Immunobiology of high-grade serous ovarian cancer: lessons for clinical translation

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Abstract | Treatment of high-grade serous ovarian cancer (HGSOC) remains challenging. Although HGSOC can potentially be responsive to immunotherapy owing to endogenous immunity at the molecular or T cell level, immunotherapy for this disease has fallen short of expectations to date. This Review proposes a working classification for HGSOC based on the presence or absence of intraepithelial T cells, and elaborates the putative mechanisms that give rise to such immunophenotypes. These differences might explain the failures of existing immunotherapies, and suggest that rational therapeutic approaches tailored to each immunophenotype might meet with improved success. In T cell-inflamed tumours, treatment could focus on mobilizing pre-existing immunity and strengthening the activation of T cells embedded in intraepithelial tumour myeloid niches. Conversely, in immune-excluded and immune-desert tumours, treatment could focus on restoring inflammation by reprogramming myeloid cells, stromal cells and vascular epithelial cells. Poly(ADP-ribose) polymerase (PARP) inhibitors, low-dose radiotherapy, epigenetic drugs and anti-angiogenesis therapy are among the tools available to restore T cell infiltration in HGSOC tumours and could be implemented in combination with vaccines and redirected T cells.

Epithelial ovarian cancer is the most deadly gynaecological cancer and the eighth-leading cause of cancer deaths in women[1]. This cancer is diagnosed in most patients when it is already at an advanced stage, and relapse after first-line chemotherapy is common, resulting in 5-year survival of less than 30% in the USA[1,2]. The most common histological subtype of epithelial ovarian cancer is high-grade serous ovarian cancer (HGSOC)[3], which accounts for 60–80% of all cases.

The discovery of intratumoural versus intraepithelial patterns of $CD8^+$ T cell infiltration in patients with chemotherapy-naive HGSOC[4] drew attention to the importance of lymphocyte infiltration in human tumours, and ultimately inspired a working classification of all solid tumours into three groups: T cell inflamed (also dubbed 'hot'), in which T cells infiltrate deposits (islets) of malignant cells as well as the surrounding and intervening stroma; excluded, in which T cells remain confined to the stroma and are absent from deposits of malignant cells, and non-inflamed, also called 'immune-desert' or 'cold' tumours[5] (FIG. 1). The Ovarian Tumour Tissue Analysis consortium showed that among all ovarian cancers, HGSOC is the type most likely to show substantial $CD8^+$ T cell infiltration, and that the presence of $CD8^+$ intraepithelial tumour-infiltrating lymphocytes (TILs) is a favourable prognostic factor regardless of the extent of surgical cytoreduction, chemotherapy or the presence of germline *BRCA1* mutation[6]. However, less than half of patients with epithelial ovarian cancer have intraepithelial TILs within tumour islets, which have been associated with prolonged survival in numerous studies[4,6,7], an association later confirmed in numerous other solid tumours[8]. Gene and protein expression analysis has molecularly classified HGSOC into four distinct yet overlapping subtypes: immunoreactive, differentiated, proliferative and mesenchymal[9,10]. Meta-analysis of The Cancer Genome Atlas data[11] led to the development of subtype-specific gene expression signatures associated with patient survival that, when combined, provided a new prognostic classification of HGSOC[12]. The Classification of Ovarian Cancer (CLOVAR) analysis further showed that the immunoreactive subtype had the best prognosis, whereas the mesenchymal subtype was associated with the worst outcomes[13]. The immunoreactive subtype is so named because these tumours display prominent T cell infiltration.

Despite these different disease classifications, at present, all patients with HGSOC are initially treated with the same first-line carboplatin–paclitaxel combination and continue to receive platinum-based treatments for as long as they continue to demonstrate a benefit.

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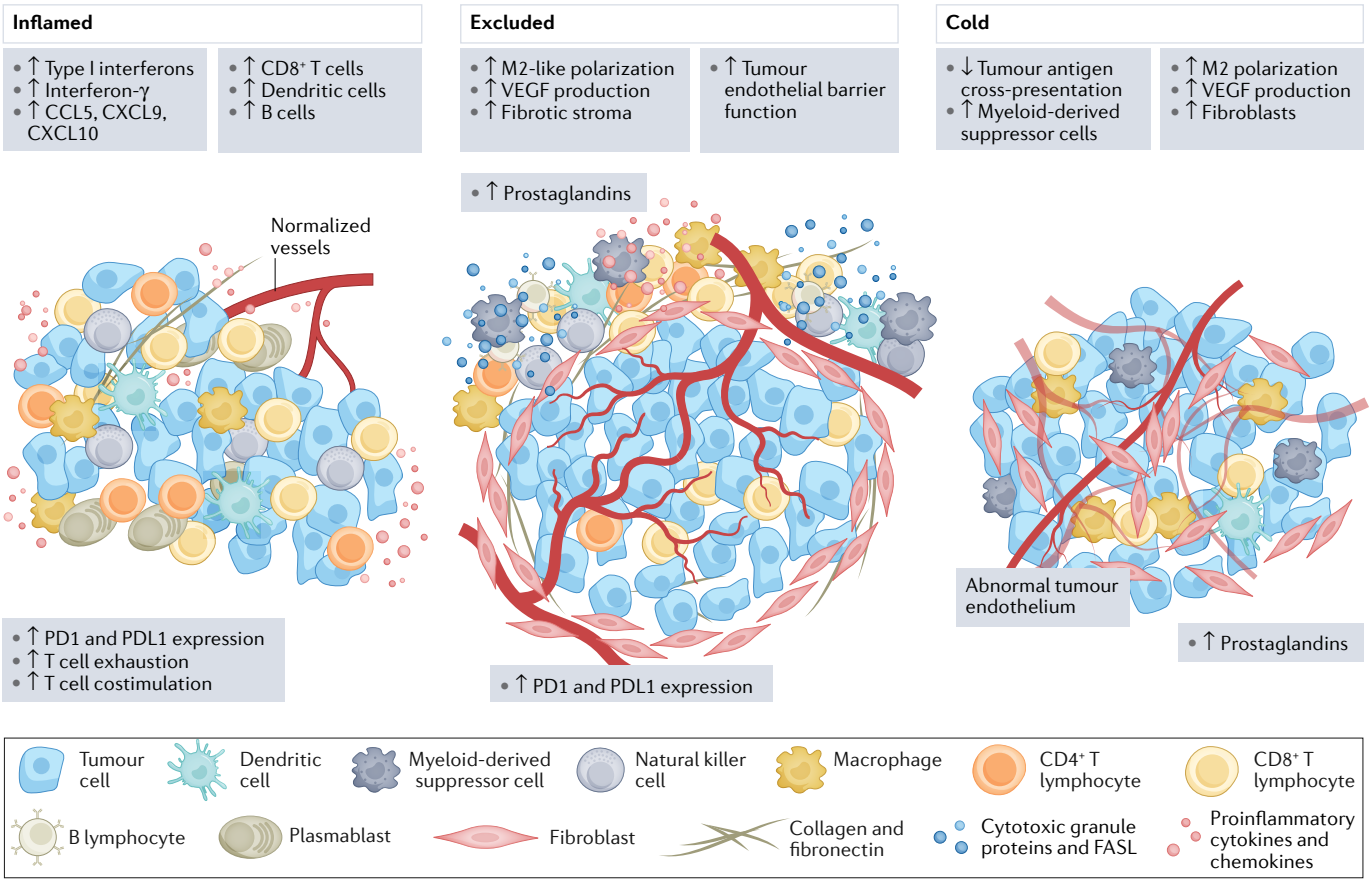

Fig. 1 | **Immunophenotypic classification of high-grade serous ovarian cancer.** Cellular and molecular composition of the tumour microenvironment for each of the three immunophenotypes of high-grade serous ovarian cancers: T cell inflamed (also termed 'hot'), immune excluded and cold. CCL5, C-C motif chemokine 5; CXCL, C-X-C motif chemokine; FASL, FAS ligand; PD1, programmed cell death protein 1; PDL1, programmed cell death 1 ligand 1; VEGF, vascular endothelial growth factor.

Many patients also receive poly(ADP-ribose) polymerase (PARP) inhibitors or vascular endothelial growth factor A (VEGFA) blockers along with bevacizumab in the adjuvant setting (BOX 1). Numerous second-line chemotherapy regimens are used, but continuing chemotherapeutic treatment beyond three failed regimens might be futile[14]. Lastly, bevacizumab has shown activity in platinum-resistant HGSOC[15,16]. Reported responses to PD1 or PDL1 blockade in patients with immunoreactive HGSOC tumours[4] have been disappointing. An initial study reported an encouraging objective response rate of 15% for treatment with the anti-PD1 antibody nivolumab, with a disease control rate of 45% and a durable complete response rate of 10%[17]. However, results from the KEYNOTE-100 phase II study (NCT02674061) in 378 patients reported an objective response rate of only 9% for treatment with the anti-PD1 antibody pembrolizumab[18]. Combinations of anti-PDL1 antibodies and chemotherapy have proved equally disappointing (discussed further in the following sections), and no clear tissue biomarkers exist for the selection of the few patients that might benefit from these combinations.

In this Review, we present current understanding of the immune aspects of HGSOC and offer insights into how emerging biological evidence could drive new therapeutic developments.

Tumour-infiltrating lymphocytes

Intraepithelial TILs have long been assumed to be a histological hallmark of immune recognition in HGSOC tumours.

