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REVIEW

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Recent developments in immunotherapy for gastrointestinal tract cancers

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Abstract

The past few decades have witnessed the rise of immunotherapy for Gastrointestinal (GI) tract cancers. The role of immune checkpoint inhibitors (ICIs), particularly programmed death protein 1 (PD-1) and PD ligand-1 antibodies, has become increasingly pivotal in the treatment of advanced and perioperative GI tract cancers. Currently, anti-PD-1 plus chemotherapy is considered as first-line regimen for unselected advanced gastric/gastroesophageal junction adenocarcinoma (G/GEJC), mismatch repair deficient (dMMR)/microsatellite instability-high (MSI-H) colorectal cancer (CRC), and advanced esophageal cancer (EC). In addition, the encouraging performance of claudin18.2-redirected chimeric antigen receptor T-cell (CAR-T) therapy in later-line GI tract cancers brings new hope for cell therapy in solid tumour treatment. Nevertheless, immunotherapy for GI tumour remains yet precise, and researchers are dedicated to further maximising and optimising the efficacy. This review summarises the important research, latest progress, and future directions of immunotherapy for GI tract cancers including EC, G/GEJC, and CRC.

Keywords Gastrointestinal tract cancers, Immunotherapy, Biomarkers, Resistance, Precise treatment

Background

Worldwide, gastrointestinal (GI) tract cancers are the most prevalent malignancies with high morbidity and mortality [1]. According to the Global Cancer Observatory 2022 estimates, East Asia registered 1,469,225 new patients with GI tract cancers in 2022, accounting for 43.1% of the global incidence of GI tract cancers and 837,360 deaths, amounting to 41.7% of cancer-related deaths of GI tract cancers. Notably, the incidence of GI tract cancers is sharply increasing among young adults [2]. However, the etiological factors for GI tract cancers encompass infectious agents (such as HP, EBV infections), genetic factors (such as CDH1 mutations), and environmental factors (such as poor dietary habits) which have not been completely eliminated. In certain high-risk regions, routine endoscopic screening has not yet been universally implemented, severely affecting the health of populations and imposing economic burdens globally. Immunotherapy, one of the most recently developed therapies based on the theory of cancer immunoediting which indicated the crucial role of immune escape in tumour development and growth, has wrought a profound revolution in cancer treatment [3]. Immunotherapy was defined as therapeutic methods restoring

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the normal anti-tumour immune response, restarting the tumour-immune and further eliminating tumour cells [4]. Immune checkpoint inhibitors (ICIs), cancer vaccines, cell therapy, oncolytic virus (OVs) were in the category of immunotherapy.

Recent clinical trials, especially of ICIs targeting programmed death protein 1 (PD-1) and PD ligand-1(PD-L1), have shown their noteworthy efficacy in GI tract cancers and have contributed to a paradigm shift in treatment principles. Notwithstanding the anti-PD-1/PD-L1 treatment patterns of second- or later-line GI tract cancers, the success of Chekmate-649 [5], ORIENT-16 [6], Keynote-177 [7], and Keynote-590 [8] enabled anti-PD-1 antibodies plus chemotherapy be involved into the first-line mainstay treatment for esophagogastric junction (EGJ)/gastric cancer (GC), mismatch repair-deficient (dMMR)/microsatellite instability-high (MSI-H) colorectal cancer (CRC) and advanced oesophageal cancer (EC). Besides, Keynote-811 promoted the combination of trastuzumab, pembrolizumab, and chemotherapy as the standard first-line treatment for human epidermal growth factor receptor 2 (HER-2) positive GC [9]. Moreover, the long-lasting survival benefit of above large-scale clinical trials is under observation. The remarkable improvement in response to first-line therapy has boosted a surge of clinical trials focused on perioperative anti-PD-1/PD-L1 therapy in GI tract cancers [10–23]. Additionally, chimeric antigen receptor T-cell (CAR-T) therapy, exerts potent anti-tumour efficacy against GI cancers. According to phase I results in claudin18.2-redirected CAR-T cells (CT041) in patients with GI cancers who failed to respond after at least two prior lines of therapy, CT041 reached an objective response rate (ORR) and Disease control rate (DCR) of 48.6% and 73.0%, respectively. What's more, the 6-month response rate was 44.8% [24]. The initial triumph of the CT041 phase I clinical trial was a milestone marking the entry of CAR-T therapy into GI tumour treatment. Therefore, an increasing number of clinical trials and translational studies concerning novel immunotherapies, combination treatments, and therapy modes in distinct lines for GI are being performed.

Despite the significant therapeutic progress in immunotherapy, many challenges persist: the biomarkers with definite cut-off values remain unknown; the majority of patients with GI tract cancers still suffer from primary or secondary resistance; the strategies for patients with specific subtypes have not been identified; and the management of adverse effects is yet to be standardized. Here, we focused on the EC, G/GEJC, and CRC and systematically summarised the pivotal clinical trials and discussed the latest advances in the management of these cancers. Furthermore, we outlined the progress and challenges in the realisation of precision immunotherapy, including the exploration of biomarkers, investigation of resistance mechanisms, and strategies to optimise the development and efficacy of novel immunotherapies.

Current immunotherapy landscape

Oesophageal cancer

History and current situation of immunotherapy for EC

Approximately 70% of patients with EC are diagnosed at an advanced stage, with a median overall survival (OS) of 7–13 months under standard chemotherapy [25, 26] and a 5-year survival rate of 15–20% [27].

The histological subtypes of EC vary significantly across different regions. Oesophageal squamous cell carcinoma (ESCC) accounted for 85% of all EC worldwide, predominating in Eastern Europe and Asia. In contrast, oesophageal adenocarcinoma (EAC) comprised about 14% of cases and is more prevalent in Western Europe and North America [28]. EAC typically involves the lower third of the oesophagus and the GEJ, associated with Barrett's oesophagus, history of gastroesophageal reflux disease, obesity, and tobacco usage, sharing molecular characteristics similar to those in GC [27–30]. Conversely, ESCC generally affects the upper two-thirds of the oesophagus and is linked with both smoking and alcohol consumption, exhibiting molecular features more resemble those observed in head and neck squamous cell carcinoma. Despite the advances in treatment, there remains a lack of phase III clinical trials focusing exclusively on immunotherapy for EAC. Currently, the efficacy data for immunotherapy in EAC is primarily derived from subgroup analyses of related studies. Previous studies have compared second-line single-agent immunotherapy with chemotherapy and reported positive outcomes, preliminarily establishing the role of immunotherapy in ESCC [31]. Immunotherapy has therefore advanced towards first-line treatment (Table 1).

To date, data from several phase III clinical trials, including KEYNOTE-590 [8], ESCORT-1st [26], CheckMate-648 [45], ORIENT-15 [37], JUPITER-06 [38], RATIONALE-306 [39], GEMSTONE-304 [46] and ESCORT-RWS [47] demonstrated that the first-line combination of chemotherapy and PD-1/PD-L1 blockade (chemo + anti-PD-1/PD-L1) could extend the OS of patients with ESCC from less than 12 months with chemotherapy alone to approximately 16 months. These findings have solidified the premier role of immunochemotherapy as the first-line treatment for advanced ESCC. And subsequently led to the exploration of immunotherapy for locally advanced diseases. In terms of adjuvant treatment, the phase III study CheckMate-577 [43] revealed significant benefits for patients with EC or GEJC (EAC accounting for 71%) underwent neoadjuvant chemoradiotherapy (nCRT) followed adjuvant

Table 1 Summary of key clinical trials of immunotherapy in EC

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
EC	KEYNOTE-181 [32]	III	Second-line	Pembrolizumab vs Chemotherapy (Paclitaxel, Docetaxel, or Irinotecan)	OS (PD-L1 CPS ≥ 10, ESCC, all patients)	ORR: Total population: 13.1% vs 6.7% PD-L1 CPS ≥ 10: 21.5% vs 6.1% ESCC: 16.7% vs 7.4% OS: Total population: 7.1 vs 7.1 m, HR 0.89 (0.75–1.05) PD-L1 CPS ≥ 10: 9.3 vs 6.7 m, HR 0.69 (0.52–0.93) ESCC: 8.2 vs 7.1 m, HR 0.78 (0.63–0.96) ESCC PD-L1 CPS ≥ 10: 10.3 vs 6.7 m, HR 0.64 (0.46–0.90) PFS: Total population: 2.1 vs 3.4 m, HR 1.11 (0.94–1.31) PD-L1 CPS ≥ 10: 2.6 vs 3.0 m, HR 0.73 (0.54–0.97) ESCC: 2.2 vs 3.1 m HR 0.92 (0.75–1.13)	18.2% vs 40.9%
ESCC	ATTRACTION-3 [31]	III	Second-line	Nivolumab vs Chemotherapy (Paclitaxel or Docetaxel)	OS	ORR: 19% vs 22% OS: 10.9 vs 8.4 m, HR 0.77 (0.62–0.96) PFS: 1.7 vs 3.4, HR 1.08 (0.87–1.34)	18% vs 63%
ESCC	ESCORT [33]	III	Second-line	Camrelizumab vs Chemotherapy (Docetaxel or Irinotecan)	OS	ORR: 20.2% vs 6.4% OS: 8.3 vs 6.2 m, HR 0.71 (0.57–0.87) PFS: 1.9 vs 1.9 m, HR 0·69 (0.56–0.86)	19% vs 40%
ESCC	ORIENT-2 [34]	II	Second-line	Sintilimab vs Chemotherapy (Paclitaxel or Irinotecan)	OS	ORR: 12.6% vs 6.3% OS: 7.2 vs 6.2 m, HR 0.70 (0.50–0.97) PFS: 1.6 vs 2.9 m, HR 1.00 (0.72–1.39)	20.2% vs 39.1%
ESCC	RATIONALE-302 [35]	III	Second-line	Tislelizumab vs Chemotherapy (Paclitaxel, Docetaxel, or Irinotecan)	OS	ORR: 20.3% vs 9.8% OS: 8.6 vs 6.3 m, HR 0.70 (0.57–0.85) PFS: 1.6 vs 2.1 m, HR 0.83 (0.67 to 1.01)	18.8% vs 55.8%

Table 1 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
EC/GEJC (Siewert type 1)	KEYNOTE-590 [8]	III	First-line	Pembrolizumab + Chemotherapy(5-Fluorouracil + Cisplatin) vs Placebo + Chemotherapy(5-Fluorouracil + Cisplatin)	PFS(PD-L1 CPS ≥ 10, ESCC, all patients); OS(ESCC PD-L1 CPS ≥ 10, PD-L1 CPS ≥ 10, ESCC, all patients)	ORR: Total population: 45.0% vs 29.3% OS: Total population: 12.4 vs 9.8 m, HR 0.73 (0.62–0.86) PD-L1 CPS ≥ 10: 13.5 vs 9.4 m, HR 0.62 (0.49–0.78) ESCC: 12.6 vs 9.8 m, HR 0.72 (0.60–0.88) ESCC PD-L1 CPS ≥ 10: 13.9 vs 8.8 m, HR 0.57 (0.43–0.75) PFS: Total population: 6.3 vs 5.8 m, 0.65 (0.55–0.76) PD-L1 CPS ≥ 10: 7.5 vs 5.5 m, HR 0.51 (0.41–0.65) ESCC: 6.3 vs 5.8 m, HR 0.65 (0.54–0.78)	72% vs 68%

Table 1 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
ESCC	CheckMate-648 [36]	III	First-line	Nivolumab + Chemotherapy / Nivolumab + Ipilimumab vs Chemotherapy	PFS; OS	ORR: nivolumab + chemotherapy vs chemotherapy Total population: 47% vs 27% PD-L1 TPS ≥ 1%: 53% vs 20% nivolumab + ipilimumab vs chemotherapy Total population: 28% vs 27% PD-L1 TPS ≥ 1%: 35% vs 20% OS: nivolumab + chemotherapy vs chemotherapy Total population: 13.2 vs 10.7 m, HR 0.74(0.58–0.96) PD-L1 TPS ≥ 1%: 15.4 vs 9.1 m, HR 0.54(0.37–0.80) nivolumab + ipilimumab vs chemotherapy Total population: 12.7 vs 10.7 m, HR 0.78(0.62–0.98) PD-L1 TPS ≥ 1%: 13.7 vs 9.1 m, HR 0.64(0.46–0.90) PFS: nivolumab + chemotherapy vs chemotherapy Total population: 5.8 vs 5.6 m, HR 0.81(0.64–1.04) PD-L1 TPS ≥ 1%: 6.9 vs 4.4 m, HR 0.65(0.46–0.92) nivolumab + ipilimumab vs chemotherapy PD-L1 TPS ≥ 1%: 4.0 vs 4.4 m, HR 1.02(0.73–1.43)	nivolumab + chemotherapy: 47% nivolumab + ipilimumab: 32% chemotherapy: 36%
ESCC	ESCORT-1st [26]	III	First-line	Camrelizumab + Chemotherapy(Paclitaxel + Cisplatin) vs Placebo + Chemotherapy(Paclitaxel + Cisplatin)	PFS; OS	ORR: 72.1% vs 62.1% OS: 15.3 vs 12.0 m, HR 0.70 (0.56–0.88) PFS: 6.9 vs 5.6 m, HR 0.56 (0.46–0.68)	63.4% vs 67.7%

Table 1 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
ESCC	ORIENT-15 [37]	III	First-line	Sintilimab + Chemotherapy(Paclitaxel/5-Fluorouracil + Cisplatin) vs Placebo + Chemotherapy(Paclitaxel/5-Fluorouracil + Cisplatin)	OS(PD-L1 CPS ≥ 10, all patients)	ORR: Total population: 66% vs 45% PD-L1 CPS ≥ 10: 68% vs 49% OS: Total population: 16.7 vs 12.5 m, HR 0.63 (0.51–0.78) PD-L1 CPS ≥ 10: 17.2 vs 13.6 m, HR 0.64 (0.48–0.85) PFS: Total population: 7.2 vs 5.7 m, HR 0.56 (0.46–0.68) PD-L1 CPS ≥ 10: 8.3 vs 6.4 m, HR 0.58 (0.45–0.75)	60% vs 55%
ESCC	JUPITER-06 [38]	III	First-line	Toripalimab + Chemotherapy(Paclitaxel + Cisplatin) vs Placebo + Chemotherapy(Paclitaxel + Cisplatin)	PFS; OS	ORR: 69.3% vs 52.1% OS: 17.0 vs 11.0 m, HR 0.58 (0.43–0.78) PFS: 5.7 vs 5.5 m, HR 0.58 (0.46–0.74)	73.2% vs 70.0%
ESCC	RATIONALE-306 [39]	III	First-line	Tislelizumab + Chemotherapy(Cisplatin/Oxaliplatin + Fluorouracil / Capecitabine or Paclitaxel) vs Placebo + Chemotherapy(Cisplatin/Oxaliplatin + Fluorouracil /Capecitabine or Paclitaxel)	OS	ORR: 63% vs 42% OS: 17.2 vs 10.6 m, HR 0.66 (0.54–0.80) PFS: 7.3 vs 5.6 m, HR 0.62 (0.52–0.75)	67% vs 64%
ESCC	ASTRUM-007 [40]	III	First-line	Serplulimab + Chemotherapy(5-Fluorouracil + Cisplatin) vs Placebo + Chemotherapy(5-Fluorouracil + Cisplatin)	PFS; OS	ORR: 57.6% vs 42.1% OS: 15.3 vs 11.8 m, HR 0.68 (0.53–0.87) PFS: 5.8 vs 5.3 m, HR 0.60 (0.48–0.75)	53% vs 48%
ESCC	GEMSTONE-304 [41]	III	First-line	Sugemalimab + Chemotherapy(5-Fluorouracil + Cisplatin) vs Placebo + Chemotherapy(5-Fluorouracil + Cisplatin)	PFS; OS	ORR: 60.1% vs 45.2% OS: 15.3 vs 11.5 m, HR 0.70 (0.55–0.90) PFS: 6.2 vs 5.4 m, HR 0.67 (0.54–0.82)	51.3% vs 48.4%
ESCC	SKYSCRAPER-08 [42]	III	First-line	Tiragolumab + Atezolizumab + Chemotherapy(Paclitaxel + Cisplatin) vs Placebo + Chemotherapy(Paclitaxel + Cisplatin)	PFS; OS	ORR: 59.7% vs 45.5% OS: 15.7 vs 11.1 m, HR 0.70 (0.55–0.88) PFS: 6.2 vs 5.4 m, HR 0.56 (0.45–0.70)	62.2% vs 57.3%

Table 1 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
EC/GEJC	CheckMate-577 [43]	III	Adjuvant therapy (stage II or III)	Nivolumab vs Placebo	DFS	DFS: 22.4 vs 11.0 m, HR 0.69 (0.56–0.86)	13% vs 6%
ESCC(thoracic locally advanced ESCC)	ESCORT-NEO [44]	III	Neoadjuvant therapy(stage I-IVa)	Camrelizumab + Chemotherapy(Albumin-bound Paclitaxel + Cisplatin) vs Camrelizumab + Chemotherapy(Paclitaxel + Cisplatin) vs Chemotherapy(Paclitaxel + Cisplatin)	pCR; EFS	pCR: 28.0% vs 15.4% vs 4.7% MPR: 59.1% vs 36.2% vs 20.9%	34.1% vs 28.5% vs 28.8%

nivolumab therapy, showing improved disease-free survival (DFS) (22.4 months vs. 11.0 months, HR 0.69, $P<0.001$). Currently, most clinical trials of different neoadjuvant immunotherapy combined with chemotherapy (CT) or chemoradiotherapy (CRT) regimens were phase I or II studies, which demonstrated favourable pathologic complete response (pCR) rates ranging from 17 to 56% [48–59]. ESCORT-NEO [44] was the first phase III study to evaluate the role of neoadjuvant immunotherapy for resectable locally advanced ESCC (LA-ESCC) demonstrated that neoadjuvant camrelizumab plus chemotherapy can significantly improve pCR rate from 4.7 to 28% ($P<0.0001$) compared to chemotherapy alone. Moreover, the phase II study NICE [60] recently updated its 2-year follow-up data, showing that 37.3% of patients experienced disease recurrence with distant metastasis accounting for 27.4% of these cases, being the predominant recurrence pattern. The 2-year OS and progression-free survival (PFS) rates were 78.1% and 67.9%, respectively. Although these trials underline the short-term benefits of neoadjuvant immunotherapy plus chemotherapy, further data are essential for evaluating long-term survival outcomes. Accordingly, National Comprehensive Cancer Network (NCCN) guidelines recommend multiple immunotherapy combinations with chemotherapy as the first-line treatment for ESCC, with nivolumab specifically endorsed for adjuvant treatment.

Challenges in immunotherapy for ESCC

Perioperative immunotherapy necessitates comprehensive investigation Despite ongoing research into various regimens, there are currently no standard guidelines for neoadjuvant immunotherapy. Based on CROSS [61] and NEOCRTEC5010 [62], nCRT could improve survival over surgery alone among LA-ESCC patients and increase pCR rate to 29.0–43.2%. Further, combining nCRT with PD-1 blockade, as demonstrated in the phase II PALACE-1 [59] and NEOCRTEC1901 [63] studies, can increase the pCR rate to 50.0–55.6%. Neoadjuvant PD-1 blockade with chemotherapy also showed a significant increase in pCR rates over chemotherapy alone [44]. However, whether these higher pCR rates contribute to prolonged survival remains unclear and requires further validation in phase III randomized controlled trials (RCTs). Moreover, there is a notable absence of comparative studies between immunotherapy combined with nCRT versus nCT alone. Additionally, the role of neoadjuvant radiotherapy in the era of immunotherapy requires further clarification. Currently, there was only trials compared the nCRT and nCT. A Japanese study [64] showed that nCRT yielded superior OS compared to nCT in patients undergone R0 resection (mOS: not reached vs. 20.2months, $P=0.028$).In contrast, a Chinese study [65] indicated that nCRT did not significantly improve OS over nCT (HR 0.82, 95%CI 0.58–1.18, $P=0.28$), which may attribute to the inconsistency to differences in T stage and radiotherapy dosage among the study populations. Considering that combined radiotherapy may not substantially improve efficacy and that treatment-related deaths have been reported in studies including PALACE-1 [59], NEOCRTEC1901 [63] and EC-CRT-001 [66], the treatment regimens of immunotherapy combined with nCRT require further optimisation. For example, researchers are exploring whether reducing the dose of radiation or chemotherapy could achieve effective outcomes with reduced toxicity. As phase I SCALE-1 [67] study presented at the 2022 ASCO indicated, a short course of nCRT combined with toripalimab exhibited promising efficacy and tolerability in LA-ESCC.

To improve the effectiveness of the above-mentioned treatments, several promising directions are being explored: (1) Optimising combination chemotherapy regimens. Different chemotherapy agents affect tumour immune microenvironment (TIME) differently. For example, a meta-analysis, showed that the combination of taxane plus platinum (TP) with ICI exert higher survival rates compared to fluorouracil plus platinum [68]. Studies showed that taxanes could induce immunogenic death of tumour cells and activate TIME [69] making the taxane-based regimen a frequent choice in ongoing studies. Taxanes such as albumin-bound paclitaxel and paclitaxel are commonly used in EC clinical trials. The ESCORT-NEO [44] study compared the differences between albumin-bound paclitaxel and paclitaxel: group A (camrelizumab, albumin-bound paclitaxel, and cisplatin) and B (camrelizumab, paclitaxel, and cisplatin). Results showed that group A exhibited a higher pCR rate of 28.0%, compared to 15.4% in group B. This superior outcome in group A may be attributed to the "selective tumour local enrichment" effect of albumin-bound paclitaxel, which allows higher concentration in tumour tissues, potentially minimizing immune system damage. Additionally, the application of albumin-bound paclitaxel avoids the negative immune regulatory effects caused by glucocorticoids. (2) Optimising the sequence of administering chemotherapy and immunotherapy. Delaying PD-1 blockade until 3 days after administrating chemotherapy, as reported in a phase II study [51] could possibly enhance pCR rates by allowing chemotherapeutic agents to clear from the body before receiving anti-PD-1 agents, thus sparing T cells and maximizing cancer cell eradication. Thus, it is essential to explore the sequence of regimens in combination strategies by RCTs. (3) Determining the optimal duration of neoadjuvant therapy. The optimal mumber of neoadjuvant immunotherapy cycles remains uncertain. A prospective study investigating the combination

of nCT and camrelizumab for the treatment of ESCC revealed that the pCR rates within the four-cycle group (50.0%, 3/6) were not significantly superior to the two-cycle group (46.7%, 7/15) [70]. Excessively prolonged treatment may result in missing the optimal surgical timing, whereas insufficient treatment could result in inadequate tumour shrinkage. Thus, it is essential to conduct prospective RCTs to determine the most effective treatment cycle and customise the number of treatment cycles for each patient. (4) Identifying patients who would most benefit from immunotherapy. Notably, the 2-year OS rate for patients treated with neoadjuvant adebrelimab is 92% [71], higher than that of immunotherapy plus nCT. Thus, it is also vital to identify whether it is enough to receive neoadjuvant monoimmunotherapy for patients with specific features.

