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Tumour Review

Understanding the immuno-biology of oesophageal adenocarcinoma: Towards improved therapeutic approaches

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ABSTRACT

With an incidence that is constantly rising, oesophageal adenocarcinoma (OAC) is becoming an increasing health burden worldwide. Although significant advances in treatment regimens have improved patient outcomes, survival rates for this deadly cancer remain unsatisfactory. This highlights the need to improve current therapeutic approaches and develop novel therapeutic strategies for treating OAC patients. The advent of immunotherapy has revolutionised treatment across a range of malignancies, however outcomes in OAC show modest results. The inherent resistance of OAC to treatment reflects the complex genomic landscape of this cancer, which displays a lack of ubiquitous driver mutations and large-scale genomic alterations along with high tumour and immune heterogeneity. Research into the immune landscape of OAC is limited, and elucidation of the mechanisms surrounding the immune responses to this complex cancer will result in improved therapeutic approaches. This review explores what is known about the immuno-biology of OAC and explores promising therapeutic avenues that may improve responses to immunotherapeutic regimens.

Introduction

Oesophageal adenocarcinoma (OAC) is a lethal cancer with a rising incidence globally [1]. Although oesophageal squamous cell carcinoma (OSCC) remains the dominant subtype worldwide due to its predominance across the "oesophageal cancer belt" through China, Central Asia, and Northern Iran as well as Eastern Africa, the incidence of OAC is rising in these populations also [2,3]. The main risk factors for OAC include advancing age, male sex, obesity, and gastro-oesophageal reflux disease (GORD), and changes in these epidemiological risk factors across both Western populations and through the oesophageal cancer belt may explain changing incidence rates [2]. Exposure of the distal oesophagus to refluxed gastric acid and bile can trigger a state of chronic inflammation and subsequent development of intestinal metaplasia (Barrett's Oesophagus), the precursor lesion to OAC [4]. Despite global differences in epidemiology, management of OAC remains largely the same and is dependent on tumour staging and patient suitability for surgery. Early T stage cancers may be directed to endoscopic therapy or may proceed straight to surgery, however the mainstay of management for resectable disease involves multimodal treatment combining surgical resection with perioperative chemotherapy (CT) or neoadjuvant chemoradiotherapy (CRT) [5,6]. The outcomes for operable patients with current therapeutic approaches, although significantly improved compared to surgery alone, remain poor, with over 50% of patients experiencing disease recurrence. Furthermore, only ~20% of patients present with disease confined to the primary site, and ~40% of patients present with distant metastatic disease. For patients with distant metastases five-year survival falls to <5% [7].

The advent of immunotherapy has revolutionised cancer care. To date, most immunotherapy trials have revolved around immune checkpoint inhibitors (ICIs), which have shown durable outcomes in a range of cancers [8]. Despite the broad and growing utility of ICIs, only a subset of patients show response to these treatments [9], thus discovery of biomarkers determining who will benefit most is paramount. Most oesophageal cancer (OC) and gastro-oesophageal cancer (GOC) immunotherapy trials have focussed on second- or third-line treatment of advanced/metastatic disease, and although encouraging, results have only shown modest survival increases compared with CT (Table 1). Recently however, results from the CheckMate-577 trial, where patients treated with adjuvant Nivolumab following CRT and surgical resection, showed significantly increased disease-free survival (DFS) compared to placebo [10] (Table 1).

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Table 1
Selected immunotherapy trials in OAC and GOJ cancer.

Clinical Trial/ Phase	Disease Setting/ Prior Line(s) of Therapy	Treatment	Results + Comments
Resectable Disease			
CheckMate-577 [10] Phase III	Neoadjuvant CRT + resected Stage II/III OC or GOJC (*n = 749*) with residual pathologic disease/NA	Nivo 240 mg or placebo q2w for 16 weeks, followed by Nivo 480 mg or placebo q4w	• 71% adenocarcinoma • OAC mDFS 19.4mo vs. 11.1mo (HR 0.75) • First study to report efficacy and safety of ICI in the adjuvant setting for OC/GOJC
Ku, et al [133] Phase Ib/II NCT02962063	Durvalumab + PET directed CRT after induction FOLFOX in resectable OAC or GOJC (*n = 36*)/NA	Induction mFOLFOX6, PET/CT. PET responders – FU or capecitabine _ oxaliplatin with RT 50.4 Gy. PET non-responder's carboplatin + paclitaxel + RT. All patients durvalumab neoadjuvant and R0 patient's adjuvant.	• Demonstrated safety and feasibility • pCR – 24% • near pCR 52% • Awaiting full study accrual for further results
Locally Advanced or Metastatic Disease – No Prior Lines of Therapy			
KEYNOTE-062 [150] Phase III NCT02494583	PD-L1 CPS ≥ 1, untreated locally advanced or metastatic GC or GOJC. (*n = 763*)/0	Pembro 200 mg q3w vs. pembro 200 mg qw3 + CT (cisplatin + FU or capecitabine) vs. CT + placebo	• Pembro vs CT o PD-L1 CPS ≥ 1: mOS 10.6mo vs. 11.6mo NS o PD-L1 CPS ≥ 10: mOS 17.4mo vs. 10.8mo NS • Pembro + CT vs. CT o PD-L1 CPS ≥ 1: mOS 12.5mo vs. 11.1mo mPFS 6.9mo vs. 6.4mo • PD-L1 CPS ≥ 10: mOS 12.3mo vs. 10.8mo NS
KEYNOTE-590 [151] Phase III NCT03189719 Published Abstract	Untreated locally advanced or metastatic OAC (*n = 202*) or OSCC (*n = 547*)/0	Pembro 200 mg q3w + CT (cisplatin + FU) vs. placebo + CT	• ORR 45% vs. 29.3% • mDOR 8.3mo vs. 6.0mo • mOS 12.4mo vs 9.8mo • mPFS 6.3 m vs. 5.8mo • Primary endpoints focussed on OSCC
CheckMate-649 [152] Phase III NCT02872116 Published Abstract	Untreated locally advanced or metastatic GC or GOJC or OAC (*n = 1581*)/0	Nivo 360 mg q3w or 240 mg q2w + ipi vs. nivo + CT (XELOX or FOLFOX) vs. CT	• PD-L1 CPS ≥ 5 (*n = 955*) o mOS 14.4mo vs. 11.1mo o mPFS 7.7mo vs. 6.1mo • PD-L1 CPS ≥ 1 (*n = 1296*) o mOS 14.0mo vs. 11.3mo • All patients (*n = 1581*) o mOS 13.8mo vs. 11.6mo • First study to report superior OS and PFS in combination with CT vs CT alone in the first line setting
ATTRACTION-4 [153] Phase II/III NCT02746796 Published Abstract	Untreated locally advanced or recurrent GC (~90%) or GOJC (*n = 724*)/0	Nivo + CT (SOX or XELOX) q3w vs. placebo + CT q3w	• ORR 57.5% vs. 47.8% • mPFS 10.5mo vs. 8.3mo • mOS 17.5mo vs. 17.2mo NS
Locally Advanced or Metastatic Disease - ≥1 Prior Lines of Therapy			
ATTRACTION-2 [154] Phase III NCT02267343	Locally advanced or recurrent GC (*n = 308*) or GOJC (*n = 42*)/≥2	Nivo 3 mg/kg vs. placebo q2w	• ORR 11.2% vs 0% • mOS 5.26mo vs. 4.14mo • mPFS 1.61mo vs. 1.45mo
KEYNOTE-028 [155] Phase IB NCT02054806	PD-L1^{+}, Locally advanced/ metastatic OAC/ GOJC (*n = 27*), or OSCC (*n = 65*)/≥1	Pembro 10 mg/ kg q2w	• ORR 30% • mDOR 15mo
CheckMate-032 [156] Phase I/II NCT01928394	Locally advanced or metastatic GC (*n = 59*), OAC (*n = 26*), or GOJC (*n = 75*)/ ≥1	Nivo 3 mg/kg vs. nivo 1 mg/kg + ipi 3 mg/kg vs. nivo 3 mg/kg + ipi 1 mg/kg	• ORR 12% vs. 24% vs. 8% • 12-mo PFS 8% vs. 17% vs. 10% • 12-mo OS 39% vs. 35% vs. 24%
KEYNOTE-059 [47] Phase II NCT02335411	Recurrent or metastatic GOJC (*n = 133*) or GC (*n = 126*)/≥2	Pembro 200 mg q3w	• ORR 11.6% • CR 2.3% • PD-L1^{+} ORR 15.5% • PD-L1^{-} ORR 6.4%
KEYNOTE-061 [157] Phase III NCT02370498	Locally advanced or metastatic GOJC (*n = 185*) or GC (*n = 407*)/≥1	Pembro 200 mg q3w vs. paclitaxel	• PD-L1 CPS ≥ 1 o mOS 9.1mo vs. 8.3mo o mPFS 1.5mo vs. 4.1mo
KEYNOTE-180 [158] Phase II NCT02559687	Locally advanced or metastatic OAC (*n = 58*) or OSCC (*n = 63*)/ ≥2	Pembro 200 mg q3w	• ORR 9.3%, OAC ORR 5.2%, OSCC ORR 14.3%, PD-L1^{+} ORR 13.8%, PD-L1^{-} ORR 6.3% • Pembro favours OSCC over OAC
KEYNOTE-181 [159] Phase III NCT02564263	Locally advanced or metastatic OAC (*n = 227*) or OSCC (*n = 401*)/1	Pembro 200 mg q3w vs. investigator choice CT (paclitaxel or docetaxel or irinotecan)	• PD-L1 CPS ≥ 10 o mOS 9.3mo vs. 6.7mo o 12-mo OS 43% vs. 20.4% • Primary endpoints focussed on OSCC • Pembro favours OSCC over OAC