Infiltrating T cells

$CD8^+$ T cells in inflamed tumours exhibit transcriptional signatures of cytotoxicity (expression of *GZMB*, *GZMA*, *GZMH*, *GNLY* and *IFNG*), exhaustion (expression of *CTLA4, TOX, PDCD1*, *LAG3* and *ENTPD1*) and tissue residence (expression of *CD69*, *ITGAE* and *CXCL13*)[19–21]. Indeed, although not all accumulating TILs are necessarily tumour specific[22], a variable fraction of $CD8^+$ TILs in intraepithelial locations bear surface markers of tumour reactivity and cytoxicity[19], including the activation marker CD137 (REF.[23]), intraepithelial homing integrin-αE (also known as CD103)[24], the exhaustion markers CD39 (encoded by *ENTPD1*) and PD1 (REFS.[25,26]), and the effector molecules interferon-γ (IFNγ), tumour necrosis factor (TNF), perforin, IL-2 and granzyme B[27]. $CD8^+$ T cells with a tissue-resident memory (T_{RM})-like phenotype exhibit superior trogocytic

Box 1 | **The genomic landscape of ovarian cancers**

Integrated genomic and transcriptomic analyses by The Cancer Genome Atlas Research Network revealed that *TP53* is almost ubiquitously mutated in high-grade serous ovarian cancer (HGSOC)[11]. About half of *TP53*-mutant HGSOC tumours exhibit DNA homologous recombination deficiency (HRD) owing to germline or somatic mutations in *BRCA1* and/or *BRCA2* (~20%) or loss of *BRCA1* function (~5–7%) owing to promoter methylation or alterations in other HRD-related genes (including *ATM*, *ATR* and genes linked to Fanconi anaemia)[11,207,208]. HGSOC cells might also harbour dysregulation of retinoblastoma 1 (RB1; 67%), phosphoinositide 3-kinase (PI3K)–RAS (45%) or neurogenic locus Notch homologue (22%) signalling pathways[11]. Owing to genomic instability, gene copy number variations have a more prominent role than mutations in HGSOC, and gene amplifications of *CCNE1*, *EMSY*, *MYC* and *MECOM* are frequently reported (more than 20%)[30].

Analysis of mutational signatures has emerged as a powerful approach for investigating the processes that generate somatic mutations in solid tumours. Single-base substitution signature 3 comprehensively captures HRD in ovarian[209,210] and breast[209,211] cancers, and can be used to stratify patients for therapeutic agents that directly target HRD, such as poly(ADP-ribose) polymerase (PARP) inhibitors[76,212]. Some HRD-positive HGSOCs exhibit tandem duplication-induced and/or unbalanced rearrangement-induced gene amplifications and have increased proportions of deletions and loss of heterozygosity across their genomes. Another distinct group of HGSOCs exhibit foldback inversions associated with high-level gene amplifications[213]. As foldback inversions with regions of microhomology are reflective of active microhomology-mediated end joining DNA repair processes, these HGSOC tumours could have an increased capacity to repair the damage induced by genotoxic chemotherapy and, therefore, might be non-responsive to PARP inhibitor therapy. Therefore, foldback inversions are likely to represent a class of non-immunogenic genomic aberrations linked to treatment resistance and poor prognosis, which suggests that patients with HGSOC tumour genomes containing foldback inversions are probably not optimal candidates for immunotherapy[29].

Copy number aberrations represent a subset of structural variations that dominate the genomic landscape of HGSOC. Seven copy number signature patterns that predict both overall survival and the probability of platinum-resistant relapse have been described in HGSOC tumours on the basis of low-coverage whole-genome sequencing coupled with a new statistical method for computing copy features[207]. Poor outcome is strongly associated with HGSOC genomic copy number signature 1 (which is characterized by foldback inversions, oncogenic RAS signalling and telomere shortening). By contrast, good outcome with standard-of-care therapy is predicted by HGSOC genomic copy number signature 3 (which is characterized by *BRCA1* and/or *BRCA2* mutations linked to HRD)[207]. In contrast to the previously reported prognostic implications of single-nucleotide polymorphism and structural variant signatures associated with HGSOC outcomes, HGSOC copy number signatures confer a continuous spectrum of clinical implications and might offer the potential to provide more-refined stratification of patients with HGSOC[207,214].

and antitumoural activities compared with their circulating counterparts, and stem-like T_{RM} cells can replenish effector T_{RM} cells as they become exhausted[28].

$CD8^+$ intraepithelial TILs have indeed been proven to be a hallmark of immune attack[19]. TILs within tumour islets engage in antigen recognition, as shown by their immunohistochemical positivity for nuclear-localized NFAT2c (a hallmark of T cell receptor (TCR) activation) and the proliferation marker Ki-67; oligoclonal expansion (shown by TCR sequencing); cytotoxicity (production of granzyme B); exhaustion (PD1); and secretion of effector cytokines such as IL-2, IFNγ and TNF. Corroborating this evidence, the density of $CD8^+$ intraepithelial TILs was inversely correlated with malignant clone diversity, neoantigen depletion and subclonal loss of heterozygosity at human leukocyte antigen (HLA) loci, associations that collectively suggest that some tumour clones undergo immunological pruning[29].

The identity of ovarian tumour antigens recognized by TILs is still under investigation. Although HGSOCs have a fairly low mutational burden[30], $CD8^+$ TILs that recognize one or more tumour neoantigens were isolated from 75% of patients[31]. Similarly, $CD4^+$ neoantigen-specific T cells have been identified[32]. Importantly, $CD8^+$ T cell clones with high-affinity TCRs that recognize tumour neoepitopes tend to localize to the tumour, whereas low-affinity clones that recognize the same epitopes can be found in blood[31]. In addition, among TILs, both $CD4^+$ and $CD8^+$ populations with specificities for shared tumour-associated antigens (such as cancer testis antigen 1 (also known as NY-ESO-1), HER2 (also known as NEU), mesothelin, folate receptor and hTERT were detected at high frequencies[19]. Some of these antigens, such as NY-ESO-1, can drive tumour rejection in patients treated with T cell-based adoptive cell therapy (ACT)[33], and NY-ESO-1-specific TILs drive rejection of autologous tumour xenograft tumours in mice[19], suggesting that these clones do participate in tumour control. Indeed, NY-ESO-1-specific T cell clones with tumour-rejecting capacity accumulate specifically in tumour islets[19], and transfer of these cells to mice bearing autologous patient-derived xenograft tumours led to tumour rejection[19].

T cells in myeloid cell niches

Tumour-reactive $CD8^+$ TILs are now well established to engage constantly with tumour antigens and to undergo T cell exhaustion in HGSOC tumours. However, the prolonged survival of patients with intraepithelial $CD8^+$ TILs suggests that these cells are still able to exert tumour-suppressive activity. How these cells maintain their function within tumours is a matter of ongoing investigation. In mouse and human HGSOC tumours, self-renewing pre-exhausted T cells that express the stemness-related gene *TCF7* (encoding transcription factor 7, also known as T cell factor 1 (TCF1)) seem to replenish the pool of exhausted TILs, and also account for TIL reinvigoration upon PD1 blockade in mice[34,35]. In mouse models, *Tcf7*-expressing cells are located within tumours as well as in lymph nodes, and might also be present in tertiary lymphoid structures; in ovarian cancer, the presence of tertiary lymphoid structures has been associated with increased survival[36]. Some evidence indicates that the state of terminally exhausted $CD8^+$ intraepithelial TILs can vary depending on their cellular associations. Strikingly, terminally exhausted $CD8^+$ intraepithelial TILs can be critically supported by tumour-resident dendritic cells (DCs), which can cluster with intraepithelial TILs within tumour islets. Heterotypic interactions between various antigen-presenting cells (APCs) and T cells in intraepithelial myeloid niches have proved essential to provide CD28 co-stimulation signals to exhausted $CD8^+$ TILs in situ, which thereby are more likely to be polyfunctional and to maintain superior effector fitness, which is associated with activation of transcriptional programmes consistent with improved effector function, improved memory, increased survival and increased proliferation[19]. Thus, we speculate that the presence and appropriate activation of tumour-resident DCs seems to be essential for sustaining anticancer immune responses initiated by close interactions between DCs and T cells within the tumour islets, and occurs at the

same time as the engagement of tumour cell targets by CD8⁺ TILs[19] (FIG. 2). Immunostimulatory (M1-like) macrophages can also participate in these niches. Indeed, macrophages in inflamed tumours exhibit transcriptomic states characterized by overexpression of *SIGLEC1* (encoding CD169) or *CX3CR1* (REF.[21]).

However, APCs isolated from most patients with ovarian cancer have functional deficiencies[37]. DCs accumulate intracellular reactive oxygen species that provoke endoplasmic reticulum stress and increased lipid peroxidation[38] and can promote upregulation of prostaglandin E_2 (PGE_2), which suppresses

