

# Targeting tumor-associated macrophages for successful immunotherapy of ovarian carcinoma

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## ABSTRACT

Epithelial ovarian cancer (EOC) is among the top five causes of cancer-related death in women, largely reflecting early, prediagnosis dissemination of malignant cells to the peritoneum. Despite improvements in medical therapies, particularly with the implementation of novel drugs targeting homologous recombination deficiency, the survival rates of patients with EOC remain low. Unlike other neoplasms, EOC remains relatively insensitive to immune checkpoint inhibitors, which is correlated with a tumor microenvironment (TME) characterized by poor infiltration by immune cells and active immunosuppression dominated by immune components with tumor-promoting properties, especially tumor-associated macrophages (TAMs). In recent years, TAMs have attracted interest as potential therapeutic targets by seeking to reverse the immunosuppression in the TME and enhance the clinical efficacy of immunotherapy. Here, we review the key biological features of TAMs that affect tumor progression and their relevance as potential targets for treating EOC. We especially focus on the therapies that might modulate the recruitment, polarization, survival, and functional properties of TAMs in the TME of EOC that can be harnessed to develop superior combinatorial regimens with immunotherapy for the clinical care of patients with EOC.

and serous membranes lining the peritoneal organs.<sup>4</sup>

The standard first-line treatment comprises cytoreductive surgery coupled with platinum/taxane doublet-based chemotherapy, which enables complete remission (CR) in the majority of patients. Nonetheless, more than 50% of patients with EOC develop resistance to chemotherapy and eventually experience recurrence.<sup>5,6</sup> Over the past decade, several targeted agents have been introduced for the routine clinical management of EOC. These include various poly (ADP-ribose) polymerase inhibitors (PARPi) (eg, niraparib, olaparib, and rucaparib),<sup>2,7</sup> which mainly block DNA damage repair (DDR) and DNA replication in cancer cells<sup>8</sup> and angiogenesis inhibitors, such as bevacizumab, a monoclonal antibody (mAb) targeting vascular endothelial growth factor (VEGF).<sup>9</sup> Homologous recombination DNA-repair pathway defects imposed by germline or somatic *BRCA1* or *BRCA2* mutations are key determinants of platinum sensitivity in patients with EOC,<sup>10</sup> and provide a robust rationale for maintenance therapies based on PARPi.<sup>11</sup> As such, maintenance therapy with PARPi has extended progression-free survival (PFS) in patients with advanced EOC that has initially responded to platinum irrespective of HR proficiency.<sup>12</sup> However, improved overall survival (OS) is only seen in individuals with *BRCA1/2* mutations.<sup>7</sup> The combination of olaparib and bevacizumab was recently shown to improve 5-year OS in OC patients with homologous recombination deficiency (HRD) in a phase III PAOLA-1/ENGOT-ov25 trial (NCT02477644). Therefore, developing novel therapeutic strategies alongside with improved understanding of immunocompetent and immunosuppressive components of the TME is of paramount importance to increase the effectiveness of EOC therapy.

Ongoing research into immunotherapeutic strategies, including immune checkpoint inhibitors (ICIs), adoptive cell therapies, and

## INTRODUCTION

Ovarian cancer (OC) is the second most common gynecologic cancer in developed countries and the leading cause of gynecologic cancer mortality. Epithelial OC (EOC) accounts for more than 95% of ovarian tumors and high-grade serous ovarian carcinoma (HGSOC) is the most common subtype.<sup>1–3</sup> Due to inefficient screening methods for early detection and the absence of specific early warning symptoms, most patients with EOC are diagnosed at an advanced stage, which is characterized by a highly immunosuppressed tumor microenvironment (TME) and distant metastases. The formation of metastatic lesions in HGSOC occurs soon after the primary disease is established and is facilitated by an accumulation of ascites fluid in the peritoneal cavity, which allows the tumor cells to adhere to the omentum



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cancer vaccines has changed the field of oncology by putting the host immune response under the spotlight as target for anticancer therapeutic interventions. Notably, ICIs have revolutionized cancer treatment by their enormous success in the clinical management of a wide variety of cancer types.<sup>13</sup> However, only a small proportion of patients with EOC respond to ICIs as a stand-alone immunotherapeutic intervention.<sup>14,15</sup> The mechanisms of treatment failure in EOC are complex and involve genomic factors, altered metabolism, abnormal neovascularization and, most importantly, robust infiltration of immunosuppressive immune cells into the TME, particularly tumor-associated macrophages (TAMs).<sup>16</sup>