Efficacy-enhancing strategy remains unclear At present, several first-line combined immunotherapy treatments have shown promising outcomes, yet they failed to meet clinical demands. The challenges of improving the efficacy persist. In recent years, the number of clinical studies related to immunotherapy combination therapy has increased rapidly. CheckMate-648 [36] showed that nivolumab plus ipilimumab extended OS compared to chemotherapy alone, without new safety concerns identified, providing new chance for ESCC patients with chemotherapy intolerance. Additionally, the feasibility of combining PD-1/PD-L1 inhibitors with TIGIT monoclonal antibody has also been explored. The phase I study GO30103 [72] and phase II study AdvanTIG-203 [73] initially assessed the safety and efficacy of this combination. Furthermore, the phase III study SKYSRAPER-08 [42] compared the efficacy of tiragolumab (tira) + atezolizumab (atezo) + chemotherapy (CT) and placebo (pts) + CT, suggesting significantly improved PFS (6.2 months vs. 5.4 months) and OS (15.7 months vs. 11.1 months). The MORPHEUS-EC study [74] included the comparison of mono-immunotherapy (tira + atezo + CT vs. atezo + CT vs. CT alone) and showed that the OS in three groups were 16, 13.1, and 9.9 months, respectively. Although the combination of PD-1/PD-L1 blockade and TIGIT mAb did not demonstrate a clear numerical advantage over the 16-month OS of the first-line immunotherapy, there are notable differences in the efficacy and prognosis between monotherapy and combination immunotherapy in the phase Ib/II MORPHEUS-EC study [74]. Therefore, blockade of PD-1/PD-L1 combined with TIGIT mAb presents a promising strategy for ESCC. Additionally, given the unique anatomical site of EC, investigation into first-line systemic therapy combined with radiotherapy is warranted. Both the ESO-Shanghai 13 study [75] and the 2023 ESMO 1576P study [76] showed improvements in prognosis. More ongoing clinical studies on immunotherapy for esophageal cancer have been summarized in Table 2.

In summary, immunotherapy strategies of EC vary by tumour sites. The management of precise perioperativeimmunotherapy, the strategies to improve efficacy of diverse tumour sites, potential beneficiary population features in perioperative and advanced-stage settings are important directions for future development.

Gastric and gastroesophageal junction cancer

History and current situation of immunotherapy for G/GEJC

Despite the continuous advancement and optimisation of chemotherapy regimens for advanced G/GEJC, the efficacy of first-line chemotherapy for advanced G/GEJC remains poor, with an OS of no longer than 12 months [77]. Immunotherapy has achieved satisfactory results in the treatment of advanced G/GEJC (Table 3), breaking through the long-standing treatment bottleneck of short survival with traditional chemotherapy.

Immunotherapy initially began after the reporting of positive results from the ATTRACTION-2 [79] and KEYNOTE-059 [95] studies, which suggested that the single-agent immunotherapy in the later-line (≥3) treatment of advanced G/GEJC could significantly improve ORR and OS. Despite the KEYNOTE-061 [80] in the second-line treatment of advanced G/GEJC were failed to meet the primary endpoint, however the post-hoc analysis found pembrolizumab in PD-L1 CPS ≥ 10 patients had a better outcome than chemotherapy group, suggesting a potential benefit from immunotherapy. KEYNOTE-062 [96] initially explored the role of first-line immunotherapy in (HER-2-negative G/GEJC across three cohorts, including pembrolizumab monotherapy, pembrolizumab combined with chemotherapy and chemotherapy alone, with primary endpoints being OS and PFS in patients with PD-L1 CPS ≥ 1 or ≥ 10. Despite failing to meet its primary endpoint, an OS benefit was observed for pembrolizumab monotherapy over chemotherapy in PD-L1 CPS ≥ 10 patients (17. 4 months vs 10. 8 months; HR 0. 69; 95%CI 0. 49–0. 97). However, this finding was not statistically tested. The study's shortcomings are presumably attributed to its complex statistical design and the variability in chemotherapy regimens. Concurrently, the CheckMate-649 study [5] of first-line treatment using nivolumab for HER-2-negative G/GEJC enrolled 2687 patients across three cohorts, focusing on OS and PFS in the PD-L1 CPS ≥ 5 patients. This study demonstrated that nivolumab combined with chemotherapy increased mOS by 3.3months and decreased the risk of death by 29% compared to chemotherapy alone in the CPS ≥ 5 patients, with similar trends across the entire cohort. Subsequently, ORIENT-16 [6], KEYNOTE-859 [82], RATIONALE-305 [97], and GEMSTONE-303 [85]

Table 2 Summary of ongoing key phase III clinical trials of immunotherapy in EC

Disease	Population selection	Line	Regimen	Primary endpoint	Status	Sample size(estimated)	NCT number
ESCC	-	First-line	Active Comparator: Camrelizumab + Chemotherapy Experimental: Radiotherapy + Camrelizumab + Chemotherapy	OS	Recruiting	436	NCT06086457
ESCC	locally advanced	-	Experimental: Arm A Camrelizumb + Radiotherapy + Chemotherapy Placebo Comparator: Arm B Placebo + Radiotherapy + Chemotherapy	PFS	Unknown status	396	NCT04426955
ESCC	-	First-line	Experimental: Atezolizumab + Tiragolumab + Chemotherapy Placebo Comparator: Placebo + Chemotherapy	OS PFS	Active, not recruiting	461	NCT04540211
ESCC	Unresectable; have not progressed following definitive concurrent chemoradiotherapy	-	Experimental: Arm A: Tiragolumab + Atezolizumab Experimental: Arm B: Tiragolumab Placebo + Atezolizumab Placebo Comparator: Arm C: Tiragolumab Placebo + Atezolizumab Placebo	PFS OS	Active, not recruiting	760	NCT04543617
ESCC	-	Second-line	Experimental: Camrelizumab + Apatinib Active Comparator: Camrelizumab	OS	Unknown status	234	NCT05049681
ESCC	Stage T1-4aN1-3M0 or T3-4aN0M0	Neoadjuvant	Experimental: Radiotherapy + Sintilimab + Chemotherapy	OS	Recruiting	422	NCT05357846
			Active Comparator: Radiotherapy + Chemotherapy				
ESCC	PD-L1 CPS < 10	First-line	Experimental: Chemoradiation + Tislelizumab	PFS	Recruiting	155	NCT05919030
			Active Comparator: Chemotherapy + Tislelizumab				
ESCC	resectable cT1-4aN + M0 or T3-4aN0M0 and residue disease is found after neoadjuvant chemotherapy plus surgery or cT1-2N0M0 and pathologically proven T1-2N + M0 after upfront surgery	Adjuvant	Experimental: Sintilimab	DFS	Active, not recruiting	219	NCT05495152
			No Intervention: Observation Arm				
ESCC	R0 resectable thoracic esophageal cancer, cT1-3N1-2M0, cT2-3N0M0	Perioperative	Experimental: Pembrolizumab + Chemotherapy + Surgery + Pembrolizumab	EFS	Recruiting	342	NCT04807673
			Experimental: neoadjuvant chemoradiotherapy + Surgery				
ESCC	PD-L1 positive	First-line	Experimental: HLX10 + Chemotherapy	PFS	Active, not recruiting	489	NCT03958890
			Placebo Comparator: Placebo + Chemotherapy	OS			
ESCC	cT1N2-3M0 or cT2-4aN0-3M0 thoracic ESCC	Neoadjuvant	Experimental: Sintilimab + Chemotherapy	pCR rate	Not yet recruiting	420	NCT05244798
			Experimental: Sintilimab + Chemoradiotherapy				
			Other: Chemoradiotherapy				

Table 3 Summary of key clinical trials of immunotherapy in G/GEJC

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
G/GEJC	KEYNOTE-059(cohort 1) [78]	II	≥Tird-line	Pembrolizumab	ORR(all patients; PD-L1CPS ≥ 1)	ORR: Total population: 11.6% PD-L1CPS ≥ 1: 15.5% PFS: 2.0 m OS: 5.6 m	4.6%(immune-mediated)
G/GEJC	ATTRACTION-2 [79]	III	≥Tird-line	Nivolumab vs Placebo	OS	ORR: 11.2% vs 0% OS: 5.26 vs 4.14 m, HR 0.63 (0.51–0.78) PFS: 1.61 vs 1.45 m, HR 0.60 (0.49–0.75)	10% vs 4%(Grade 3 or 4)
G/GEJC	KEYNOTE-061 [80]	III	Second-line	Pembrolizumab vs Chemotherapy(Paclitaxel)	PFS (PD-L1 CPS ≥ 1); OS (PD-L1 CPS ≥ 1)	ORR: PD-L1 CPS ≥ 1: 16% vs 14% OS: PD-L1 CPS ≥ 1: 9.1 vs 8.3 m, HR 0.82(0.66–1.03) PFS: PD-L1 CPS ≥ 1: 1.5 vs 4.1 m, HR 1.27 (1.03–1.57)	14% vs 35%
G/GEJC	ATTRACTION-4 [81]	III	First-line	Nivolumab + Chemotherapy (Oxaliplatin + S-1/Capecitabine) vs Placebo + Chemotherapy (Oxaliplatin + S-1/Capecitabine)	PFS; OS	ORR: 57% vs 48% OS: 17.45 vs 17.15 m, HR 0.90 (0.75–1.08) PFS: 10.45 vs 8.34 m, HR 0.68 (0.51–0.90)	18% vs 9%
G/GEJC	ORIENT-16 [6]	III	First-line	Sintilimab + Chemotherapy(Capecitabine + Oxaliplatin) vs Placebo + Chemotherapy(Capecitabine + Oxaliplatin)	OS (PD-L1 CPS ≥ 5, all patients)	ORR: Total population: 58.2% vs 48.4% PD-L1 CPS ≥ 5: 63.6% vs 49.4% OS: Total population: 15.2 vs 12.3 m, HR 0.77 (0.63–0.94) PD-L1 CPS ≥ 5: 18.4 vs 12.9 m, HR 0.66 (0.50–0.86) PFS: Total population: 7.1 vs 5.7 m, HR 0.64 (0.52–0.77) PD-L1 CPS ≥ 5: 7.7 vs 5.8 m, HR 0.63 (0.49–0.81)	59.8% vs 52.5%

Table 3 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
G/GEJC	KEYNOTE-859 [82]	III	First-line	Pembrolizumab + Chemotherapy(Fluorouracil + Cisplatin or Capecitabine + Oxaliplatin) vs Placebo + Chemotherapy(Fluorouracil + Cisplatin or Capecitabine + Oxaliplatin)	OS (PD-L1 CPS ≥ 1, PD-L1 CPS ≥ 10, all patients)	ORR: Total population: 51% vs 42% PD-L1 CPS ≥ 1: 52% vs 43% PD-L1 CPS ≥ 10: 61% vs 43% OS: Total population: 12.9 vs 11.5 m, HR 0.78 (0.70–0.87) PD-L1 CPS ≥ 1: 13.0 vs 11.4 m, HR 0.74 (0.65–0.84) PD-L1 CPS ≥ 10: 15.7 vs 11.8 m, HR 0.65 (0.53–0.79) PFS: Total population: 6.9 vs 5.6 m, HR 0.76 (0.67–0.85) PD-L1 CPS ≥ 1: 6.9 vs 5.6 m, HR 0.72 (0.63–0.82) PD-L1 CPS ≥ 10: 8.1vs 5.6 m, HR 0.62 (0.51–0.76)	59% vs 51%

Table 3 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
EAC/GC/GEJC	CheckMate-649 [5, 83, 84]	III	First-line	Nivolumab + Chemotherapy (Capecitabine + Oxaliplatin or Leucovorin + Fluorouracil + Oxaliplatin) /Nivolumab + Ipilimumab vs Chemotherapy(Capecitabine + Oxaliplatin or Leucovorin + Fluorouracil + Oxaliplatin)	nivolumab + chemotherapy vs chemotherapy PFS (PD-L1 CPS ≥ 5); OS (PD-L1 CPS ≥ 5)	OS: nivolumab + chemotherapy vs chemotherapy Total population: 13.7 vs 11.6 m, HR 0.79(0.71–0.88)(4 year follow-up) PD-L1 CPS ≥ 5: 14.4 vs 11.1 m, HR 0.70(0.61–0.81)(4 year follow-up) nivolumab + ipilimumab vs chemotherapy Total population: 11.7 vs 11.8 m, HR 0.91(0.77–1.07) PD-L1 CPS ≥ 5: 11.2 vs 11.6 m, HR 0.89(0.71–1.10) PFS: nivolumab + chemotherapy vs chemotherapy Total population: 7.7 vs 6.9, HR 0.80(0.71–0.89)(4 year follow-up) PD-L1 CPS ≥ 5: 8.3 vs 6.1 m, HR 0.71(0.61–0.82)(4 year follow-up) nivolumab + ipilimumab vs chemotherapy Total population: 2.8 vs 7.1 m, HR 1.66(1.40–1.95) PD-L1 CPS ≥ 5: 2.8 vs 6.3 m, HR 1.42(1.14–1.76)	nivolumab + chemotherapy: 60%(Grade 3 or 4) nivolumab + ipilimumab: 38%(Grade 3 or 4) chemotherapy: 45%, 46%
G/GEJC	GEMSTONE-303 [85]	III	First-line	Sugemalimab + Chemotherapy(Capecitabine + Oxaliplatin) vs Placebo + Chemotherapy(Capecitabine + Oxaliplatin)	PFS; OS	ORR: 68.6% vs 52.7% OS: 15.64 vs 12.65 m, HR 0.75 (0.61–0.92) PFS: 7.62 vs 6.08 m, HR 0.66 (0.54–0.81)	31.1% vs 28.7%

Table 3 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
G/GEJC	RATIONALE-305 [86, 87]	III	First-line	Tislelizumab + Chemotherapy(Capecitabine + Oxaliplatin or Fluorouracil + Cisplatin) vs Placebo + Chemotherapy (Capecitabine + Oxaliplatin or Fluorouracil + Cisplatin)	OS(PD-L1-TAP ≥ 5%, all patients)	ORR: Total population: 47.3% vs 40.5% PD-L1-TAP ≥ 5%: 50.4% vs 43.0% OS: Total population: 15.0 vs 12.9 m, HR 0.80 (0.70–0.92) PD-L1-TAP ≥ 5%: 17.2 vs 12.6 m, HR 0.74(0.59–0.94) PFS: Total population: 6.9 vs 6.2 m, HR 0.78 (0.67–0.90) PD-L1-TAP ≥ 5%: 7.2 vs 5.9 m, HR 0.67(0.55–0.83)	53.8% vs 49.8%(Total population) 64.7% vs 62.9%(PD-L1-TAP ≥ 5%)
G/GEJC	KEYNOTE-811 [88]	III	First-line	Pembrolizumab + Trastuzumab + Chemotherapy (Fluoropyrimidine and Platinum-based) vs Placebo + Trastuzumab + Chemotherapy(Fluoropyrimidine and Platinum-based)	PFS; OS	ORR(third interim analysis): Total population: 72.6% vs 60.1% OS(third interim analysis): Total population: 20.0 vs 16.8 m, HR 0.84(0.70–1.01) PD-L1 CPS ≥ 1: 20.0 vs 15.7 m, HR 0.81(0·67–0·98) PFS(third interim analysis): Total population: 10.0 vs 8.1 m, HR 0.73(0.61–0.87) PD-L1 CPS ≥ 1: 10.9 vs 7.3 m, HR 0.71(0.59–0.86)	58% vs 51%(second interim analysis)

Table 3 (continued)

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
G/GEJC	KEYNOTE-585 [89, 90]	III	perioperative	Pembrolizumab + Chemotherapy (Cisplatin-based doublet Chemotherapy or Fluorouracil + Docetaxel + Oxaliplatin) vs Placebo + Chemotherapy (Cisplatin-based doublet chemotherapy or Fluorouracil + Docetaxel + Oxaliplatin)	pCR; EFS; OS	pCR: main cohort: 12.9% vs 2.0% main + FLOT cohort: 13.0% vs 2.4% EFS: main cohort: 44·4 vs 25.3 m, HR 0·81(0.67–0.99) main + FLOT cohort: 45.8 vs 25.7 m, HR 0.81(0.68–0.97) OS(third interim analysis): main cohort: 60·7 vs 58.0 m, HR 0·90(0.73–1.12) main + FLOT cohort: 60.7 vs not reached, HR 0.93(0.76–1.12)	65%(main cohort) vs 67%(main + FLOT cohort) vs 63%(chemotherapy)
G/GEJC	MATTERHORN [91]	III	Perioperative	Durvalumab + Chemotherapy(Fluorouracil + Docetaxel + Oxaliplatin) vs Placebo + Chemotherapy (Fluorouracil + Docetaxel + Oxaliplatin)	EFS	pCR: 19% vs 7%	58% vs 56%
G/GEJC	ATTRACTION-5 [92]	III	Adjuvant(pStage III)	Nivolumab + Chemotherapy(S-1 or Capecitabine + Oxaliplatin) vs Placebo + Chemotherapy(S-1 or Capecitabine + Oxaliplatin)	RFS	RFS(3-year RFS rates): 68.4% vs 65.3%	54.4% vs 46.8%
G/GEJC	GERCOR NEONIPIGA [93]	II	Neoadjuvant	Nivolumab + Ipilimumab	pCR	pCR: 58.6%	19%
G/GEJC	INFINITY(cohort 1) [94]	II	Neoadjuvant	Tremelimumab + Durvalumab	pCR	pCR: 60%	16.7%

showed that combination chemotherapy with sindilizumab, pembrolizumab, tirelizumab, and suglizumab (PD-L1 mAb) significantly increased mOS to 13 ~ 15 months in first-line treatment for HER-2-negative G/GEJC, especially in patients with high PD-L1 expression, where mOS could reach 15 ~ 18 months, and ORR was approximately 60%. Notably, as a biomarker-driven clinical trials, GEMSTONE-303 [85] specifically enrolled individuals with PD-L1 expression ≥ 5%, thus resulting in relatively high efficacy and survival benefits.

Based on these promising results, PD-1/PD-L1 mAb combined with platinum-containing chemotherapy has become the standard recommended regimen for the first-line treatment of G/GEJC, as incorporated into NCCN guidelines. Besides, it is noteworthy that long-term follow-up data from Checkmate-649 revealed a marked survival advantage in the Chinese population when compared to the global average, particularly notable in patients with a CPS ≥ 5, where the 3-year OS rates are 31% [98] and 17% [99] respectively. This disparity likely stems from genetic and lifestyle differences between Eastern and Western populations. Additionally, molecular characteristics, the TME, and health economics concerns should also be considered. The shared dining customs among Chinese may increase the transmission of Helicobacter pylori (HP), a known precursor for G/GEJC [100, 101]. A comprehensive meta-analysis involving 11 studies showed that G/GEJC cases with HP positivity are associated with higher PD-L1 expression levels, which correlates to better responses to immunotherapy [102, 103]. A study by Jia et al. [104] demonstrated that patients with HP-positive, EBV-negative, and MSS-positive G/GEJC achieved significantly longer immune-related PFS (irPFS) (6.97 months vs 5.03 months, HR 0.76, $p < 0.001$) and showed a tendency toward four months extended irOS in comparison to those in the HP-negative group (18.3 months vs 14.2 months, $p = 0.105$). Therefore, considering the characteristics of GC populations in different regions, the high spatiotemporal heterogeneity of GC, and the complexity of the microenvironment, researchers around the world have been exploring personalized and precise immunotherapy strategies for GC patients with specific features in recent years.

Current situation of immunotherapy for G/GEJC with specific subtypes

G/GEJC is characterized by significant heterogeneity and a diversity of molecular subtypes. Several studies concentrating on specific molecular subtypes of G/GEJC have demonstrated that combination immunotherapy may offer survival advantages, especially in patients with MSI-H and EBV infection. These insights are often derived from subgroup analyses in combination immunotherapy clinical trials [80, 96], or from retrospective evaluations of clinical data [105]. The discussion below will detail the investigation of immunotherapy for particular subtypes of G/GEJC.

HER-2 HER-2 is a classic target for G/GEJC. ToGA study had established trastuzumab combined with chemotherapy as the first-line standard treatment for HER-2-positive G/GEJC [106]. Since then, despite numerous failures in the development of new drugs targeting HER-2, the emergence of immunotherapy and antibody–drug conjugates (ADCs) has dramatically altered the treatment landscape for HER-2-positive GC. KEYNOTE-811 evaluated trastuzumab plus chemotherapy with or without pembrolizumab in the first-line treatment of HER-2-positive G/GEJC. The third interim analysis (follow-up 38.5 months) [88] showed significant improvement in mPFS and mOS for patients with ITT and PD-L1 CPS ≥ 1 when combined with ICIs. However, for patients with CPS < 1, there was no difference in mPFS and mOS was worse when combined immunotherapy.Given the promising results, NCCN guidelines have restricted the indication for combined immunotherapy to HER-2-positive patients with CPS ≥ 1. The lack of survival benefit of combination immunotherapy in patients with CPS < 1 may stem from a higher percentage of patients in the control group receiving subsequent treatments (47% vs 39%). Additionally, HER-2 may up-regulate PD-L1 expression [107], and the status of immunotherapy included in follow-up treatment after progression in the control group is unknown, which could also affect the outcome. Besides trastuzumab combined with immunotherapy, HER-2-targeted ADCs have also achieved satisfying efficacy in clinical application of G/GEJC. The results from the Chinese mul-ticenter phase I study C103 [108] showed that in HER-2-expressing G/GEJC who progressed after at least first-line treatment, the ORR for disitamab vedotin (RC-48) combined with toripalimab was 43%, with an mPFS of 6.2 months, and an mOS of 16.8 months. Clinical benefits were also observed in HER-2-positive and low-expressing patients at the recommended phase II dose, with ORR of 56% and 46%, respectively, and mPFS of 7.8 months and 5.8 months, respectively. These outcomes represent a significant improvement compared over previous data for RC-48 monotherapy. In addition, duration of response (DoR) in second-line treatment was significantly outperformed that in third-line and subsequent treatments, 15.6 months and 3.6 months respectively, suggesting that the advance of the combined treatment regimen could yield superior benefits. A clinical trial evaluating RC-48 combined with toripalimab and chemotherapy or trastuzumab in first-line HER-2-expressing G/GEJC is ongoing (NCT05980481), with results eagerly anticipated.

Claudin18. 2 According to the SPOTLIGHT [109] and GLOW [110], Zolbetuximab, a CLDN18.2 mAb in combination with chemotherapy provided a survival benefit in first-line Claudin18.2-positive (≥75% of tumor cells exhibit moderate-to-strong membranous CLDN18.2 staining) and HER-2-negative G/GEJC. Preclinical study [111] demonstrated that zolbetuximab combined with chemotherapy can significantly promote CD8 + T cell infiltration. Moreover, when combined with PD-1 mAb, zolbetuximab more effectively inhibited tumour growth, and significantly increased the number of mice achieving complete response (CR), indicating a synergistic effect. The phase 1 study of QLS31905 [112] a CLDN18.2/CD3 bispecific antibody, in advanced solid tumours, presented at the 2023 ESMO, included 31 patients with G/GEJC and initially reported safety and efficacy data. Further clinical studies focusing on CLDN18.2 combination immunotherapy are currently underway (NCT06206733, NCT05964543).

dMMR/MSI-H Notable characteristics of dMMR/MSI-H G/GEJC include a favourable prognosis, chemotherapy insensitivity and obvious benefits from immunotherapy [113]. Due to the low incidence, high-level evidence from large sample studies is absent. Subgroup analyses from the KEYNOTE-059, KEYNOTE-061 and KEYNOTE-062 studies initially suggested that PD-1 mAb exhibits a high response rate and prolonged survival in dMMR/MSI-H G/GEJC [114]. Preliminary findings from the phase II prospective single-arm NO LIMIT study [115] indicate that nivolumab combined with ipilimumab as a first-line treatment results in an ORR of 62.1%, with 3 patients (10%) achieving CR, a DCR of 79.3%, and a mPFS of 13.8 months, aligning with the established safety profile of dual immunotherapy. In addition, results from multiple prospective phase II studies have shown therapeutic efficacy of envafolimab [116], tislelizumab [117], serplulimab [118], and pembrolizumab [119] in the second-line treatment of dMMR/MSI-H advanced G/GEJC patients who have not previously received immunotherapy..

