of neoantigen-specific T cells. These data provide a strong rationale for further development of this approach alone and in combination with checkpoint blockade or other immunotherapies.\(^{47}\) Simultaneously, the first in-human application of this concept was carried out in melanoma using an RNA-based poly-neo-epitope approach comprising computational prediction of neo-epitopes, and design and manufacturing of a vaccine unique to each patient. All patients developed T-cell responses against multiple vaccine neo-epitopes at up to high single-digit percentages. Cumulative rate of metastatic events was significantly reduced after the start of vaccination, resulting in sustained progression-free survival (PFS). Two of the five patients with metastatic disease experienced vaccine-related objective responses. A third patient developed CR to vaccination in combination with PD-1 blockade therapy.\(^{48}\) Although these reports involved melanoma, this strategy holds promise for the treatment of gastrointestinal cancers.

### 3 IMMUNE CHECKPOINT INHIBITORS

Recently, the effectiveness of immunotherapy targeting immune checkpoints in the treatment of numerous forms of cancer has been studied. In 2011, the Food and Drug Administration in the USA approved ipilimumab, an anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) treatment for metastatic melanoma. In 2012, Topalian et al reported results for PD-1 therapy in nearly 300 people, and an update was provided in 2013.\(^{39}\) Shrunk by about half or more in 31% of those with melanoma, in 29% with kidney cancer, and in 17% with lung cancer. Immune checkpoint inhibitors have also been applied to gastrointestinal cancers (Tables 2-4).

#### 3.1 Esophageal cancer

Compared with other solid tumors, esophageal squamous cell carcinoma (ESCC) has a very high somatic mutation rate.\(^{51,62}\) The high mutation load in esophageal tumors has been associated with the clinical benefit of PD-1 blockade.\(^{63}\) Nivolumab is a human monoclonal immunoglobulin (Ig)G4 antibody that seals PD-1 expressed on activated T cells. This drug was applied to treatment-refractory esophageal cancer in an open-label, multicenter, phase II trial (Table 2). Nivolumab showed promising activity with a manageable safety profile.\(^{51}\) PD-1/PD-L1 blockade alone or in combination with radiotherapy and chemotherapy will be a direction for future research in the treatment of advanced esophageal cancer (Table 4).

#### 3.2 Gastric cancer

To assess the efficacy and safety of nivolumab in patients with advanced gastric cancer (GC) or gastroesophageal junction cancer (GEJC) refractory to, or intolerant of, two or more previous regimens of chemotherapy, a randomized, double-blind, placebo-controlled, phase III trial was carried out (Table 2). In that phase III study, survival benefits indicated that nivolumab might represent a new treatment option for heavily pretreated patients with advanced GC or GEJC. Based on that study, nivolumab was approved in Japan for unresectable advanced or recurrent gastric cancer that has progressed after chemotherapy (Table 3).\(^{52}\)