Here, we review the clinical relevance of TAMs in EOC. We first discuss how the ovarian TME recruits TAMs and modulates their polarization, as well as the mechanisms by which TAMs contribute to the development and progression of EOC. We then summarize the current knowledge on the impact of distinct TAM states and/or TAM-related signatures on the prognosis and response to ICI-based immunotherapy in EOC. Finally, we describe the recent advances of TAM-targeting agents and combinatorial strategies, as well as the rationale for their use in EOC therapy.

## PHENOTYPIC AND FUNCTIONAL DIVERSITY OF MACROPHAGES

Macrophages represent a diverse set of highly plastic mononuclear phagocytic cells which, in response to various microenvironmental stimuli, such as cytokines and chemokines, polarize into distinct phenotypes with specific functionality. Multiple populations of macrophages are known to be present within the same

microenvironment and each phenotype has a different combination of expressing receptors and secreting cytokines/chemokines. Their diversity has long been recognized and thus, terms such as the so-called M1- and M2-like phenotypes were introduced to define the possible *in vitro* polarization extremes.<sup>17,18</sup> M1-like macrophages (classically activated, proinflammatory) play a major role in the host defense against infection in the context of T<sub>H</sub>1 immunity after exposure to proinflammatory cytokines such as interferons (IFNs) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), toll-like receptor (TLR) ligands, and bacterial products such as lipopolysaccharide. Once activated, they produce proinflammatory cytokines (eg, interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, IL-23, and TNF- $\alpha$ ), generate reactive oxygen species and nitric oxide, and exhibit increased expression of major histocompatibility complex (MHC) class II and costimulatory molecules CD80 and CD86, thereby contributing to the removal of pathogens and tumor cells (table 1).<sup>18–21</sup> By contrast, M2-like macrophages (alternatively activated, anti-inflammatory), which are induced by immunoregulatory cytokines (eg, IL-4, IL-10, IL-13, and transforming growth factor  $\beta$  (TGF- $\beta$ )), glucocorticoids, or colony-stimulating factor 1 (CSF1), mainly support T<sub>H</sub>2-related tissue repair, remodeling, and tumor promoting processes mediated by IL-10, TGF- $\beta$ , prostaglandin E2, VEGF, matrix metalloproteinases (MMPs), and arginase 1 (ARG1) secretion (table 1).<sup>17,19,22</sup>

However, this binary classification is greatly oversimplified because the polarization/activation of macrophages *in vivo* in the local tissue microenvironment is far more complex process, as they are exposed to M1 and M2 signals of different origins and context. Thus,

**Table 1** The characteristics of M1-like versus M2-like macrophages

	M1-like macrophages	M2-like macrophages
Inducers	IFN- $\gamma$ , TNF- $\alpha$ , TLR ligands, bacterial products (such as LPS)	IL-4, IL-10, IL-13, TGF- $\beta$ , CSF1, PGE2, glucocorticoids
Phenotypic markers	HLA-DR, CD80, CD86	CD163, CD204, CD206
Secreted/produced molecules	IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, IL-23, TNF- $\alpha$ , iNOS, ROS	IL-10, TGF- $\beta$ , PGE2, VEGF, MMPs, ARG1
Metabolism	Highly glycolytic—dependent on the stabilization of HIF-1 $\alpha$ ; increased generation of lactic acid	Glycolysis is dispensable when OXPHOS is intact
	$\uparrow$ PPP flux $\rightarrow$ $\uparrow$ NADPH production $\rightarrow$ generation of NO and ROS; lipid biosynthesis	$\downarrow$ PPP flux
	$\uparrow$ lipid synthesis $\rightarrow$ membrane biogenesis, granule formation	$\uparrow$ FAO
	Impaired TCA cycle $\rightarrow$ accumulation of citrate and succinate $\rightarrow$ fatty acid synthesis and generation of inflammatory effector molecules—for example, NO	Intact TCA cycle driven by FAO and glutamine catabolism
	Impaired OXPHOS and ETC $\rightarrow$ $\uparrow$ ROS generation	$\uparrow$ OXPHOS and mitochondrial biogenesis
	$\uparrow$ iNOS expression $\rightarrow$ arginine is preferentially catabolized into NO	$\uparrow$ ARG1 expression $\rightarrow$ arginine is preferentially catabolized into ornithine and urea $\rightarrow$ production of polyamines and proline $\rightarrow$ cell growth and collagen synthesis
Functions	Proinflammatory, pathogen clearance, antitumor properties	Anti-inflammatory, tissue repair and remodeling, protumorigenic properties—immunosuppression, angiogenesis, tumor cell invasion and metastasis