Abbreviations: CPS, combined positive score; CR, complete response; CRT, chemoradiotherapy; CT, chemotherapy; DFS, disease free survival; FU, fluorouracil; FOLFOX, oxaliplatin + leucovorin + fluorouracil; GC, gastric cancer; GOJ, gastro-oesophageal junction; GOJC, gastro-oesophageal junction carcinoma; HR, hazard ratio; ICI, immune checkpoint inhibitor; Ipi, Ipilimumab; mDFS, median disease free survival; mDOR, median duration of response; mo, month; mOS, median overall survival; mPFS, median progression free survival; NCT, ClinicalTrials.gov Number; Nivo, Nivolumab; OAC, oesophageal adenocarcinoma; NS, not statistically significant; OC, oesophageal cancer; ORR, objective response rate; OS, overall survival; OSCC, oesophageal squamous cell carcinoma; pCR, pathologic complete response; Pembro, pembrolizumab; PFS, progression free survival; q2w, every two weeks; q3w, every three weeks; SOX, S-1 + oxaliplatin; Tx, therapy; XELOX, oxaliplatin + capecitabine.

The inherent resistance of OAC to treatment, and failure to develop accurate, biomarker-driven immunotherapy thus far, reflects the complexity of the underlying genomic landscape, associated immune responses, and heterogeneity of this cancer. However, with the

increasing understanding of the genomic features of OAC, and the increasing recognition of the role of the immune system in the anti-tumour response, novel therapeutic regimens are being explored. In this review, we highlight what is known about the immunogenic features of OAC and summarize available evidence of the anti-tumour immune responses in these patients as critical knowledge to best determine ways to overcome current therapeutic resistance, and improve outcomes in this complex and deadly cancer.

Genomic features of OAC and associated immune responses

The two main subtypes of OC are OAC and OSCC, and although inherently different in their biological and clinical features, they are often studied as a combined disease. Advances in the understanding of the molecular biology of OC and gastric cancer (GC) have shown that OAC should be considered separately from OSCC and be considered along a single disease spectrum with GC. OSCC more resembles head and neck SCC, whereas OAC is more similar to chromosomally instable (CIN) GC [11,12].

OAC is characterised by a high tumour mutation burden (TMB), high numbers of copy number aberrations (CNAs), a high prevalence of aneuploidy, and chromosomal instability [12–20]. Furthermore, complex genomic rearrangements, chromothripsis, breakage-fusion-bridges (BFBs), and kataegis are also key features of OAC that give rise to a genomically instable cancer, further supporting the similarity to CIN GC [11,20–22]. CIN GOCs have been found to be highly infiltrated by tumour associated macrophages (TAMs) and display T-cell exclusion, with $CD8^+$ T-cells predominately found at the invasive margin [23]; and have been shown to display suppression of pro-inflammatory cytokines such as interleukin-2 (IL-2), interleukin-3 (IL-3), and interferon-gamma (IFN-γ) [24]. These findings are supported by Davoli et al, who showed high CNA/aneuploidy is associated with reduced expression of the immune signature score and weakened MHC antigen presentation [25]. A recent study assessing the mutational landscape in Chinese OAC patients using whole genome sequencing (WGS) found an absence of the hallmark acid reflux A > C mutational signature, lower TMB, and no chromothripsis or genome doubling [26]. Although this study only included 10 tumour samples, the results are in stark contrast to Western tumour findings and lay the groundwork for further comparative studies between Asian and non-Asian patients. Outcome differences between Asian and non-Asian patients have been conflicting across immunotherapy trials, with CheckMate 649 reporting an overall survival (OS) benefit for Asian patients, whereas no difference was seen for DFS in CheckMate 577 [10,27]. No studies to date have compared the tumour microenvironment (TME) of OAC between Asian and non-Asian patients, and integrative studies comparing the TME and underlying genomic features may inform geographically relevant biomarkers and therapeutic strategies.

Another common genomic feature of OAC is somatic mobile element insertions, particularly long interspersed nuclear element-1 (L1) retrotransposons, which are the most frequent type of somatic structural variation in OAC [18,28,29]. L1 overexpression can contribute to genomic instability and tumour progression through mutagenic insertions, and in OAC L1 was found to induce *CCND1* oncogene amplification through inducing BFBs [29]. L1 activity is normally contained by the restrictive action of p53 [30,31]. However, *TP53* is the most common mutated gene in OAC [11], and in a recent analysis, gastrointestinal cancers with *TP53* mutations were found to have frequent L1 insertions [32]. A significant inverse correlation between the number of L1 insertions and immune pathway activity has been shown in GOCs, with analysis of immune pathways revealing "high" and "low" immune signature groups [32]. The immune-low group contained mainly OCs with enrichment in *TP53* mutations, increased L1 expression and insertion frequency, and higher CNA numbers, further supporting the findings of Davoli et al [25,32]. L1 hypomethylation results in its activation and has been associated with poor survival in a range of cancers, including OAC [31,33]. DNA methyltransferase (DNMT) is an important enzyme involved in catalysing DNA methylation, and the use of DNMT-inhibitors (DNMTis) can effectively inhibit L1 expression [31]. DNMTis have been shown to improve tumour immunogenicity and promote $CD8^+$ T-cell and NK-cell function by inducing a type I interferon response [31,34]. Targeting L1 through DNMTis and potentially also in combination with immunotherapeutic approaches is an attractive area of research in OAC [34].