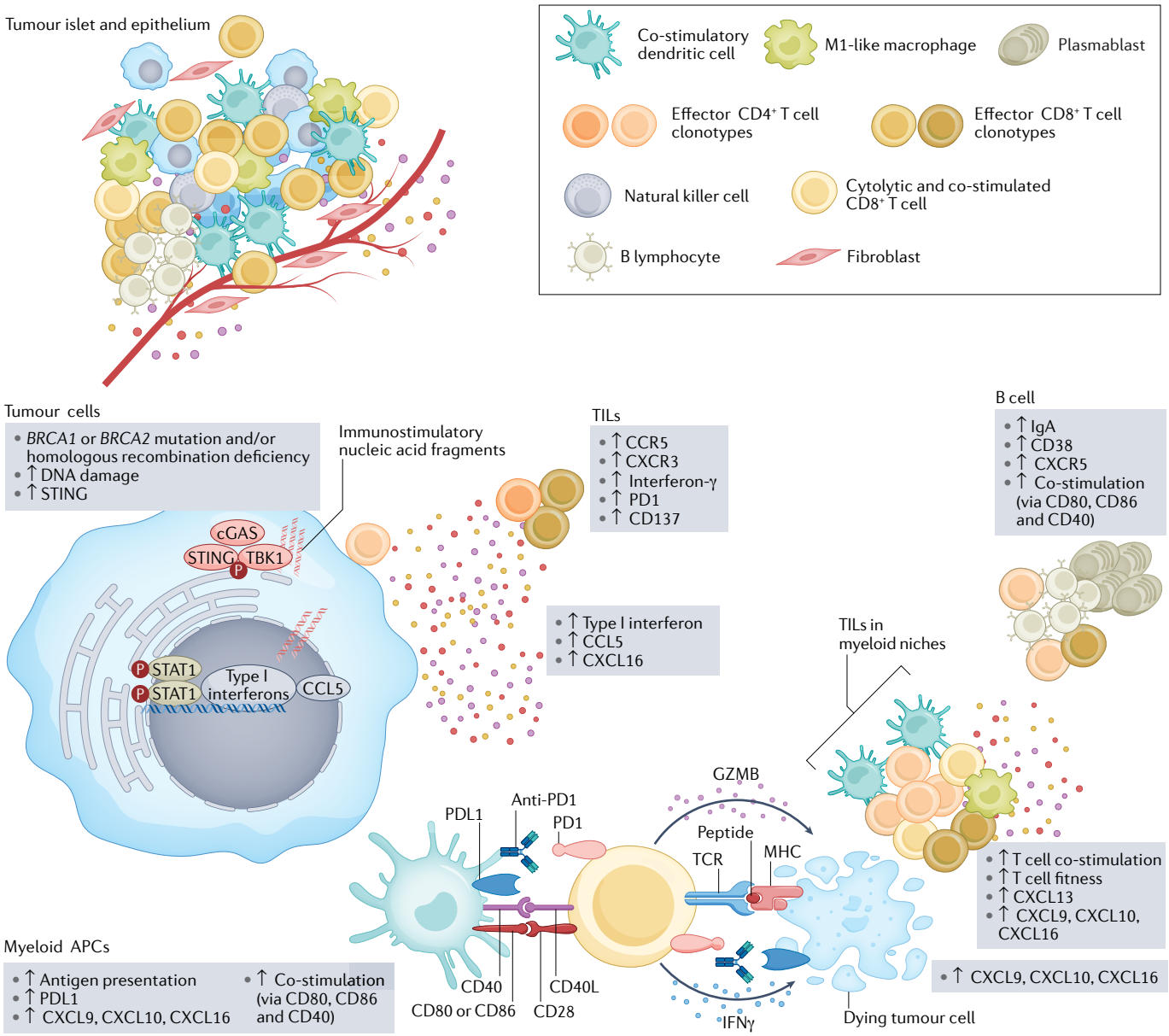

Fig. 2 | **Cellular crosstalk in the tumour microenvironment orchestrates the T cell-inflamed immunophenotype of high-grade serous ovarian cancer.** Tumour cells with increased DNA damage (for example, those with homologous recombination deficiency) are constitutively inflamed through DNA damage sensing and upregulation of stimulator of interferon genes (STING) and tumour-intrinsic type I interferon signalling pathways. This tumour cell inflammation leads to constitutive upregulation of chemokine C-C motif chemokine 5 (CCL5), which is secreted by ovarian cancer cells to attract T cells into tumour islets. Patrolling T cells that recognize tumour antigens produce interferon-γ, which polarizes adjacent myeloid dendritic cells and macrophages to secrete C-X-C motif chemokine 9 (CXCL9) and/or CXCL10 and attract tumour-specific T cells to the tumour site. Tumour-specific CD8⁺ tumour-infiltrating lymphocytes (TILs) within tumour islets can simultaneously engage tumour cell targets and co-stimulatory myeloid antigen-presenting cells (APCs). In response to antigen stimulation, TILs upregulate programmed cell death 1 ligand 1 (PDL1). Intraepithelial CD8⁺ TILs embedded in APC niches might receive CD28 co-stimulation through interactions between CD80 and CD86, which sustain the polyfunctional phenotype of exhausted TILs. Programmed cell death protein 1 (PD1) blockade unlocks the capacity for T cell receptor (TCR) signalling and CD28 co-stimulation in exhausted TILs; in particular, restoration of CD28 co-stimulation in these cells ensures their activation and anticancer response. Co-stimulated, CD8⁺ TILs with PD1 expression are activated polyfunctional T cells that secrete CXCL13. CXCL13 recruits B cells, which can populate not only tumour-associated tertiary lymphoid structures but also tumour islets. B cells can undergo class switching to produce IgA, which mediates antitumour humoral responses and enhances TIL performance. cGAS, cyclic GMP–AMP synthase; GZMB, granzyme B; MHC, major histocompatibility complex.

T cell-mediated anticancer immunity[39]. Ovarian cancers recruit immature myeloid cells and alternatively activated (M2-polarized) macrophages, which provide inadequate stimulation to effector T cells owing to their low expression of *CD80* and *CD86* and diminished production of IL-12. Ovarian cancer cells also actively inhibit tumour-infiltrating macrophages through their production of immune checkpoint ligands, suppressive cytokines, transforming growth factor-β (TGFβ), PGE_2 and enzymes such as arginase and indoleamine 2,3-dioxygenase 1 (IDO1) that deplete key amino acids from the tumour microenvironment[40,41]. B7-homologue 4 (B7-H4; also known as V-set domain-containing T cell activation inhibitor 1), a co-inhibitory molecule expressed by ovarian macrophages[42], also suppresses T cell proliferation, reactivity and IL-2 production[43,44].

Interestingly, whereas T cell-excluded tumours are dominated by *TREM2*-overexpressing $CD169^+$ macrophages characterized by tumour-associated macrophage-like signatures, immune-desert tumours are infiltrated by *TREM1*-overexpressing, *FCN1*-expressing monocytes and *MARCO*-expressing macrophages with myeloid-derived suppressor cell-like signatures[20], indicating that specialized immune networks underlie distinct HGSOC phenotypes. These networks establish complex cell interactions that secure immune tolerance. For example, suppressive myeloid cells further co-opt the tumour microenvironment by promoting the engraftment of regulatory T cells (T_{reg} cells)[45,46], which promote tumour progression through immunosuppression[46] and angiogenesis[47]. Repolarization of tumour myeloid cells is, therefore, essential to provide support for $CD8^+$ TILs and is a high priority for immunotherapy (discussed further later).

Tumour-infiltrating B cells

Other immune cell interactions might be also important for $CD8^+$ T cell function. Interestingly, ovarian tumours that contain both $CD8^+$ T cells and $CD20^+$ B cells are associated with improved outcomes[48]. T cell-inflamed ovarian cancers are enriched in both $PRDM1^+SDC1^+$ B cells and *IFNG*-expressing $PRDM1^+CD38^+MKI67^+$ plasmablasts[21], whereas the detection of plasma cells in tertiary lymphoid structures predicted enhanced cytolytic features of TILs[36]. The discovery that CD28-co-stimulated exhausted $CD8^+$ TILs express high levels of C-X-C motif chemokine 13 (CXCL13)[19], a B cell-recruiting chemokine, further supports the role of crosstalk between B cells and T cells (FIG. 2). These observations were extended in a study of 575 treatment-naive patients with HGSOC, among whom intraepithelial TILs were associated with increased survival only when they were present along with intraepithelial $CD138^+$ plasma cells that displayed a dominant IgA switch. These two cell types are thought to cooperate in the tumour microenvironment. Besides indirect tumour cell killing by macrophages, via antibody-dependent cell-mediated cytotoxicity, binding of IgA to the polymeric immunoglobulin receptor on ovarian cancer cells enabled its internalization by tumour cells, which induced profound transcriptional changes. This process also sensitized ovarian tumour cells to T cell-mediated cytolysis, thereby unveiling a novel synergy between intratumoural B cells and intratumoural T cells[49]. Another study demonstrated that patient-derived tumour cells were frequently coated with IgG and that tumour-reactive autoantibodies either were naturally occurring or had evolved through an antigen-driven selection process via somatic hypermutation[50]. Additionally, recruited B cells might provide co-stimulatory signals to tumour-resident CXCL13-secreting T cells[51] (FIG. 2). These interactions could also be important drivers of the response to PD1 blockade, as patients with triple-negative breast cancer who responded to combination treatment with PD1 blockers and paclitaxel showed an influx of *CXCL13*-expressing TILs as well as B cells in their tumours[51].

Other T cell subtypes and stromal cells

The presence of high numbers of γδ T cells within HGSOC tumours predicts increased survival, and their modulation by targeting CD277 (also known as butyrophilin subfamily 3 member A1 (BTN3A1)) leads to increased activity of tumour-specific αβ TILs[52]. Moreover natural killer (NK)-like $CD3^-CD56^+$ innate lymphoid cells negatively regulate the proliferation of $CD8^+$ TILs in response to IL-2 (REF.[53]). The presence of decidual-like NK cells (which are distinguished by the surface marker CD9) was correlated with increased tumour cell abundance in HGSOCs of fallopian tube origin[54]. NK cells that acquire CD9 from ovarian tumour cells by trogocytosis develop immunosuppressive properties[54]. Inflamed HGSOC tumours are also associated with activated $CD4^+$ T cells and T_{reg} cells[20]. Interestingly, IL-1-activated fibroblasts were also associated with tumour infiltration of *GZMB*-expressing $CD8^+$ T cells, in addition to activated $CD4^+$ T cells and T_{reg} cells[20]. The results of these studies suggest that additional crosstalk between these cell types regulates the retention and function of TILs. Mapping these interactions by high-dimensional immunostaining and spatial transcriptomic analysis is expected to unveil the hallmarks of effective immune attack and reveal new therapeutic targets.