EBVaGC EBV associated GC (EBVaGC) patients, characterized by highly active immune-active TME [120], may benefit from immunotherapy. However, the efficacy of immunotherapy in EBV-positive G/GEJC are inconsistent [105, 121, 122]. At present, a clinical trial investigating double-immunotherapy in EBV-positive G/GEJC patients is nearing completion (NCT04202601), with results eagerly anticipated.

AFPGC *Alpha fetoprotein-producing G/GEJC* (AFPGC), a distinct subtype of G/GEJC, comprises approximately 6% and is known for its predisposition to liver and lymph node metastases, elevated AFP levels, and a poor prognosis [123]. A prospective phase II study [124] demonstrated that a combination of camrelizumab, apatinib and SOX achieved promising outcomes in advanced G/GEJC patients with AFP > 2 × ULN or AFP positive, with an ORR of 55.6%, a 12-month PFS rate of 42.1%, and a 12-month OS rate of 63.7%.

The results presented above indicate that G/GEJC, which expresses numerous specific targets, can be effective in combination with immunotherapy. The subsequent steps involve further validating the efficacy through additional phase III studies and conducting translational research to elucidate the precise mechanisms impacting immunotherapy, aiming to facilitate its clinical application. Furthermore, it is important to note that while G/GEJC expressing different targets can receive targeted therapy, multiple targets are often expressed simultaneously. In this regard, determining the appropriate weighting and sequencing of different targeted therapies is crucial, necessitating a deeper understanding of tumours and the TME.

With the continuous exploration of new targets, molecular typing is becoming increasingly precise, and treatment options tending to be diversified. Compared to EC and CRC, molecular targets that have been discovered and successfully translated into clinical practice are enriched in G/GEJC. Therefore, an increasing number of biomarker-driven gastric cancer-related clinical studies are underway, covering populations with dMMR/MSI (NCT 06346197), HER-2 positive (NCT05152147), CLDN18.2 positive (NCT06093425, NCT06206733), PD-L1 positive (NCT06346197, NCT06093425, NCT06206733), FGFR2b positive (NCT05111626) GC. For patients expressing specific targets, a major goal of biomarker-driven research is to further explore whether immune combination targeted therapy produces synergistic anti-tumour effects and whether combination therapy can help these patients achieve a chemo-free state. Additionally, accompanying clinical research to further explore biomarkers of patient efficacy and survival, and to uncover resistance mechanisms and reversal strategies, will aid in achieving a sustained refinement of GC stratification (Table 4).

Challenges in immunotherapy for G/GEJC

Efficacy-enhancing strategy remains unclear

Despite the advancements of ICIs as first-line regimen in G/GEJC, approximately 40% of patients still could not benefit from combination therapy with single-agent ICIs. The mOS shows a modest extension of merely 1–2 months for intention-to-treat (ITT) patients and 4–5 months for those with high PD-L1 expression, which is insufficient to meet clinical expectations.

Table 4 Summary of ongoing key phase III clinical trials of immunotherapy in G/GEJC

Disease	Population selection	Line	Regimen	Primary endpoint	Status	Sample size(estimated)	NCT number
G/GEJC	D2/R0 resected	Adjuvant	Experimental: Chemotherapy + PD-1 Inhibitors + Chemoradiotherapy	3-year DFS	Recruiting	433	NCT04997837
	pN3		Active Comparator: Chemotherapy				
G/GEJC	D2/R0 resected	Adjuvant	Experimental: JS001 + Chemotherapy	DFS	Recruiting	680	NCT05180734
	pathological stage II (T4aN0M0) and stage III		Placebo Comparator: Placebo + Chemotherapy				
G/GEJC	MSI/dMMR	First-line	Experimental: Botensilimab + Balstilimab	OS	Not yet recruiting	124	NCT06346197
	HER-2 negative		Active Comparator: Chemotherapy + Nivolumab				
	PD-L1 CPS ≥ 5						
G/GEJC	HER-2 negative	First-line	Experimental A: SHR-1210 + Apatinib + XELOX	OS (all patients, PD-L1 positive)	Unknown status	887	NCT03813784
			Active Comparator B: XELOX				
			Experimental C: SHR-1210 + XELOX				
G/GEJC	Claudin18.2 positive	First-line	Active Comparator: TST001 + PD-1 Inhibitors + Chemotherapy	PFS	Not yet recruiting	950	NCT06093425
	HER-2 negative		Placebo Comparator: Placebo + PD-1 Inhibitors + Chemotherapy				
	PD-L1 positive						
G/GEJC	CLDN 18.2 positive	First-line	Experimental: ASKB589 + Tislelizumab + Chemotherapy	PFS	Recruiting	780	NCT06206733
	Suitable for chemotherapy combined with PD-1 inhibitor		Placebo Comparator: Placebo + Tislelizumab + Chemotherapy				
	Not suitable for anti-HER-2 therapy						
G/GEJC	FGFR2b positive	First-line	Experimental: Bemarituzumab + Nivolumab + Chemotherapy	OS	Recruiting	528	NCT05111626
	HER-2 negative		Placebo Comparator: Placebo + Nivolumab + Chemotherapy				
G/GEJC	HER-2 negative	Second-line	Experimental: Cadonilimab + pulocimab + paclitaxel	PFS	Recruiting	506	NCT06341335
			Active Comparator: Placebo + paclitaxel	OS			
G/GEJC	HER-2 negative	First-line	Experimental: AK104 + Chemotherapy	OS	Active, not recruiting	610	NCT05008783
			Placebo Comparator: Placebo + Chemotherapy				
G/GEJC	HER-2 negative	First-line	Experimental: Domvanalimab + Zimberelimab + Chemotherapy	OS	Active, not recruiting	1040	NCT05568095
			Active Comparator: Nivolumab + Chemotherapy				

Table 4 (continued)

Disease	Population selection	Line	Regimen	Primary endpoint	Status	Sample size(estimated)	NCT number
G/GEJC	HER-2 negative	First-line	Experimental: mFOL-FIRINOX + nivolumab Active Comparator: mFOL-FOX + nivolumab	OS	Recruiting	382	NCT05677490
G/GEJC	HER-2 positive	First-line	Active Comparator: Arm A Trastuzumab + Chemotherapy Experimental: Arm B Zanidatamab + Chemotherapy Experimental: Arm C Zanidatamab + tislelizumab + Chemotherapy	PFS OS	Recruiting	918	NCT05152147
G/GEJC	-	≥ Third-line	Experimental: Regorafenib + nivolumab Active Comparator: Standard of Care	OS	Active, not recruiting	450	NCT04879368

Clinical trials investigating combination therapy never stop. The dual blockade of nivolumab plus ipilimumab cohort in CheckMate-649 [5] did not demonstrate prolonged survival compared to chemotherapy alone, indicating that chemo-free regimens may not be suitable for all patients with advanced G/GEJC. COMPASSION-15 [125] showed that cadonilimab (AK104, a PD-1/CTLA bispecific antibody) combined with chemotherapy significantly improved mOS compared with placebo combination chemotherapy (15.0 months vs 10.8 months, HR 0.62), even in PD-L1 low expression patients (PD-L1 22C3 CPS < 5), indicating that cadonilimab combined with chemotherapy benefits patients regardless of PD-L1 expression status, providing a new treatment approach for HER-2 negative and PD-L1 low expression G/GEJC. Additionally, The sequence of medications in treatment protocols warrants careful consideration. The JAVELIN Gastric 100,103 study [126] evaluated the effect of fusing Avelumab, a PD-L1 monoclonal antibody, as maintenance therapy after a minimum of 12 weeks of first-line chemotherapy. While OS benefits were not observed universally, notable differences were noted among patients without metastatic sites after induction chemotherapy and a small subset with MSI-H. What's more, avelumab showed favourable safety profiles compared to continuous chemotherapy, offering new insights for maintenance therapy in selected subgroups of G/GEJC patients.

Perioperative immunotherapy strategies were yet established

Encouraged by the results of immunotherapy in advanced G/GEJC [98], the integration of immunotherapy in frontline treatments is under investigation recently.

Several phase II studies have demonstrated that neoadjuvant chemotherapy combined with immunotherapy can improve pCR or major pathologic response (MPR) rate [127–129]. KEYNOTE-585 [16] revealed that neoadjuvant pembrolizumab combined with chemotherapy significantly improved pCR rate. Although long-term follow-up did not demonstrate statistical improvement in event-free survival (EFS), there was a noticeable trend towards delaying disease recurrence. The near-significant *P*-value highlights the importance of statistical design in clinical trial research. Further long-term follow-up data from MATTERHORN [91] and HLX-10 (NCT04139135) neoadjuvant studies are expected.

ATTRACTION-5 [92] evaluated the efficacy of nivolumab in combination with chemotherapy as adjuvant therapy for phase III G/GEJC, revealing no significant improvement in relapse-free survival (RFS). However, subgroup analysis indicated benefits from immunotherapy for patients with ECOG 1, postoperative pathology stage IIIc, and PD-L1 tumour proportion score ≥ 1.

For the perioperative treatment for the special MSI-H G/GEJC, GERCOR NEONIPIGA [11] and INFINITY [130] preliminarily explored the feasibility of perioperative immunotherapy alone for locally advanced MSI-H G/GEJC without chemotherapy, and achieved satisfactory pCR rates of 58.6–60.0%.

All in all, the significant heterogeneity of G/GEJC and the complexity of its microenvironment present formidable challenges in advancing immunotherapy. In the era of precision medicine, developing strategies to overcome this heterogeneity and achieve "high efficiency and low

toxicity", comprehensive management of immunotherapy will be crucial for future advancements.

Colorectal cancer

History and current state of immunotherapy for CRC

Immunotherapy has been utilised against dMMR/MSI-H CRC (Table 5). The efficacy of this cancer subtype is largely due to the high tumour mutational burden, abundance of neoantigens, and increased immune cell infiltration [131, 132]. Compared with that of other tumour types, the incidence of MSI-H/dMMR in CRC is relatively high (~ 15%) [132, 133]. MSI-H/dMMR CRC tends to be less sensitive to chemotherapy-based treatments and has a lower ORR than that of microsatellite stable (MSS)/proficient mismatch repair (pMMR) CRC [134–137]. For later-line treatment of MSI-H/dMMR metastatic CRC (mCRC), multiple studies have confirmed the efficacy of immunotherapy, with PD-1 monotherapy showing an ORR of 32.8–40% [138–140]. The results from these clinical studies have facilitated the exploration of immunotherapy as a first-line option against MSI-H/dMMR mCRC. The KEYNOTE-177 evaluated the antitumor activity of pembrolizumab (pembro) vs. chemotherapy ± bevacizumab or cetuximab (chemo) and showed that pembro was superior to chemo for mPFS (16.5 m vs. 8.2 m; HR 0.60) [141]. Additionally, CheckMate-8HW compared nivolumab (NIVO) + ipilimumab (IPI) with NIVO or chemotherapy (chemo); NIVO + IPI demonstrated clinically meaningful and statistically significant improvement in PFS vs. chemo, with a 79% reduction in the risk of disease progression or death [142]. These trials demonstrated that both mono- and dual-immunotherapy were superior to chemotherapy in terms of efficacy and reducing associated AEs.

Challenges in immunotherapy for MSI-H/dMMR CRC

Efficacy-enhancing strategy remains unclear The retrospective study by Chen et al. demonstrated that chemo-anti–PD-1/PD-L1 therapy was more effective than anti–PD-1/PD-L1 alone in treating MSI/dMMR GI cancers, offering better survival benefits [145]. Another phase III trial, COMMIT, is currently assessing the safety and effectiveness of atezolizumab alone versus its combination with mFOLFOX6 and bevacizumab as a first-line treatment for dMMR/MSI-H mCRC [146]. Wu et al. investigated the efficacy of combining COX inhibitors with ICIs for the treatment of dMMR/MSI-H mCRC. The results indicated that the mPFS and mOS were not reached, with an ORR of 73.3% [147]. Additionally, the results of ongoing CheckMate-8HW study comparing NIVO + IPI to Nivolumab monotherapy will provide insight into whether first-line NIVO + IPI can enhance the therapeutic efficacy over

Table 5 Summary of key clinical trials of immunotherapy in CRC

Disease	Trial	Stage	Line	Therapy	Primary endpoint	Outcomes	TRAEs
CRC	CheckMate-142(cohort 1 and 2) (5-year follow-up) [143]	II	≥ Second-line	Cohort 1: Nivolumab Cohort 2: Nivolumab + Ipilimumab	ORR	ORR: cohort 1: 39% cohort 2: 65% OS: cohort 1: 44.2 m cohort 2: not reached PFS: cohort 1: 13.8 m cohort 2: not reached	cohort 1: 27% cohort 2: 32%
CRC	KEYNOTE-164 [140]	II	≥ Third-line(cohort A) ≥ Second-line(cohort B)	Pembrolizumab	ORR	ORR: cohort A: 32.8% cohort B: 34.9% OS: cohort A: 31.4 m cohort B: 47.0 m PFS: cohort A: 2.3 m cohort B: 4.1 m	≥ 2: 12.7% ≥ 3: 16.4%
CRC	KEYNOTE-177 [136]	III	First-line	Pembrolizumab vs Chemotherapy(5-Fluorouracil–based therapy ± Bevacizumab or Cetuximab)	PFS; OS	ORR: 43.8% vs 33.1% OS: 77.5 vs 36.7 m, HR 0.73(0.53–0.99) (5-year follow-up) PFS: 16.5 vs 8.2 m, HR 0.60 (0.45–0.80)	21.6% vs 67.1%(5-year follow-up)
CRC	CheckMate-142(cohort 3)(64-month follow-up) [144]	II	First-line	Nivolumab + Ipilimumab	ORR	ORR: 71% OS: not reached PFS: not reached	20%

NIVO for dMMR/MSI-H mCRC [142]. More clinical trials on ongoing are detailed in the Table 6.

Optimal treatment strategies following progression necessitates explored A retrospective study analysed 51 patients with dMMR/MSI-H GI cancers who continued to receive antitumor therapy after progression on immunotherapy. Of these patients, 35 cases (68.6%) were mCRC. The study concluded that continuing with anti-PD-1/PD-L1 therapy along with other drugs significantly improved the disease control rate, PFS, and OS compared with receiving chemotherapy alone or with targeted therapy [148]. The ongoing NIPIRESCUE study (NCT05310643) evaluates the efficacy of nivolumab and ipilimumab in MSI/dMMR mCRC patients following resistance to PD-1 monotherapy. The results are eagerly anticipated.

Perioperative immunotherapy strategies require further validation Despite no statistical difference in OS between the Pembrolizumab group and the Chemotherapy group in KEYNOTE-177 [141], a trend towards decreased mortality risk was observed, indicating that immunotherapy should be utilised early and encouraging further investigation of immunotherapy in the perioperative treatment of CRC. The NICHE-1 [149] and NICHE-2 trial [150], demonstrated effective neoadjuvant outcomes for NIVO + IPI, showing pCR rates of 60–67% and MPR rates of 95–97%.

Table 6 Summary of ongoing key phase III clinical trials of immunotherapy in CRC

Disease	Population selection	Line	Regimen	Primary endpoint	Status	Sample size(estimated)	NCT number
CRC	dMMR/MSI-H	First-line	Experimental: Pembrolizumab Active Comparator: Standard of Care Chemotherapy	PFS	Recruiting	100	NCT05239741
CRC	dMMR/MSI-H	First-line	Active Comparator: Arm I (bevacizumab + mFOLFOX6) Experimental: Arm II (atezolizumab) Experimental: Arm III (atezolizumab + bevacizumab + mFOLFOX6)	PFS	Recruiting	120	NCT02997228
CRC	dMMR/MSI-H	all lines (Part 1 enrollment) First-line (Part 2 enrollment)	Experimental: Arm A: Nivolumab Experimental: Arm B: Nivolumab + Ipilimumab Active Comparator: Arm C: Investigator's Choice Chemotherapy	PFS	Recruiting	831	NCT04008030
CRC	MSS/MSI-low	Progressed or intolerant to standard-of-care	Experimental: XL092 + atezolizumab Active Comparator: regorafenib	OS	Recruiting	874	NCT05425940
CRC	PD-L1 positive pMMR/MSS	Progressed on or after or could not tolerate standard treatment	Experimental: Favezelimab/Pembrolizumab Active Comparator: Regorafenib or TAS-102	OS	Active, not recruiting	432	NCT05064059
CRC	pMMR/MSS	previously treated and has shown disease progression on or after or could not tolerate standard treatment	Experimental: lenvatinib + pembrolizumab Active Comparator: regorafenib OR TAS-102	OS	Active, not recruiting	480	NCT04776148
CRC	dMMR/MSI-H Stage III (TanyN + , M0)	Adjuvant	Experimental: Sintilimab Experimental: XELOX	DFS	Recruiting	323	NCT05236972

Eventhough the long-term follow-up data was absent, the high pCR rates notably preserved organ function and enhanced Quality of Life, making this regimen widely accepted. Nevertheless, there are still challenges. Given the AEs associated with dual immunotherapy, the reliability of mono-immunotherapy and the appropriate treatment duration remain unclear. Efforts to resolve these challenges never stop. A phase II MSKCC study explored the PD-1 antibody, dostarlimab, for a total of 6-months neoadjuvant the results showed that all patients achieved cCR (12/12), with manageable safety [151].

Explorations of other combined strategies are ongoing. For example, The phase II study PICC showed the possibility of toripalimab in combination with celecoxib (COX-2 inhibitor) [152]. What's more, research is also being conducted on adjuvant immunotherapy regimens for dMMR/MSI-H CRC [153, 154], and the findings from these studies will shed light on whether adjuvant immunotherapy can boost survival rates in stage III dMMR/MSI-H or POLE-mutated CRC, which will significantly influence future treatment approaches.

Challenges in immunotherapy for pMMR/MSS CRC

Most patients with mCRC have pMMR/ MSS tumours, which are considered "cold tumours".The low immunogenicity of these tumours makes them less recognizable by CD8 + T cells. Cold tumours are characterized by the overexpression of innate immune inhibitory oncogenic pathways and the presence of numerous immunosuppressive factors in the tumour microenvironment [155]. Consequently, single-agent immunotherapy often yields unsatisfactory results, necessitating the exploration of new treatment strategies and the identification of populations likely to benefit from immunotherapy.

Treatment strategies remain controversial

The primary current explorations mostly focus on combined treatments enhancing the sensitivity of immunotherapy [156]. Nevertheless, most of the research is still in the early exploratory stage with small samples, and the results remain further validation. The LEAP-017 study suggested that the lenvatinib plus pembrolizumab showed no improvement in survival in the pMMR/MSS mCRC patients compared with the standard of care group, with OS being 9.8 vs. 9.3 months (HR 0.83, 95% CI 0.68–1.02, $P = 0.0379$) [157]. A study included 39 pMMR/MSS mCRC patients who experienced progression following standard chemotherapy and received the RIN regimen (regorafenib + ipilimumab + nivolumab). The RP2D cohort's patients had an ORR, mPFS, and mOS of 27.6%, 4 months, and 20 months, respectively, which demonstrated promising prospective of clinical application [158]. Segal et al. included 24 chemotherapy-refractory pMMR mCRC patients and administered durvalumab and tremelimumab with concurrent radiotherapy, with an ORR of 8.3%. The mPFS and mOS were 1.8 month and 11.4 months correspondingly, which did not meet the prespecified endpoint [159]. Meanwhile, Thibaudin et al. included 57 patients with RAS-mutated mCRC treated with first-line durvalumab and tremelimumab plus mFOLFOX6. The 3-month PFS for MSS patients was 90.7%, with a response rate of 64.5%; mPFS was 8.2 months, while OS was not reached [160]. The CAPability-01 study evaluated the efficacy of romidepsin, a histone deacetylase inhibitor, plus sintilimab and bevacizumab or romidepsin plus sintilimab in treating patients with advanced or metastatic pMMR/MSS CRC who have failed at least two lines of prior treatment and reported an ORR of 44.0% and a PFS of 7.3 months for the triplet group [161]. More clinical trials on ongoing are detailed in the Table 6.

Features of potential beneficiaries remain unclear

A study that explored the efficacy of treatment with PD-1, BRAF, and MEK inhibition in BRAFV600E CRC patients exhibited an ORR of 25% and a DCR of 75%, with a mPFS of 5 months in MSS CRC patients [162]. Acquired resistance to temozolomide may be associated with the onset of hypermutation, facilitating immune sensitisation. The MAYA study included 33 previously treated O^6-methylguanine–DNA methyltransferase silenced MSS mCRC patients who received temozolomide followed by combination with low-dose ipilimumab and nivolumab. The results reported mPFS and mOS of 7.0 and 18.4 months, respectively, and an ORR of 45% [163]. The biomarker analysis from the AtezoTRIBE study indicated that patients with high immunomodulatory signature scores within the pMMR subgroup derived superior benefits from atezolizumab [164]. The CheckMate-9X8 study reported that MSS/pMMR mCRC patients with consensus molecular subtype (CMS) 1 and CMS3 at baseline had a higher probability of being progression-free at 12 months when treated with nivolumab in combination with the standard treatment regimen [165].

Anal carcinoma Anal carcinoma (AC) is a rare malignant tumour of the digestive system, accounting for approximately 2% of all GI cancers, with anal squamous cell cancer (ASCC) constituting the majority (approximately 80%) of AC [166]. The treatment options for advanced ASCC are limited, predominantly relying on chemotherapy; therefore, new therapeutic approaches are warranted [167–169]. Unlike EC, G/GEJC, and CRC, human papillomavirus (HPV) infection is closely associated with the occurrence of ASCC. The HPV oncoproteins are immunogenic and can trigger the host's anti-tumour immune

response. Therefore, immunotherapy holds promise as a treatment strategy for ASCC. However, due to its low incidence, only preliminary exploration has been conducted. The results of the studies KEYNOTE-158, POD1UM-202, and CARACAS, have demonstrated the efficacy of single-agent ICIs in treating metastatic ASCC that has failed previous treatments. The ORR ranged from 10–24%, PFS from 2.0–4.1 months, and OS from 10.1–12.8 months in these studies [170–173]. There has also been some preliminary exploration into combination therapies in populations with failed prior treatments.