CSF1, colony-stimulating factor 1; ETC, electron transport chain; FAO, fatty acid oxidation; HIF-1 $\alpha$ , hypoxia-inducible factor 1 $\alpha$ ; HLA-DR, human leukocyte antigen-DR; IFN, interferon; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; OXPHOS, oxidative phosphorylation; PGE2, prostaglandin E2; PPP, pentose phosphate pathway; ROS, reactive oxygen species; TCA cycle, tricarboxylic acid cycle; TLR, toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

macrophages found in the TME (ie, TAMs) may display a spectrum of phenotypes with a mixture of proinflammatory and alternatively activated macrophages, coexpressing M1 and M2 gene signatures,<sup>18 23 24</sup> or may show expression patterns distinct from the M1/M2 states, such as *FABP5*- and *APOE*-expressing macrophages, recently identified in the TME of breast cancer. These two populations bear close transcriptomic similarity to a population of lipid-associated macrophages (LAMs).<sup>25</sup> Nevertheless, it seems that during early carcinogenesis, TAMs exhibit a higher degree of similarity to M1-like subtypes and in later stages the majority of tumors, including EOC, recruit macrophages with M2-like phenotypes possessing low tumoricidal activity and high potential to promote immunosuppression, tumor cell invasion, angiogenesis and metastasis.<sup>26–31</sup>

Given the fact, that most of the results discussed in this review, have been obtained based on the binary M1/M2 classification, a critical view is necessary for proper assessment of the impact of TAMs on the TME and every aspect of tumor growth and progression in EOC.