Chemokine circuits in T cell crosstalk

Tumours spontaneously recruit T cells because T cell trafficking and interactions between immune cells within tumours are both regulated by chemokine circuits[47,55–59]. These circuits, therefore, precisely control the infiltration and retention of intraepithelial $CD8^+$ TILs in the tumour microenvironment, the presence of which is associated with slower tumour progression and prolonged survival, and is essential for responses to immunotherapy. Extensive pan-cancer studies have revealed a close association between TIL density and the constitutive expression of T cell-recruiting chemokines, usually C-C motif chemokine 5 (CCL5; also known as RANTES), across all solid tumours interrogated. In HGSOC, CCL5 is produced by tumour cells, and its absence accurately predicts the absence of intraepithelial TILs[59]. However, CCL5 production alone is not sufficient to ensure high levels of intraepithelial TIL recruitment and engraftment, as synergy with the inducible cytokine CXCL9 seems to be required to produce the

Box 2 | **Preclinical models of ovarian cancer**

The syngeneic ID8 mouse model of high-grade serous ovarian cancer (HGSOC) is the most widely used. The ID8 cancer cell line was developed through repeated in vitro propagation of mouse ovarian surface epithelial cells, which underwent spontaneous malignant transformation. Intraperitoneally implanted orthotopic ID8 carcinomas show histopathologic and molecular features similar to those of human advanced HGSOCs[215]. This model has been used to investigate the molecular pathways and pathogenetic mechanisms underlying HGSOC as well as therapeutic strategies, including vaccination. ID8 cell lines with deletion of *Trp53*, *Brca1*, *Brca2* or other genes have been generated via CRISPR–Cas9 gene editing[216,217].

However, ID8 cell lines represent ovarian cell-derived models, whereas the presumed normal cells of origin of HGSOC are fallopian tube epithelial cells.

A series of genetically defined mouse HGSOC cell lines derived from fallopian tube epithelial cells of C57BL/6 mice carry constellations of mutant alleles that are present in human HGSOC genomes[218]. These new models capture some of the most prominent pathways dysregulated in homologous recombination-deficient and homologous recombination-proficient HGSOC patient-derived tumour samples and recapitulated the histologic features and clinical behaviour of human HGSOCs in their spread through the peritoneal cavity, their preferential adhesion to intraperitoneal sites (including the omentum), and their responsiveness to both DNA-damaging agents and poly(ADP-ribose) polymerase inhibitors. Comparisons of the tumour microenvironment in human HGSOC samples with ovarian and fallopian tube tumours in different genetically engineered mouse models[219] identified common cellular and molecular features that reflect similarities in innate and adaptive immune responses. However, it is still not clear whether the tumour microenvironment of these mouse models completely reflects that seen in human HGSOCs.

Patient-derived xenograft models are generated by implantation of human HGSOCs into immunocompromised mice. The assumption is that such models preserve tumour heterogeneity and the molecular features of human HGSOC. Patient-derived xenograft models are useful for testing drug treatments but are of limited value for testing immunotherapy, as the host mice lack a functional immune system. Nevertheless, patient-derived xenograft models can be useful to evaluate the efficacy of adoptive T cell strategies and vaccine-primed T cells[196,220]. Efforts are under way to improve humanized mouse models through engineering of a functional human immune system[221,222], although the resulting immune lineages may still have important biases.

Organoids are three-dimensional cell cultures derived from stem cells that provide a novel in vitro platform used to predict drug responses[223] and to increase the efficiency of personalized therapy. However, the use of organoids to assess immunotherapy strategies in HGSOC has proven challenging owing to the intrinsic limitations of current organoid platforms (namely, lack of high-fidelity cell types, limited maturation, atypical physiology and lack of arealization). Several attempts are ongoing to develop improved organoid systems for immunotherapy testing[224].

'hot' tumour immunophenotype[59]. Interestingly, myeloid cells are the main source of CXCL9, production of which is specifically dependent on the presence of IFNγ in the tumour microenvironment. This link reveals the potential existence of a feedforward loop whereby T cells recruited by CCL5 infiltrate tumour islets, where they secrete IFNγ upon encountering their cognate antigen and consequently elicit CXCL9 secretion by myelocytes in the vicinity of the tumour, thereby recruiting further effector T cells to the site. Accordingly, coexpression of *CCL5* and *CXCL9* heralds the presence of tumour-specific TILs, and unsurprisingly also predicts responses to immune checkpoint blockade, in patients with melanoma[60]. Consequently, effective expression of *CXCL9* requires antigen recognition by infiltrating T cells, which explains why tumour antigen presentation is a prerequisite for TIL infiltration in HGSOC[61]. Indeed, the loss or downregulation of HLA class I antigens and loss of heterozygosity in HLA genes[62] drive immune evasion in ovarian cancers[29].

The necessity for constitutive, tumour cell-intrinsic expression of *CCL5* (and genes encoding other chemokines that drive recruitment of $CD8^+$ T cells) appears paradoxical, given that CCL5 also plays a key role in orchestrating T cell-mediated attacking of tumour cells. Importantly, HRD, a genetic alteration important in ovarian oncogenesis, might be responsible for both triggering and maintaining these chemokine circuits. Indeed, *BRCA1*-mutated HGSOCs (BOX 1) often exhibit intraepithelial accumulation of $CD8^+$ TILs[63,64] along with a prominent immunoreactive gene expression signature[10], including *CCL5*, *CXCL9* and *CXCL10*, which encode interferon-induced chemokines[65]. Tumour cell-intrinsic expression of these chemokine genes is driven by HRD via the stimulator of interferon genes (STING) pathway and activation of type I interferon signalling[65], which locks these HGSOC tumour cells into a cell-autonomous inflamed phenotype associated with T cell recruitment. Furthermore, sensing of tumour-derived extracellular DNA results in paracrine activation of intratumoural DCs via the cyclic GMP–AMP synthase (cGAS)–STING pathway, leading to further secretion of type I interferons[66–69]. This pathway can be prevalent in HGSOC cells with HRD[70–72] (BOX 1). Unsurprisingly, some HGSOC tumours with widespread HRD can dampen $CD8^+$ T cell infiltration by epigenetic silencing of *CCL5*, with consequent loss of stromal *CXCL9* expression[59,65]. This pathway has been identified as a mechanism of immune escape in ovarian cancer[58] (FIG. 2).

Given the role of T cells in driving antitumour immunity, it is not surprising that tumours can evolve to exclude them. For example, in the ID8 mouse model of ovarian cancer, intraperitoneal tumours are initially infiltrated by $CD8^+$ T cells following inoculation, but these T cells are eliminated spontaneously from the tumour microenvironment[59,73] (BOX 2). As expected, emerging 'cold' tumours that become resistant to immune checkpoint blockade[73] are characterized by epigenetic silencing of *CCL5* in tumour cells and loss of *CXCL9* expression in tumour-resident myeloid cells[59]. Furthermore, in the ID8 mouse model of ovarian cancer, although *Brca1*-deficient tumours are highly T cell inflamed, knockdown of *Ccl5* in tumour cells leads to loss of TILs and aggressive tumour growth. These mouse results are helpful to interpret the findings of human clinical trials: among HGSOC tumours with *BRCA1* deficiency or HRD, those with a 'cold' immunophenotype frequently exhibit copy number loss or promoter methylation of *CCL5*, supporting the key role of this chemokine in orchestrating T cell inflammation. Ovarian tumour cells may also epigenetically silence *CXCL9* and *CXCL10* expression, further leading to the development of immune desertification[58].

Opportunities for immunotherapy

T cell-inflamed tumours

Overcoming resistance to immune checkpoint blockade. T cell-inflamed HGSOC tumours should, in theory, be suitable candidates for immune checkpoint blockade, as their tumour microenvironment is already conducive to immune attack. However, responses to

PD1 or PDL1 blockade have been disappointing in HGSOC. The tumour mutational burden predicts responses to immune checkpoint blockade in some HGSOCs[74], but only when this parameter is correlated with TIL density[74]. Therefore, a low mutational burden in HGSOC tumours predicts a lower response rate than would be seen in generally more immunoresponsive tumour types, such as melanoma or non-small-cell lung cancer (NSCLC). Importantly, clinical studies of PD1 or PDL1 blockade in patients with HGSOC have to date treated unselected populations irrespective of the tumour immune phenotype. Efforts should thus focus on developing biomarkers that could help identify which patients with HGSOC are most likely to respond to PD1 or PDL1 blockade. Standardized TIL measurements and $CD8^+$ immune-classification algorithms would be helpful for this purpose. Such selection can be accomplished using digital pathology and computational analysis tools that calculate the density of intraepithelial $CD8^+$ TILs using stringent cut-offs, and also take into account the extent of intraepithelial $CD8^+$ TIL heterogeneity across the tumour tissue. As well as increasing the accuracy of patient selection, this approach could improve the identification of long-term survivors[6] and help to predict which patients are likely to respond to immune checkpoint blockade. Indeed, in the phase III IMagyn050 randomized clinical trial, first-line treatment with the anti-PDL1 antibody atezolizumab in combination with carboplatin, paclitaxel and bevacizumab seemed to benefit only the ~20% of patients whose tumours showed notable PDL1 positivity (that is, containing more than 5% PDL1-expressing immune cells)[75]. In addition, TILs from patients with ovarian cancer with HRD (BOX 1) who responded to combination treatment with a PARP inhibitor and an anti-PDL1 agent had an exhausted phenotype before treatment initiation, marked by type I interferon activation[76]. Therefore, biomarkers that reveal myeloid niches or CD28 co-stimulation[19] could be helpful to select patients for immune checkpoint blockade (FIG. 2). Indeed, niche-embedded TILs are thought to benefit from treatment with an anti-PD1 antibody, because in these cells PD1 blockade strengthens both TCR signalling and CD28 co-stimulation, thereby enabling the appropriate activation and ultimately clonal persistence of these TILs. Conversely, nicheless or solitary exhausted TILs respond to anti-PD1 treatment solely by strengthening their TCR signalling, which potentially leads to their activation-induced cell death in the absence of CD28 co-stimulation signals. This differential effect could explain both the lack of clinical response in many tumours with pre-existing TILs (which might lack APC niches) and the clonal replacement of TILs with an exhausted phenotype by newly infiltrating clones observed during anti-PD1 treatment in vitro[19] and in vivo[77]. Strikingly, HGSOCs typically display a markedly lower CD28 co-stimulation signature at baseline than is seen in tumours that respond well to PD1 blockade[19]. Only a small fraction of HGSOCs exhibit a CD28 co-stimulation signature intensity similar to that of immunoresponsive tumours[19].