The NCI ETCTN (NCI9673 Part B) study showed a trend towards prolonged PFS and OS with dual immunotherapy (nivolumab + ipilimumab) compared with nivolumab monotherapy for metastatic ASCC with failed previous treatments. However, the differences were not statistically significant(PFS: 2.9 vs. 3.7 months, HR 0.80, 95% CI 0.51–1.24; OS: 15.4 months vs. 20.0 months, HR 0.86, 95% CI 0.51–1.47) [174]. The CARACAS study also indicated that for advanced ASCC with failed previous treatments, the ORR for PD-L1 antibody avelumab combined with cetuximab was 17%, with PFS at 3.9 months and median OS at 7.8 months [171]. Atezolizumab, another PD-L1 antibody, combined with bevacizumab, showed no superior results to single-agent ICI in treating advanced ASCC with failed previous treatments, with an ORR of 10%, PFS of 4.1 months, and OS of 11.6 months [175]. In the first-line treatment setting, the SCARCE study was the first to validate the combination of ICI with chemotherapy for first-line treatment of advanced ASCC, although failed to meet the primary endpoint, with 1-year PFS rates of 45% for atezolizumab + mDCF (docetaxel + cisplatin + fluorouracil) and 43% for mDCF alone, 12-month OS rates of 77% and 81% respectively, and ORRs of 75% and 78% [176]. Several phase III RCT studies are currently ongoing [177, 178] to evaluate the role of chemotherapy combined with ICI in the first-line treatment of advanced ASCC.

In summary, ICI therapy can improve survival for patients with advanced ASCC who have previously received treatment. However, the role of ICI in the first-line treatment of advanced ASCC remains unclear. Furthermore, identification of the population that would benefit from the treatment remains necessary, as does exploration of the optimal combination treatment strategy. For the treatment of locally advanced ASCC, several studies are currently in the early stages of exploration (NCT04230759 and NCT03233711).

Collectively, immunotherapy has advanced the treatment for EC and G/GEJC with significant breakthroughs in CRC, although several challenges remain to be addressed, including the diversity and complex molecular types in GC, the optimal treatment regimen for MSI-H/dMMR CRC, the potential for immunotherapy in MSS/pMMR, and nutritional issues in EC. In addition to the specific limitations associated with each tumour type, other challenges, including identifying biomarkers and the mechanisms underlying resistance to immunotherapy, optimisation of methods for assessing the efficacy of immunotherapy, determination of the best combination treatment modalities, and development of new immunotherapeutic agents remain to be addressed. With more studies, the application of immunotherapy in the field of GI cancers will be further optimised, thereby improving clinical outcomes.

Biomarkers of immunotherapy

The exploration of biomarkers is crucial for realising precise and individualised immunotherapy and has witnessed significant advancements in recent years. This progress is primarily evident in two aspects. First, the predictive efficacy of classical markers has been validated and discussed in large-scale, multi-dimensional studies, thereby elucidating their strengths and weaknesses. Second, with the development and cost reduction of cutting-edge technologies, including high-throughput detection, visualisation, liquid biopsy, and artificial intelligence, researchers have shifted their focus from tumour cells to the entire tumour microenvironment, from single to multiple dimensions, and from a static to a dynamic pattern. Here, we elaborate on the progress of both classical and novel biomarkers based on these two perspectives (Fig. 1).

Classic biomarkers

PD-L1

PD-L1 positivity in EC, G/GEJC, CRC, and immune cells has shown variability, ranging from 17.4% to 43.5%, 12.0% to 43.6%, 8.1% to 44.0%, and 15.3% to 69.0%, respectively [179]. While the predictive utility of PD-L1 expression assessed using the CPS appears limited in CRC immunotherapy, it demonstrates a robust predictive value in EC and G/GEJC. The cut-off values of CPSs in clinical trials are commonly defined as 1, 5, and 10. The ATTRACTION-03 and CheckMate-648 trials revealed nivolumab benefited the entire population for advanced EC regardless of CPS [31, 180]. Conversely, the KEYNOTE series trials of EC demonstrated that pembrolizumab treatment predominantly benefited patients with high PD-L1 expression (CPS ≥ 10) [181]. Similar to studies on EC, the KEYNOTE series of trials on advanced GC has highlighted the therapeutic advantages of immunotherapy in populations with high PD-L1 expression (CPS ≥ 10) [80], while the ChecMate-649 [5, 182] and ORIENT-16 trials [6] validated CPS ≥ 5 for identifying patients that would

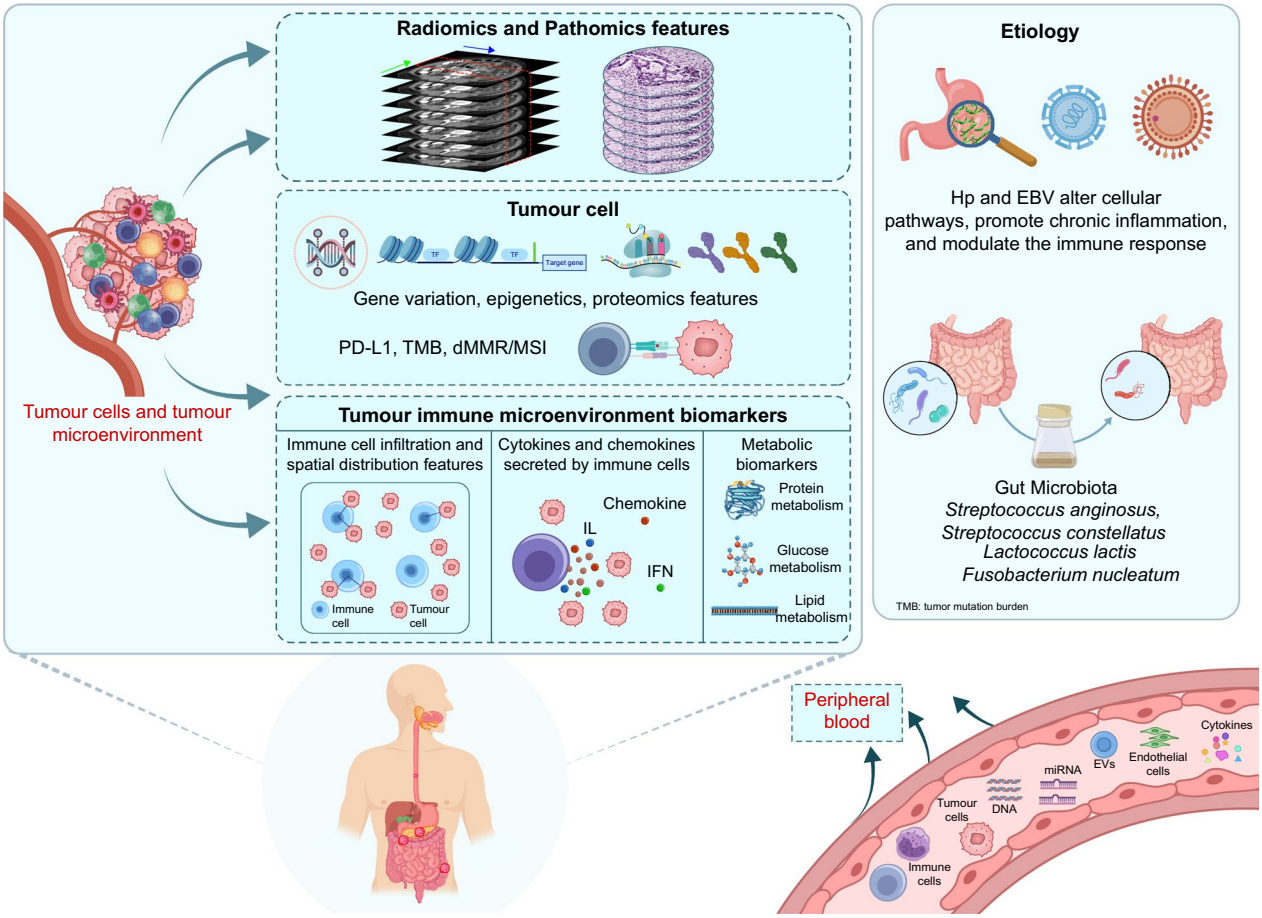

Fig. 1 Biomarkers under-investigation for immunotherapy in GI tract cancers. The pattern diagram summarized immunotherapeutic biomarkers under-investigations in GI tract cancers from three perspectives including tumour cells and tumour microenvironment, peripheral components and etiology

benefit from chemotherapy combined with nivolumab or sintilimab, respectively.

However, the use of different detection antibodies and assay platforms in various clinical trials has led to confusion and a lack of uniformity in the CPS cut-off values used to identify the beneficiary population, and conclusions drawn from existing studies have limited applicability. Several studies have attempted to address this variability. A meta-analysis incorporating 6,488 cases of advanced GC immunotherapy for PD-L1 demonstrated significant survival benefits in patients with CPS ≥ 1, regardless of whether they underwent monotherapy or combination immunotherapy (single-agent immunotherapy: OS (hazard ratio (HR) 0.84, 95% confidence interval (CI) 0.74–0.96); combination immunotherapy: OS (HR 0.81, 95% CI 0.71–0.92) and PFS (HR 0.77, 95% CI 0.69–0.86)) [183]. However, another retrospective study analysing low-CPS subgroups in CheckMate-649, KEYNOTE-062, and KEYNOTE-590 (CPS: 1–4; CPS: 1–9) observed that patients with low PD-L1 expression did not benefit from chemotherapy combined with ICIs [184]. Notably, these findings pertained specifically to advanced G/GEJC. Furthermore, the PD-L1 CPS faces additional challenges as a critical biomarker of immunotherapy efficacy. First, PD-L1 expression exhibits considerable heterogeneity [185] and can be dynamically influenced by various therapeutic approaches [186, 187], affecting its reliability as a biomarker of efficacy. Second, there is no standardised method for PD-L1 testing, leading to low consistency among commercially available antibodies. For examples, PD-L1 positivity with the Dako 28–8 antibody, which is used in the CheckMate-649 trial, is higher than that with the Dako 22C3 antibody, which is used in the KEYNOTE series of trials. This suggests that with a CPS cut-off value of 5, the positivity for Dako 28–8 is approximately twice that for Dako 22C3 [188].

Therefore, establishing a consensus on PD-L1 testing is essential to standardise diagnostic platforms across different clinical trials and accelerate the identification of biomarkers for different stages. Integrating various

therapeutic drugs, patient clinical characteristics and other relevant biomarkers is key to achieving breakthroughs in this field.

MSI-H/dMMR

MSI-H and dMMR are known to increase somatic cell mutations and neoantigen production, often resulting in extensive lymphocytic infiltration and elevated expression of immune checkpoints in the tumour microenvironment (TME) [189, 190]. Consequently, patients with MSI-H/dMMR exhibit poor response to cytotoxic drugs and high sensitivity to immunotherapies. Emerging evidence suggests that the predictive value of MSI-H/dMMR for immunotherapy efficacy in GI tract cancers is significantly superior to that of other biomarkers. Nevertheless, the incidence of MSI-H/dMMR in EC is relatively low. Currently, there are no large-scale clinical trial data available, and most studies have focused on GC and CRC.

A retrospective analysis [114] of three trials (KEYNOTE-059, 061, and 062) revealed that patients with MSI-H advanced G/GEJC benefited significantly from first-line to third-line pembrolizumab monotherapy, establishing MSI-H as a predictive biomarker for efficacy. Similarly, in the KEYNOTE-158 trial, all 24 patients with GC and MSI-H/dMMR benefited from pembrolizumab treatment [191]. The 3-year follow-up results from the CheckMate-649 trial [180] showed that in unselected MSI-H subgroup of patients, nivolumab combined with chemotherapy versus chemotherapy alone led to an improved median OS, further supporting MSI-H as an effective biomarker for identifying advanced patients with GC for immunotherapy. Several clinical trials on perioperative immunotherapy for GC are ongoing, and the interim results suggest that MSI status, not PD-L1 CPS, is the most crucial biomarker for predicting the benefit of perioperative pembrolizumab with chemotherapy in locally advanced GC [11, 15, 16]. Therefore, MSI-H can also serve as a biomarker for the efficacy of perioperative immunotherapy in GC, laying the foundation for the exploration of precise treatment patterns during the perioperative period with promising application prospects.

To date, MSI-H/dMMR is the most important biomarker for predicting the efficacy of immunotherapy in CRC [138, 139, 192–194], as confirmed by the 5-year OS results from KEYNOTE-177 [195] and trials such as NICHE-2 [20], NICHE-3 [196], and PICC [21] which demonstrate the significant role of MSI-H/dMMR as a biomarker of immunotherapy efficacy across different CRC stages.

However, several challenges remain for the use of MSI-H/dMMR as a biomarker of immunotherapy efficacy. First, MSI-H/dMMR is underrepresented in the population, accounting for only 15% of CRC, 7% of GC, 4% of GEJC, 0.4% of EAC, and 0% of ESCC cases [197, 198]. Moreover, although the overall percentage of patients with MSI-H/dMMR in CRC is 15%, only 5% of patients with advanced CRC have MSI-H, greatly limiting its clinical practice as a biomarker of immunotherapy efficacy. Second, the accuracy of MSI detection and interpretation needs to be improved. Currently, immunohistochemistry (IHC) is the main method for detecting MMR protein expression, whereas polymerase chain reaction (PCR) and next-generation sequencing (NGS) are the main methods used to identify MSI status. PCR is the only approved method for detecting MSI in CRC, and NGS-based MSI detection methods need to be verified using more data. Additionally, MSI detection methods for G/GEJC need to be supported by a large sample size and reliable data. In 2022, the College of American Pathologists (CAP) released the recommended examination protocols for MSI/MMR in different tumour types [199]: (1) For patients with CRC, the use of either IHC to detect MMR or PCR to detect MSI is recommended. Although these methods are preferred, an NGS assay that has been validated for dMMR could also be used to determine the MSI status. (2) For patients with gastroesophageal or small intestinal cancer, IHC should be used to identify MMR or PCR should be used to identify MSI. Although the consistency between MMR-IHC and MSI-PCR testing results for various solid cancers is 90.3–99.4% [200–203], the accuracy of MMR testing and interpretation is affected by several factors, including tumour heterogeneity (such as variability between different lesions and intra-tumoural heterogeneity), antitumour therapies, the experience of the pathologist, and detection platforms [204]. Currently, any evidence of dMMR or MSI should be interpreted as a positive result, thereby qualifying patients for immunotherapy. Additionally, inconsistent results should be rigorously reviewed to ensure they are not due to misjudgement.

Tumour mutational burden

Tumour mutational burden-high (TMB-H) is defined as 10 or more mutations per Mb, with an incidence of 5% in patients with CRC, 8% in patients with GC, 3% in GEJC, 2% in patients with EAC, and 3% in those with ESSC [198, 205]. Given its potential to generate immunogenic neoantigens, the application of TMB status as a biomarker of immunotherapy efficacy has been widely studied. However, these conclusions remain controversial [206–208].

Based on the findings of KEYNOTE-158 study [191], in 2020, the FDA approved the use of pembrolizumab in patients with TMB-H cancers. However, a retrospective analysis of data from 10,000 solid tumour samples included in The Cancer Genome Atlas Program (TCGA)

[209] revealed that TMB status is not correlated with immunotherapy efficacy in GI tract cancers, indicating distinct immune patterns of GI tract cancers. Furthermore, the study divided cancers into two categories based on whether CD8 + T cell infiltration was positively correlated with the generation of tumour neoantigens. Category I cancers were defined as cancers with CD8 + T cell infiltration positively correlated with neoantigens productions, while category II exhibited reverse relationship.TMB-H could facilitate the selection of patients that might benefit from immunotherapy for Category I tumour but not for Category II cancers; notably, most GI cancers were Category II. Therefore, the infiltration of antigen-specific immunocytes may be a decisive factor underlying the influence of TMB-H on immunotherapy efficacy. Moreover, a retrospective study of 48,606 GI tract cancers exploring the characteristics of gene mutations associated with TMB-H independent of dMMR/MSI [205] found that not all gene mutations related to TMB-H could play a role in influencing immunotherapy efficacy. Based on the mutations that influence immunotherapy efficacy, this study established an mTMB model and discovered an increase in the infiltration of cells associated with antitumour immunity, such as M1 and CD8 + T cells, among patients with mTMB-H. Collectively, further division based on immune cell infiltration and gene variation in patients with GI tract cancers and different TMB statuses is warranted.

Novel biomarkers under-investigation

Virus and microbiome

EBV

9% of patients with GC have EBVaGC, and 80% of them harbour an immune-inflamed microenvironment with enriched T-cell and B-cell infiltration [101, 210–212]. Consequently, EBV is considered a potential marker for the efficacy of GC immunotherapy.

While clinical trials with large sample sizes specifically focusing on EBVaGC are mostly ongoing, certain small-sample studies have indicated that EBVaGC has a relatively high response rate to immunotherapy. For instance, a phase II clinical study conducted a retrospective analysis on the use of pembrolizumab for treating advanced GC, reporting a 100% response rate and an mOS time of 8.5 months among six cases of EBVaGC [213]. In contrast, a prospective clinical study enrolling nine patients with advanced EBVaGC treated with a combination of PD-1 and CTLA-4 inhibitors (including two patients receiving first-line treatment, four receiving second-line treatment, and three receiving third-line treatment) found an ORR and DCR of 55.6% and 88.9%, respectively [214]. A retrospective study involving 66 patients with advanced GC treated with PD-1 and CTLA-4 inhibitors indicated that patients with EBV + /pMMR (n = 22) displayed significantly superior outcomes than those with EBV-/pMMR (n = 44) (ORR: 54.6% vs 17.7%, $P = 0.008$; mPFS: 8.5 months vs 2.0 months, $P < 0.001$; mOS: not reached vs 5.0 months, $P = 0.002$) [105]. Nevertheless, the conclusions drawn by these studies were limited in generalisability owing to factors such as sample size and the presence of other molecular markers (such as PD-L1 and MSI), necessitating further validation.

Helicobacter pylori

Helicobacter pylori (*H.pylori*) considerably contributes to the pathogenesis of GI tract cancers by producing the cytotoxins VacA and CagA [215–217]. Among patients with GI tract cancers receiving immunotherapy, those with *H.pylori* infection exhibited shorter PFS and OS [218–220]. However, these studies have several limitations, including the small size of the *H.pylori* infection samples, inconsistent methods used to detect *H.pylori* infection, and varied standards for diagnosing *H.pylori* positivity, that dramatically influenced the reliability of the conclusions. In a recent prospective observational study involving 10,122 patients with GI tract cancers [104], the incidence rates of *H.pylori* infection, as detected using the ^{13}C breath test, were 44.19%, 33.24%, and 42.35% for patients with EC, GC, and CRC, respectively. Among the 636 patients with EBV- MSS GC receiving anti-PD-1/PD-L1 treatment enrolled in the study, patients with *H.pylori* infection showed a significantly better response [PFS: 6.97 months vs. 5.03 months; median immune-related overall survival (irOS): 18.30 months vs. 14.20 months $P < 0.01$]. of note, the impact of *H.pylori* infection on immunotherapy efficacy appears to be organ-specific. Patients with *H.pylori* infection and dMMR/MSI-H CRC and ESSC displayed shorter irPFS (dMMR/MSI-H CRC: 16.13 months vs. not reached, $P = 0.042$; ESSC: 5.57 months vs. 6.97 months, $P = 0.029$). The heterogeneity of the modulation of the gut microbiome and TME may explain the contrasting role of *H.pylori* in distinct GI tract cancers. Although this study provides powerful evidence for *H.pylori* as a biomarker of immunotherapy efficacy for GI tract cancers, the underlying mechanisms remain unclear. Considering the diversity and specificity of the microbiome in GI tract cancers, further studies should focus on the *H.pylori* function on the microbiome and tumour metabolic features.

Microbiome

The human microbiome, which resides primarily in the respiratory, digestive tracts, and skin, finds its highest abundance and diversity within the gut. Recent advancements in culture-independent microbial analysis methods have deepened our understanding of the gut

microbiome's role in cancer's onset, development and treatment. Research increasingly shows that its composition, diversity, and specific communities modulate cancer patients' immune status, influencing immunotherapy outcomes. Compared to healthy individuals, patients with digestive tract cancers exhibit markedly diverse gut microbiome richness and diversity [221–223]. A reduction in microbial diversity and stability can compromise local immunity in the intestinal mucosa and trigger systemic immune responses via immune cells. This impairs both local and systemic immune functions, damages the mucosal barrier, and allows microbial elements to enter the systemic circulation, altering cytokine profiles throughout the body [224]. Moreover, metabolic byproducts from the gut microbiome, such as short-chain fatty acids (SCFAs), indole propionic acid, serotonin, and secondary bile acids, play crucial roles in regulating bacterial composition and activity [225]. These can permeate the intestinal barrier, impact host physiology, and activate immune responses, with SCFAs being particularly influential on host immunity. Studies highlight that the gut microbiome and its metabolites are pivotal in modulating innate immunity (including dendritic cells, macrophages, and NK cells), adaptive immunity (involving CD8 + T and CD4 + T cells), and tumour cell immunogenicity, thus affecting immunotherapy's effectiveness [226]. Research by Zhang et al. [227] demonstrated the impact of PD-1 inhibitors in CRC, which is highly influenced by gut microbiome diversity. Their use of broad-spectrum antibiotics to erase endogenous bacteria in mice diminished the PD-1 inhibitors' tumuor-suppressing effects, indicating a dependency on microbial diversity. Furthermore, studies have found a positive correlation between the abundance of Joshi's lactobacillus in the mouse gut microbiome and the response to anti-PD-1 immune checkpoint therapy. Enhancements in CD8 + T cell-mediated anti-PD-1 therapy efficacy were observed when supplemented with Joshi's lactobacillus or the tryptophan-derived metabolite indole-3-propionic acid [228]. An analysis of fecal samples from 106 patients with various rare cancers revealed that 22 strains, mainly from the phylum Firmicutes, significantly predict responses to combined anti-PD-1 and anti-CTLA-4 therapy. Strains that indicate better treatment responses are predominantly from the Ruminococcaceae family and the Faecalibacterium genus [229]. Additionally, the gut microbiome's predictive capabilities have also been validated in GI tract cancer cohorts. For instance, a study involving 74 patients with advanced digestive tract cancers undergoing PD-1/PD-L1 inhibitor therapy found a positive correlation between treatment response and levels of true bacteria, lactobacilli, and streptococci [230]. Similarly, another study with 117 patients suffering from HER-2-negative advanced GC or GEJC showed those with higher lactobacilli abundance experienced greater microbial diversity and improved response to anti-PD-1/PD-L1 therapy, often achieving better PFS [231]. The potential of using the gut microbiome for precise subtyping of digestive tract tumors was also explored in a study covering 77 advanced MSI-H/dMMR GI tract patients. By analyzing gut microbiomes, blood metabolomes, and cytokine and chemokine profiles, researchers identified specific microbes like B. caccae, V. parvula, V. atypica, and Clostridiales bacterium as potential subtyping markers for MSI-H/dMMR GI tract cancers, offering predictive insights into immunotherapy responses [232]. Despite its promise, the use of gut microbiome characteristics as markers for predicting the efficacy of ICIs encounters challenge of reproducibility issues, often exacerbated by factors like diet, regional differences, and ethnicity. Furthermore, the specificity of microbiome traits to particular treatment regimens [229] limits their clinical utility as reliable markers. Therefore, a comprehensive exploration of the gut microbiome spectrum and abundance in GI tumours, identification of broad-spectrum efficacy characteristic genera, and quantification of recognized beneficial or harmful bacteria proportions remain critical areas for future research, aiding in the realization of precision therapy.