TMB has been investigated extensively as a potential biomarker for the prediction of survival and response to immunotherapy across multiple cancers [35]. Some cancer types displaying high TMBs have been shown to have a higher likelihood to respond to ICIs, leading to the hypothesis that this may be due to immune responses generated by increased numbers of immunogenic tumour neoantigens [35–37]. Based on results of the KEYNOTE-158 trial, the United States Food and Drug Administration (FDA) recently approved the use of pembrolizumab for the treatment of any unresectable or metastatic TMB-high (≥10 mutations/megabase), non-mismatch repair deficient (dMMR)/microsatellite instability-high (MSI-high) cancer that has progressed on prior therapy [38,39]. OAC is a TMB-high cancer, with 8.0 mutations/megabase (range 1.53–34.56/Mb) [13,14,16], and although a recent study found that the top 20th-percentile of mutation burden GOCs displayed a trend toward survival advantage after ICI treatment [35], response to ICI in GOCs has been shown to be worse than predicted by TMB [14]. This is consistent with other studies reporting that response to immunotherapy does not correlate with high TMB in all patients [40]. The use of TMB-high status as a biomarker to predict ICI response in OAC has been challenged further by recent studies that have shown no benefit for ICI use in mismatch-repair proficient, TMB-high cancers [41,42]. In TMB-high tumours where $CD8^+$ T-cell levels are not associated with neoantigen load, such as OC, treatment with ICIs is associated with a significantly lower response rate, and trend towards worse overall survival (OS) compared to TMB-low tumours [42]. This may help to explain the modest results seen for ICI use in OAC to date. In OAC, a "mutagenic" subtype has been shown that displays a significantly higher TMB, neoantigen burden, and $CD8^+$ tumour infiltrating lymphocyte (TIL) density compared to other subtypes, with no difference however in histological response or survival outcomes [18]. While ICI therapy was not used in this study cohort, the mutagenic subtype described may represent a subgroup of OAC patients for whom ICIs may be of benefit given they may represent a subgroup of OAC patients where $CD8^+$ T-cell infiltration is positively correlated with neoantigen load. TMB-high cancers where $CD8^+$ T-cell infiltration was positively correlated with neoantigen load have been shown to have significantly higher response rates to ICIs [42]. It is clear TMB and neoantigen load are not precise enough indicators to describe the true immunogenicity of a tumour, and do not necessarily equate to an effective immune response or improved outcomes with unselected ICI therapy [36,43]. Thus, although ICI therapy has been approved for use in OAC, effective biomarker driven strategies are required for optimal patient selection when using ICIs in order for maximum patient benefit.

In OAC MSI is rare, with a reported incidence of up to 4% [11,16,18,44]. Furthermore, most MSI-high OCs occur at the gastro-oesophageal junction [44]. MSI-high status results in hyper-mutated tumours with increased neoantigen numbers and TILs [45], and has been shown to be predictive of response to immunotherapy in multiple cancers [11,46,47]. This led to the FDA approving pembrolizumab as a tumour-agnostic therapy in MSI-high or dMMR chemo-refractory, metastatic or unresectable solid tumours [48]. A recent study found a three-way association between hyper-mutation, *Wnt* pathway activation, and loss of immune signalling genes such as *B2M* in OAC [49]. While MSI hyper-mutation has been associated with higher immune activity, *Wnt*/β-catenin pathway activation has been linked to immune escape via T-cell exclusion and prevention of antigen-presenting cell recruitment, thus suggesting this may be an acquired mechanism of immune escape from hyper-mutation induced immune surveillance in OAC [46,49].

Two clinical trials are currently exploring the role of *Wnt* pathway blockade in advanced gastro-oesophageal adenocarcinoma (GOA) using a Dickkopf-1 (DKK1) neutralising monoclonal antibody, DKN-01, in combination with PD-1 blockade (Table 2). Results of these trials may provide new treatment options for DKK1 expressing GOAs.

Specific genomic alterations can also influence the anti-tumour immune response. In OAC, *PIK3CA* amplifications, present in 5% of patients, are associated with significant accumulation of T-cells in the TME and with favourable prognosis [50]. CIN GOAs with immune-cold profiles show enrichment of MYC and cell cycle pathways, including *CCNE1* amplification [23]. Both *MYC* and *CCNE1* mutations are frequent in OAC, and MYC-driven cancers may evade immune detection by multiple mechanisms including immune-suppression and programmed death ligand-1 (PD-L1) upregulation [11,51]. Other frequent driver mutations in OAC, including *TP53* and *KRAS,* have been shown to promote PD-L1 expression, immune evasion, and immunosuppressive TME remodelling [52]. The lack of common oncogenic driver mutations in OAC makes targeted therapy challenging [53], however further characterisation of specific driver mutations in OAC and their possible relationship with anti-tumour immune responses will potentially lead to the development of novel therapeutic approaches.

Tumour heterogeneity

Intra-tumour heterogeneity (ITH), the existence of genotypic and phenotypic variation within an individual tumour, is a major factor underpinning cancer progression, treatment resistance, and poor outcomes [43,54,55]. ITH can exist both spatially and temporally, with subclonal variations evolving dynamically under constant pressure from the host immune system, and as a result of cytotoxic therapy [19,56,57] (Fig. 1). ITH secondary to the clonal genomic evolution of cancer is related to genomic instability [54,56], which may be due to CIN with CNA/aneuploidy, and MSI [54,56]. While MSI results in hyper-mutated tumours with increased tumour neoantigens and TILs, tumours with high CNAs are less commonly TIL infiltrated and have a worse prognosis [25,58].

In cancers where targeted therapies have been more successful, genomic analyses of paired primary and metastatic biopsies have shown

Table 2
Ongoing combination therapy trials in OAC and GOJ cancer.

Trial Name/NCT Identifier	Phase	Enrolment/ Status	Disease Setting/Prior Line(s) of Therapy	Treatment
Resectable Disease				
ICONIC NCT03399071	II	40/Recruiting	Resectable GC or GOJA or OAC/NA	Peri-operative Avelumab + CT (FLOT)
KEYNOTE-975 NCT04210115	III	600/Recruiting	Resectable GOJC or OAC or OSCC and suitable for dCRT or ineligible for curative surgery/NA	dCRT (cisplatin + fluorouracil + 50 Gray in 25 fractions or 60 Gray in 30 fractions OR FOLFOX + 50 Gray in 25 fractions) + pembro vs. dCRT + placebo
NCT03488667	II	40/Recruiting	Resectable GC or GOJA/NA	Peri-operative mFOLFOX + pembro
NCT04221555	II	68/Recruiting	Potentially resectable MMR proficient GOJA or GC/NA	NAT durva + CT (docetaxel + oxaliplatin + S-1) then surgery then adjuvant durva + CT (S-1)
NCT02962063	II	35/Recruiting	Resectable GOJA or OAC/NA	Induction CT (fluorouracil + oxaliplatin) then durva + NAT CRT (carboplatin + paclitaxel + radiotherapy) then surgery
NCT02730546	IB/II	68/Recruiting	Resectable, locally advanced GOJC or gastric cardia cancer/NA	NAT pembro + CT (mFOLFOX-6) OR concurrent CRT (carboplatin + paclitaxel + radiotherapy), surgery, adjuvant pembro
NEO-CREATE ACTRN12619000288123	II	52/Recruiting	Resectable OAC or GOJA/NA	CRT (carboplatin + paclitaxel + radiotherapy) + avelumab
Resectable Disease or Locally Advanced/Metastatic Disease				
NCT02735239	I/II	75/Active, not recruiting	Multiple cohorts with Locally advanced or metastatic OC OR with neoadjuvant C(R)T for operable OC/NA	Durva + C(R)T (XELOX OR dose-escalation treme + XELOX OR radiotherapy + paclitaxel + carboplatin OR FLOT)
Locally Advanced or Metastatic, Unresectable Disease – No Prior Lines of Therapy				
NCT03783936	II	63/Recruiting	Metastatic HER2-amplified GC or OAC/0	mFOLFOX-6 + trastuzumab + avelumab
KEYNOTE-811 NCT03615326	III	732/Recruiting	Locally advanced or metastatic HER2-positive GC or GOJA/0	Pembro + trastuzumab + CT (fluorouracil + cisplatin OR XELOX OR SOX) vs. placebo + trastuzumab + CT
NCT03777657	III	980/Recruiting	Locally advanced unresectable or metastatic GC or GOJA/0	Tislelizumab + CT (XELOX or cisplatin + fluorouracil) vs. placebo + CT
Locally Advanced or Metastatic, Unresectable Disease – ≥1 Prior Lines of Therapy				
NCT03959293	II	105/Recruiting	Unresectable advanced GC or GOJC/1	FOLFIRI + durva OR FOLFIRI + durva + treme
NCT04594811	I/II	145/Not yet recruiting	Advanced or metastatic GC or GOJA or OAC/≥2	NT-I7 + nivo vs. nivo alone
NCT02013154	I	151/Active, not yet recruiting	Recurrent or locally advanced metastatic GC or GOJC or OC with Wnt Signalling Alterations/1	Dose-escalation study of DKN-01 as monotherapy or in combination with paclitaxel or pembro
Locally Advanced or Metastatic, Unresectable Disease – Various Categories of Prior Lines of Therapy				
NCT03802591	III	480/Recruiting	Unresectable locally advanced or metastatic GC or GOJA/Prior Tx allowed	CS1001 monoclonal antibody + CT (XELOX) vs. placebo + CT
NCT03281369	IB/II	410/Recruiting	Locally advanced unresectable or metastatic GC or GOJC or OC/GC – 1 or 2, OC – 0	Umbrella study of multiple immunotherapy-based treatment combinations (fluorouracil, leucovorin, oxaliplatin, atezolizumab, cobimetinib, ramucirumab, paclitaxel, PEGPH20, BL-8040, linagliptin, cisplatin, tiragolumab)
DisTinGuish NCT04363801	IIA	72/Recruiting	Inoperable, locally advanced, or metastatic GC or GOJA/0 or 1	First- or second-line tislelizumab + DKN-01 (+XELOX in first-line setting)