Efforts to develop effective drug treatments for tumours with pre-existing intraepithelial TILs should focus on reinforcing the molecular interactions that lead to TIL activation in myeloid niches. For example, blockade of CTLA4 strengthens CD28 signalling and synergizes with PD1 blockade to reinvigorate tumour-specific $CD8^+$ TILs both in vitro[19] and in mouse models[19,78]. Indeed, the results of the phase II randomized clinical trial NRG GY003 ($n = 100$) showed that the combination of nivolumab and ipilimumab resulted in a response rate superior to that achieved with nivolumab alone in patients with advanced recurrent epithelial ovarian cancer, although the gain in progression-free survival was limited[79]. However, given the increased toxicity resulting from the addition of ipilimumab[80], alternative combinations are desired. Reactive upregulation of alternative immune checkpoints has been observed in patients receiving PD1 blockade, and interesting preclinical work suggests a synergistic effect of combining PD1 blockers with LAG3 blockers[81]. Further TIL reinvigoration can be provided by treatment with co-stimulatory agonists[82] and/or IL-2-targeted molecules[83].

Importantly, activation of the tumour myeloid compartment seems to be essential for mobilizing $CD8^+$ TILs in response to immune checkpoint blockade, and has powerful effects in HGSOC models[84]. Indeed, the capacity to mount an optimal TIL response to immune checkpoint blockade hinges on the presence of co-stimulatory APCs in situ[19]. CD40 agonists have an extremely powerful polarizing effect on the myeloid cell and B cell compartments, as they reduce the numbers of both myeloid-derived suppressor cells and T_{reg} cells (which increases $CD8^+$ effector T cells)[85,86], and activate APCs. Treatment with an anti-CD40 agonist antibody had synergistic effects with combined PD1 and CTLA4 blockade in preclinical models of ovarian cancer in vitro and in vivo[19]. More specifically, this triple combination treatment induced TIL polyfunctionality in tumours that lacked activated myeloid cells at the baseline[19]. CD40 agonist therapy was also effective and sufficient to overcome tumour resistance to immune checkpoint blockade in a mouse model of pancreatic cancer[87], and the combination of these two interventions produced polyfunctional T cells in tumours that had previously been classed as 'cold'. Importantly, these interactions required *Batf3*$^+$ DCs[87]. Thus, although CD40L can exert important effects on B cells and $CD4^+$ T cells[88,89], activation of APCs by CD40L-mediated priming of T cells does seem to be required to overcome resistance to immunotherapy specifically in the context of immune checkpoint blockade[87]. APC activation by CD40L is expected to result in upregulation of the CD28 ligands CD80 and CD86, as well as upregulation of an array of important molecules, including major histocompatibility complex class II, additional co-stimulatory molecules of the TNF receptor family (such as TNF ligand superfamily member 9, also known as 4-1BBL) and IL-12 (REFS.[90–93]).

Toll-like receptor (TLR) agonists might also be therapeutically useful in HGSOC, although the heterogeneity of TLR expression by different myeloid populations and the differences between mouse myeloid cells and human myeloid cells complicates the clinical development of these agents[94]. Furthermore, systemic administration of TLR agonists can produce excessive

inflammation[95]. In a phase II randomized clinical trial, the addition of a TLR8 agonist to pegylated liposomal doxorubicin chemotherapy did not produce any benefit in patients with platinum-resistant HGSOC[96]; however, from a biological point of view, this approach is worth pursuing further. CD24 is another potential drug target that is highly upregulated in ovarian cancers; this molecule orchestrates a novel innate immune checkpoint through its interaction with the inhibitory receptor sialic acid-binding immunoglobulin-like lectin 10 (SIGLEC10) on tumour-associated macrophages, which highlights CD24 blockade as a promising immunotherapeutic strategy[97,98]. CD47 is also overexpressed in ovarian cancer, and provides a 'don't eat me' signal when it binds to its receptor on macrophages[99]. Anti-CD47 antibodies are being actively tested in clinical trials but, owing to the toxicity of these treatments, other CD47-targeted interventions (such as oncolytic viruses[95] and chimeric antigen receptor (CAR) T cell therapies[96]) are also under investigation[100,101].

TREM2 has been identified as a hallmark marker of tumour-associated macrophages in various solid tumours, including ovarian cancers[102], and its blockade restored response to immune checkpoint blocking monotherapy in the ID8 mouse model[103]. TREM2 is an attractive candidate for targeting myeloid cells in solid tumours, and a dose-escalation phase I clinical trial of an anti-TREM2 antibody (PY314) is under way (NCT04691375).

Importantly, T cell-inflamed HGSOCs show increased enrichment of T_{reg} cells[20], which exert a deleterious effect on immune attack[46]. Low-dose cyclophosphamide therapy decreases intratumoural T_{reg} cells in both mouse models and human tumours[104], and could be an attractive partner for combination with in vivo or in vitro vaccination. Future studies that explore immunosuppressive factors specifically associated with T cell inflammation in HGSOC could provide additional drug targets for use in combination treatment strategies.

The optimal timing of immunotherapy in the overall treatment plan for patients with HGSOC requires careful consideration. First-line chemotherapy is highly effective in most patients and cytoreduces disease to undetectable levels; nonetheless, most treated individuals ultimately relapse[2]. However, potentially curative intervention strategies could be implemented before relapse occurs. Efforts to develop adjuvant therapies need to take into account potential drug interactions with chemotherapy and PARP inhibitors (discussed later). Immunotherapy can be effective in patients with minimal residual disease after first-line chemotherapy, as shown by the favourable response rates (~25%) for intraperitoneal administration of IL-2 in two studies[105,106]. Platinum-resistant HGSOC remains an area of unmet medical need, and testing of new immunotherapy combinations is urgently needed in this setting.

Adoptive cell therapy. ACT using autologous TILs is a possible strategy for patients with T cell-inflamed tumours. TILs are isolated from autologous tumour samples and expanded ex vivo using high concentrations of IL-2. Patients are then treated with lymphodepleting chemotherapy followed by infusion of the expanded TILs and systemic IL-2 treatment to support further TIL expansion in vivo. In patients with late-stage melanoma, ACT with autologous TILs achieved durable remission in a proportion of patients[107–109], suggesting that this approach has curative potential. TILs have also been used in the treatment of NSCLC[110], cervical and head-and-neck tumours[111], and other tumour types[112]. Experience with TIL ACT in HGSOC is preliminary: TILs harvested from one metastatic deposit led to regression of the index lesions but did not suppress the appearance of new metastatic lesions, and disease progression occurred in six treated patients[113]. Following observations that the infused TILs express PD1 (REF.[113]), the combination of immune checkpoint blockade and TIL ACT was tested in another group of patients, one of whom experienced a partial response and one of whom had long-lasting stable disease[114]. The remaining four patients had progressive disease[114].

An important challenge for TIL ACT relates to intratumoural heterogeneity. Peritoneal dissemination of HGSOC is characterized by a multibranch metastatic process, in which different molecular alterations and pathways can be activated at different metastatic tumour sites, resulting in a heterogenic immunophenotype[29,115,116] (BOX 3). Such heterogeneity can be an important limitation on the effectiveness of neoantigen-based treatment

Box 3 | **Natural history, evolution and heterogeneity of ovarian cancer**

High-grade serous ovarian cancer (HGSOC) was initially believed to arise from the ovarian surface epithelium[69]. However, emerging evidence supports the fallopian tube epithelium as a potential origin of primary HGSOC tumours[225–229]. Non-invasive serous tubal intraepithelial lesions or serous tubal intraepithelial carcinomas (STICs) are thought to form preferentially in the distal fallopian tube epithelium and progress to malignant and metastatic HGSOC only after implantation on the ovary or peritoneum[12,227,228,230–232]. STICs exhibit nuclear atypia with the presence of mitosis and/or apoptotic bodies, contain either missense or deleterious *TP53* mutations and have a high Ki-67 proliferation index, whereas serous tubal intraepithelial lesions might or might not contain a p53 signature, are distinguished by a low Ki-67 proliferation index (less than 10%) and can be biologically considered to be dormant STICs[231,233].

Current evidence indicates that STICs might serve as a precursor to HGSOC in women with a high risk of cancer owing to germline *BRCA1* and/or *BRCA2* mutations[229,234]. Molecular features of HGSOCs were mostly shared by STICs in women with both types of lesion, indicating that they had a common biologic origin[235]. Although STICs are clearly associated with an increased risk of HGSOC, not all STICs progress to HGSOC, nor do all HGSOCs arise from STICs, even in women with high-risk characteristics[236]. Interestingly, marked infiltration of $CD4^+$ T cells and $CD8^+$ T cells has been reported in the perineoplastic stroma surrounding STICs, which suggests that tumour-specific antigens are already present at this pre-invasive stage. Remarkably, no regulatory T cells were seen in the stroma. Such observations could aid in the selection of preventive immune strategies[237].