## ROLE OF TAMs IN THE DEVELOPMENT AND PROGRESSION OF EOC

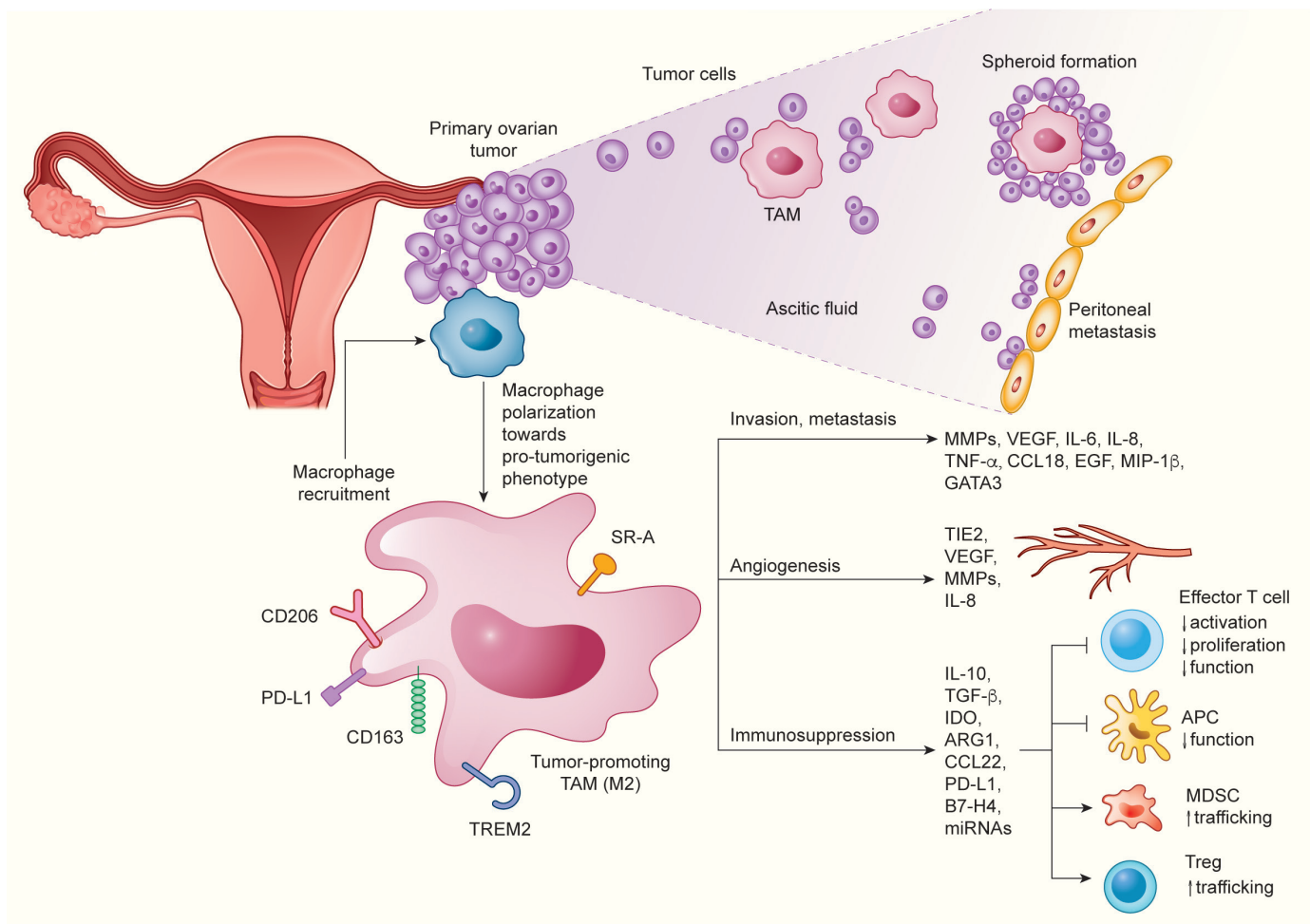
### Recruitment and polarization of TAMs in EOC

TAMs can originate from tissue-resident macrophages developed from embryonic precursors (eg, fetal yolk sac progenitors), residing in the majority of adult organs, including ovaries, and from monocytes derived from bone-marrow hematopoietic cell progenitors. Compared with physiological conditions, EOC development is characterized by increased monocyte recruitment and/or expansion of tissue-resident macrophages with both populations involved in tumorigenesis.<sup>32 33</sup> In EOC, TAMs are the most predominant population of immune cells, constituting up to 39% of total immune cell infiltrate.<sup>29 31 34–38</sup> In addition, data from The Cancer Immune Atlas showed that the majority (51%) of these TAMs display M2-like phenotype.<sup>34</sup> TAMs in EOC are not only localized in the TME of primary tumor, but are also abundantly present in ascitic fluid and metastases (eg, omental). Experimental mouse models have demonstrated that TAMs constitute a major cell fraction in intraperitoneal milieu/malignant ascites which play pivotal role in the transcoelomic dissemination of ovarian tumor cells by supporting their survival and invasiveness.<sup>39 40</sup> In addition, TAMs are frequently found in the omentum, the most common site of metastases, in areas called milky spots, where they facilitate omental colonization by ovarian tumor cells.<sup>41</sup> Various chemokines, cytokines and growth factors have been implicated in the recruitment of suppressive monocytes and macrophages to the ovarian TME, including IL-6, leukemia inhibitory factor, chemokine (C-C motif) ligand 2 (CCL2; also known as monocyte chemoattractant protein 1), CSF1, TNF, CCL22, C-X-C motif chemokine ligand 12 (CXCL12), VEGF, periostin, and semaphorin 4D.<sup>26 42–47</sup>