Abbreviations: ACTRN, Australian Clinical Trials Registry Number; CRT, chemoradiotherapy; CT, chemotherapy; dCRT, definitive chemoradiotherapy; Durva, durvalumab; FLOT, fluorouracil + leucovorin + oxaliplatin + docetaxel; FOLFIRI, fluorouracil + leucovorin + irinotecan; GC, gastric cancer; GOJ, gastro-oesophageal junction; GOJA, gastro-oesophageal adenocarcinoma; GOJC, gastro-oesophageal junction carcinoma; mFOLFOX, modified oxaliplatin + leucovorin + fluorouracil; MMR, mismatch repair; NA, not applicable; NAT, neoadjuvant; NCT, ClinicalTrials.gov Number; Nivo, nivolumab; OAC, oesophageal adenocarcinoma; OC, oesophageal cancer; OSCC, oesophageal squamous cell carcinoma; PEGPH20, PEGylated recombinant human hyaluronidase; Pembro, pembrolizumab; SOX, S-1 + oxaliplatin; Treme, tremelimumab; Tx, therapy; XELOX, oxaliplatin + capecitabine.

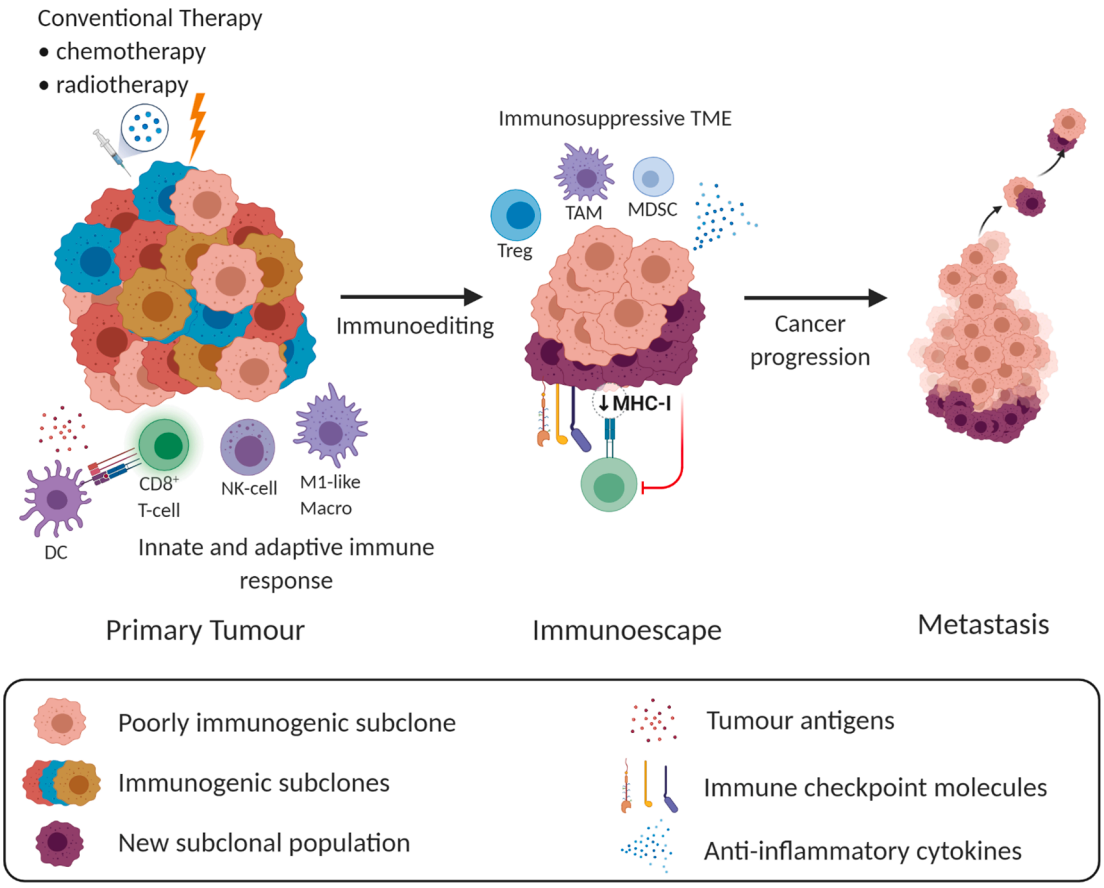

Fig. 1. OAC undergoes dynamic clonal evolution in response to chemo(radio)therapy, and immunoselection and immunoediting, ultimately leading to the emergence of resistant subclones that can escape immune killing and progress to metastatic disease. Abbreviations: OAC, oesophageal adenocarcinoma; DC, dendritic cell; M1-Macro, M1-macrophage; TAM, tumour associated macrophage; MHC-I, major histocompatibility complex-I; NK-cell, natural killer cell.

high concordance of genomic features [59]. In OAC, this concordance has not been shown. GOAs have a high ITH, with discordant oncogene amplification profiles between primary tumour and metastatic sites [59–61]. This includes vast interpatient and intrapatient molecular heterogeneity, spatially at baseline, and temporally after therapy, which importantly occurs in targetable gene mutations such as *HER2, EGFR, MET* and *FGFR2* [60,61]. Similarly, Swanton and colleagues found substantial ITH in the somatic mutation landscape of resected OAC samples, however, in contrast, they found that key oncogenic mutations were ubiquitous across all tumours [62]. Higher ITH is also associated with reduced response to NAT in OAC, which is supported by the United Kingdom MRC OE02 trial, where low ITH in pre-treatment biopsies was associated with survival benefit [62,63].

In addition to genomic factors, the TME is also known to influence tumour heterogeneity through immunoediting. The selective pressure applied by the immune response can reduce subclonal diversity via antigen targeted elimination [57]. However, evidence also suggests the adaptive immune response can promote genomic instability and subsequent increase in ITH [64]. Furthermore, the immune contexture may be heterogeneous within the TME, both temporally and spatially in the tumour secretome, receptor-ligand, and neoantigen profiles [65,66]. One example of immune cell heterogeneity exists in natural killer (NK) cell infiltration and function. Spatial heterogeneity may occur in OAC through areas of rapid tumour growth that result in hypoxic conditions [67]. Tumour cell hypoxia is associated with transforming growth factor-beta (TGF-β) and adenosine release [68], with a subsequent increase in tumour cell resistance to the cytotoxic effects of NK-cells in these areas. Functional heterogeneity can also occur via NK-cell receptor phenotypic changes, which may be regulated via the cancer mutanome and epigenetic regulation, ultimately resulting in spatially heterogeneous functional NK-cell responses across a tumour [65]. One advantage of the NK-cell anti-tumour response, however, is the ability to subvert neoantigen heterogeneity through their MHC-I unrestricted method of cytotoxicity. As a result, NK-cell based immunotherapies are emerging as an attractive armamentarium option to overcome this element of heterogeneity.