Pembrolizumab uses another development strategy to target patients with programmed death ligand 1 (PD-L1)-positive advanced gastric cancer. A phase Ib trial designed to assess the safety and
| Tumor type | Target | Key drug and trial identifier | Treatment line | Phase | Allocation | Sample size | Clinical efficacy | irAE | Reference |
|------------|--------|-------------------------------|----------------|-------|------------|-------------|------------------|------|-----------|
| ESCC       | PD-1   | Nivolumab, ONO-4538-07        | Late           | II    | Single arm | 64          | ORR, 11 of 64 (17%) | Lung infection (4), dehydration (2), IP (2) of 65 | No treatment-related deaths | 51 |
| GC or GEJC | PD-1   | Nivolumab, ATTRACTION-2       | 3rd or more    | III   | Randomized, double-blind | 493 (330 vs 163) | ORR, 30 (11.2%) of 268, 1Y OS: 26.2% (Nivo) vs 10.9% (Place) | Grade 3 or 4 irAE in 34 (10%); irAE led to death in 5 (2%) | 52 |
| PD-L1 +    | GC or GEJC | Pembrolizumab, KEYNOTE-012 | Late | Ib | Single arm | 39 | ORR, 22% (8 of 36) | Grade 3, 2 fatigue, 1 PG, 1 hypothyroidism, 1 PSN; Grade 4, 1 IP | No treatment-related deaths | 53 |
| GC or GEJC | PD-1   | Pembrolizumab, KEYNOTE-059-Cohort 1 | 3rd or more | II | Single arm | 259 | ORR, 15.5% in PD-L1+ pts, 5.5% in PD-L1– pts | Grade 3-5 irAE in 43 (16.6%), discontinuation in 2 (LFT, BDS), fatal in 2 (AKI, PE) | 54 |
| dMMR/MSI-H CRC | PD-1 | Pembrolizumab, CT01876511 | Late | II | Single arm | 28 | ORR, 40% (4 of 10) for dMMR/MSI-H CRC, 0% (0 of 18) for pMMR CRC | Grade 3 and 4 irAE, anemia (17%), lymphopenia (20%), diarrhea (5%), BO (7%) | 55 |
| dMMR/MSI-H tumors³ | PD-1 | Pembrolizumab, CT01876511 | 2nd or more | II | Single arm | 86 | ORR, 53%; CR, 21% | irAE were manageable74% had AE (Grade 1 or more), hypothyroidism (21%) managed with THR | 56 |
| dMMR/MSI-H CRC | PD-1 | Pembrolizumab, CheckMate 142 | 2nd or more | II | Single arm | 74 | ORR, 31.1% (23 of 74) | Common grade 3 or 4 irAE, elevation of lipase (6) and amylase (2). No treatment-related deaths | 57 |
| dMMR/MSI-H CRC | PD-1 | Nivolumab + ipilimumab CheckMate 142 | 2nd or more | II | Single arm | 119 | ORR, 55% (65 of 119), 4 CR, 61 PR | Grade 3 irAE in 32, AST or ALT (11%), lipase (4%), anemia or colitis (3%), hypothyroidism (1%). No treatment-related deaths | 58 |
| HCC | PD-1 | Nivolumab, CheckMate 040 | 1st or more | I/II | Dose escalation and expansion P I, 48 P II, 214 | ORR, 15% (3 CR, 4 PR of 48) in P I, 20% in P II (3 CR, 39 PR of 214) | 12 (25%) of 48 had grade 3/4 irAE; 3 (6%) had serious AE (PG, adrenal insufficiency, liver disorder) | 59 |
| PDAC | PD-L1 | BMS-936559, CT00729664 | 1st or more | I | Dose escalation Total, 207 PDAC, 16 | PDAC, 0 of 14 (0%) | MTD was not reachedirAE, 81 of 207 (39%), included rash, hypothyroidism, hepatitis, diabetes mellitus | 60 |

³dMMR tumors: 12 different tumor types.

AKI, acute kidney injury; BDS, bile duct stenosis; BO, bowel obstruction; CR, complete response; CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; dMMR, defective mismatch repair; ESCC, esophageal squamous cell carcinoma; GC or GEJC, gastric or gastroesophageal junction cancer; HCC, hepatocellular carcinoma; IP, interstitial pneumonia; irAE, immune-related adverse effect; Late, standard therapy failure; LFT, liver function test; MSI-H, microsatellite instability-high; MTD, maximum tolerated dose; Nivo, nivolumab; ORR, objective response rate; PD-1, programmed cell death 1; PDAC, pancreatic ductal adenocarcinoma; PD-L1, programmed cell death ligand 1; PE, pleural effusion; PG, pemphigoid; Place, placebo; pMMR, proficient mismatch repair; PR, partial response; PSN, peripheral sensory neuropathy; pts, patients; THR, thyroid hormone replacement.
activity of pembrolizumab was carried out in patients with PD-L1-positive recurrent or metastatic GC or GEJC. Patients received i.v. pembrolizumab at 10 mg/kg once every 2 weeks (Table 2). Pembrolizumab showed a manageable toxicity profile and promising antitumor activity. The FDA approved pembrolizumab for previously treated patients with recurrent locally advanced or metastatic GC or GEJC whose tumors express PD-L1. This decision was based on data from a global multicohort trial, KEYNOTE-059, which indicated a superior response in patients with tumors that expressed PD-L1 (Tables 2, 3).

Ongoing trials are investigating various settings and earlier treatment lines for GC or GEJC (Table 4).