Tumour microenvironment

The TME encompasses the internal environment where tumour cells develop, including all non-tumour components, metabolites, and secretions. These components include the extracellular matrix, fibroblasts, endothelial cells, immune cells, mesenchymal stem cells, cytokines, and metabolites [183]. The TME is complex, exhibiting both tumour-promoting and tumour-inhibiting effects [184–189]. Currently, identifying different TME statuses from multiple dimensions as biomarkers for the efficacy of immunotherapy is a focal area of research, with particular emphasis on the TIME.

TILs

Tumour-infiltrating lymphocytes (TILs) are integral components of the TME. With the integration of spatial information in histopathology and other technologies, the predictive value of TILs for the response to immunotherapy in GI tract cancers has gained attention, particularly focusing on CD8 + and CD4 + T cells.

The importance of CD8 + T cells in predicting therapeutic efficacy has been demonstrated in several clinical research cohorts. For example, the EC-CRT-001 study applied multipanel multiplex immunofluorescence to a cohort of patients with EC undergoing immunotherapy, revealing a significantly higher density

of CD8 + T cells in the CR group than in the non-CR group [66]. However, with advancements in multi-omics technologies, it has become clear that a single marker is insufficient to represent the functional characteristics of a cell population. Further in-depth studies have attempted to reveal the functional CD8 + T cell subsets that play an active role in the response to immunotherapy. In the PANDA study, for instance, researchers discovered significantly higher densities of PD-1 + CD8 + T cells in responders to immunotherapy than in non-responders; such differences were not observed for other subsets of CD8 + T cells [233]. The NICHE study also identified the infiltration of PD-1 + CD8 + T cells as a predictor of response to immunotherapy in pMMR cancers [19]. These findings indicate that in the context of GI tract cancers, which are highly heterogeneous, CD8 + T cells can be predictive markers for the efficacy of immunotherapy and that differentiating these cells into subsets can provide more precise guidance for predictions [71]. CD4 + T cells primarily mediate the function and maintenance of CD8 + T cells [234]. FOXP3 + CD4 + T cells (Tregs) are believed to contribute to the inhibition of antitumour immunity and maintenance of immunological tolerance via immune checkpoints and high levels of anti-inflammatory cytokines, thus exerting adverse effects on prognosis [235]. However, further research has shown that an increased abundance of Tregs is not necessarily a poor prognostic indicator in GI tract cancers; in many cases, it is predictive of a superior prognosis. The SPACE study reported a correlation between high Treg infiltration and a more favourable prognosis [236]. Conversely, other clinical studies argued that Tregs level is not an effective predictor of immunotherapy efficacy. For instance, the NICHE study found no difference in baseline Tregs densities between responders and non-responders [19], and the PANDA study illustrated a lack of correlation between FOXP3 expression and therapeutic responses [233]. As these contradictory results indicate that Tregs consist of multiple subsets that vary substantially in terms of function and influence treatment responses, further dividing Tregs into more precise subsets may be necessary to better understand their impact on treatment outcomes. For example, Masuda et al. conducted single-cell sequencing of CRC samples and found that while total Tregs are associated with a more favourable prognosis, CD38 + Tregs exhibit a highly suppressive phenotype and are associated with a poor prognosis [237]. In an early clinical study of durvalumab and tremelimumab combined with chemotherapy, FOXP3- CD25 + CD4 + T cells were the only subset of CD25 + CD4 + T cells that correlated with survival;FOXP3 + CD25 + CD4 + T cells did not correlate with treatment outcomes [238].

Despite extensive research on TILs in GI cancer immunotherapy and the identification of numerous TIL subgroups with established links to immunotherapy response, TILs have yet to be used in clinical practice like PD-L1, MMR, and TMB. This can be attributed to several factors: (1) The plethora of detection methods for TILs complicates standardization. Current techniques include hematoxylin and eosin (HE) staining, IHC, mIHC, tissue microarray technology, multiphoton microscopy. Crucial yet rare TIL subgroups require identification and detection through advanced methods such as single-cell RNA sequencing and tumor tissue flow cytometry. Although HE and IHC staining are routinely used clinically, other methods demand high sample volume and viability, are expensive, and thus limit clinical application. Additionally, these methods involve lengthy testing and analysis cycles, taking at least a month from sample collection to result interpretation, which fails to meet urgent patient treatment timelines. (2) The challenge of standardizing interpretation methods and criteria. While HE staining and IHC are commonly employed in clinical settings, standardized interpretation methods and criteria are lacking. Efforts to standardize the clinical application of TILs commenced with breast cancer, highlighted by the International TILs Working Group's 2014 consensus on manual interpretation standards based on HE staining [239]. This consensus outlined experimental procedures, calculation formulas, and interpretation criteria. Furthermore, studies indicated that stromal TILs (sTILs) are more abundant and consistently measurable than intratumoral TILs (iTILs), leading to recommendations from the International Immuno-Biological Markers Collaboration to prioritize sTILs in experimental research and clinical applications [240]. The combinatory predictive value of TILs and PD-L1 has been validated in multiple clinical trials, leading to their inclusion in the NCCN guidelines for breast cancer, offering insights for GI tract tumours. With advancements in visualization technology and artificial intelligence (AI), developing algorithms for analyzing tumour TILs based on pathological slice images and supporting pathologists in interpretation through computer assistance remains an active area of research.

Cytokines

Cytokines—including interferons (IFNs), interleukins (ILs), tumour necrosis factors (TNFs), and chemokines—produced by multiple immune cells have been suggested to be closely linked to immunotherapy for GI tract cancers.

IFNs are crucial inflammatory cytokines that activate the PD-1 signalling axis of tumour and stromal cells

by upregulating PD-L1 expression [241]. A phase Ib study on neoadjuvant adebrelimab treatment for locally advanced ESCC reported a significantly higher level of IFN-γ expression among responders, along with a notable correlation between the IFN score and pathological regression [71]. Similar conclusions were drawn for EAC based on the findings of the PERFECT study [65]. Additionally, the KEYNOTE-028 study analysed the IFN-γ gene-expression profile of six genes and demonstrated that patients with higher expression could benefit more from pembrolizumab treatment [242].

ILs are soluble proteins secreted by leukocytes that are primarily involved in regulating immune cell functions. A meta-analysis summarising 14 studies on immunotherapy involving 3,190 participants indicate that individuals with high IL-8 levels exhibited lower ORR, OS, and PFS than those with low IL-8 levels [243]. Similarly, a phase IB/II clinical study of durvalumab and tremelimumab plus chemotherapy for advanced CRC revealed that high levels of IL-6 and IL-8 were correlated with lower response rates [238]. Additionally, a phase II clinical study of combined anti-PD-1 and anti-VEGF therapy for pMMR CRC showed a significantly increased level of cytokine signalling in responders compared with in non-responders [244].

Chemokines primarily coordinate cell movement during inflammation and induce directional migration of leukocytes and endothelial cells. However, studies on the relationship between chemokines and immunotherapeutic responses in GI tract cancers are currently limited. The MEDITREME study, which included patients with mCRC undergoing immunotherapy, reported that CXCL9, CXCL10, and CXCL11 expression levels were correlated with a more favourable PFS [238]. Similarly, in the PANDA study, responders exhibited a significant increase in CXCL13 expression compared with non-responders [233].

Currently, research on cytokines and chemokines primarily uses peripheral blood from patients, which necessitates small sample sizes and relatively uncomplicated detection techniques, predominantly ELISA, ELISPOT, and microscopy. These approaches facilitate dynamic and real-time monitoring of therapeutic efficacy. However, unlike emerging efficacy biomarkers like TILs under investigation, the utility of cytokines and chemokines in guiding the clinical practice of immunotherapy for GI tract cancers is limited by their low specificity and the challenges associated with establishing abnormal cut-off values. The levels of cytokines and chemokines are affected by a variety of factors, including infectious diseases, hormonal changes, trauma, general patient status, and treatment regimen, which significantly diminishes their specificity in reflecting tumour status. Moreover, current research predominantly relies on small cohorts and lacks extensive expression profiles of cytokines and chemokines in healthy populations and cancer patients, including the range of fluctuations following different treatments. Consequently, the application prospects of cytokines and chemokines should primarily focus on supplementing mainstream biomarkers to predict therapeutic efficacy and monitor disease status.

Owing to the complexity and diverse functions of the TME, a single-marker classification is insufficient to predict the efficacy of immunotherapy in patients with GI tract cancers. To address this, researchers have utilised data from the TCGA database of GI cancers, classifying the TME based on immune cell infiltration or distinct gene expression features, and developed a TME score to predict immunotherapy outcomes. Patients who benefit from immunotherapy typically harbour TME subtypes characterised by M1 macrophages, infiltration of CD8 + T cells, elevated expression of immune checkpoints, and robust T cell activity [245–251]. Tertiary lymphoid structures (TLS), ectopic lymphoid organs comprising B cells, plasma cells, CD4 + T cells, CD8 + T cells, DCs, macrophages, and neutrophils, are associated with improved OS in patients with GI tract cancers characterised by high infiltration of CD3 + T cells, CD8 + T cells, and M1 macrophage infiltration [252]. However, conclusive evidence based on large-scale studies correlating TLS with immunotherapy efficacy in GI tract cancers is still lacking [253, 254]. Additionally, the spatial distribution of immune cells relative to the immunotherapy response has been increasingly emphasised. Chen et al. analysed immune cell infiltration and its two-dimensional spatial distribution characteristics related to immunotherapy efficacy in 80 patients with GC using multiple immunofluorescence markers (immune checkpoints including PD-1, CTLA-4, TIM-3, LAG-3, and immune cell infiltration markers such as T, B cells, and macrophages). They established an infiltrating immune cells (TIICs) model, which included CD4 + FoxP3-PD-L1 +, CD8 + PD-1 -, the density of CD8 + PD-1-LAG3 -, and CD68 + STING + cells, as well as the spatial distribution of CD8 + PD-1 + LAG3-T cells. This study revealed that patients with low TIIC scores have better immunotherapy outcomes [10]. Moreover, in dMMR CRC, the proximity between PD-1 + cells and PD-L1 + cells can also serve as a positive marker of immunotherapy efficacy [255]. Furthermore, a study on the construction of a mathematical framework for cancer immunotherapy outcomes found that the increase in oxygen content caused by vascular normalisation promoted the polarisation of M1 cells and infiltration of immune cells, thereby promoting tumour cell killing and improving immunotherapy efficacy. Hence, mathematical models incorporating

tumour cells, immune cells (M1, M2, CD4 + T, CD8 + T, Tregs), vascular and perivascular cells, and anti-angiogenic/pro-angiogenic-related molecules (including Ang1, Ang2, PDGF-B, VEGF, and CXCL12) effectively predict immunotherapy efficacy [256].

In addition to the TIME, the TME of GI tract cancers is also affected by perineural invasion, which is closely related to immunotherapy efficacy. A study involving 3,236 patients with GI cancers constructed a neuroinflammatory infiltration (NII) scoring system based on 26 PNI-related specific inflammatory genes. This study found that cancers with low NII scores infiltrated more immunosuppressive cells. Patients with PNI and high NII scores usually benefit from immunotherapy [257].

The advancement of cutting-edge techniques has enabled researchers to comprehensively characterise the TME in patients with GI. For instance, single-cell detection technology has been utilised to identify new cell subsets related to immunotherapy efficacy [212, 253]. High-resolution technology has facilitated the exploration of immune cell infiltration and spatial distribution. Moreover, the combination of multiomics detection and AI techniques has contributed to the extraction of key features influencing immunotherapy efficacy from extensive data [258, 259], aiding in the construction of predictive models.

Peripheral components

Liquid biopsy troika, consisting of circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), and extracellular vesicles (EVs), offers several advantages for longitudinal monitoring of disease progression, minimally invasive sampling, and providing a holistic reflection of whole cancers. As a result, they are widely utilised for predicting therapeutic efficacy and prognosis and monitoring disease progression in GI cancers [260–272].

Profiling gene variation using NGS of baseline ctDNA as a biomarker has been universally explored. Gene subsets, including *RAS, AKT, PTEN, PI3KCA, BRAF, ERBB2, MET, RAS, BRACA, POLE, POLD-1, TGFB2, RHOA,* and *PREX2* variations are reportedly related to immunotherapeutic efficacy in GI tract cancers [273–277].

Additionally, peripheral PD-L1is is an important biomarker for immunotherapy, with PD-L1 expression on CTCs or EVs during treatment playing diverse roles in predicting immunotherapy outcomes. The presence of PD-L1 expression on CTCs at baseline indicated a superior response to anti-PD-1/PD-L1 therapy, whereas. post-treatment PD-L1 + CTCs were specifically associated with resistance in GI tract cancers. Furthermore, pre-treatment PD-L1 + CTCs were considered to indicate the presence of druggable tumour cells, whereas post-treatment PD-L1 + CTCs contribute to immune evasion [278–282]. Baseline exosomal PD-L1 was regarded as an indicator of exhausted T cells, and thus unable to react to PD-1 inhibitors, whereas exosomal-PD-L1 released after treatment is thought to be relevant to the elevated activity of T cells [283, 284].

With the advancement of high-throughput detection of trace samples, an increasing number of studies have explored multi-omics features identified by analysing peripheral components as biomarkers for immunotherapy. For instance, Zhang et al. conducted a retrospective analysis of immune-related protein expression in plasma EVs from 112 patients with GC who received ICI therapy. They developed a predictive model (EV score) based on the expression of PD-L1, PD-L2, CD3, and Arg1 on EVs, showing that patients with higher EV-score have superior ICI outcomes [206]. Additionally, biomarkers from plasma proteomics and metabolomics analyses are currently under intense investigation. Components such as peripheral leukaemia inhibitory factor (LIF) [285], proteins associated with the complement cascade pathway, lipid metabolites [286], and glutathione metabolism [287] are closely associated with immunotherapeutic responses.

However, there are still limitations to the universal clinical application of peripheral components. Factors such as the techniques used for sampling, storage protocols, diagnostic standards, and cost should be considered for further exploration.

Epigenetics and gene variation

Epigenetics modification

Epigenetics alterations, including DNA methylation, histone modification, and regulation of non-coding RNA, significantly modulate gene expression. Hence, distinct epigenetic modifications result in various therapeutic efficacies.

Methylation is the most common epigenetic modification, with specific gene methylation levels significantly impacting immune responses. In GI tract cancers, alterations in m6A regulatory factors have been shown to modulate the infiltration of various TIICs, thereby leading to heterogeneity observed in the efficacy of immunotherapy [246, 288–290]. Furthermore, researchers have identified molecular subtypes based on epigenetically regulated gene expression profiles in CRC and GC, which exhibit varying sensitivities to immunotherapy [291, 292]. Notably, methylation levels change with treatment; in a single-arm phase II clinical trial involving mCRC, treatment with pembrolizumab plus azacitidine led to a decrease in global methylation, particularly at promoter sites, enhancing universal gene expression [293]. Beyond methylation, the glycosylation and ubiquitination levels of certain genes have also been shown

to influence immunotherapy outcomes in GI tract cancers [294, 295]. Numerous studies have explored non-coding RNA, including lncRNA, miRNA and circRNA, as biomarkers for predicting immunotherapy outcomes. These non-coding RNAs may exert their effects through mechanisms such as modulating m6A regulatory factor expression [296], PD-L1 expression [297], immune cell infiltration [298], and cell proliferation [299, 300].

Genetic variation

In advanced GC, *CDH1*, *JAK2*, *AXIN1,* and *PTCH1* mutations have been identified as adverse factors for immunotherapy efficacy [301]. Conversely, in EC, *ERBB2* mutation has been negatively associated with neoadjuvant immunotherapy response [302]. *POLE/POLD1* mutations in patients with CRC exhibited a better response to immunotherapy, even in patients with MSS CRC, highlighting their strong predictive value [303, 304]. Furthermore, approximately 11–25% of patients with CRC harbour *KRAS* mutations. However, several analyses (such as KEYNOTE-177 and CheckMate-142) demonstrated that pembrolizumab has low efficacy in patients with mCRC harbouring *KRAS/NRAS* mutations [7, 139]. Nonetheless, findings from the KEYNOTE-164 study showed that the ORR among patients with mCRC receiving pembrolizumab is similar regardless of *RAS* status (37.0% vs. 42.0%) [305]. Therefore, further large-scale explorations are warranted.

Given the intricate nature and spatial–temporal heterogeneity of GI tract cancers, the dysfunction of a single gene cannot be applied as a general biomarker. Therefore, it is essential to develop prediction models based on subsets of epigenetic alterations or gene variations. For instance, in GI tract cancers, a study analysed the genomic profiles of 227 patients treated with immunotherapy across multiple centres and devised a GIPS model based on the status of six crucial genes (*RNF43*, *CREBBP*, *CDKN2A*, *TP53*, *SPEN*, *NOTCH3*). GIPS can independently serve as an excellent prognostic factor for immunotherapy [306].

Resistance to immunotherapy

Resistance to immunotherapy is one of the main challenges associated with GI tract cancers owing to the insufficient response to immune checkpoint blockade (ICB)-based immunotherapy. Even in patients who initially benefited from immunotherapy, 46.4% of them developed acquired resistance, most of which occurred within 24 months [307]. In this section, we discuss the recent research progress. First, we discuss emerging advancements in immune, metabolism, microbiota and epigenetics-mediated immunotherapy resistance mechanisms, mainly in GI tract cancers. Second, we review strategies to overcome immunotherapy resistance by combining them with other regimens and aligning efforts with clinical evidence (Fig. 2).

Mechanisms of resistance

Suppressed activation of T cells

Loss of PD-L1 expression

Considering the reactive anti-tumour immunity of ICIs by blocking the binding of PD-1 and PD-L1, the loss of PD-L1 expression is a contributing factor to ICI resistance. Genetic alterations, such as mutations or loss of function in the JAK/STAT pathway, can diminish PD-L1 expression in cancer cells, leading to both primary and acquired resistance to anti-PD-1 antibodies [307]. Especially in MSI-H cancers, JAK1 frameshifts (loss of function alterations) were observed in 6% (9/158) and 15% (4/27) of MSI-H colorectal and gastric cancers in the TCGA database, respectively, and exhibited decreased expression of IFN-associated genes [308]. In addition to external signalling alterations, internal modifications in CD274 also contributed to the loss of PD-L1 expression.

Among 13 patients with MSS metastatic CRC possessing high-affinity Fcγ receptor 3a (FcγR3a), 3 developed tumour subclones harbouring PD-L1 mutations selectively. These mutations led to the loss of tumour PD-L1 expression following treatment with the PD-L1 antibody avelumab, either through nonsense-mediated RNA decay in the case of PD-L1 K162fs mutation or protein degradation in the case of PD-L1 L88S mutation [309].

Downregulation of IFN-γ signalling

Alterations in the IFN-γ-JAK/STAT signalling pathway at the epigenetic, transcriptional, posttranscriptional, and posttranslational levels are associated with ICI resistance [310]. In CRC, mutations of JAK1/2 and B2M and lack of the IFN-γ receptor [311, 312] have been identified as contributors to ICI resistance. Research on therapeutic strategies that target the genetic, epigenetic, and metabolic elements regulating IFN-γ signalling is underway.

Expression of immune checkpoints beyond PD-1/PD-L1

The compensatory upregulation of alternative checkpoint proteins, such as lymphocyte-activation gene-3 (LAG-3), is a potential mechanism involved in acquired resistance to anti–PD-L1 therapy [313, 314]; LAG-3 is an immunomodulatory receptor that regulates effector T-cell (Teff) homeostasis, proliferation, activation and the suppressive activity of Treg cells [315]. A pioneering human study demonstrated that a combination therapy of favezelimab, an anti-LAG-3 antibody, plus pembrolizumab improved exploratory efficacy in terms of survival and DOR among patients with MSS mCRC [316]. Klapholz et al. found that patients with MSS CRC displayed

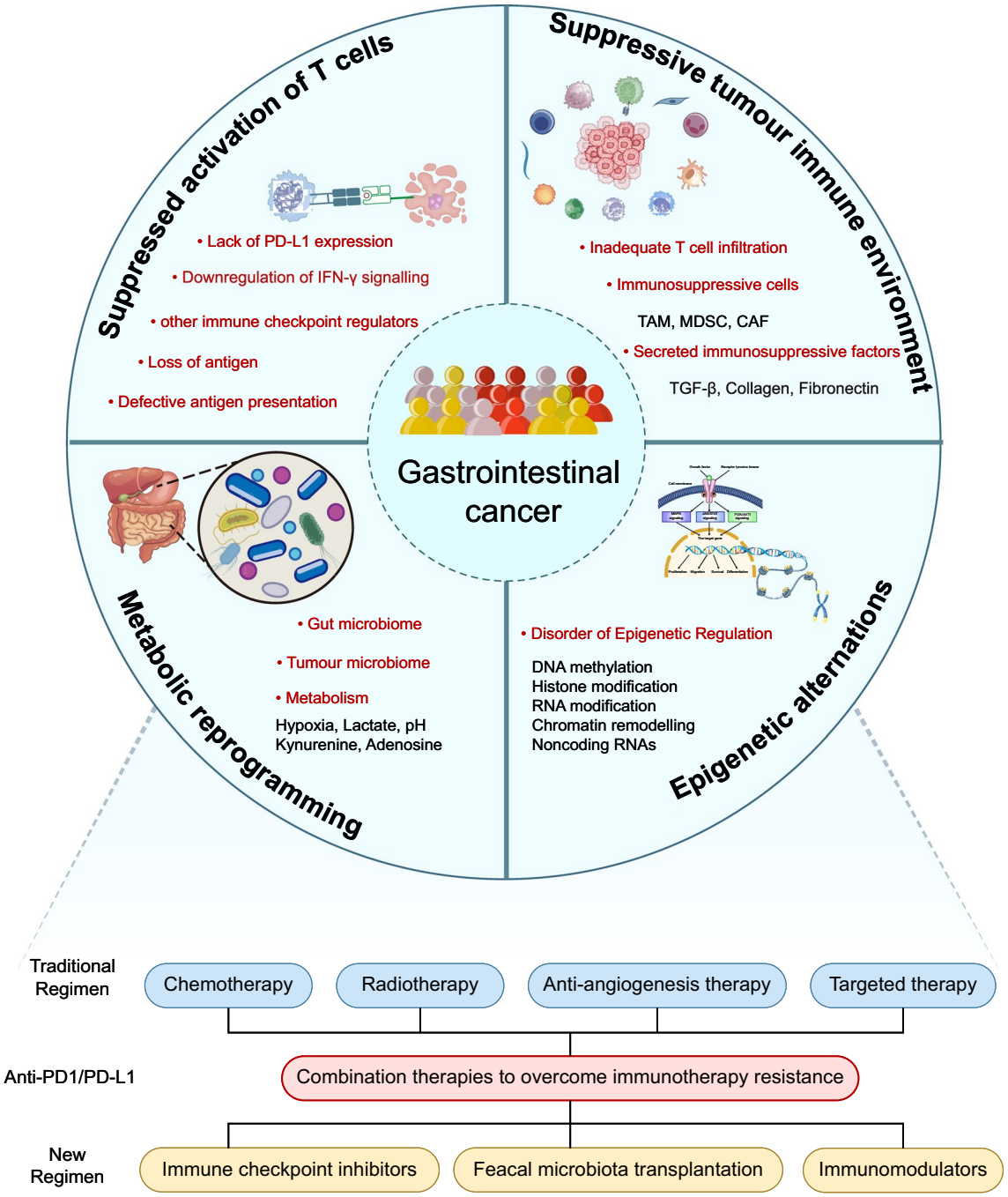

Fig. 2 The summary of immunotherapy resistant mechanisms and overcoming strategies in patients with GI tract cancers. This algorithm provides guidance for developing strategies overcoming resistance in patients with GI tract cancers

immune exhaustion signatures (MSS-ImmEx) characterised by the accumulation of Tim-3 + PD-1 + CD8 + TILs, which exhibited a dysfunctional or "exhausted" phenotype [317]. Therefore, dual blockade of Tim-3 and PD-1 may have additive or synergistic anti-tumour effects on patients with GI. Another immune checkpoint, T-cell immunoglobulin and ITIM domain (TIGIT), is associated with CD8 + T-cell exhaustion. Exhausted T cells develop resistance to anti-PD-1 when completing the exhausted transcriptional program [318, 319]. A study involving patients with CRC showed that anti-TIGIT combined with anti-PD-L1 therapy restores the functions

of intratumoural CD4 and CD8 T cells, irrespective of microsatellite status, in either primary tumour or liver metastasis [320]. Moreover, an alkaline phosphatase (ALP)-responsive and transformable supramolecular bis-specific cell engager (Supra-BiCE), consisting of both SA-P (a phosphorylated peptide targeting and blocking PD-L1) and SA-T (a phosphorylated peptide targeting and blocking TIGIT), achieved a tumour suppression rate of 98.27% in a CRC model, providing a promising tool for engaging NK and T-cells for cancer immunotherapy [321].