The high genomic and immune heterogeneity seen both among and within OAC tumours can affect the discovery of predictive biomarkers as well as potential targets for immunotherapy and is also a barrier to the development of novel therapeutic strategies [69]. The issues related to ITH may potentially be subverted by integrating the principles of clonal evolution into clinical treatment protocols. This strategy may identify subclonal populations who have escaped host immune/treatment pressures. Future trials incorporating molecular testing via multi-region biopsies, repeat biopsies or circulating tumour DNA testing could allow for the development of precision therapies in OAC.

The tumour microenvironment

The immune cell population within the TME is vital to the overall fate of a tumour. Described by Galon et al, the immune contexture describes the nature, density, functional orientation and distribution of immune cells within a tumour [70]. By analysing the TME and immune contexture in individual cancer types, the prognostic significance of various immune cell phenotypes can be determined, and a thorough understanding of the immune contexture of OAC patients will aid future predictive/prognostic assessments and therapeutic developments (Fig. 2). Across cancers, tumour infiltration by immune cells able to

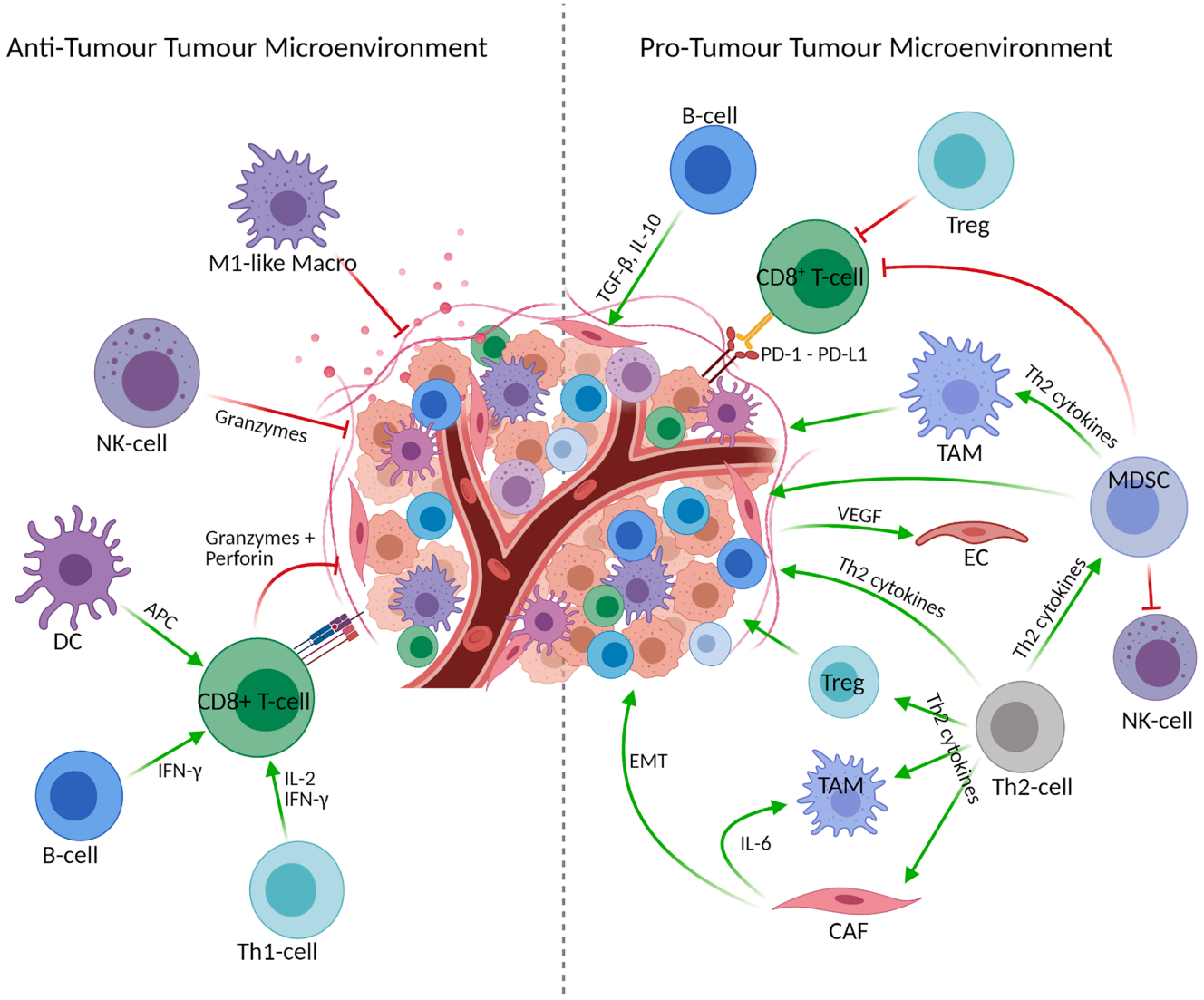

Fig. 2. The tumour immune microenvironment of OAC can either suppress tumour growth and progression or promote tumourigenesis. This is dependent on the complex interactions between tumour cells and the surrounding immune cell phenotypes, stromal cells and their respective cytokine responses. OAC specific TME responses are shown in this figure, and highlight the complexity and pro-tumour dominance of the TME in OAC. Th2 cytokines include interleukin-4, interleukin-6, interleukin-10, and interleukin-13. Abbreviations: APC; antigen presenting cell; CAF, cancer associated fibroblast; DC, dendritic cell; EC, endothelial cell; EMT epithelial-to-mesenchymal transition; IFN-γ, interferon-gamma; IL-2, interleukin-2; IL-6, interleukin-6; IL-10, interleukin-10; M1-like Macro, M1-like macrophage; TAM, tumour associated macrophage; MDSC, myeloid derived suppressor cell; NK-cell, natural killer cell; PD-1/PD-L1, programmed cell death protein-1/ligand-1; Th1, T-helper 1 cell; Th2, T-helper 2 cell; TGF-β, transforming growth factor-beta; Treg, T-regulatory cell; VEGF, vascular endothelial growth factor.

exert anti-tumour activities such as cytotoxic T-cells, dendritic cells (DCs) and NK-cells is generally associated with good prognoses, whereas infiltration with pro-tumour immune cell types such as T-regulatory cells (Tregs), myeloid derived suppressor cells (MDSCs), and TAMs is generally associated with poorer prognoses [71]. Macrophages within the TME can be roughly divided into two dichotomous subtypes: anti-tumour M1-like macrophages (M1) which secrete pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), and interleukin-12 (IL-12) and drive a potent Th1 response; and pro-tumour M2-like macrophages (M2), which secrete immunosuppresive cytokines such as interleukin-10 (IL-10) and TGF-β, along with arginase 1 [72]. Both M1's and M2's are $CD14^{low}$ $CD16^{high}$ $CD68^{+}$ cells, however they can be distinguished via differential expression of specific markers such as nitric oxide synthase 2 (NOS2) and CD86 for M1 and CD163 and CD206 for M2 [73,74]. TAMs are a heterogenous and highly plastic cell type that can polarise between these subtypes, with this balance thus affecting their prognostic signifcance [75]. Generally, TAMs are predominately M2 which are associated with tumour initiation, progression, angiogenesis and metastasis [75]. Individual studies aiming at assessing the prognostic significance of TILs abundance have given mixed results in OAC. A recent meta-analysis of over 5,000 OC patients found that a high density of unspecified TILs was associated with better OS, but not DFS [76]. A detailed analysis of immune cell subsets disclosed a significant correlation between high numbers of $CD8^{+}$ and $CD4^{+}$ T-cells in OAC and improved OS, while results for Tregs were non-significant, but trended towards improved survival [76]. A more recent study not included in the Gao et al meta-analysis found that Treg infiltration correlates with reduced patient survival, indicating that further efforts are required to determine the biological significance of different TIL subsets and outcomes in OAC [76,77].