### 3.3 Colorectal cancer

In early-phase studies, responses of CRC to PD-1/PD-L1 inhibitors were not promising. No objective response was seen in patients treated with PD-1 inhibitors (0/19) or PD-L1 inhibitors (0/16). Surprisingly, updated reports have indicated that patients with metastatic CRC who harbored the microsatellite instability-high (MSI-H) genotype achieved objective responses after disease progression on an intermittent dosing regimen of PD-1 inhibitors and finally achieved complete responses. The majority of colorectal cancers are proficient mismatch repair (pMMR) tumors, and approximately 15% show defective mismatch repair (dMMR), which can be measured by either the five-marker panel with fluorescent multiplex assay or by the lack of DNA mismatch repair proteins. Tumors with dMMR can have MSI-H and a somatic mutation frequency of more than 10- to 100-fold that of pMMR tumors. Hence, dMMR (MSI-H) tumor is thought to have the potential to encode “non-self” immunogenic antigens and predict responsiveness to the immune checkpoint blockade (Tables 2–4).

Based on this perspective, a phase II study was conducted to evaluate the clinical activity of pembrolizumab, an anti-PD-1 immune checkpoint inhibitor, and 32 patients with progressive metastatic carcinoma with or without dMMR were enrolled and received i.v. pembrolizumab. Objective response rates (ORR) were 40% for dMMR colorectal cancers and 0% for pMMR colorectal cancers. Median PFS and OS were not reached in the cohort with dMMR CRC, but were 2.2 months and 5.0 months, respectively, in the cohort with pMMR CRC. Whole-exome sequencing showed a mean of 1782 somatic mutations per tumor in dMMR tumors, as compared with 73 in pMMR tumors, and high somatic mutation loads were associated with prolonged PFS ($P = .02$). This study showed that patients with dMMR are good candidates for receiving immune checkpoint blockade (Table 2).

Next, this study was expanded to evaluate the efficacy of PD-1 blockade in patients with advanced dMMR cancers across 12 different tumor types. Responses were durable with median PFS and OS still not reached. These data support the hypothesis that the large proportion of mutant neoantigens in dMMR cancers make them sensitive to immune checkpoint blockade, regardless of the tissue of origin for the cancer, and the FDA approved the use of pembrolizumab in the treatment of patients with MSI-H or dMMR (Tables 2, 3).

Similar results were obtained from an open-label, phase II study of nivolumab in patients with dMMR/MSI-H metastatic CRC. Patients were given nivolumab at 3 mg/kg every 2 weeks until disease progression or unacceptable toxic effects (Table 2). The FDA approved nivolumab use in the treatment for MSI-H or dMMR metastatic colorectal cancer that has progressed following treatment (Table 3). Studies of immune checkpoint inhibitors are ongoing to indicate potential efficacy as first-line agents (Table 4).