Loss of antigen

Cancer immunoediting occurs during tumour evolution and in the anti-PD-1 therapy process, leading to the downregulation or loss of target antigen, a common mechanism of resistance to immunotherapies [322–324]. Likewise, cancers with low TMB and immunogenicity cancers, such as MSS CRC and pancreatic ductal adenocarcinoma (PDAC), tend to be primary refractory to ICIs [325, 326]. Therapeutic combinations with agonistic antibodies against the CD40 receptor (αCD40) are efficacious in preclinical mouse models, which can rescue and generate new T-cell responses against weak affinity or poorly expressed neoantigens or against tumour-associated self-antigens that lack high-affinity T-cell clones owing to central tolerance. Hence, there is therapeutic potential in combining αCD40 with ICI, particularly for treating MSS CRC and other immunologically cold cancers, especially cancers that developed resistance to immunotherapy through antigen loss [325, 326].

Defective antigen presentation

Patients with MSI-H CRC exhibit a favourable response to ICIs due to the TMB-H and the presence of neoantigens [327–330]. Contrastingly, frequent mutations in the B2M and HLA-ABC genes cause defects in antigen presentation [328, 331, 332], thus impairing the recognition of tumour cells by cytotoxic CD8 + T cells and resulting in acquired resistance to ICIs [333–335]. Currently, CAR-T-based therapy that bypasses self-MHC restriction and targets nonrestricted cell surface antigens is the viable immunotherapeutic option for overcoming genome-level defects during antigen processing and presentation pathway components [336].

Suppressive tumour immune environment

Inadequate T-cell infiltration

The critical function of ICIs is to reinvigorate the exhausted T cells. A notable resistance mechanism in the TME is the absence of T cells, indicating a target-missing situation [337]. Several factors, including the presence of abundant cancer-associated fibroblasts (CAF), regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) [338], expression of immune checkpoints beyond PD-1/PD-L1 (such as TIM-3) [339], and elevated lactate level, can induce T-cell exhaustion [340]. Additionally, specific genetic mutations and alterations in signalling pathways impact T-cell infiltration in GI tract cancers. For instance, mutations in the phosphatase domain of PTEN, which reduce protein expression, have been linked to the reduced levels of intratumoral CD8 + T cells and resistance to anti-PD-1 therapy in dMMR/MSI-H GI tract cancers [341]. Interestingly, the immunosuppressive effect of PTEN loss may not be through the regulation of the PI3K/AKT pathway but rather related to the release of cytokines and chemokines that promote the proliferation and differentiation of MDSC and M2 macrophages and expansion of Tregs and reduce NK cell infiltration [342–344]. Furthermore, WNT signalling activation through CTNNB1 mutation, which encodes β-catenin, is associated with a low T-cell infiltration rate [345].

A retrospective analysis of data from the KEYNOTE 177 trial revealed that the highly cytotoxic CD8 infiltration in hypermutated CRC is facilitated by the low activation of the WNT pathway, resulting in an immune hot environment favourable for immune response [346].

Immunosuppressive cells

MDSCs are one of the primary immunosuppressive factions in the TME, as they directly target and release soluble mediators to regulate immune responses [347–349], thereby influencing therapeutic efficacy and the prognosis of patients with GI tract cancers [350–354]. Tsutsumi et al. analysed single-cell RNA sequencing (scRNA-seq) data derived from human GC specimens following anti-PD-1-antibody therapy and proposed that tumour-infiltrating MDSCs, particularly those expressing immediate early response 3 (IER3), play a predominant role in the development of the immunosuppressive and ICI-resistant GC tumour immune microenvironment (TIME) [355]. IER3 is involved in protecting cells from apoptosis [356, 357], which suggests that IER3 + M-MDSCs are less susceptible to apoptosis and subsequently differentiate into immunosuppressive macrophages. Although MDSCs represent promising therapeutic targets, developing MDSC-specific treatments remains challenging.

The proportions of circulating and tumour-infiltrating MDSCs could be reduced by chemotherapy [358, 359], targeted therapy [360, 361], all-trans retinoic acid (ATRA) [362], and blockade of chemokine receptors on MDSCs. MDSCs infiltrate into the cancers induced by various cytokines. Under the extreme conditions within cancers, such as low oxygen levels, high oxidative stress, and nutrient deficiency, the MDSCs undergo functional

and differentiation changes, transforming into tumour-associated macrophages (TAMs). TAMs play a significant role in promoting the occurrence and progression of GC and are inversely associated with patient prognosis. In particular, CD204-positive TAMs, M2-polarized macrophages, are a critical risk factor for the progression from gastric adenoma to adenocarcinoma [363–366]. Existing studies have focused on the "reprogramming" of TAMs from "tumour-support cells" to "tumour-killer cells" [367, 368]. For instance, Cao et al. investigated the reprogramming of TAM through V-domain immunoglobulin suppressor of T-cell activation (VISTA), an immune checkpoint associated with immunotherapeutic resistance, across eight independent cohorts involving a total of 1,403 patients with GC. The blockade of VISTA successfully reprogrammed TAMs to a proinflammatory phenotype, enhanced T cell-mediated antitumour immunity, and improved the efficacy of PD-1 inhibitors [369].

Tregs modulate immune homeostasis and, at the same time, inhibit immune responses in cancer patients. Tregs secrete IFN-γ, which enhances the efficacy of ICIs [370] but produces inhibitory cytokines, such as IL-10 and transforming growth factor-β (TGF-β) [371], which are correlated with ICI resistance [372]. The opposite functions of Tregs can be attributed to the heterogeneity of their subsets [373]. C–C motif chemokine receptor 8 (CCR8) is a marker of activated Tregs expressed on Tregs infiltrated in tumour rather than peripheral Tregs [372].

The inhibition of CCR8 expression is a promising strategy in cancer immunotherapy, specifically for enhancing anti-tumour immunity in colon cancer by modulating tumour-resident Tregs. [374]. Furthermore, LM-108, a monoclonal antibody targeting CCR8, is currently being evaluated for its efficacy in treating patients with advanced solid cancers. The treatment groups involved anti-CCR8 monotherapy or in combination with anti-PD-1 therapy (CTR20221680).

Tumour-associated neutrophil (TAN) populations are associated with unfavourable prognosis in patients with cancer [375]. Single-cell transcriptomic analyses have unveiled the striking cellular heterogeneity of neutrophils under pathological conditions and identified their diverse roles in cancer progression. Specifically, pro-tumour TAN-expressing markers, such as CCL3, CCL4, SPP1, and PD-L1, are promising targets for immunotherapy. These TAN subtypes can potentially be targeted either alone or in combination with ICIs to devise more effective cancer treatment strategies [375, 376].

The role of mesenchymal stem cells (MSCs) in cancer remains controversial, as evidence of both pro- and anti-tumour effects has been reported [377, 378]. A potential explanation for these conflicting observations, as suggested by Cascio et al., may be the origin of MSCs and the degree of "cancer education" [379]. In particular, MSCs from local tissues can be epigenetically reprogrammed by the TME into cancer-associated MSCs (CA-MSCs), which drive tumour immune exclusion and resistance to immunotherapy. In GC, MSCs mediate immunosuppression via the CXCR2/HK2 (Hexokinase II)/PD-L1 pathway, blocking GCMSCs-derived IL-8/CXCR2 pathway can reduce PD-L1 expression and lactate production, thereby improving the anti-tumour efficacy of anti-PD-1 immunotherapy; therefore, it may be a promising target for treating advanced GC [380]. MSCs modulate multiple biological processes in cancer, extending beyond immune modulation to immunotherapeutic resistance. Specifically, the exosomes secreted by MSCs can facilitate resistance to immunotherapy [381–384].

In conclusion, the intimate crosstalk between cancer cells and their surrounding immunosuppressive cells plays a critical role in cancer progression and resistance to therapies. Understanding these interactions will help advance cancer research and treatment. Future studies may ultimately enhance the efficacy of anti-PD-1 therapy by identifying and targeting a particular cell subset or specific inhibitory molecule that broadly neutralises the effect of anti-PD-1 therapy on GI cancer patients [385, 386].

Secreted immunosuppressive factors and extracellular matrix

Immunosuppressive factors secreted by tumour, immune, and stromal cells significantly contribute to the formation of an immunosuppressive TME [387, 388]. Notably, the TGF-β released by cancer cells and CAFs is pivotal to facilitating tumour immune evasion [389], while that released into the TME acts as a chemoattractant factor for fibroblasts to induce the formation of CAFs [390]. Therefore, the repression of TGF-β signalling is critical to enhancing the efficacy of current and forthcoming immunotherapies, although potential adverse effects should be carefully monitored [391]. The composition of the extracellular matrix within the TME also influences resistance [392, 393], as the stiffened extracellular matrices could impede the infiltration of drugs and immune cells into the tumour and induce disorganised neovascular vessels with low drug-delivery efficiency [394]. The interleukin (IL) family plays an important role in the immune cell signalling of GI tract cancers. High levels of IL-6 promote epithelial-mesenchymal transition (EMT), clonogenicity, and immunosuppressive phenotype of EC cells. IL-10, potentially in conjunction with IL-35, can be derived from Tregs, promoting the exhaustion of CD8 + TILs and impeding anti-tumour immunity [395, 396].

Metabolism-mediated resistance

Reprogramming of cellular metabolism is a hallmark of cancers impacting TME, immune landscape and therapy resistance [397, 398]. Microenvironmental ammonia induces T-cell exhaustion in CRC, while the accumulation of lactate suppresses the activity of cytotoxic T lymphocytes [399]. The metabolic processes of tumour cells contribute to an immunosuppressive TME through various mechanisms, including nutrient competition, hypoxia, and acidity. Immunotherapy reshapes the features of metabolism. For instance, anti-PD-1 therapy restores glucose levels in the TME, thereby facilitating T-cell glycolysis and IFN-γ production [400]. Recent advancements in single-cell technologies and analytical algorithms have enhanced the exploration of immune metabolism. Hartmann et al. uncovered the spatial organisation of metabolic programs in human CRC and revealed that T cells expanding within cancers exhibit distinct metabolic profiles compared with excluded T cells at the tumour-immune boundary [401]. Despite these insights, the efficacy of targeted metabolic therapies in enhancing T-cell function and promoting anti-tumour immune responses remains unproven. Unfortunately, the most promising metabolic therapy targeting IDO1 failed in a phase III clinical trial, even when combined with anti-PD-1 therapy [402]. Contrastingly, beyond ICIs, preclinical studies that incorporated metabolic targeting alongside adoptive transfer protocols of autologous T-cells and oncolytic viruses have shown potential, necessitating further exploration [403].

Epigenetics-mediated resistance

Epigenetic alterations in cancer cells affect the TIME. Sundar et al. showed that epigenetic promoter alterations in GI tract cancers mediate immune editing and resistance to immune checkpoint inhibition by generating 5′ truncated protein isoforms missing immunogenic N-terminal peptides. Moreover, a high alternate promoter burden resulted in an immune-depleted TME and contributed to the resistance to ICIs [404]. Xu et al. found that IL-1β-associated nicotinamide nucleotide transhydrogenase (NNT) acetylation leads to iron-sulphur cluster maintenance and immunotherapy primary resistance, both in advanced and locally advanced GC. The blockage of NNT acetylation by IL-1β neutralisation synergises with anti-PD-1 therapy in vivo [405]. Epigenetic modification can also affect the efficacy of immunotherapy by modifying tumour cells [406]. Current research suggests that aberrant alterations in the activities of histone-modifying enzymes [407] and epigenetic modification by histone deacetylase 8 [408, 409] overexpression play a crucial role in resistance to hepatocellular carcinoma immunotherapy. Epigenetic alterations are potential biomarkers for predicting the efficacy of immunotherapy and promising targets for overcoming ICI resistance. The mechanisms driving the resistance remain to be elucidated.

However, there are heterogeneity among the possible mechanisms of resistance to immunotherapy in different GI cancers or in different histological types. In the previous study of our center, H. pylori infection is a beneficial factor for GC immunotherapy by shaping hot tumor microenvironments. However, in dMMR/MSI-H colorectal adenocarcinoma and ESCC patients, H. pylori adversely affects the efficacy of immunotherapy [104]. Besides, EC, especially ESCC, is an extremely high TMB tumour, comparable to lung cancer and melanoma, generating specific neoantigens [410]. But the incidence of EAC is rapidly rising worldwide [411], which are highly heterogeneous and surrounded by a largely immunosuppressive TME, resulting disparate sensibility to immunotherapy [412]. Therefore, GI cancers from different regions, histological types, molecular subtypes as GC TCGA and CRC CMS, microbial infection status may have disparate TME, sensitivity and resistance to ICB.

Interestingly, we consider that unique microenvironment of GI tract cancer, corresponding therapy strategies and treatment resistance of which may due to the particular molecular subtypes when compared to other systems. Most GCs are immunologically 'cold', in comparison, EBV (+) GC represents a unique subgroup of GC which is heavily infiltrated by active T/B cells associated with antitumour immunity, making it more sensitive to ICB. In refractory EBV (+) GC tumours after standard chomoimmunotherapy, LAG-3 is upregulated on exhausted T cells, underscores a new promising immunotherapeutic target for EBV (+) GC [120].

AFPGC and HAS are special and rare subtypes of gastric cancer [413]. ScRNA-seq on HAS tumour showed that cytotoxic CD8 + T cells exhibited remarkable heterogeneity in their functional states, with a vast majority of cells following the trend of activation-coupled exhaustion, exemplified by high expression of activation markers GZMA and IFNG and of exhaustion markers PDCD1 and CTLA-4. This suggests that immunotherapy may have a good therapeutic effect on HAS. In a real-world study, anti-PD-1 plus chemotherapy could benefit AFPGC and HAS patients. Because of the small sample size, it is difficult to analyze the efficacy-related predictors such as PD-L1. However, one patient with high expression of PD-L1 exhibited hyperprogressive disease, suggesting the existence of particular resistant mechanism and necessity of further investigation [414].

A negative association between CLDN18.2 expression and the prognosis of anti-PD-1/PD-L1 therapy was

reported in a study. This correlation might be due to the unique tumour microenvironment of CLDN18.2-positive GC, specifically the lack of PD-1/PD-L1-positive lymphocytes in CLDN18.2-positive GC limited its chance to benefit from PD-1/PD-L1 inhibitors, while infiltrating neutrophils may also augment this negative therapeutic response. But CLDN18.2-targeted CAR-T cell therapy may be a promising treatment strategy in CLDN18.2-postive patients because of the non-exhausted CD8 + T cells surrounding tumour cells [415].

Higher expression of PD-L1 has been found in trastuzumab-resistant HER-2-positive cells [416], but development of resistance during anti-HER-2 plus anti-PD-1 therapy remains unclear. Based on liquid biopsy, the presence of PD-L1 + CTCs/CECs and their impact on therapy were explored using longitudinal analyses in patients receiving anti-HER-2 plus anti-PD-1 therapy. Study showed that triploid-PD-L1 + CTCs participated in primary and acquired therapeutic resistance before and after treatment. As for PD-L1 + CECs, intratherapeutically detected multiploid PD-L1 + CECs demonstrated a superior clinical response, when triploidy and tetraploidy contributed to acquired resistance [282].

Efforts to overcome resistance to immunotherapy

While efforts have been made to classify the resistance mechanisms of immunotherapy into distinct categories, resistance is a complex and dynamic process with various interrelated mechanisms in the real world [385]. The most promising strategy appears to reverse resistance through strategic combination with other treatments [417] (Table 7). Overcoming resistance to immunotherapy is accomplished by understanding the specific resistance mechanisms rather than relying on arbitrary combinations.

Combination with chemotherapy

Chemotherapy increases the TMB through DNA damage, subsequently enhancing antigen presentation, eliminates immunosuppressive cells, and enhances the function of effector cells, thereby reprograming the TME and augmenting the immune response [418, 419]. The combination of chemotherapy with anti-PD-1/PD-L1 is a standard-of-care option for GI owing to the synergistic effects.

Combination with radiotherapy

Radiotherapy eliminates local lesions, stimulates the systemic antitumor immune response (also known as abscopal effects) [420], and synergises with anti-PD-1/PD-L1 therapy. Furthermore, radiotherapy promotes T-cell infiltration and expands T-cell receptor (TCR) repertoire [421], upregulates PD-L1 on tumour cells targeted by anti-PD-1/PD-L1 [422], increases MHC-I expression on tumour cells, and alleviates resistance to anti-PD-1/PD-L1 [423]. These promising pre-clinical attributes have been substantiated in prospective studies. A phase II trial (NCT03104439) that combined radiation with ipilimumab and nivolumab to treat primary immunotherapy-resistant cancers as MSS CRC and PDAC demonstrated notable clinical benefits and prolonged disease control [424]. Furthermore, as a local therapy and an immunomodulator, radiotherapy can also synergise with immunotherapy in oligometastatic ESCC patients who have either failed previous immunotherapy or acquired immunotherapy resistance. This combination has shown responses even in unirradiated lesions [425]. By reprogramming the TME in cancers with minimal immune infiltration, radiotherapy, combined with immunotherapy, induces a potent mobilisation of both innate and adaptive immunity [426]. However, the optimal combination strategies, including precise timing, optimal dose, fractionation schedule and target sites, remain to be determined [425].

Combination with anti-angiogenesis therapy

Combined antiangiogenic and anti-PD-1/PD-L1 therapy has demonstrated a synergistic effect [427, 428] in enhancing antitumor immunity by inducing vascular normalisation [429]. Following the promising findings from the REGONIVO study, the combination of regorafenib, a VEGFR inhibitor, and PD-1 inhibitors has been considered as a treatment for refractory pMMR/MSS mCRC patients, particularly those with CPS < 1 and low TMB [430]. Nevertheless, patients with liver metastasis could not benefit from this treatment strategy, which necessitates further investigations. A combination of VEGFR inhibitors can potentially extend the applicability of PD-L1/PD-1 inhibitors beyond patients with dMMR/MSI-H mCRC. Moreover, patients with AFPGC, which is characterised by high invasiveness, early metastasis, rapid progression [431], and elevated VEGF-C expression [432], may benefit from targeting the VEGF-C-VEGFR2 pathway. Recent data presented at the 2024 ASCO meeting highlighted the efficacy of apatinib, a VEGFR2 tyrosine kinase inhibitor, combined with anti-PD-1 and chemotherapy in patients with AFPGC (NCT04609176). Encouragingly, the study reported an ORR of 55.6%, a DCR of 86.1%, and an improved prognosis with a 1-year PFS of 42.1% and a 1-year OS of 63%.

Combination with targeted therapy beyond VEGF

Intracellular signal transduction pathways that mediate resistance to immunotherapy are potential targets for combination therapy. TGF-β not only suppresses the immune response but also promotes angiogenesis and

Table 7 Summary of the clinical trials of anti-PD-1/L1 combined with other agents in GI tract cancers

Combined strategy	ICI	Combined target	Agent	Tumour	Sample size	Outcome/ ongoing	References
Radiotherapy	PD-1	low dose radiotherapy	LDRT	ESCC	49	ORR:40.8%, DCR:75.5%, mPFS:6.9 m, mOS:12.8 m	ChiCTR2000040533
	PD-1 + CTLA-4	low dose radiotherapy	LDRT	MSS CRC, PDAC	65	CRC ORR:10%, DCR:25%, mPFS:2.4 m, mOS:7.1	NCT03104439
	PD-L1	Stereotactic Body Radiotherapy	SBRT	PDAC	59	ORR:5.1%	/
Targeted therapy	PD-L1	TGF-βRII	M7824	EAC	30	ORR:20%, DCR:33.3%, mDOR:4.3 m	NCT02517398
	PD-L1	TGF-βRII	M7824	ESCC	30	ORR:10%, DCR:30%, DOR:7.0 m	NCT02699515
	PD-L1	TGF-βRII	M7824	GC/GEJ	31	ORR:16%, DCR:26%, DOR:8.7 m	NCT02699515
	PD-1	Histone deacetylase inhibitor	CXD101	MSS CRC	55	ORR:9%, DCR:48%, mOS:7.0 m	EudraCT NUMBER 2017–004509-42
	PD-L1	PARP	Olaparib	MSS CRC, PDAC	90	/	NCT03851614
	PD-L1	ATR	ceralasertib	GC	31	ORR:22.6%, DCR:58.1%, mPFS:3.0 m, mOS:6.7 m	NCT03780608
	PD-1	VEGFR	apatinib	GC/GEJC	25	ORR:17.4%, DCR:78.3%, mPFS:2.9 m, mOS:11.4 m	NCT02942329
	PD-1	VEGFR	Regorafenib	GC, CRC	50	GC: ORR 44%, mPFS 5.6 m, CRC: ORR 36%, mPFS 7.9 m	NCT03406871
	PD-1	VEGFR	Regorafenib	MSS CRC	70	ORR:7%, mPFS:1.8 m, mOS: 11.9 m	NCT04126733
	PD-1	VEGF	bevacizumab	MSS CRC	29	ORR:9%, DCR:61%	NCT03396926
Other ICIs	PD-L1	CTLA-4	tremelimumab	CRC	117	ORR:0%, DCR:22.7%, mPFS:1.8 m, mOS:6.6 m	NCT02870920
	PD-L1	CTLA-4	cadonilimab	ESCC, HCC	46	ESCC: ORR 18.2%, DCR 50%, mDOR 10.2 m, mPFS 3.5 m, mOS 9.4 m	NCT03852251
	PD-L1	CTLA-4	cadonilimab	GC/GEJ	610	/	NCT05008783
	PD-1	LAG3	relatlimab	GC/GEJ	16	pCR:21.4%, MPR:57.1%	NCT03044613
	PD-L1	LAG3	FS118	solid tumour	43	DCR:46.5%	NCT03440437
	PD-1	TIM3	Sabatolimab	solid tumour	86	ORR:6%, DCR:44%	NCT02608268
Immuno-modulators	PD-1	TLR9 activator	pixatimod	MSS CRC, PDAC	58	CRC: ORR 12%, DCR 44%, PDAC: ORR 0%, DCR:11%	NCT05061017
	PD-L1	IL-17 + TGF-βRII	AIN457	MSS CRC		/	NCT04298320
	PD-L1	4-1BB	GEN1046	solid tumor	61	DCR:65.6%	NCT03917381
	PD-1	ICOS	KY1044	advanced malignancies	65	ORR:6.25%	NCT03829501

Table 7 (continued)

Combined strategy	ICI	Combined target	Agent	Tumour	Sample size	Outcome/ ongoing	References
	PD-1	GITR	GWN323	solid tumor	53	ORR:7.5%, DCR:34%	NCT02740270
FMT	PD-1	feacal microbial transplantation	FMT	MSS CRC	20	ORR:20%, DCR:95%, mDOR:8.1 m, mPFS:9.6 m, mOS:13.7 m	/

activates CAFs. M7824 (also known as Bintrafusp alfa) is an anti-PD-L1/TGF-β receptor II (TGF-βRII) trap agent [433]. Despite the limited efficacy of first-line treatment for post-platinum biliary tract cancer patients [434], M7824 has shown remarkable results in post-chemotherapy EAC [434] and ESCC [435], with ORR of 83% and 100% in patients with immune-excluded phenotype, respectively. Similarly, another dual PD-L1/TGF-β inhibitor, Retlirafusp alfa (SHR-1701), is undergoing evaluation in phase III studies in patients with G/GEJC.