TME heterogeneity also exists among GOC subtypes, with genome stable GOCs showing enrichment for $CD4^{+}$ T-cells, macrophages, B-cells, and tertiary lymphoid structures (TLSs). CIN cancers, on the other hand, display T-cell exclusion, with $CD8^{+}$ T-cells found only at the invasive margin, while TAMs show high intra-tumour infiltration [23]. TLSs are non-encapsulated, ectopic lymphoid structures including B-cell

containing active germinal centres, and surrounding T-cell zones, that are directly exposed to the TME [78]. Mature TLSs can contribute to the anti-tumour immune response by sustained antigen presentation and antibody dependent cellular cytotoxicity, and their presence has been associated with favourable prognosis in a variety of cancers [78]. While immature TLSs may suppress anti-tumour immunity through expression of IL-10, TGF-β, and PD-L1 [78,79]. In OAC, presence of B-cells and TLSs has been associated with improved prognosis [78,80,81]. Furthermore, the presence of B cells in TLSs has been associated with favourable response to immunotherapy in multiple cancers [82], especially when combined with immunogenic CT [79]. With further research, these findings may be applied to improve ICI responses in OAC patients.

One issue that arises is the presence of TIL subsets identified by phenotypic markers does not adequately inform about functionality. $CD8^+$ TILs in patients with advanced cancer, including OAC, have been found to be functionally deficient or impaired [83,84]. Transcriptomic analyses can be used to assess functional immune responses within the TME, and a recent paper combining methylation profiling, WGS and RNA-sequencing uncovered a subtype of OAC enriched for both innate and adaptive immune cell types along with increased enrichment of immune regulation pathways [85]. Interestingly, this subtype was associated with significantly poorer OS compared to the other subtypes, which may be related to the increased enrichment of TAMs and cancer associated fibroblasts (CAFs) seen alongside the enrichment of cytotoxic T-cells and B-cells [85]. Transcriptomic analyses have also been used to develop scores to characterise and classify anti-tumour immune responses in pan-cancer studies and predict response to immunotherapy [86,87]. The Tumour Inflammation Signature (TIS) is an 18-gene signature measuring pre-existing adaptive immunity that has been peripherally suppressed [86], with tumours with known responses to ICIs showing higher TIS scores on average [87]. OC has a moderate TIS score, which may reflect the variable responses seen with ICIs in OC (Table 1) [87]. The results of TIL infiltration and survival outcomes need to be interpreted alongside the complex interactions between tumour cells and their TME. A thorough understanding of this complex interplay may help the development of novel therapeutic strategies that can promote a pro-inflammatory TME with high levels of functional cytotoxic immune effectors.

The malignant transformation from Barrett's Oesophagus to OAC is accompanied by a change from a Th1-type immune response associated with IFN-γ and IL-2 expression, to a Th2-type chronic inflammatory response with production of interleukin-4 (IL-4) and interleukin-13 (IL-13). This results in an immunosuppressive, pro-tumour microenvironment, with a mixed Th1/Th2 type response and reduced number and impaired function of T-cells [88,89] (Fig. 2). IL-4 and IL-13 interact with immunosuppressive cells such as MDSCs [90]. MDSCs are a heterogeneous population of cells that are produced in response to chronic inflammatory states such as cancer [90]. They exhibit a strong inhibitory effect on anti-tumour immunity in OC via inhibition of T-cell and NK-cell function, Treg and TAM activation, and promotion of fibroblast evolution to CAFs [91,92] (Fig. 2). MDSCs with high CD38 expression can strongly inhibit the cytotoxic effect of activated T-cells in OC, and the anti-CD38 monoclonal antibody Daratumumab can inhibit OC cell growth both *in vitro* and *in vivo* [93]. Daratumumab has been safely used in the treatment of multiple myeloma [94], although there is limited data in solid tumours.

IL-4 and IL-13 along with other Th2- and CAF-derived cytokines, such as interleukin-6 (IL-6), have also been shown to polarise macrophages into the immunosuppressive M2 phenotype [95]. In OC, TAM infiltration is associated with a poor prognosis [91,96–98]. M1-like macrophages on the other hand, display an anti-tumour, pro-inflammatory phenotype, and are associated with a Th1 response [96]. The exploration of the complex pathways and interactions between TAMs and tumour cells is a promising research area that is being investigated [96]. IL-6 has also been shown to activate the Jak1-STAT3 signalling pathway in GC [99], and drive epithelial-to-mesenchymal (EMT) transition in OAC [100]; changes which are associated with chemotherapy resistance, and decreased DC activity and T-cell immunity. Monoclonal antibodies targeting IL-6, such as Tocilizumab, have been trialled in various cancer types with limited success, however a recent *in vivo* trial using GC models showed that Tocilizumab effectively enhanced responsiveness to chemotherapy, thus warranting further exploration [99].

High levels of pro-tumour, immunosuppressive cytokines such as interleukin-8 (IL-8) and TGF-β have also been reported in OAC [101]. IL-8 is produced by myeloid cells and has multiple functions including myeloid-immune cell recruitment, decreased T-cell responsiveness, stimulation of angiogenesis (via increased VEGF expression), and stimulation of tumour-cell proliferation [102,103]. Increased plasma IL-8 levels correlated with poor prognosis and limited response to ICIs and with further research IL-8 may become a validated predictive/prognostic biomarker [103]. It may also represent a potential treatment target via direct inhibition or inhibition of its receptors CXCR1 and CXCR2, either as monotherapy or in combination with ICIs [103]. TGF-β is a cytokine with contrasting roles in tumourigenesis across gastrointestinal cancer types. In OAC, TGF-β has been shown to be a SMAD4-independant tumour promoter and may rely on p53 dysfunction in its tumourigenic effect [104]. TGF-β is overexpressed compared to normal tissue in up to 70% of OAC tumours and has been associated with increased metastasis and decreased survival [68,104]. As such, novel TGFβR1 inhibitors have been tested in preclinical models with encouraging results, suggesting a rationale for further testing in clinical trials [104].

Immune checkpoint molecules

Immune checkpoint molecules, such as programmed death protein-1 (PD-1), its ligands (PD-L1 and PD-L2), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have been studied extensively in cancer. Tumour cells can invoke inhibitory signalling of T-cells for immune evasion by (over)expressing these inhibitory molecules or by inducing their (over)expression by immune cells. The development of antibodies targeting immune checkpoint inhibitory pathways has revolutionised modern oncology practice. ICIs have shown effective and durable benefits in a range of cancers [105]. However, despite extensive efforts, effective biomarker-driven patient selection remains elusive. In OAC, the benefits of ICI therapy in the advanced/metastatic setting are more modest. However recent results from the CheckMate-577 trial have shown promise in the adjuvant setting for resectable tumours with residual disease post-surgery (Table 1). A thorough understanding of the landscape of immune checkpoint molecule expression in OAC may provide evidence for reliable predictive biomarker discovery and allow for improved patient selection.