### 3.4 Hepatocellular carcinoma

The only evidence-based systemic treatment option is sorafenib, a small-molecule multikinase inhibitor, for patients with advanced...
| Tumor type       | Target | Key drug                      | Trial design (arm)                      | Trial identifier     | Treatment line | Phase | Allocation | Status               | Sample size | Study start date | Estimated primary completion date |
|------------------|--------|-------------------------------|----------------------------------------|----------------------|----------------|-------|------------|----------------------|-------------|------------------|-----------------------------------|
| dMMR or MSI-H CRC | PD-1   | Pembrolizumab                 | Pembrolizumab Standard 1st-line therapy for CRC | Keynote 177 NCT02563002 | 1st            | III   | Randomized | Active, not recruiting | 300         | 30 Nov 15        | 15 Aug 19                         |
| dMMR or MSI-H CRC | PD-1   | Pembrolizumab                 | Pembrolizumab                           | Keynote 164 NCT02460198 | 2nd or more     | II    | Single arm | Active, not recruiting | 124         | 25 Aug 15        | 09 Sep 19                         |
| GC or GEJC       | PD-1   | Nivolumab                     | Nivolumab + chemotherapy (SOX or CapeOX) | ONO-4538-37 NCT02746796 | 1st            | III   | Randomized | Recruiting           | 680         | Mar 16           | Aug 20                            |
| GC or GEJC       | PD-1, CTLA-4 | Nivolumab + ipilimumab       | Nivolumab + ipilimumab                  | CheckMate649 NCT02872116 | 1st            | III   | Randomized | Recruiting           | 1349        | 04 Oct 16        | 12 Mar 20                         |
| GC               | PD-1   | Nivolumab                     | Nivolumab + chemotherapy (S-1 or CapeOX) | ONO-4538-38 NCT03006705 | adjuvant        | III   | Randomized | Recruiting           | 700         | Jan 17           | Jun 21                            |
| GC or GEJC       | PD-1   | Pembrolizumab                 | Pembrolizumab monotherapy Pembrolizumab + cisplatin + 5-FU Placebo + cisplatin + 5-FU | KEYNOTE-062 NCT02494583 | 1st            | III   | Randomized | Active, not recruiting | 764         | 31 Jul 15        | 05 Feb 19                         |
| GC or GEJC       | PD-1   | Pembrolizumab                 | Pembrolizumab Paclitaxel                | KEYNOTE-063 NCT03019588 | 2nd            | III   | Randomized | Active, not recruiting | 360         | 16 Feb 17        | 17 Aug 19                         |
| GC or GEJC       | PD-1   | Pembrolizumab                 | Pembrolizumab + XP or FP Placebo + XP or FP | KEYNOTE-585 NCT03221426 | Neoadjuvant     | III   | Randomized | Recruiting           | 860         | 09 Oct 17        | 26 Jul 23                         |
| GC or GEJC       | PD-L1  | Avelumab                      | Induction phase: FOLFOX or CapeOX       | JAVELIN Gastric 100 NCT02625610 | 1st            | III   | Randomized | Active, not recruiting | 499         | 24 Dec 15        | 13 Mar 19                         |

(Continues)
| Tumor type | Target | Key drug | Trial design (arm) | Trial identifier | Treatment line | Phase | Allocation | Status | Sample size | Study start date | Estimated primary completion date |
|------------|--------|----------|-------------------|-----------------|----------------|-------|-----------|--------|-------------|-----------------|----------------------------------|
| ESCC       | PD-1   | Nivolumab | Nivolumab         | ONO-4538-24     | 2nd            | III   | Randomized | Active, not recruiting | 390           | Dec 15              | Sep 19                           |
|            |        |           | Docetaxel or paclitaxel | NCT02569242     |                |       |           | Recruiting |                          |                                 |
| ESCC       | PD-1   | Nivolumab | Nivolumab + ipilimumab | CheckMate 648   | 1st            | III   | Randomized | Recruiting | 939           | 19 Jun 17           | 25 May 20                       |
|            |        |           | Nivolumab + cisplatin + fluorouracil | NCT03143153     |                |       |           |                      |                           |                                 |
| ESCC       | PD-1   | Pembrolizumab | Pembrolizumab + cisplatin + 5-FU | KEYNOTE-181     | 2nd            | III   | Randomized | Recruiting | 720           | 01 Dec 15           | 25 Sep 19                       |
|            |        |           | Paclitaxel or docetaxel or irinotecan | NCT02564263     |                |       |           |                      |                           |                                 |
| EC or EGJC | PD-1   | Pembrolizumab | Pembrolizumab + cisplatin + 5-FU | KEYNOTE-590     | 1st            | III   | Randomized | Recruiting | 700           | 25 Jul 17           | 22 Aug 21                       |
|            |        |           | Placebo + cisplatin + 5-FU | NCT03189719     |                |       |           |                      |                           |                                 |
| HCC        | PD-1   | Nivolumab | Nivolumab         | CheckMate 9DX   | Adjuvant       | III   | Randomized | Recruiting | 530           | 18 Dec 17           | 17 Apr 22                       |
|            |        |           | Placebo           | NCT03383458     |                |       |           |                      |                           |                                 |
| HCC        | PD-1   | Nivolumab | Nivolumab         | CheckMate 459   | 2nd            | III   | Randomized | Active, not recruiting | 726           | 25 Nov 15           | 16 Oct 18                       |
|            |        |           | Sorafenib         | NCT02576509     |                |       |           |                      |                           |                                 |
| HCC        | PD-1   | Pembrolizumab | Pembrolizumab + BSC | KEYNOTE-394     | 2nd or more    | III   | Randomized | Recruiting | 330           | 27 Apr 17           | 23 Dec 19                       |
|            |        |           | Placebo + BSC     | NCT03062358     |                |       |           |                      |                           |                                 |
| HCC        | PD-1   | Pembrolizumab | Pembrolizumab + BSC | KEYNOTE-240     | 2nd or more    | III   | Randomized | Active, not recruiting | 408           | 26 May 16           | 01 Feb 19                       |
|            |        |           | Placebo + BSC     | NCT03062358     |                |       |           |                      |                           |                                 |