Moreover, DNA damage repair defects may enhance cancer sensitivity to ICI treatment [189]. Therefore, the combination of ICIs and DNA-damaging therapy could theoretically reduce immunotherapy resistance and improve efficacy [436]. Ceralasertib (AZD6738), an ataxia telangiectasia and Rad3-related protein kinase inhibitor, plus durvalumab displayed promising efficacy with an ORR of 22.6% and a DCR of 58.1% in patients with refractory advanced GC. Accompanying translational research during the treatment highlighted the activation of both innate and adaptive immune responses in responders [437].

Combination with other ICIs

Dual blockade of two checkpoints could potently reduce the probability of resistance and has been demonstrated in abundant preclinical studies [438]. Anti-PD-1 plus anti-CTLA-4 has achieved a prolonged mOS of 6.6 months in patients with "cold" cancers, such as pMMR/MSS CRC, but without ORR and significant improvement in PFS. Further subgroup analyses indicated that patients with TMB > 28 and those classified as CMS2 may benefit from the therapy [439]. Cadonilimab (AK104), an anti-PD-1/CTLA-4 bispecific antibody, showed an encouraging tumour response rate with a manageable safety profile in ESCC and HCC [440]. A phase III study of cadonilimab in first-line treatment of G/GEJ adenocarcinoma is underway (NCT05008783). Moreover, a phase I first-in-human study evaluating the activity of FS118, a bispecific antibody targeting LAG-3 and PD-L1, showed clinical benefit in 43 patients (including 5 patients with CRC) resistant to anti-PD-(L)1-based therapy earlier. SD was also observed in patients with previous ICI as their most recent therapy and/or co-expression of LAG-3 and PD-L1. An increase was observed in the counts of CD4 + and CD8 + T cells following treatment in patients with SD (NCT03440437) [441].

Combination with immune modulators

Combining immune modulators with PD-(L)1 targeting agents is a promising strategy for patients with PD-(L)1–refractory disease [442]. In addition to co-inhibitory pathways such as PD-1 and CTLA-4, co-stimulatory pathways, including CD40/CD40L, CD27/CD70, 4-1BB/4-1BBL, GITR/GITRL, and ICOS/ICOSL, are crucial in regulating T-cell function [443]. Agonists targeting co-stimulatory pathways have demonstrated the potential to boost T-cell activity and elicit antitumor immune responses [444]. The TNF receptor superfamily member 9 (CD137 or 4-1BB) is an inducible T-cell costimulatory receptor expressed on activated CD4 + , CD8 + T cells, and NK cells [442]. In a phase I trial involving 12 (19.7%) CRC and 6 (9.8%) PDAC patients, a bispecific antibody targeting PD-L1 and 4-1BB (GEN1046) achieved a DCR of 65.6%. Notably, two patients who had previously progressed on anti-PD-(L)1 therapy achieved PR. IL-17 affects the infiltration and exhaustion of immune cells that contribute to the formation of an immunosuppressive microenvironment [445, 446]; therefore, targeting IL-17 is a promising strategy for overcoming immune suppression and enhancing the sensitivity of anti-PD-1 therapy [447, 448]. The synergistic effect of anti-IL-17 and anti-PD-1 is being verified in several ongoing clinical trials (NCT05061017).

Combination with faecal microbiota transplantation

Findings have proved the efficacy of responder-derived faecal microbiota transplantation (FMT) in reversing resistance to ICIs in patients with melanoma, induced an ORR up to 30% after anti-PD-1 was restarted [449, 450]. Responding patients had an increased frequency of activated dendritic cells, type I interferon signalling and CD8 + T cells in the TME. In GI tract cancers, the attempts of FMT are concentrated in colorectal cancers.

In a small sample size, phase II trial (RENMIN-215), responder-derived FMT plus anti-PD-1 and fruquintinib as third-line or above treatment showed inspiring mPFS with 9.6 months in refractory MSS mCRC. Peripheral blood expanded TCRs exhibited the characteristics of antigen-driven responses in responders [451]. There are a few points need to discuss, first, FM is usually derived from the responders, but an additional study found that FMT from untreated healthy donors led to an ORR of 65% in ICB-naive patients with melanoma [452]. Secondly, mechanisms by which FMT improves efficacy or overcome resistance. Based on the elevated DC in melanoma and the expanded TCRs in CRC, we hypothesized that FMT might promote the antigen presentation process. Besides, dosage forms, administration timing et al. warrant further study yet.

New attempts to overcome immunotherapy resistance

Treatment strategy following the true progression of immunotherapy is yet to be elucidated, given the limited evidence currently available. In a study of acquired immunotherapy resistance in GI cancer, chemotherapy was the main post-resistant regimen (23.7%), followed by maintaining the original immunotherapy (12.7%) [307]. In addition to combination strategies to overcome immunotherapy resistance, targeted intervention of the key links of immunotherapy tolerance is another potentially effective strategy. To date, this attempt has mainly included CAR-modified cell therapies, herbal medicines, monomeric drugs, ovs, and other biological agents.

Novel immunotherapy for GI tract cancers

Although immunotherapy of GI has made breakthroughs, the conventional strategies focused on immune cells alone, especially immune checkpoints, have limited benefits. Therefore, the development of novel immunotherapy has explored the whole process of anti-tumour response. First, the transfusion of expanded immune cells has shown great potential in GI tract cancers recently. Second, tumour antigens must be presented to T cells by antigen-presenting cells (APCs) so that they can be recognised by T cells, which makes cancer vaccines an attractive strategy to elicit anti-tumour response. Third, T cells are inhibited by immune checkpoints in cancers. Novel ICIs to rescue the second signal, which is essential to T-cell activation, remains to be identified. Furthermore, oncolytic viruses killing tumour cells in versatile ways, which is far beyond directly lysing tumour cells (Fig. 3).

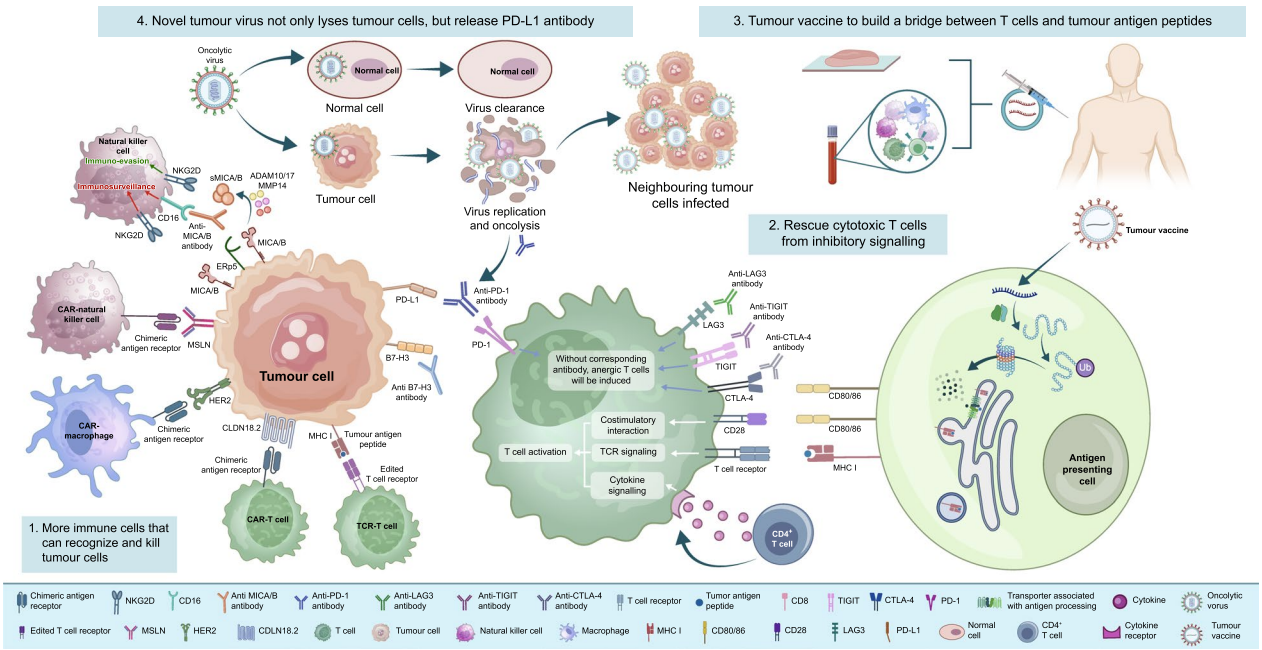

Fig. 3 Novel strategies of GI immunotherapy. We summarized four directions of the development of novel immunotherapy including 1. Transfusion of immune cells, including T cells and innate immune cells, which are edited by gene engineering to recognize tumour antigen such as HER-2, CLDN18.2, and exert potent killing effect;2. Novel immune checkpoint inhibitors can rescue cytotoxic T cells from inhibitory signalling induced by the combination of legend with PD-1, LAG3, TIGIT;3. cancer vaccines, including peptide vaccine and dendritic cells vaccine, can present tumour antigen peptides to activate T cells; 4. Novel tumour virus not only lyses tumour cells, but also provide a versatile platform to kill cancer cells, such as encoding PD-L1 antibody

Transfusion of killer cells

T cells beyond classic cytotoxic T cells

One form of adoptive immunotherapy is the use of viruses to introduce genes encoding novel types of receptors, known as CAR, into the T cells of patients. T-cells expressing CAR have made breakthroughs in the treatment of GI tract cancers.

Qi et al. reported the interim analysis of a phase I clinical trial of Claudin18.2 (CLDN18.2)-targeted CAR-T cells (CT041) in patients with previously treated CLDN18.2-positive digestive system cancers (NCT03874897). The author reported promising efficacy against CLDN18.2-positive digestive cancers, particularly in GC [24]. In another study, CT041 was administered to two patients with metastatic pancreatic cancer. They responded remarkably to CT041. One of the patients achieved partial regression (PR), whereas the other achieved CR of lung metastasis [453].

However, autologous CAR-T cell therapies necessitate an extended period prior to transfusion due to manufacturing time. In contrast, allogeneic donor-derived CAR-T cells, which can be banked for immediate use, address the critical temporal limitation. CYAD-101, an allogeneic CAR-T using a non-gene edited, peptide-based technology to mitigate graft versus host disease (GvHD) is combined with a NKG2D-based CAR. Pre-clinical studies have confirmed that CYAD-101 could maintain CAR-directed anti-tumour activity without inducing GvHD [454]. Clinical grade CYAD-101 cells were produced for the phase I trial (NCT03692429) enrolling fifteen patients with refractory mCRC patients, among whom, two patients achieved PR, nine patients achieved stable disease (SD), and the mPFS was 3.9 months [455]. Based on the encouraging results, KEYNOTE-B79, a phase Ib clinical tiral (NCT04991948) conducted in MSS/pMMR mCRC patients with CYAD-101 is under investigation.

Another challenge faced by CAR-T cell therapy is the limited number of safely targetable cancer antigens on the cell surface. Several tumour antigens, including neoantigens and shared antigens, are derived from intracellular proteins. Therefore, the development of TCR-T cell targeting intracellular antigens may be a prospective treatment option. KRAS is one of the most frequently mutated proto-oncogenes in human cancers. The prevailing oncogenic mutations observed in KRAS involve single amino acid substitutions at codon 12, specifically G12D and G12V. These mutations are highly prevalent, accounting for approximately 60% to 70% of PDAC and 20% to 30% of CRC. Neoantigens related to KRAS mutation can elicit a strong immune response, making it a potential target for TCR. Wang et al. employed HLA-peptide prediction algorithms and determined the potential of HLA-A*11:01 to present mutated variants of KRAS [456]. The researchers extracted murine T cells and subsequently isolated TCR specific to the mutated KRAS variants G12V and G12D. Peripheral blood lymphocytes (PBL), upon transduction with these specific TCR, exhibited a remarkable ability to discern multiple HLA-A*11:01(+) tumour cell lines harbouring the KRAS G12D and G12V. Adoptive transfusion of these transduced PBLs showed potent killing effects of G12D-mutated pancreatic cancer in vivo, which facilitated relevant clinical trials.

A phase I/II study (NCT03745326) involving the administration of PBLs transduced with a murine TCR recognising the G12D variant of mutated RAS in HLA-A*11:01 patients is currently recruiting at National Cancer Institute. Similarly, Chen et al. evaluated the preliminary efficacy and safety of HLA-A*11:01 KRAS G12V, G12D, and G12C mutant antigen-specific TCR-T cells in the treatment of patients with advanced pancreatic cancer, lung cancer, and colorectal cancer (ChiCTR2200057171).

Innate immune cells

Innate immune cells are responsible for nonspecific immune responses, whereas the adaptive anti-tumour response is T-cell-dependent. Although cytotoxic T-cells are the most important killer cells in fighting against cancer, innate immune cells play an irreplaceable role in anti-tumour processes. Mesothelin (MSLN) is an immunotherapeutic target in GC. Cao et al. constructed anti-MSLN CAR NK cells which could specifically kill MSLN-positive GC cells in vitro and in vivo, showing excellent potential for clinic application [457].

Recently, rapid progress has been made in adoptive cell therapy using macrophages as effector cells owing to their phagocytotic, antigen presentation, and high penetration capabilities. Dong et al. generated a novel CAR-Macrophage (CAR-M) based on genetically modified human peritoneal macrophages (PMs) expressing a HER-2-FcεR1γ-CAR (HF-CAR). The researchers observed that HF-CAR-PMs specifically targeted the HER-2-expressing GC cells and that intraperitoneal administration of HF-CAR-PMs significantly facilitated HER-2-positive tumour regression in a peritoneal carcinomatosis (PC) mouse model and prolonged the OS [458]. However, the clinical trial (NCT06224738) has not yet been conducted.

Cancer vaccines

T cells exert killing effects following induction by APCs carrying tumour antigen peptides, which is the first signal of T-cell activation. Cacner vaccines include cancer peptide vaccines and cell vaccines. Peptide-based cancer vaccines typically consist of 20–30 amino acids containing specific epitopes from highly immunogenic antigens to induce the desired immune response by stimulating

specific immune responses against cancer cells. Dendritic cells (DCs) are APCs with a unique ability to induce primary immune response. DCs play an important role in adaptive immunity. Neoantigen-based DC vaccines present tumour neoantigens to naïve cytotoxic T cells and stimulate potent immune response. Taken together, therapeutic vaccines hold promise for providing long-term clinical benefits to patients with cancer.

Peptide-based cancer vaccines

EBV latent proteins are expressed in multiple EBV-associated cancers, play a significant role in carcinogenesis and thus represent vital therapeutic targets for these malignancies. Zhao et.al developed mRNA-based therapeutic vaccines designed to express the T-cell-epitope-rich domain of truncated EBV latent proteins. These vaccines effectively activated both cellular and humoral immunity in tumour-bearing mice, leading to suppressed tumour progression [459]. Peng et. al conducted a clinical trial (NCT05714748) to investigate the efficacy of EBV mRNA vaccine against tumours. What's more, wGc-043 as a mRNA cancer vaccine, recently received FDA approval for clinical trials which was the first granted EBV related mRNA therapeutic cancer vaccine, representing a milestone advancement in the research of future cancer treatments.

An ongoing phase III study (NCT03639714) is assessing the safety, tolerability, and recommended phase II dose of an individualised heterologous chimpanzee adenovirus (ChAd68) and self-amplifying mRNA-based neoantigen vaccine in combination with nivolumab and ipilimumab in patients with advanced metastatic solid cancers. The individualised vaccine regimens were well tolerated, with no dose-limiting toxicities. In addition, the vaccine induces long-lasting neoantigen-specific CD8 + T-cell responses. Despite limitations due to the small study size, the observed increase in OS in MSS CRC warrants further exploration in large-scale randomized studies [460].

However, challenges remain before the vaccine can be widely applied in clinical practice owing to their high level of individualisation. In an ongoing phase I/II study (NCT03953235), Rappaport et al. constructed a therapeutic vaccine encoding 20 shared neoantigens derived from selected common oncogenic driver mutations. The vaccine was administered in combination with the ICIs ipilimumab and nivolumab to patients with advanced/metastatic solid cancers expressing one of the human leukocyte antigen-matched tumour mutations included in the vaccine. Almost all patients (18/19) harboured KRAS mutations. Unfortunately, the ORR was 0%, and the mPFS and OS were 1.9 and 7.9 months, respectively. A notable preference was observed for TP53 neoantigens encoded in the vaccine, suggesting an unidentified immunodominance hierarchy which might influence the efficacy of multi-epitope-shared neoantigen vaccines. Therefore, a more effective vaccination specifically targeting KRAS-derived neoantigens is currently under development and assesment in a subset of patients in a phase II trial [461].

Neoantigen-based dendritic cell vaccines

Tumour antigens include tumour-associated antigens (TAAs) and tumour-specific antigens (TSAs). As they are expressed in both tumour and normal cells, TAAs rarely provoke evident cellular immune responses. In contrast, TSAs exclusive to cancer cells can trigger a robust immune response. Tumour antigens must be captured by DCs and cross-presented to CD8 + T cells to initiate their activation. Subsequently, antigens must be directly presented to tumour cells for recognition by cytotoxic T-cells. Therefore, neoantigen-based DC vaccines hold the potential to induce robust anti-tumour immune responses, with the efficacy and safety confirmed.

Guo et al. documented a case in which a patient with metastatic GC who received a personalised neoantigen-loaded monocyte-derived DC (Neo-MoDC) vaccine followed by combination therapy of the Neo-MoDC and an ICI. The patient developed T-cell responses against neoantigens after receiving the Neo-MoDC vaccine alone. Subsequent combination therapy triggered a stronger immune response and mediated the complete regression of all cancers for over 25 months. Peripheral blood mononuclear cells recognised most of the vaccine neoantigens. The frequency of neoantigen-specific T-cell clones significantly increased post-vaccination (NCT03185429) [462].

Whole-cell vaccines

The whole-cell cancer vaccine, which incorporates a comprehensive array of TSAs, shows great potential in preventing tumour development, progression, and recurrence. Its primary function is to provoke the immune system into identifying and eradicating tumour cells instead of cytotoxic effects on the tumour cells. Researchers have developed a whole-cell tumour vaccine named GVAX. This allogeneic vaccine is modified to generate granulocyte–macrophage colony-stimulating factor (GM-CSF), crucial for triggering immune responses. A phase II study (NCT02981524) evaluated the combination of GVAX, cyclophosphamide, and pembrolizumab in patients with advanced pMMR CRC. Results indicated that the combination of GVAX/cyclophosphamide and pembrolizumab is well tolerated and induces carcinoembryonic antigen (CEA) decrease, without radiographic changes. In this small cohort, PFS and OS rates

appeared favourable compared to controls. Notably, CEA responses were absent in the anti-PD-1 monotherapy group, suggesting GVAX's potential to enhance the anti-tumour immune response.

Chen et al. employed the CRISPR-Cas9 system to disable the interferon-β (IFN-β) specific receptor in live tumour cells, subsequently engineering them to produce IFN-β. This strategy effectively inhibited tumour growth and angiogenesis. Furthermore, these engineered tumour cells were manipulated to express GM-CSF to modulate immune responses. This bifunctional tumour cell vaccine directly induced caspase-mediated tumour cell apoptosis while activating and sustaining long-term immunity. Nonetheless, the safety of using living tumour cell vaccines remains under verification, necessitating further evidence and precautions against potential secondary tumours [463].

Tumour cell-derived cancer nano vaccines

Tumour cell-derived cancer nano vaccines introduce tumour cell-derived components as functional units that endow the nano vaccine systems with antitumor function. Liang et. al generated an endoplasmic reticulum stress inducer α-mangostin (αM) into tumour cells and harvested biologically self-assembled tumour cell-derived cancer nano vaccines (αM-Exos) based on the biological process of tumour cell exocytosing nanoparticles through exosomes. Following subcutaneous injection, αM-Exos efficiently migrated to lymphonodes and was expeditiously endocytosed by DCs, delivering tumour antigens and adjuvants to DCs synchronously, which then powerfully triggered antitumor immune responses and established long-term immune memory [464].

ICIs beyond PD-1/PD-L1

T-cell immunoreceptors with Ig and ITIM domains

T-cell immunoreceptors with Ig and ITIM domains (TIGIT) are immunosuppressive receptors expressed on immune cells, predominantly found on the surface of T- and NK cells. TIGIT is significantly overexpressed in tumour-infiltrating lymphocytes across various malignancies [465, 466]. The ligands CD155, CD112, and CD113 are associated with TIGIT, with CD155 and CD112 being notably overexpressed in many tumour types. Zhu et al. observed that the TAMs from CRC showed robustly higher expression of CD155 than the macrophages from adjacent normal tissues. TAM-specific CD155 contributes to M2-phenotype transition, immunosuppression, and progression [467]. CD155/TIGIT signalling regulates CD8 + T-cell metabolism, promoting the progression of GC [468]. TIGIT exerts its immunosuppressive function by competitively binding to CD155, which is also a ligand of CD226, a co-stimulatory receptor on T cells [469]. Specifically, the novel ligand Nectin-4 (PRR4 and PVRL4) exclusively interacts with TIGIT, thereby inactivating NK cells [470].