PD-L1 upregulation occurs in up to 75% of OACs [89,106], with evidence of PD-L1 expression on OAC tumour cells ranging from 1.7% to 43.5% [107–110]. Several studies reporting low expression of PD-L1 on OAC cells found that expression occurs mainly on tumour-associated immune cells and stromal cells [111]. In contrast, others have found high tumour cell expression of PD-L1, but similarly, a high prevalence of PD-L1-positive TILs was also observed [109]. A significant positive correlation between the density of $CD8^+$ T-cells and PD-L1 positivity can be seen in OAC patients, although this was not associated with a survival benefit in some studies [108], and worse survival in others [112]. This may be due in part to the variable pattern of TIL infiltration, with a significantly higher number of PD-L1-positive TILs being seen at the invasive margin [107,108]. For PD-L1-negative cases, mean survival for patients with dense $CD8^+$ T-cell infiltrates is almost four-times longer than for patients without T-cell infiltration [108,110]. The above inconsistencies in the pattern of PD-L1 expression in OAC demonstrate that the clinical utility of PD-L1 status as a predictive biomarker for both survival outcomes and response to ICI therapy is suboptimal. These inconsistencies arise in part due to the lack of consensus in assessment

criteria for PD-L1 status. There is an abundance of available immunohistochemistry antibodies and staining platforms for PD-L1, partly attributable to the development of proprietary diagnostic tests used in ICI drug trials. A lack of knowledge about the comparative performance of these assays has led to variability of results between ICI trials (Table 1). Furthermore the definition of "positive" staining varies, ranging from >1% to >50% based on percent of tumour cells stained, which in itself may be confounded by ITH [113]. Recently, two scoring systems for PD-L1 have been developed. The tumour proportion score (TPS), which was developed for lung cancer, and represents the ratio of PD-L1-expressing tumour cells to all tumour cells, was subsequently found to be inadequate in GOC due to the need to measure PD-L1 expression on surrounding immune cells [114]. This resulted in the development of the combined positive score (CPS), which represents the ratio of all PD-L1-expressing cells (tumour and immune cells) to all tumour cells. The CPS was developed using the PD-L1 22C3 (223C) pharmDx assay as a companion diagnostic test for pembrolizumab and has subsequently been used in multiple GOC KEYNOTE trials for selection criteria or in subset analysis [114] (Table 1). Trials involving Avelumab or Nivolumab used different assays measuring tumour PD-L1 staining only [114]. A direct comparison of the 22C3 assay (CPS $\geq$ 1) to the 73–10 assay ($\geq$1% tumour cell staining) used in the JAVELIN-100 trial showed survival benefit for avelumab over CT only when using the CPS [115]. This highlights the heterogeneity of outcomes when using different PD-L1 assays, and further demonstrates the need for careful biomarker driven patient selection.

Other immune checkpoint molecules such as V-domain Ig suppressor of T cell activation (VISTA), CTLA-4, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), and indoleamine 2,3-dioxygenase 1 (IDO1) can be detected in OAC [107,116,117]. VISTA is a negative checkpoint regulator expressed on T-cells and myeloid cells, which functions to suppress T-cell activation, proliferation and cytokine production, and shares homology with PD-L1 [118]. In solid tumours, VISTA expression has been associated with improved OS and with high numbers of $CD8^+$ TILs [118]. In OAC, VISTA expression on TILs occurs in 22.2% of patients and is associated with increased OS in pT1 or pT2 cancers only [119]. CTLA-4 is usually expressed on immune cells, but in OAC, positivity can be also found on tumour cells, and at significantly higher levels post-CRT [111]. The clinical relevance of CTLA-4 in OAC remains unexplored. LAG-3 is a transmembrane molecule of the immunoglobulin superfamily that structurally resembles the CD4 co-receptor. It is expressed on activated T-cells, Tregs, NK-cells, B-cells and plasmacytoid DCs, and plays an important role in negative regulation of T-cell proliferation via binding to MHC class II with high affinity [120]. LAG-3 expression has been shown in approximately 50% of treatment naïve primary GOAs and lymph node metastases [121], with expression increasing over four-fold post-NAT in OAC patients [111], and is significantly correlated with PD-L1 expression on both tumour cells and TILs in OAC [111,121]. LAG-3 has also been shown to be an independent factor for increased survival in GOCs [121]. Co-expression of LAG-3 with PD-1 has been shown to mark dysfunctional or exhausted $CD8^+$ T-cells; and in pre-clinical models, LAG-3 and PD-1 co-blockade improved the proliferation and cytokine production of tumour-antigen-specific $CD8^+$ T-cells [122,123]. IDO1 is an intracellular enzyme that oxidises tryptophan into kynurenine and modulates the immune response by inhibiting $CD8^+$ T-cells by promoting cell cycle arrest and apoptosis, as well as inducing Tregs in the TME [124]. The level of IDO1 gene expression is significantly higher in OAC tumours post-neoadjuvant CRT [111] and its expression significantly correlated with lower survival compared with IDO1-negative patients [116]. Despite mixed trial results for IDO1 inhibition in solid cancers, further clinical trials are currently assessing their use in combination with ICI and other therapies [125]. Thus, with the increasing knowledge of the interactions between immune checkpoint molecules, combination blockade may be a promising avenue to improve outcomes for OAC patients.

Neoadjuvant therapy and the immune response

Potentially curable OAC patients are treated with perioperative CT or neoadjuvant CRT as standard of care [6]. Pathological response to neoadjuvant therapy (NAT) is variable, with tumour stage following NAT being a reported determinant of patient outcome [126]. Along with their direct cytotoxic effects, CT and radiotherapy (RT) can potentiate tumour immunogenicity [127]. Available evidence indicates that some C(R)T regimens may induce immunogenic tumour cell death resulting in the release of tumour-antigens and pro-inflammatory mediators, whose concerted action promotes the recruitment and activation of immune cells and enhances the activity of professional antigen presenting cells (APCs) [128]. In post-NAT OAC patients, the number of $CD8^+$ T-cells infiltrating the tumour has been shown to significantly increase after CRT, while only a small increase was observed after CT alone [111,129]. The impact of this increase is conflicting, with some studies showing a positive association for increased $CD8^+$ TILs with survival [130], whereas others showed no survival impact for $CD8^+$ T-cell density post-CRT, and a negative survival impact for increased Treg density [131]. Following neoadjuvant CRT, OAC surgical specimens have also been found to have dense IgG4+ plasma cell infiltration, with high numbers of $IgG4^+$ plasma cells within CRT induced ulcers being associated with pathological response and improved survival [132]. $IgG4^+$ plasma cells were present in CRT patients in significantly higher numbers than in those who went straight to surgery, a phenomenon potentially related to and dependent on the inflammatory response associated with CRT [132].

Platinum containing CT drugs, commonly used in OAC treatment, have been shown to leave a platinum-signature post therapy through their DNA damage mechanism, and the subsequent erroneous DNA polymerase repair [62]. As a result, these drugs can impact on tumour subclonal diversity through selective pressures that can directly induce a high proportion of subclonal mutations with a subsequent increase in total neoantigen burden. Subclonal mutations may however fail to drive a clinically effective anti-tumour immune response, which usually targets tumour cells carrying clonal mutations [43]. To date, this critical aspect has not been studied specifically in OAC and represents a promising avenue of research.

The immunomodulatory effects of C(R)T make their use in combination with ICIs of high therapeutic relevance. In OAC, PD-L1 positivity has been shown in 45% of pre-NAT tumours, which increased to 77% post-CRT [111]. Furthermore, 50% of tumours changed status from PD-L1-negative to PD-L1-positive post-therapy [111]. In contrast, another study found no statistically significant difference between OAC PD-L1 expression and the administration of neoadjuvant therapy, although of note, these patients received neoadjuvant CT only, further supporting a possible role of RT in potentiating tumour immunogenicity [108]. The immunomodulatory effects described may help explain the encouraging results of CheckMate-577 [10] (Table 1). Furthermore, the addition of durvalumab to induction FOLFOX chemotherapy and positron emission tomography (PET) scan-directed CRT in the treatment of resectable OAC has been shown to be safe and feasible, with early results suggesting an encouraging rate of pathologic complete response [133]. In OC and GOC patients, multiple trials are currently investigating combination therapies in the advanced/metastatic setting, and as part of curative regimens (Table 2). Results of these trials are anticipated and may help overcome the current limitations of variable pathological responses and survival outcomes in OAC.