The TAM compartment undergoes extensive remodeling of core energy metabolism during tumor progression (table 1).<sup>48–50</sup> In hypoxic TMEs, including the TME in EOC, the cells prefer to use glycolytic metabolism. The accumulation of lactic acid in TAMs and other cells present in the TME that use glycolysis results in the differentiation of TAMs into cells characterized by a tumor-promoting phenotype.<sup>48 51 52</sup> Because TAM polarization can be altered by lipid metabolism, the expression of 5-lipoxygenase (5-LOX) and upregulation of 5-LOX metabolites in EOC cells are associated with TAM recruitment into hypoxic areas of the ovarian TME in a process mediated by increased MMP-7 expression.<sup>53</sup> Moreover, lipidomic analysis of EOC-associated ascites revealed high concentrations of polyunsaturated fatty acids (PUFAs), particularly linoleic acid, which are potent inducers of peroxisome proliferator-activated receptor  $\beta/\delta$  in TAMs. Fatty acids accumulate in lipid droplets in TAMs and contribute to their protumorigenic polarization.<sup>54</sup> In similar line, results from mouse model of metastatic EOC showed that ovarian tumor cells can promote membrane-cholesterol efflux and depletion of lipid rafts from TAMs. Increased cholesterol efflux promote IL-4-mediated reprogramming of macrophages, including inhibition of IFN- $\gamma$ -induced gene expression.<sup>55</sup> Moreover, the depletion of glutamine, due to its increased consumption by EOC cells, causes upregulation of glutamine synthetase expression in TAMs and promotes their polarization toward an immunosuppressive, proangiogenic, and prometastatic M2-like phenotype.<sup>56 57</sup> Overall, it has become clear that TAMs are regulated by the dynamic nature of the TME, which drives changes in their functional phenotypes and distribution as a response to tissue-specific and tumor-specific stimuli. As the tumor grows, the stimuli in the TME alter, eliciting changes in TAM infiltration and polarization.

### TAM-mediated immunosuppression in the ovarian TME

TAMs suppress antitumor immune responses in ovarian TME via multiple mechanisms (figure 1). Perhaps the most studied of these mechanisms is the production of cytokines and chemokines, which inhibit the antitumor properties of immunocompetent immune cells, including cytotoxic T cells (CTLs) and antigen-presenting cells (APCs), while supporting the immunosuppressive immune components, such as regulatory T cells (Tregs). For instance, CCL22 produced by TAMs enables the trafficking of Tregs and myeloid-derived suppressor cells, leading to their accumulation in the ovarian TME.<sup>58</sup> In a mouse model of lung carcinoma, TAM-derived IL-10 was shown to inhibit the functional capacity of APCs by downregulating the expression of IL-12 and costimulatory molecules.<sup>59</sup> M2-like TAMs found in EOC and other solid tumors, such as melanoma, lung carcinoma and multiple myeloma, suppress effector T cells via increased secretion of TGF- $\beta$ <sup>60</sup> and by depleting the amino acids essential for T cell activation, such as tryptophan and L-arginine, reflecting the high expression of the catabolic enzymes



**Figure 1 Roles of TAMs in the TME of EOC.** Ovarian TME drives the polarization of TAMs predominantly toward M2-like phenotype. M2-like TAMs display limited tumoricidal activity and promote immunosuppression, tumor cell invasion and metastasis, and angiogenesis, by producing a variety of cytokines, chemokines, growth factors and other molecules. APC, antigen-presenting cell; ARG1, arginase 1; CCL, chemokine (C-C motif) ligand; EGF, epidermal growth factor; EOC, epithelial ovarian carcinoma; GATA3, GATA binding protein 3; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; MDSC, myeloid-derived suppressor cell; MIP-1 $\beta$ , macrophage inflammatory protein 1 $\beta$ ; miRNA, microRNA; MMP, matrix metalloproteinase; PD-L1, programmed death-ligand 1; SR-A, scavenger receptor A; TAM, tumor-associated macrophage; TGF- $\beta$ , transforming growth factor  $\beta$ ; TME, tumor microenvironment; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; Treg, regulatory T cell; TREM2, triggering receptor expressed on myeloid cells 2.

indoleamine 2,3-dioxygenase (IDO)<sup>61 62</sup> and ARG1<sup>63 64</sup> by TAMs. Interestingly, exosomes containing ARG1 found in ascites and plasma of EOC patients were found to inhibit the proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells by distributing this enzyme from tumor cells to APCs in secondary lymphoid organs.<sup>65</sup>