Studies of spatial and temporal tumour evolution reveal both linear and parallel forms of clonal evolution, and both can be present at different tumour sites within a single patient[236,238–242]. Multiple discrete tubal lesions from the same individual carrying non-identical *TP53* mutations are possible, even at very early stages of tumorigenesis[236]. In most patients with HGSOC, clonal diversity emerges at a primary lesion site, followed by unidirectional and monoclonal seeding to distal intraperitoneal sites. Only a minority of patients exhibit a high degree of polyphyletic clonal mixing and reseeding of clones at distant tumour foci[238–240,243]. Thus, metastasis-to-metastasis spread and tumour heterogeneity is generated through ongoing clonal evolution, which can be identified by multisite sequencing. Increased rates of clonal evolution are linked to worse outcomes and to treatment-resistant relapse[244]. Neoadjuvant chemotherapy induced minor but detectable changes in tumour heterogeneity when compared with the overall changes captured from disease onset[244].

strategies, especially if tumour sampling is restricted to limited areas, which might not be representative. Furthermore, tumour cell evolution can lead to — and is probably sculpted by — heterogeneity of the tumour microenvironment. Genomic and immunological studies of several lesions in a single patient revealed that the various metastases were molecularly heterogeneous and differentially infiltrated by immune cells: regressing and stable metastases were infiltrated by oligoclonal $CD8^+$ T lymphocytes and $CD4^+$ T lymphocytes, whereas progressing metastases were characterized by immune cell exclusion[117]. Thus, within-patient spatial variation in the immune microenvironment shapes intraperitoneal malignant spread[29]. This circumstance could explain in part the heterogeneous fates of metastatic lesions after neoadjuvant chemotherapy[117]. Further disease evolution might occur in the context of chemotherapy that causes progressing metastases to be characterized by immune cell exclusion. In support of this contention, neoadjuvant chemotherapy induces increases in the numbers of NK cells and TCR clonality in site-matched samples but not in site-unmatched samples[116]. Therefore, a sample from a single tumour lesion might not include the entire T cell repertoire required to induce regression of all distant tumour lesions. In NSCLC, for example, the TIL repertoire (as assessed by TCR sequencing) in each tumour region reflected local variations of the mutational landscape and revealed substantial intratumoural heterogeneity, with some clonotypes being ubiquitous and others only regional[118]. The success of TIL ACT in this context hinges largely on the abundance of ubiquitous clones.

Thus, TIL ACT strategies must be developed into curative treatments. In patients with HGSOC, primary or interval tumour debulking offers a unique opportunity to harvest TILs from multiple tumour sites. Ascites fluid samples obtained at presentation also contain abundant tumour-specific T cells[119]. Patients with residual disease after first-line chemotherapy could also benefit from TIL ACT. In an intriguing early study, consolidation TIL ACT was given to all patients who had a complete response to first-line platinum-based therapy, as long as their TILs could be expanded; overall survival at 3 years was 100% in patients who received TILs, versus 54.5% in those who did not[120]. Although this was not a randomized study, these provocative findings encourage more efforts in this direction.

Cold or excluded tumours

Restoration of T cell inflammation. Pre-existing intratumoural T cell inflammation is required for the success of immune checkpoint blockade, vaccines and T cell-based immunotherapies. Accordingly, the restoration of T cell inflammation within tumours is an important ingredient of immunotherapy for cold or immune-excluded tumours. The application of low-dose radiotherapy to tumour masses can create convenient windows of opportunity to induce T cell inflammation in tumours, and thereby render immune checkpoint blockade effective. In ID8 mice treated to exclude T cells from tumour islets, application of 1 Gy to the whole abdomen resulted in considerable intratumoural T cell inflammatory responses[121]. Furthermore, low-dose radiotherapy upregulated several druggable immune checkpoints in the tumour microenvironment and created a basis for intervention with immune checkpoint blockers (to reinvigorate TILs), CD40 agonists (to activate myeloid cells) and low-dose cyclophosphamide therapy (to counter the effects of T_{reg} cells). Combinations of these different approaches with low-dose radiotherapy resulted in complete tumour eradication and cure of a fraction of mice into which orthotopic ID8 tumours had been implanted[121]. The inflammatory effect of low-dose radiotherapy was transient, and repeated administration was required to retain its therapeutic benefit. Furthermore, relapse of ID8 tumours in these mice occurred only after discontinuation of combination therapy, which suggested that the combination therapy should be continued as long as it is tolerated.

Mechanistically, low-dose radiotherapy mimicked the steady-state DNA damage response induced by HRD. Such tumours show upregulation of CCL5 in tumour cells and reactive upregulation of CXCL9 in tumour myeloid cells, in association with profound reprogramming of tumour-infiltrating immune cells. Importantly, administration of low-dose radiotherapy once weekly to the entire abdominal cavity is safe in patients with HGSOC[122]. Inspired by these results, a phase I study conducted in patients with cold solid tumours, including two with HGSOC, tested the combination of 1 Gy stereotactic radiotherapy plus ipilimumab, nivolumab, low-dose cyclophosphamide and aspirin therapy (given to attenuate biosynthesis of PGE_2 and its immunosuppressive effects)[121]. The patients with HGSOC received low-dose radiotherapy targeting large abdominal tumour volumes without radiation toxicity, proving that this approach is feasible. Low-dose radiotherapy resulted in de novo inflammation and regression of metastatic solid tumours when combined with orthogonal immunotherapy. Importantly, in the patients who responded, the combination treatment triggered intratumoural T cell infiltration, predominantly of $CD4^+$ cells, which reflected similar findings in mice[121].

Whether chemotherapy (and specifically which chemotherapeutic drugs, doses and schedules) can also induce T cell inflammation and render tumours susceptible to immunotherapy remains a matter of investigation[123]. Several chemotherapy agents, including oxaliplatin, paclitaxel and doxorubicin, induce immunogenic cell death that exposes tumour antigens and activates DCs to elicit in situ immunization[124], which might trigger T cell inflammation under the right conditions (FIG. 3). For example, doxorubicin treatment increases the number of tumour-infiltrating NK cells and T cells in patients with breast cancer[125], and neoadjuvant carboplatin with paclitaxel chemotherapy induced T cell infiltration in ovarian cancers[126,127]. However, second-line combination treatment consisting of the anti-PDL1 antibody avelumab with pegylated liposomal doxorubicin did not show any benefit over treatment with pegylated liposomal doxorubicin alone in a phase III randomized clinical trial[128]. Similarly, in another phase III randomized clinical trial, the combination of the anti-PDL1 antibody atezolizumab with carboplatin and paclitaxel chemotherapy plus bevacizumab therapy