BSC, best supportive care; CRC, colorectal cancer; dMMR, defective mismatch repair; EC or EGJC, adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the esophagogastric junction; ESCC, esophageal squamous cell carcinoma; FP, cisplatin + 5-fluorouracil; GC or GEJC, gastric or gastroesophageal junction cancer; HCC, hepatocellular carcinoma; MSI-H, microsatellite instability-high; XP, cisplatin + capecitabine.
Immunotherapies that target the tumor microenvironment and promote the growth of immunogenic tumors are emerging as promising treatments for PDAC. One such approach combines immune checkpoint inhibitors with other therapies, such as chemotherapy or radiation. Studies have shown that the combination of anti-PD-L1 antibodies with anti-CTLA-4 antibodies can achieve durable responses in patients with PDAC, particularly those with high tumor mutation burden.

For patients with advanced PDAC, immune checkpoint inhibitors can be used as a monotherapy or in combination with other treatments. The addition of platinum-based chemotherapy to pembrolizumab improved overall survival compared to pembrolizumab alone, with a median OS of 13.1 months in the combination arm versus 11.1 months in the monotherapy arm.

In addition, the use of immune checkpoint inhibitors in combination with vaccines or other targeted therapies is being explored. One such study evaluated the combination of nivolumab with a checkpoint inhibitor and a T-cell vaccine in patients with advanced PDAC. The combination was well-tolerated and showed promising efficacy, with an ORR of 36% and a DCR of 60%.

Other strategies being investigated include the use of combination immunotherapies with other drugs, such as PD-1 blockers and chemotherapy, to further enhance antitumor activity. These studies are ongoing and show promise in improving outcomes for patients with PDAC. Overall, the use of immune checkpoint inhibitors in combination with other therapies is a promising area of research for the treatment of PDAC.
immunity, and patients with immune exhaustion (Figure 1E) might require a multidisciplinary approach (Table 5).

The immunosuppressive environment surrounding PDAC might be one of the major obstacles to the development of successful therapies for this fatal disease.69 Advances in our understanding of the immunosuppressive mechanisms in PDAC might lead to promising immunotherapeutic approaches. PDAC patients displayed an increased number of Treg and MDSC.71 Cyclophosphamide72 and metformin73 could downregulate the number and function of Treg, and cyclooxygenase (COX)-2 inhibitors74 and cimetidine75 could regulate MDSC. Metformin is also reported to have a direct effect on CD8+ T cells for protection against the inevitable functional exhaustion marker T-cell immunoglobulin and mucin domain-containing protein-3 (Tim-3) in the tumor microenvironment.77 The production of secreted suppressive molecules such as IL-6 leads to the limited efficacy of immune checkpoint inhibitors in PDAC, so IL-6 blockade would modulate the immunological features of PDAC. An experimental study of combined IL-6 and PD-L1 blockade elicited efficacy in mice bearing s.c. tumors, accompanied by increased intratumoral effector T lymphocytes. These preclinical results indicate that targeted inhibition of IL-6 may enhance the efficacy of anti-PD-L1 in PDAC (Table 5).78 Other inhibitory factors in the tumor microenvironment, such as TGF-β80 and PGE275,81 and its inhibitors, are summarized in Table 5.