The functional impairment of T cell responses by TAMs can also be mediated by direct cell–cell interactions via immune checkpoint receptors and their ligands. Thus, programmed death-ligand 1 (PD-L1), a ligand of the inhibitory checkpoint receptor PD-1, and the coinhibitory molecule B7-H4, which are expressed at higher levels on TAMs than on EOC cells, induce T cell exhaustion and inactivation of cytotoxic T cell responses.<sup>66–68</sup> Recently, it was demonstrated that miRNAs (miR-29a-3p and miR-21–5p) transferred from TAMs to CD4<sup>+</sup> T cells through exosomes synergistically induce imbalance of Treg/T<sub>H</sub>17 ratio in EOC TME by targeting signal transducer

and activator of transcription 3 in CD4<sup>+</sup> T cells.<sup>69</sup> Taken together, these effects of TAMs on antitumor immune responses strongly foster the immunosuppressive TME in EOC.

### Role of TAMs in angiogenesis, invasion and metastasis

Neovascularization, a highly complex process, is crucial for tumor growth, metastasis, and immune evasion. TAMs are emerging as major promoters of angiogenesis in EOC, as in other cancers.<sup>26 70</sup> Thus, increased microvessel density and vascular permeability in EOC were correlated with increased intratumoral density of TAMs, Tregs, and T<sub>H</sub>17 cells.<sup>71</sup> In this context, reducing the infiltration of protumorigenic M2-like TAMs and blocking their function by CSF1 receptor (CSF1R) inhibitors, led to the normalization of disrupted peritoneal vasculature and a decrease in ascites volume.<sup>72</sup> The tumor foci, ascites, and peripheral blood of EOC patients also contain high frequencies

of proangiogenic TAMs characterized by the expression of TIE2, a tyrosine kinase receptor. The proportion of TIE2<sup>+</sup> TAMs was positively correlated with increased microvessel density. Supporting this notion, functional studies revealed that angiopoietin-2 (Ang2), a ligand of TIE2, enhanced the recruitment of TIE2<sup>+</sup> TAMs in the TME. Recruited TAMs promoted angiogenesis via insulin-like growth factor 1 signaling pathway.<sup>73</sup> TAMs are also involved in the regulation of EOC angiogenesis by producing a variety of proangiogenic factors, including VEGF, platelet-derived growth factor, epidermal growth factor (EGF), MMPs, osteopontin, osteonectin, cathepsins, and fibronectin.<sup>74</sup> Supporting this notion, high VEGF expression in ovarian primary tumors and peritoneal ascites was associated with poor disease outcome.<sup>75–76</sup> In addition to its direct effects on tumor cells, VEGF sustains a permissive environment supporting metastasis of EOC cells by acting on peritoneal ECs to promote angiogenesis and vascular permeability, leading to ascites and cyst formation in EOC xenograft models.<sup>77–78</sup>

TAMs promote extracellular matrix (ECM) remodeling by secreting MMPs, a process that contributes to the invasion of multiple tumor cell types, including EOC cells.<sup>26–29</sup> By producing metastasis-promoting cytokines and chemokines (eg, IL-6, TNF- $\alpha$ , CCL6, and CCL18), TAMs play a central role in the EOC cytokine network supporting the adhesion and invasion of EOC cells.<sup>41–80</sup> For instance, TAM-derived TNF- $\alpha$  enhances the invasiveness of ovarian and breast tumor cells by upregulating macrophage migration inhibitory factor, extracellular MMP inducer, and MMP secretion.<sup>79</sup> In addition, TAMs found in the omentum and malignant ascites of EOC patients sustain metastasis dissemination by secreting CCL6<sup>41</sup> and CCL18.<sup>81</sup> Interestingly, CCL6 and its human homolog CCL23 promote EOC cell migration into the omentum by activating CCR1 signaling.<sup>41</sup> There is also strong evidence that the formation of metastases in EOC is facilitated by inflammation through a mechanism largely mediated by TAMs, presumably involving stromal VEGF production. Thus, depletion of peritoneal TAMs was shown to reduce ascites formation, peritoneal metastasis, and tumor progression.<sup>82</sup> Moreover, macrophage inflammatory protein 1 $\beta$ , which is secreted by TAMs via the CCR5-phosphatidylinositol 3-kinase (PI3K) signaling pathway, mediates the upregulation of P-selectin expression on mesothelial cells resulting in an increased rolling under ascites flow and adhesion between EOC cells and mesothelial cells.<sup>83</sup>