Domvanalimab, an Fc-silenced IgG1 monoclonal antibody targeting TIGIT, blocks the interaction between CD155 and TIGIT, so that CD155 in turn binds to the CD226 protein and rescues immune activation signalling. An ongoing global multi-arm EDGE-Gastric study (NCT05329766) investigates the safety and efficacy of the combination of domvanalimab (D) and zimberelimab (Z) in patients with locally advanced unresectable or metastatic G/GEJC. As the primary results revealed at 2023 ASCO, the ORR and 6-month PFS rate in the ITT patients was 59% and 77% respectively. Notably, patients with high-PD-L1 expression(TAP ≥ 5%) exhibited superior response (ORR:80% vs.46%;6 m-PFS rate in ITT:93% vs.68%). The regimen was well-tolerated, with a comparable AEs profile to that of anti-PD-1 + FOLFOX. In conclusion, the D + Z + FOLFOX regimen showed encouraging ORR and 6 months-PFS rates, particularly in patients with high PD-L1 expression(TAP ≥ 5%). STAR-221,a randomised phase III first-line clinical trial comparing D + Z + chemotherapy with Nivolumab + chemotherapy is currently in progress [471].

B7-H3

B7-H3, a notable target for cancer owing to its restricted expression in normal cells, is typically overexpressed in multiple solid cancers [472–476]. In addition, B7-H3 participates in the metabolism, migration, invasion, and endothelial-to-mesenchymal transition of cancer cells [477–481]. Deregulated B7-H3 expression is closely associated with worse outcomes of GI cancers [482]. In addition, emerging evidence points to an immune-evasive phenotype due to B7-H3 overexpression, such as inhibition of CD4 + and CD8 + T-cell activation and proliferation, reduction in IL-2 and IFN-γ production [483, 484], and promotion of tumour immune evasion [485].

Zekri et al. generated B7-H3xCD3 bispecific antibody (bsAb) that showed superior tumour cell elimination, enhanced T-cell activation, proliferation, and memory formation in vitro and in vivo [486]. Unfortunately, the clinical trial of B7-H3xCD3 (NCT02628535) was terminated.

LAG-3

Recently, Kelly et al. reported a phase Ib trial (NCT03044613) that evaluated the efficacy of neoadjuvant nivolumab (Arm A, n = 16) or nivolumab-relatlimab (Arm B, n = 16) in combination with chemoradiotherapy CRT in 32 patients with resectable stage II/stage III GEJC. The results showed overall 2-year RFS and OS rates of 72.5% and 82.6%, respectively. Baseline PD-L1

and LAG-3 expression were positively associated with pathological responses. These findings provide valuable insights into the safety profile and promising efficacy of combining PD-1 and LAG-3 inhibitors in neoadjuvant immune therapy for GEJC [487, 488].

MICA/B

NKG2D is an activation receptor expressed on natural killer (NK) cells, natural killer T (NKT) cells, γδT cells, and naïve CD8 + T cells that plays a vital role in the killing of tumour cells. Eight different NKG2D ligands (NKG2DL), including the MICA/MICB (MICA/B) and ULBP1–6 proteins, have been described in humans [489]. NKG2D/NKG2DL axis is important for tumour immunity, as NKG2DL are upregulated by DNA damage and cGAS-STING signalling, which are rarely observed in healthy cells [490, 491]. Evidence suggests that NKG2DL is frequently detected in malignant cancers. Generally, upregulation of NKG2DLs results in the tagging of stressed cells for elimination by cytotoxic lymphocytes. However, proteolytic shedding of MICA/B on tumour cells results in immune escape [492, 493]. Evidence shows that deficient signal of NKG2D leads to an increased susceptibility to spontaneous tumour development and progression in mice models [494].

As illustrated above, MICA/B is a suitable target for cancer therapy. Due to its complicated mechanisms in immune evasion, MICA/B monoclonal antibody (mAb) is another strategy, besides cell therapy, targeting the NKG2D/NKG2DL axis. Treating human cancer cell lines with MICA/B mAb substantially increases the surface density of these NKG2DLs and induces their killing by human NK cells [495]. Capuano et al. illustrated that the CD16 receptor on NK cells could further enhance the therapeutic activity of MICA/B mAb by inducing NK-cell activation through both NKG2D and CD16 receptors [496], while Courau et al. observed that MICA/B mAb enhanced the destruction of CRC tumour spheroid by increasing NK-cell infiltration and activation. NKG2A expression was increased after anti-MICA/B treatment, and the combination of anti-MICA/B and anti-NKG2A was synergistic [497]. A phase I dose-escalation study (NCT05117476) investigating the safety and efficacy of CLN-619 (anti-MICA/B antibody) alone and in combination with pembrolizumab in patients with advanced solid cancers is currently recruiting.

Novel immune modulators

CD8 + T cells require a third signal, along with the first signal and co-stimulating signal, to generate an active response and avoid death and/or tolerance induction. IL-12 and Type I IFN (IFNα/β) are the predominant contributors to the third signal in various responses. Priming CD8 + T-cells in the absence of IL-12 renders them unresponsive to the same antigen [498]. Curtsinger et al. illustrated that the third signal regulates the CD8 + T cells by promoting chromatin remodelling to maintain the transcription of numerous genes needed for differentiation and effector functions [499].

Razak et al. administered the anti-colony-stimulating factor 1 receptor (anti-CSF1R) monoclonal antibody AMG 820 in combination with pembrolizumab to pMMR patients with refractory in CRC and PDAC. The primary endpoints were the incidence of dose-limiting toxicities and AEs and the ORR per immune-related response evaluation criteria in solid cancers at the recommended combination dose. Although pharmacodynamic effects were observed, the anti-tumour activity was insufficient for further evaluation of this combination in larger patient populations (NCT02713529) [500].

Novel oncolytic viruses

Oncolytic viruses (Ovs) are natural or modified viruses that effectively and selectively infect and lyse cancer cells. Natural viruses have limited tumour specificity. With the advancement of gene engineering, modified Ovs can attack cancer cells with high selectivity and versatility. Ovs directly eliminate cancer cells through their cytocidal effects and activate the immune system directly by stimulating immune cells and indirectly by releasing tumour antigens from dead cancer cells. A phase II clinical trial illustrated that the oncolytic H-1 parvovirus significantly activated the immune system with excellent tolerability in patients with metastatic PDAC [501].

Additionally, novel Ovs reshape the TME through anti-angiogenesis, metabolic reprogramming, and decomposition of the extracellular matrix. Novel Ovs have been adopted as delivery systems for other anti-tumour drugs. LOAd703 is an oncolytic adenovirus loaded with a cytomegalovirus-driven transgene cassette encoding CD40L and 4-1BBL (manufactured by Baylor College of Medicine, Houston, TX, USA on behalf of Lokon Pharma). Preclinical models have illustrated that LOAd703-mediated oncolysis is cancer-selective and that LOAd703 can infect adjacent immune and stromal cells, leading to the expression of CD40L and 4-1BBL and the secretion of chemokines [502]. A non-randomised, single-centre, phase I/II study conducted by the same team combining LOAd703 with chemotherapy in patients with advanced PDAC (LOKON001) concluded that this regimen is feasible and safe. Arm 2 of this trial, which combines LOAd703, chemotherapy, and ICIs, is ongoing [503]. VG161 is the first recombinant oncolytic herpes simplex virus type 1 that carries multiple synergistic anti-tumour immunomodulatory factors (IL12, IL15/15RA, and PD-L1-blocking peptide). VG161 can systematically activate

acquired and innate immunity in PDAC models and remodel the TIME, indicating strong anti-tumour potential. The anti-tumour effects and safety of VG161 require further investigation prior to clinical application [504].

Although novel immunotherapy has demonstrated substantial outcomes in preclinical studies, as indicated in Table 8, it is hindered by prolonged research duration and high resource consumption. Furthermore, despite some drugs advancing to early clinical trials, challenges such as high toxicity, poor efficacy, and elevated failure rates persist. Consequently, most novel immunotherapies remain confined to the preclinical and early clinical trial phases. Moreover, the majority of current studies predominantly designed for solid cancers, including lung, breast, and prostate cancer, with few novel methods developed based on the characteristics of GI cancers. Therefore, developing efficient novel immunotherapy strategies with a high clinical translation rate, tailored to GI features, within a truncated timeframe, represents a critical challenge that must be addressed in future research.

Challenges and Future directions

The immunotherapy of GI tract cancers has made monumental progress in recent years and the efficacy is encouraging and promising. However, opportunities are always accompanied by challenges in realising precise and individual immunotherapy for GI tract cancers.

Despite numerous explorations on novel immune related targets in GI tract cancers, there are still few druggable targets available. Digestive tract cancers including EC, G/GEJC and CRC, are hollow organ cancers, characterized by significant genetic and molecular spatiotemporal heterogeneity and complexity, thus identification of universal targets is extremely limited. Mutations of targets and activation of alternative signalling pathways commonly occur in GI tract cancers which remarkably impair the efficacy. Besides, TME in GI tract cancers tends to be highly suppressive making it challenging to find targets that reliably evoke anti-tumour immunity. In addition, the absence of organ-specific and tumour-specific antigens resulting in on-target, off-tumour toxicities, further limiting the clinical application of targets. Therefore, to improve the success rates of targets transformation, integrating multi-omics massive data for target mining and employing preclinical research models that more accurately mirror the overall characteristics of the human TME for validation, represent the pivotal research directions for future exploration of new targets.

Progress has been made in identifying immunotherapeutic markers for GI tract cancers, with PD-L1 and MSI/dMMR serving as primary biomarkers for EC, G/GEJC and CRC. While substantial potential beneficiaries are still unidentified, there is still a long journey ahead in selecting patients precisely. Relying on a single biomarker fails to stratify patients accurately and precisely. In recent years, research on GI immunotherapy biomarkers has expanded across multiple dimensions with attempts to construct predictive models for outcomes and AEs that integrate multi-omics data using AI techniques. Moreover, current research utilizing baseline specimens does not adequately capture the transformative effects of immunotherapy on the TME. Therefore, incorporating specimens collected post-therapy for dynamic detection represents a trend in future biomarker exploration necessitating an increase in specimen collection frequency. Develop minimally invasive sampling methods and multi-omics detection using trace specimens techniques, such as nuclear marker-labelled PET-CT, liquid biopsy, and gut microbiota analysis, that reflect the TME from multi-dimensions are urgently anticipated.

Additionally, the integration of clinical trials and translational research with high efficiency and quality is an important project worthy of further exploration. As shown in Fig. 4, "Dynamic-Recycle-Closed loop" research model is recommended. A biomarker-driven clinical trial involves patients with biomarker-positive expression, therefore, optimising efficacy and reducing the waste of medical resources. Moreover, the results from the Rationale 305 trial, which focused on OS in the PD-L1-positive population (tumour area positivity ≥ 5) as the primary endpoint, suggest that tislelizumab combined with chemotherapy leads to statistically and clinically significant OS improvements, with the final results highly anticipated [87]. Developing new treatments based on resistance mechanisms, integrated analysis of datasets, and multi-omics information of specimens will contribute to the rapid mutual transformation between clinical and laboratory data. Patients will be more precisely distinguished and ultimately enrolled in corresponding clinical trials. All research in the model is closely connected, time-transformed, and mutually optimised to form a closed loop.

In the era of precision and individual immunotherapy, the comprehensive management mode for patients with GI tract cancers is undergoing revolutionary changes. Traditional and classic treatment modalities and management are facing challenges. In the context of significantly increased rates of successful transformation surgery for patients with locally advanced GI tract cancers undergoing neoadjuvant immunotherapy, issues that still need to be explored include: (1) whether it is possible for patients with rectal cancer to achieve clinical CR and further adopting "Watch and Wait" treatment strategy to avoid surgery-related adverse; (2) for patients with EC or G/GEJC benefit to neoadjuvant immunotherapy, it is necessary to evaluate whether extensive lymph nodes

Table 8 Summary of under-investigation novel immunotherapies involving GI tract cancers

Strategy	Category	Target	Regimen	Status	Sample size(estimated)	NCT number
Cell therapy	CAR-T cells	CLDN18.2	AZD6422	Recruiting	96	NCT05981235
		EpCAM	EPCAM CAR-T cells	Recruiting	48	NCT05028933
		GCC	IM96 CAR-T cells	Recruiting	19	NCT05287165
		GCC	LCAR-G08 cells	Recruiting	42	NCT06197178
		CEA	CEA CAR-T cells	Recruiting	36	NCT06010862
		LGR5	LGR5-targeted CAR-T Cells	Recruiting	45	NCT05759728
		MSLN	αPD-1-MSLN-CAR T Cells	Recruiting	30	NCT05089266
		B7-H3	anti-CD276 CAR T cells	Recruiting	100	NCT04432649
	CAR-T/TCR-T cells	NY-ESO-1, DR5, EGFR vIII, Mesothelin	CAR-T/TCR-T cells	unknown	50	NCT03941626
		Muti-targets	Cyclophosphamide/ Fludarabine + CAR-T/ TCR-T cells	unknown	73	NCT03638206
	TCR-T cells	NY-ESO-1	TBI-1301 + Cyclo-phosphamide/TBI-1301 + Cyclophospha-mide + Fludarabine	unknown	9	NCT02366546
		PRAME	IMA203/IMA203CD8 product	Recruiting	186	NCT03686124
	CAR-NK92 cells	NKG2D	NKG2D-CAR-NK92 cells	Recruiting	20	NCT05528341
	CAR-macrophage cells	HER-2	HER-2-targeted CAR-M cell	Not yet recurting	9	NCT06224738
Tumour vaccine	mRNA vaccine	Personalized Neoan-tigen	Neoantigen tumor vaccine with or without PD-1/L1	Recruiting	30	NCT05192460
		Personalized Neoan-tigen	SW1115C3	Recruiting	30	NCT05198752
		Personalized Neoan-tigen	RO7198457	Recruiting	229	NCT04486378
		Personalized Neoan-tigen	Cyclophospha-mide + personalized neoantigen vac-cine + Pembrolizumab	Recruiting	36	NCT05269381
	Dendritic Cell Vaccine	Neoantigen	Neoantigen DC Vaccine and Nivolumab	Recruiting	60	NCT04912765
ICIs Beyond PD-1/L1	ICIs Beyond PD-1/L1	TIGIT	A: Tislelizumab plus Oci-perlimab B: Tislelizumab plus Placebo	Completed	125 (actual)	NCT04732494
		TIGIT	Pembrolizumab/Vibos-tolimab	Recruiting	610	NCT05007106
		TIGIT	BAT6005	Recruiting	36	NCT05116709
		LAG3	BMS-986213 + chemo-therapy	Completed	274 (actual)	NCT03662659
		LAG3	IBI 110 ± sintilimab	Recruiting	268	NCT04085185
		CTLA-4	Pembrolizumab/ Quavonlimab	Recruiting	320	NCT04895722
		MICA/B	CLN619 + Pembroli-zumab	Recruiting	410	NCT05117476
Oncolytic viruses	Oncolytic viruses	Oncolytic virus	IDOV-SAFE	Recruiting	60	NCT06380309
		Oncolytic virus	HX008	Recruiting	300	NCT03866525
		Oncolytic virus	R130	Recruiting	20	NCT05961111
		Oncolytic virus	T3011	Recruiting	233	NCT05602792

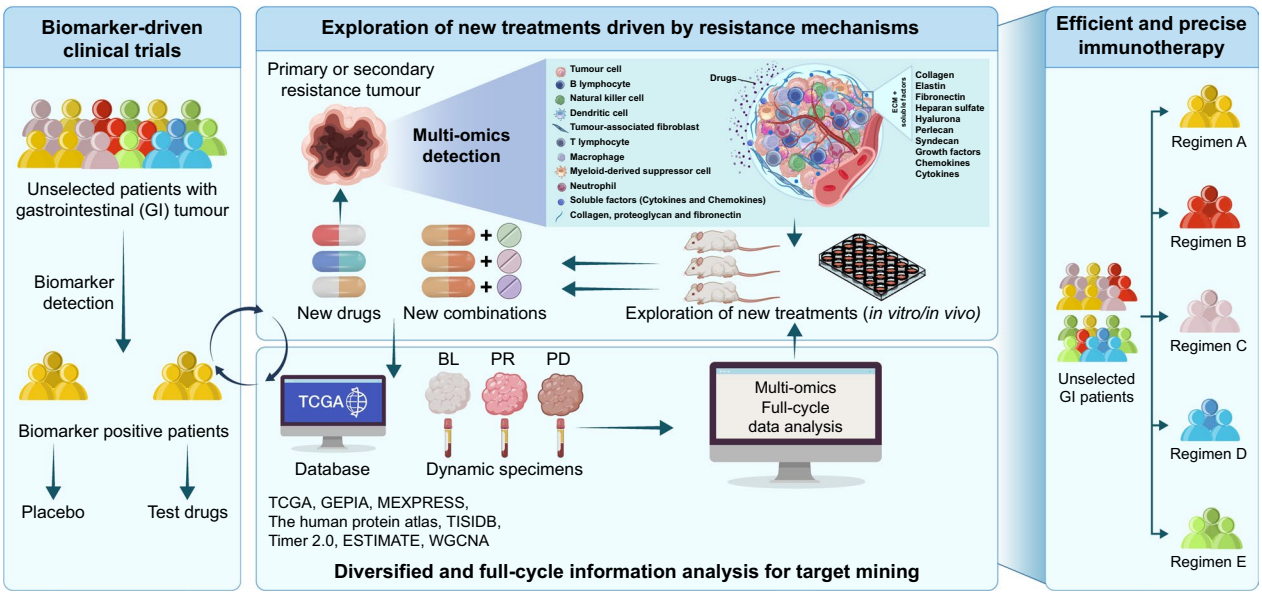

Fig. 4 Dynamic-Recycle-Closed loop research model. We designed a novel research pattern especially for the precise and individual immunotherapy in GI tract cancers. Three parts "biomarker-driven clinical trials", "exploration of new treatments driven by resistance mechanisms" and "diversified and full-cycle in formation analysis for target mining" are mutually linked, and formed a recycle closed loop. The final goal is achieving "precise and efficient immunotherapy"

dissection is still required and whether the scope of surgery can be minimized to preserve organ function. Additionally, given the notable improvement in long-term survival rates in patients with advanced GI tract cancers receiving immunotherapy, it is crucial to integrating multidisciplinary wisdom for future development. Establish joint decision-making platforms for physicians and patients based on Chat GPT 4.0 and utilize intelligent techniques to realize long-term AE management and follow-up of survival. Moreover, leveraging technologies such as Digital Twins in the intelligent design of clinical trials advocates for conducting prospective multi-cohort clinical studies more efficiently, cost-effectively, and with minimal patient involvement while maintaining the reliability of outcomes, which represents a promising direction for future development.

Conclusion

Collectively, immunotherapy has pioneered a new chapter in the treatment of GI tract cancers. However, significant challenges, including limited treatment options, undefined beneficiary groups, complex drug resistance mechanisms, and low success rates of new drug research and development, remain in achieving precise immunotherapy. We anticipate breakthroughs in basic, translational, and early clinical research on new drugs.

Abbreviations

AC	Anal carcinoma
ADCs	Antibody-drug conjugates
ASCC	Anal squamous cell cancer
GI	Gastrointestinal
PD-1	Programmed death protein 1
ICIs	Immune checkpoint inhibitors
CRC	Colorectal cancer
CAR-T	Chimeric antigen receptor T-cell
ESCC	Oesophageal squamous cell carcinoma
GERD	Gastroesophageal reflux disease
HNSCC	Head and neck squamous cell carcinoma
OS	Overall survival
DFS	Disease-free survival
pCR	Pathologic complete response
PFS	Progression-free survival
FGFR	Fibroblast growth factor receptor
EGFR	Epidermal growth factor receptor inhibitors
CPS	Combined positive score
EFS	Event-free survival
RFS	Recurrence-free survival
DOR	Duration of response
CR	Complete response
MPR	Major pathologic response
ORR	Objective response rate
MDSCs	Myeloid-derived suppressor cells
ATRA	All-trans retinoic acid
TAMs	Tumour-associated macrophages
VISTA	V-domain immunoglobulin suppressor of T-cell activation
TGF-β	Transforming growth factor-β
CCR8	C-C motif chemokine receptor 8
MSCs	Mesenchymal stem cells
IL	Interleukin
EMT	Epithelial-mesenchymal transition
PDAC	Pancreatic ductal adenocarcinoma
NNT	Nicotinamide nucleotide transhydrogenase
NK	Natural killer
TCR	T-cell receptor
PMs	Peritoneal macrophages
PC	Peritoneal carcinomatosis
APC	Antigen-presenting cells
TIGIT	T-cell immunoreceptors with Ig and ITIM domains

CKs	Chemokines
Ovs	Oncolytic viruses
AI	Artificial Intelligence
BRAF	B-Raf proto-oncogene
CDH1	Cadherin 1
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CRC	Colorectal Cancer
CREBBP	CREB-binding protein
CT	Chemotherapy
CTC	Circulating Tumour Cell
CTLA-4	Cytotoxic T-Lymphocyte-Associated protein 4
CRT	Chemoradiotherapy
CXCL	Chemokine (C-X-C motif) ligand
DC	Dendritic Cell
DCR	Disease Control Rate
DNA	Deoxyribonucleic Acid
dMMR	Deficient Mismatch Repair
EC	Oesophageal Cancer
EAC	Oesophageal Adenocarcinoma
EBV	Epstein-Barr Virus
EBVaGC	Epstein-Barr Virus-associated Gastric Cancer
EV	Extracellular Vesicle
FOXP3	Forkhead box P3
GC	Gastric Cancer
GEJC	Gastric and gastroesophageal junction cancer
GIPS	Genomic Immunotherapy Prognostic Score
H.pylori	Helicobacter pylori
ICIs	Immune Checkpoint Inhibitors
IFN	Interferon
IL	Interleukin
irOS	Immune-related Overall Survival
JAK2	Janus Kinase 2
KRAS	Kirsten Rat Sarcoma viral oncogene homolog
LIF	Leukaemia Inhibitory Factor
LA-ESCC	Resectable locally advanced ESCC
lncRNA	Long non-coding RNA
m6A	N6-methyladenosine
mCRC	Metastatic Colorectal Cancer
MET	MET proto-oncogene
mOS	Median Overall Survival
mPFS	Median Progression-Free Survival
MSS	Microsatellite Stable
MSI	Microsatellite Instability
MSI-H	Microsatellite Instability-High
nCRT	Neoadjuvant chemoradiotherapy
NICHE	Neoadjuvant Immunotherapy for Colon Cancer Patients with Mismatch Repair Deficiency
NGS	Next-Generation Sequencing
NII	Neuroinflammatory Infiltration
ORR	Objective Response Rate
PD-L1	Programmed Death-Ligand 1
PD-L2	Programmed Death-Ligand 2
PDGF-B	Platelet-Derived Growth Factor-B
PI3KCA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PNI	Perineural Invasion
POLE	DNA Polymerase Epsilon
POLD-1	DNA Polymerase Delta 1
PTCH1	Patched 1
PTEN	Phosphatase and Tensin Homolog
RAS	Rat Sarcoma viral oncogene homolog
RCTs	Randomized controlled trials
RHOA	Ras Homolog Family Member A
RNF43	Ring Finger Protein 43
SPEN	Spen Family Transcriptional Repressor
TP	Taxane plus platinum
TCGA	The Cancer Genome Atlas
TIL	Tumour-Infiltrating Lymphocyte
TIME	Tumour Immune Microenvironment
TIIC	Tumour-Infiltrating Immune Cell
TLS	Tertiary Lymphoid Structure
TME	Tumour Microenvironment
TMB	Tumour Mutational Burden
TMB-H	Tumour Mutational Burden-High
TNF	Tumour Necrosis Factor
VEGF	Vascular Endothelial Growth Factor

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