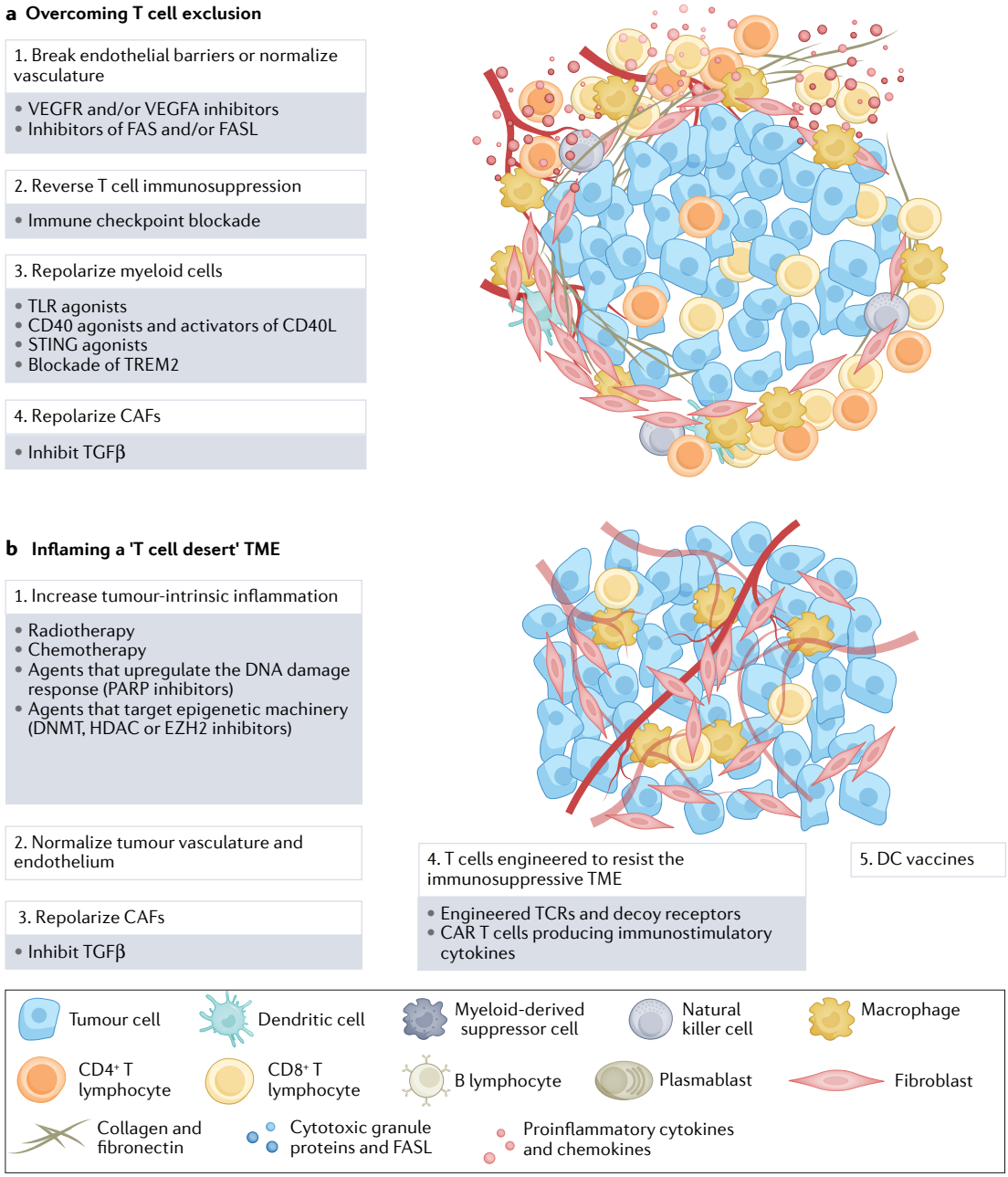

Fig. 3 | **Inducing anticancer immune responses in high-grade serous ovarian cancer tumours with an immune-excluded or cold immunophenotype. a** | Combinatorial therapeutic strategies for use in tumours with an immune-excluded immunophenotype aim to overcome T cell exclusion and promote T cell infiltration into the tumour cores. **b** | Combinatorial therapeutic strategies for use in tumours with a cold ('immune-desert') immunophenotype aim to activate and inflame T cells present within these tumours. CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; DC, dendritic cell; DNMT, DNA (cytosine 5) methyltransferase; FASL, FAS ligand; HDAC, histone deacetylase; PARP, poly(ADP-ribose) polymerase; STING, stimulator of interferon genes; TCR, T cell receptor; TGFβ, transforming growth factor-β; TME, tumour microenvironment; VEGFA, vascular endothelial growth factor A; VEGFR, vascular endothelial growth factor receptor.

(although successful in the NSCLC setting[129]) fell short of expectations as a first-line treatment in patients with HGSOC; however, this combination seemed to be more beneficial in patients with PDL1$^+$ tumours[75]. This discrepancy between HGSOC and NSCLC is presently hard to explain. Importantly, paclitaxel might antagonize the immune-activating effects of blocking PD1 and/or PDL1, and curtail anticancer immune responses by suppressing effector cells[51]. Therefore, the benefits derived from using combinations of these agents with paclitaxel might be produced through independent and complementary effects of each drug, rather than a mechanistic synergy[130]. Important differences in the immune milieu and chemosensitivity of these two tumour types might offer alternative hypotheses to interpret these findings.

Given the increased T cell inflammation observed in tumours that exhibit HRD, as well as the important clinical efficacy of PARP inhibition, we and others have

explored the immune mechanisms associated with the use of PARP inhibitors to treat HGSOC. In HGSOC tumours with *BRCA1* loss, PARP inhibition highly potentiated tumour-intrinsic DNA damage pathways and the DNA-sensing type I interferon pathway, which ultimately increased the expression of T cell-recruiting chemokines and T cell-stimulatory cytokines[65]. DNA damage is also sensed by myeloid cells in the tumour microenvironment, where it amplifies the inflammatory response[72]. PARP inhibition alone also triggers the STING–type I interferon pathway in homologous recombination-proficient tumours, albeit to a lesser extent (probably due to the lower level of DNA damage generated in such tumours)[131]. In vivo preclinical data showed that PARP inhibition synergizes with anti-PDL1 or anti-PDL1 plus anti-CTLA4 immune checkpoint blockade in ovarian cancer[65]. Therefore, the use of PARP inhibitors to treat patients with minimal residual disease after front-line chemotherapy could activate important immune responses that might partly mediate the sustained benefits observed with this agents of this class. The results of ongoing clinical trials of PARP inhibition combined with immune checkpoint blockade in the maintenance setting of HGSOC treatment are awaited (NCT03522246)[123,132].

Given the epigenetic mechanisms involved in silencing of CCL5, CXCL9 and/or CXCL10 ligands and other key inflammatory mediators, the immunomodulatory properties of epigenetic therapies could prove helpful in restoring T cell inflammation[133–135]. Reversion of epigenetic silencing could derepress DNA-sensing or RNA-sensing and type I interferon signalling pathways, upregulate production of T helper 1 cell-recruiting chemokines[136], class I and class II antigen presentation and priming of T cell inflammation in tumours (FIGS. 2,3), and downregulate MYC[137]. Furthermore, unique tumour antigens, including cancer testis antigens and endogenous retroviral elements, might be unmasked by treatment with DNA methyltransferase inhibitors[136,138]. Despite the low rates of response to single-agent epigenetic therapy[134], combinations of these agents with other forms of immunotherapy have shown increased efficacy in preclinical and clinical studies[135,139] (FIG. 3).

Overcoming the stromal barrier. Immune-excluded tumours are characterized by infiltration of $CD8^+$ T cells in the stroma between tumour islets, although these immune cells are unable to engraft within the tumour islets (FIG. 1). These TILs have predysfunctional and/or effector memory transcriptional states indicated by *GZMK* expression[20] (a marker of T cell senescence[140]) and reduced exhaustion signatures[20], although so far these characteristics seem to have no prognostic value[141]. Interestingly, the tumour cell status did not differ between $CD8^+$ T cell-excluded ovarian cancers and $CD8^+$ T cell-inflamed ovarian cancers, indicating that TIL exclusion could be driven by non-tumour cells[20]. T cell exclusion is associated with upregulation of TGFβ and activated stromal cell signatures in bulk transcriptional analyses[27]. Multiple mechanisms might drive cancer-associated fibroblast (CAF)-mediated T cell exclusion, including remodelling of the extracellular matrix that prevents access of $CD8^+$ TILs to the densely encapsulated tumour epithelium[142,143] and/or shaping the immunosuppressive environment through their secretome and ligandome[144,145]. Stromal fibroblasts become CAFs upon their activation, a programme driven epigenetically by the master metabolic regulator nicotinamide *N*-methyltransferase (NNMT)[146], which is capable of modifying tumour behaviour[147,148]. TGFβ, WNT, platelet-derived growth factor (PDGF) and inflammatory cytokines also play important roles in the generation of CAFs, and clinical efforts to target these molecules are ongoing. Specifically, the combination of TGFβ inhibition and immune checkpoint blockade was motivated by evidence that TGFβ drives TIL exclusion and resistance to anti-PDL1 (atezolizumab) treatment in patients with urothelial carcinoma, and by encouraging results in preclinical models of colon cancer[142,143]. Such treatment combinations could also be attempted in ovarian cancer because CAFs constitute an important component of its tumour microenvironment[141] (FIG. 3).

Overcoming the endothelial barrier. The discovery that the tumour vasculature plays a role in limiting the entry of T cells into tumour islets came as a surprise, given the leaky nature of these vessels. However, upregulation of endothelin B receptor (ET_BR) is a hallmark of tumour endothelial cells in tumours with an immune-excluded or immune-desert immunophenotype[149]. ET_BR enables tumour endothelial cells to sense endothelin 1 (ET1) secreted by tumour cells and, via upregulation of nitric oxide, to suppress the inflammatory activation of tumour endothelial cells. In turn, this suppression leads to dysregulation of intercellular adhesion molecule 1 (ICAM1) clustering on tumour endothelial cells, which prevents T cell adhesion and transendothelial migration[149]. In addition, VEGFA can directly downregulate ICAM1 and vascular cell adhesion molecule 1 (VCAM1)[150] on tumour endothelial cells. Furthermore, VEGFA, IL-10 and PGE_2 produced by ovarian tumour cells[151] remain present in the tumour microenvironment[152], where they cooperatively upregulate death-inducing FAS ligand (FASL) on tumour endothelial cells, thereby killing transmigrating activated (that is, FAS^+) T cells but not T_{reg} cells, leading to tumour immune desertification[153]. Tumour endothelial cell positivity for FASL is a hallmark of T cell exclusion in a variety of solid tumours, which show upregulation of FASL in capillary endothelium within tumour islets but not in the adjacent stroma. This situation explains why circulating effector (FAS^+) T cells cannot infiltrate tumour islets but can freely extravasate into the adjacent stroma, thereby leading to the exclusion phenotype[153]. Complementing the immunoregulatory role of tumour endothelial cells in solid tumours is a panoply of immunomodulatory molecules that have been identified in these cells, including PDL1 (REF.[154]), PDL2 (REF.[154]), TIM3 (REFS.[155,156]), B7-H3 (REFS.[157–159]), B7-H4 (REF.[160]), IL-6 (REFS.[161–163]), PGE_2 (REFS.[161–163]), IL-10 (REFS.[161–163]) and TGFβ[161–163].

Therefore, reprogramming the tumour vasculature has emerged as an important strategy to restore immune attack[164,165]. Indeed, combinations of angiogenesis inhibitors and immune checkpoint blockers have

now proven successful in many tumour types[129,166–168]. In ID8 mice, treatment of cold ovarian tumours with anti-VEGFA antibody induced substantial infiltration of $CD8^+$ T cells and achieved tumour control; anti-VEGFA treatment was even more effective when combined with aspirin treatment to block PGE_2. $CD8^+$ T cells were entirely responsible for the therapeutic effect of this combination[153]. Anti-VEGF therapy also enhances T cell infiltration in human tumours, and might potentiate responses to immune checkpoint blockade[169]. The anti-VEGFA antibody bevacizumab has shown a benefit in patients with recurrent HGSOC[170,171], and is approved for first-line treatment of HGSOC in combination with carboplatin and paclitaxel[172]. Although the addition of atezolizumab did not improve the combination of carboplatin and paclitaxel plus bevacizumab in a randomized phase III study, additional atezolizumab did seem to benefit the subgroup of patients with immunoreactive (that is, $PDL1^+$) tumours[75]. Furthermore, the combination of nivolumab and bevacizumab achieved an objective response rate of 21% and a median progression-free survival time of 9.4 months in patients with recurrent epithelial ovarian cancer in a phase II study[173], whereas the anti-PDL1 agent durvalumab in combination with the mixed VEGF receptor tyrosine kinase inhibitor cediranib showed an objective response rate of 50% and a disease control rate of 75%[174]. These encouraging observations point towards a potential benefit of combining bevacizumab treatment with anti-PD1 or anti-PDL1 therapy, especially in patients with platinum-sensitive recurrent ovarian cancer. The successful results of the OCEANS[175] and GOG-213 (REF.[176]) studies led to the approval of the use of bevacizumab in this setting.