Another problem is the presence of a uniquely desmoplastic stroma that functions as a barrier to T-cell infiltration (Figure 1B,C). Hyperactivated focal adhesion kinase (FAK) activity in PDAC cells is an important regulator of the fibrotic and immunosuppressive microenvironments. FAK activity is elevated in human PDAC tissues and correlates with high levels of fibrosis and poor CD8+ cytotoxic T-cell infiltration.82 Single-agent FAK inhibition using the selective FAK inhibitor VS-4718 substantially limited tumor progression, resulting in a doubling of survival in a mouse model of human PDAC. This delay in tumor progression was associated with markedly reduced CAF and decreased numbers of tumor-infiltrating immunosuppressive cells.82 TAM83 are also an inhibitory factor in tumor microenvironments, and associated therapeutic strategies are shown in Table 5.

Tumor tissues that lack expression of many immunological markers may indicate a non-immunogenic tumor microenvironment (Figure 1C), which may require combination therapies consisting of an agent to create an immunogenic tumor microenvironment plus an immune checkpoint agent to further enhance immune responses for clinical benefit.84 Conventional cancer therapies such as chemotherapy or radiation may also lead to tumor cell death and release of antigens to initiate activation of T cells, which may then migrate into tumor tissues. Combination studies using conventional agents and immune checkpoint therapies should thus clarify the conditions needed to create an “immunogenic” tumor microenvironment with subsequent clinical benefit for patients.85 We have reported that cetuximab strongly enhances immune cell infiltration into liver metastatic sites in CRC.46 Cetuximab induces antibody-dependent cell-mediated cytotoxicity and immunogenic cell death. We assessed immune cell infiltration into liver metastatic sites of 53 CRC patients treated with chemotherapy and cetuximab, chemotheray without cetuximab, and no chemotherapy. Of note, inflammatory cells were found in intratumoral areas, and the destruction of cancer cell foci was observed in the cetuximab group. Moreover, higher infiltration of CD8+ (P = .003) and CD56+ (P = .001) cells was observed in the cetuximab group. The immune-related mechanism of cetuximab may enhance the efficacy of combination therapy using immune checkpoint inhibitors and/or therapeutic peptides.46

Although no objective responder to pembrolizumab is seen in pMMR/MSS-CRC, some patients obtained SD lasting more than 3 months according to tumor markers as well as radiographical evaluation,55 indicating that some groups of MSS-CRC could respond to
PD-1 blockade. Galon et al\textsuperscript{89} attested that MSS colon cancer is divided into tumors with or without massive CD8\textsuperscript{+} T-cell infiltration. The reason why metastatic MSS-CRC did not respond well to immune checkpoint inhibitors might be divided into two patterns, owing to the highly immunosuppressive state of the “dark tumor” microenvironment (Figure 1B) and the low immunogenicity in the “cold tumor” microenvironment (Figure 1C). For dark tumors, a combination of immune checkpoint inhibitors and the modalities summarized in Table 5 to resolve the suppressive immunity might prove effective. For cold tumors (Figure 1C), additional use of methods to induce immune cells to the tumor site would be needed, as described above. The combination of neoantigen-derived vaccination and cetuximab might be one of the most promising strategies.

Hence, combination immunotherapy should be selected as a precision medicine based on comprehensive analyses using whole-exome sequencing and RNA sequences. Moreover, novel immune checkpoints might not yet have been detected. Absolutely effective combination strategies might be just around the corner.

**FIGURE 1** Concept of immunological status of various tumors or patients and implications for immunotherapy. A, Hot tumor might respond well to immune checkpoint inhibitors. B, Dark tumor might need immune checkpoint inhibitors and agents to resolve suppressive immunity. C, Cold tumor might require combination therapies comprising an agent to create an immunogenic tumor microenvironment plus immune checkpoint inhibitors and agents to resolve suppressive immunity. D, Patients without immune exhaustion might not need additional treatment. E, Patients with immune exhaustion might need additional treatment to resolve exhaustion. CAF, cancer-associated fibroblasts; CRP, C-reactive protein; IDO, indoleamine-2,3-dioxygenase; IL, interleukin; MDSC, myeloid-derived suppressor cells; NLR, neutrophil-to-lymphocyte ratio; PD-1, programmed cell death 1; PD-L1, programmed death ligand 1; PGE2, prostaglandin E2; TAM, tumor-associated macrophage; TGF-β, transforming growth factor beta; TIM-3, T-cell immunoglobulin and mucin domain-containing protein-3; Treg, regulatory T cell.
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