Personalised immunotherapies

To date, immunotherapeutic strategies in cancer have mainly relied on passive therapies such as monoclonal antibodies targeting cell surface molecules such as HER2, and active therapies such as ICIs [134]. The clinical success of ICI-therapy relies on an effective T-cell response, with evidence indicating that immune-hot tumours correlate with

clinical responses to ICIs [86]. In contrast, immune-cold tumours are associated with poorer responses to ICIs [48]. With improved knowledge into the determinants of tumour immunogenicity and tumour-antigen profiles, and the mechanisms driving effective anti-tumour T-cell responses, personalised therapies are being progressively investigated [134]. Treatment with therapeutic cancer vaccines and adoptive cellular therapies (ACTs) may be utilised to increase the anti-tumour immune response and convert an immune cold TME into an inflamed microenvironment via utilising immunogenic tumour-antigens.

Cancer vaccines

Therapeutic cancer vaccines work by priming the host immune system to target tumour cells via antigen recognition, and aim to create durable tumour-specific memory T-cell responses [135]. Cancer vaccination strategies can employ the use of tumour-associated-antigens (TAAs) or may involve tumour neoantigens. While TAAs are present across all tumour cells and are generally cancer specific, they are usually poorly immunogenic owing to central tolerance mechanisms. In contrast, tumour neoantigens are highly tumour specific and display no central tolerance, however the vast majority are unique to an individual tumour and may show marked spatial heterogeneity [37].

Although OAC may provide actionable tumour neoantigens, the feasibility, safety and efficacy of cancer vaccines have been mainly investigated in OSCC. In this subset of oesophageal tumours, clinical trials investigating therapeutic cancer vaccines have been undertaken utilising immunogenic TAAs [136–142]. Although phase I and II clinical trials confirmed feasibility and safety of multi-peptide vaccination, only a small subset of patients demonstrated clinical responses [140,141]. Encouragingly, a follow-up Phase II trial, whereby patients with pathological positive lymph nodes following oesophagectomy were given an HLA-A*24:02-restricted multi-peptide vaccine, found peptide-specific cytotoxic T-cell responses, as well as improved 5-year cancer-specific survival in the vaccinated group [142]. These results have led to the investigators undertaking a subsequent large-scale, double-blind, placebo-controlled phase III trial (UMIN000016954).

Cellular based vaccinations induce an adaptive immune response by directly exploiting the central role of APCs and have been studied in a range of cancers [143]. In OC, the infusion of autologous DCs presenting tumour-antigens has shown safety and effectiveness [144]. A Phase I clinical trial combining a DC vaccine with RT, where autologous DCs were loaded with heat-shock proteins from apoptotic OC cells, showed significant increases in serum Th1-cytokines and $CD8^+$ T-cells, as well as significant increases in survival [145]. These results are encouraging; however larger scale trials are required. Neoantigen-based vaccines have shown promise in multiple cancer types, with evidence showing the generation of neoantigen-specific T-cell responses, and ability to induce vaccine-related tumour regression. Recently, the combination of personalised neoantigen vaccination with ICI in patients with advanced solid cancers demonstrated regime safety, neoantigen-specific cytotoxic TIL responses, TIL trafficking to the tumour, and objective response rates of up to 59% [146]. Although cancer vaccines have shown promise in other cancer types, the tumour-antigen landscape of OAC has not been reported, making this an attractive area of research given the encouraging preliminary results reported for other cancer types thus far.

Adoptive cellular therapy

Adoptive cell therapy (ACT), which encompasses the peripheral re-infusion of autologous or allogeneic immune cells that have been primed and expanded *ex vivo*, has shown durable responses in some cancer types [135]. ACT may involve the isolation of autologous T-cells, that are subsequently genetically engineered to express conventional alpha–beta T-cell receptors (TCRs) or chimeric antigen receptors (CARs) encoding antigen-specific variable domains. Although robust clinical responses have been seen for haematological malignancies, successful application of CAR T-cell therapy in solid tumours remains elusive [135]. Significant challenges with the application of CAR T-cell therapy in solid malignancies remain, however a thorough discussion of this is outside the scope of this review.

In OC, a trial utilising loco-regional administration of tumour-stimulated autologous T-cells showed clinical response in 50% of patients, while the other half showed progressive disease [147]. More recently, a CAR-T cell trial targeting the MAGE-A4 antigen in recurrent OSCC patients, found 70% of patients suffered disease progression within two months, while 30% survived over two years post treatment [148]. Currently no studies investigating ACT therapy in OAC patients have been reported to date. Characterisation of the tumour-antigen landscape in OAC alongside the optimisation of CAR T-cell engineering, will allow for trials investigating their clinical utility in OAC.

Bispecific T-cell engagers

Bispecific T-cell engager (BiTE) therapy induces T-cell killing via the use of recombinant bispecific antibodies that have two linked single-chain variable fragments consisting of an antigen-recognition domain and a T-cell engaging domain [149]. Similarities can be drawn between CAR T-cell therapy and BiTE therapy in that they are both TCR and MHC-independent, however there are several key differences. BiTEs bind simultaneously to the CD3 domain of the TCR complex and the tumour-antigen, without need for the Fc domain, potentially mediating Fc receptor-mediating toxicity [149]. BiTEs can be produced by mammalian cell lines, negating the need for exogenous T-cell engineering and expansion, thus improving efficiency and costs [149]. Furthermore, BiTEs can be expanded to a checkpoint-inhibitory T-cell engager (CiTE), that simultaneously engages a tumour-antigen, CD3, and PD-L1, potentially obviating the need for additional ICI therapy [149]. Like CAR T-cells, BiTE therapy has mainly shown promise in haematological malignancies, however a Phase Ib trial utilising BiTE therapy for castration-resistant prostate cancer showed encouraging results with manageable toxicity [149]. Another phase I study investigating advanced-stage solid cancers was precluded from progression due to severe on-target, off-tumour toxicity, highlighting the need for careful target antigen selection [149]. Two phase 1 trials are currently investigating the safety and tolerability of BiTE directed therapy to MUC17 (NCT04117958) and EpCAM (NCT0063556) in metastatic or locally advanced, unresectable MUC17-positive, or EpCAM-positive, GOJ cancer. Results of these trials are anticipated and may represent new treatment options for patients with MUC17 or EpCAM expressing OAC tumours.

Conclusion

It is clear that the genomic and immunogenic profiles of OAC are complex and incompletely understood, and research to piece them together is a significant challenge. OAC is driven by complex large-scale genomic events with chromosomal and genomic instability. Furthermore, a lack of recurrent targetable oncogenic mutations combined with vast inter-patient and ITH in OAC complicates the development of novel therapeutic strategies further and highlights the need for personalised therapy. With improvements in next-generation sequencing and bioinformatics pipelines, and a shift towards treatment based on genomic rather than histopathologic features that integrates principles to subvert ITH, personalised, precision therapy for individual tumours may be realised. In addition to targeting genomic features, it is necessary to further characterise the immunogenic features of OAC and the associated anti-tumour immune responses. This will allow the development of successful combination regimens that harness current therapies along with development of novel personalised approaches that augment the anti-tumour immune response to improve outcomes in this deadly cancer. Augmenting the anti-tumour immune response must occur through several approaches: identification of patients with pro-tumour

or immune-cold TMEs and elucidating mechanisms to shift this balance towards a T-cell inflamed, anti-tumour immune-cell phenotype; improving strategies for attenuating immune-checkpoints and regulatory circuits; amelioration of immunogenicity and antigen presentation, potentially through combination of C(R)T and novel tumour-antigen based therapies; and generation of effective and durable tumour-antigen-specific T-cell responses. Successful strategies explored in other tumour types showing similar genomic and immunogenic features may be helpful to design novel and potentially effective clinical trials. By further characterising and integrating the immunogenic features and anti-tumour immune responses in OAC to the development of biomarker driven novel therapeutic approaches, the development of personalised therapy will allow for improved outcomes where current "one-size-fits-all" approaches have failed.

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CRediT authorship contribution statement

James M. Lonie: Conceptualization, Visualization, Writing - original draft. **Andrew P. Barbour:** Conceptualization, Supervision, Writing - review & editing. **Riccardo Dolcetti:** Conceptualization, Supervision, Writing - review & editing.

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