Interestingly, in a mouse model of *Brca1*-deficient ovarian cancer, PARP inhibition plus immune checkpoint blockade was ineffective, but therapeutic resistance in these mice could be overcome by also blocking VEGFA. VEGFA was strongly upregulated at baseline in these *Brca1*-deficient ovarian tumours, and its production further increased by PARP inhibition via STING, whereas VEGFA blockade produced vascular reprogramming and T cell infiltration that enabled immune checkpoint blockade to be effective[65]. These data might explain the important synergy seen with PARP inhibitor and bevacizumab treatment specifically in patients with HGSOC tumours with HRD treated in the adjuvant setting in the PAOLA-1 study[177].

Effective anti-angiogenesis therapy might induce tumour hypoxia. Hypoxia-inducible factor 1α (HIF1α), the key transcription factor associated with hypoxia response, upregulates PDL1 on myeloid-derived suppressor cells, thereby leading to T cell exhaustion and the generation of T_{reg} cells[178]. In addition, HIF1α induces recruitment of T_{reg} cells into the tumour microenvironment via CCL18 (REF.[47]). The hypoxic tumour microenvironment can also become metabolically non-permissive owing to lactate accumulation and the consequent reduction in pH[179,180], which causes effector T cells and NK cells to undergo first anergy, then apoptosis[181–184], although immunosuppressive myeloid cells and T_{reg} cells continue to function[178,185]. An attractive strategy, therefore, is to combine anti-angiogenesis agents and immune checkpoint blockers with drugs that could counter T_{reg} cells. Cyclophosphamide used at a low dose is an example of a drug that can reduce the numbers of tumour suppressive T_{reg} cells[104]. Interestingly, the combination of pembrolizumab, bevacizumab and low-dose cyclophosphamide yielded an objective response rate of 47.5% and a median progression-free survival time of 10.0 months in a phase II study involving patients with platinum-resistant HGSOC[186].

Vaccines

The generation of competent tumour-specific T cells is an important task in cancer immunotherapy. Immune checkpoint blockade can rejuvenate immune responses by mobilizing T cells in the circulation. Furthermore, stereotactic radiotherapy or other physical tumour ablation methods can release relevant tumour antigens and induce in situ vaccination[187]. However, cancer vaccines are a direct and effective means for generating T cell priming[188,189]. Cancer vaccines based on shared tumour-associated antigens, including cancer testis antigens such as NY-ESO-1 or cell lineage antigens such as HER2, hTERT, MUC1 and WT1, are often a convenient strategy[190–192]. However, the molecular heterogeneity of HGSOC presents a challenge for approaches that target a single antigen. Integration of neoantigens derived from non-synonymous somatic mutations might improve cancer vaccine strategies for HGSOC, as these antigens have the potential to activate tumour-specific T cell clones with high avidity[193]. However, the development of such vaccines can be technically challenging, and they have not yet been fully investigated in ovarian cancer, a malignancy with a fairly low mutational burden. The use of whole-tumour lysate to provide tumour antigens for vaccination provides a reasonable alternative to single antigen or neoantigen approaches. Personalized DC vaccines manufactured using oxidized autologous tumour lysate as the tumour antigen source are universally applicable, and effectively mobilize antitumour T cell immunity in patients with HGSOC[194,195], and such vaccines had a tangible clinical benefit in combination with bevacizumab and low-dose cyclophosphamide therapy[196,197]. Importantly, these DC vaccines elicit immune responses against numerous tumour antigens, including tumour-associated antigens and private neoepitopes, and mobilize new T cell clones with high avidity and polyfunctional features[196]. This scenario is ideal for their combination with immune checkpoint blockade[78].

Engineered T cells

Finally, ACT based on genetically engineered T lymphocytes could be a potential therapeutic intervention, including in cold tumours. TCR or CAR T cell approaches have been reviewed in detail elsewhere[198]. The target antigen of TCRs or CAR T cells must be chosen carefully to achieve efficient antitumour responses without on-target organ toxicity. Many cancer testis antigens, particularly NY-ESO-1, are not expressed in healthy adult tissues and are overexpressed in HGSOC. T cells engineered to recognize NY-ESO-1 have been successfully used to treat patients with melanoma and synovial

sarcoma[33,199] without organ toxicity, and several clinical trials of T cell transfer are ongoing in patients with ovarian cancer, given as monotherapy (NCT01567891 and NCT02869217) or in combinations with either a vaccine (NCT01697527) or the DNA methyltransferase inhibitor decitabine (NCT03017131 and NCT02366546).

However, most of the targets used for engineered CAR T cell therapy are overexpressed lineage antigens that are usually not tumour restricted. To date, CAR T cells that have been tested in clinical trials as a therapy for HGSOC have targeted folate receptor-α and mesothelin, which are both almost ubiquitously expressed in HGSOC. Firm conclusions regarding the utility of this approach in HGSOC cannot be drawn from the results of these trials, owing to technological shortcomings specific to the two clinically tested CAR T cells: the folate receptor-α CAR T cell was a first-generation CD3ζ construct lacking co-stimulatory modules, and the infused cells did not persist in vivo or localize to tumour sites[200]; the second-generation anti-mesothelin CAR T cell endowed with 4-1BB co-stimulation also exhibited limited persistence in vivo, which was attributed to the xenogeneic origin of its extracellular domain — a mouse single-chain variable antibody fragment that elicited anti-chimeric antibodies, which probably mediated CAR T cell elimination[201]. CAR T cells presently under development for the treatment of ovarian cancer include a CD28–CD3ζ CAR T cell that targets the MUC16 ectodomain and has been engineered to secrete human IL-12 (NCT02498912)[202], and a CAR T cell that targets HER2 (NCT02713984).

Importantly, many of the antigens that can be targeted in these approaches are associated with poor prognosis in patients with HGSOC[203,204], and their presence might herald poorly immunoreactive or immunoresistant tumours[205]. Therefore, drug combinations or further T cell engineering approaches that address the tumour microenvironment barrier[206] will be necessary to overcome these challenges.

Conclusions

Although early studies indicated that ovarian cancers might be immunogenic, clinical trials of immunotherapy have not fulfilled this promise to date. In this Review, we have described how implementation of a systematic, immune phenotypic classification of ovarian cancers based on the presence or absence of intraepithelial $CD8^+$ T cells can shed light on appropriate immunotherapy approaches and facilitate the development of tailored therapeutic combinations that build on the lessons learned from studies of cancer immunobiology. Indeed, we recommend that these three phenotypes — hot, cold and excluded — are used as a basis to personalize therapeutic decisions for patients with HGSOC in the future. With regard to this point, further research is direly needed, not only to characterize the different subsets of HGSOC but also to determine ideal biomarkers and digital methods for use in the analysis of pathology specimens that are capable of stratifying patients into the $CD8^+$ T cell immunophenotypes described in this Review. These biomarkers and digital methods will also need to take into consideration intratumoural heterogeneity of $CD8^+$ T cell infiltration to offer accurate selection of patients and prediction of treatment response. These $CD8^+$ T cell immunophenotypes could be further combined with genomic markers that define HRD status (that is, BRCA gene mutations), which are commonly used to select patients for PARP inhibitor therapy.

We also propose that more efforts should focus on the development of first-line treatment strategies that target minimal residual disease after completion of first-line chemotherapy, to maximize the chances of such patients having durable responses. Although platinum-resistant HGSOC remains an area of unmet medical need, for which testing of new treatment combinations remains a priority, potentially curative intervention strategies could helpfully be investigated earlier in the disease course. Immunotherapy can be effective in the setting of minimal residual disease after first-line chemotherapy, as shown by the durable responses of a proportion of patients to intraperitoneal administration of IL-2 (REFS.[105,106]).

Interactions with chemotherapy and PARP inhibitors need to be taken into account when one is considering the options for adjuvant therapy. Indeed, consideration must also be given to the effect of chemotherapeutic agents or targeted therapies on the tumour microenvironment. Single-cell technologies that analyse the evolution of the whole tumour microenvironment are expected to provide comprehensive information about the effects of chemotherapy and targeted therapy, not only on the tumour compartment but also on immune cells. We believe that if treatments and patients are carefully selected on the basis of the parameters discussed in this Review, such as disease stage, immune fitness (that is, having an immune system capable of returning and healthy enough to return to homeostasis), appropriate choices of combination therapy and appropriate routes of administration, adjuvant treatments could constitute a promising and valuable option for management of HGSOC in the future. Finally, clinical studies that integrate different types of immunotherapies into standard-of-care treatments for patients with ovarian cancer are warranted, to investigate the personalization of combinatorial therapy for this difficult-to-treat population of patients.

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Competing interests
G.C. declares that he has received grants or research support from or is a co-investigator in clinical trials conducted by Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Iovance, Kite, Roche and Tigen. Lausanne University Hospital has received honoraria for advisory services provided by G.C. to AstraZeneca, Bristol-Myers Squibb, Hoffmann-La Roche, MSD and Geneos Therapeutics. G.C. holds patents related to antibodies and vaccines targeting the tumour vasculature as well as technologies related to T cell expansion and engineering of T cell therapy, for which G.C. receives royalties from the University of Pennsylvania. L.E.K. declares that she has received honoraria for advisory services provided to Geneos Therapeutics and AstraZeneca. D.D.L. declares no competing interests.

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