Additional mechanism by which TAMs support malignant cell dissemination and metastasis is mediated by the formation of primary tumor cell-TAM spheroids. During EOC progression, tumor cells detach from the primary tumor and interact with TAMs to survive in the peritoneal fluid as free-floating spheroids. In an EOC mouse model of early planting metastasis, it was observed that 80% of infiltrating macrophages were located in the peritoneal spheroids. These TAMs displayed a predominantly M2-like phenotype providing matrix support and growth

factors to tumor cells. In this model TAM-secreted EGF activated the EGF receptor (EGFR) in surrounding tumor cells, thereby supporting their proliferation and protection against anoikis. TAM-tumor cell crosstalk induced autocrine VEGFC-VEGFR3 signaling in neoplastic cells, which further upregulated  $\alpha$ M $\beta$ 2 integrin and intercellular adhesion molecule 1 expression, and thus maintained cell-to-cell contacts and stabilized tumor spheroids.<sup>40</sup> Taken together, TAMs employ multiple strategies to promote invasion and metastasis of EOC, representing potential targets for promising EOC therapy.

## CLINICAL RELEVANCE OF TAMs IN EOC

### Impact of TAMs on EOC prognosis

TAM polarization has a pronounced effect on survival and the response to therapy in EOC patients. A high M1-like/M2-like TAM ratio is associated with favorable disease outcomes in EOC, whereas a low M1-like/M2-like TAM ratio is correlated with poor OS.<sup>38–84–87</sup> M1-like TAMs, defined as CD86<sup>+</sup>human leukocyte antigen-DR<sup>+</sup>inducible nitric oxide synthase<sup>+</sup> cells, are associated with good prognosis in women with EOC, largely reflecting their ability to promote robust inflammatory responses that limit disease progression.<sup>88–90</sup> Conversely, CD163<sup>+</sup>CD206<sup>+</sup>CD204<sup>+</sup> M2-like TAMs promote disease progression and their frequency increases with tumor stage, ascites volume, lymphatic invasion, and metastasis.<sup>31–88–89</sup> M2-like CD68<sup>+</sup>CD163<sup>+</sup> TAMs are highly enriched in the metastatic TME of HGSOE, where they dictate clinically relevant immunosuppression, which is correlated with a poor clinical outcome.<sup>35</sup> Interestingly, the poor prognosis of HGSOE patients was recently linked to the presence of CD68<sup>+</sup>CD163<sup>+</sup> TAMs surrounding dying or dead adipocytes within the adipose tissue of the omentum, forming a ‘crown-like structure’.<sup>91</sup> In addition, the expression of CD163 in TAMs is correlated with elevated IL-6, IL-10, TGF- $\beta$  and PUFA levels, which mediate protumor signaling and negatively affect the prognosis in EOC.<sup>80–92–94</sup> Similarly, the upregulation of PD-L1 on TAMs is associated with higher disease grade and reduced survival of EOC patients.<sup>66–67–95</sup>

However, traditionally used histological approaches and flow cytometry rely on a limited set of markers to classify TAMs. Thus, defining TAM-related gene signatures and/or their functions that are involved in tumor progression or regression could be a more sophisticated way to reveal clinically relevant TAM states. For instance, protumorigenic TAM gene signatures encompassing high expression of *SLAMF7*, *GNAS*, *TBX2-ASI*, and *LYPD6*,<sup>96</sup> *CD163*, *PCOLCE2*, and *IL6*,<sup>97</sup> or *CD163*, *VSIG4* and *MS4A7*<sup>98</sup> were correlated with poor prognosis of patients with EOC. Similarly, an analysis of single-cell transcriptomic data in multiple tumor types, including EOC, identified *SPPI*-expressing TAM state, which upregulates proangiogenic M2-associated genes, to be linked with worse prognosis.<sup>23</sup> By contrast, the upregulation of genes linked to IFN signaling<sup>99</sup> or other TAM-related

genes, including *CD3E*, *IGKV4* and *TAPI*,<sup>98</sup> was associated with favorable clinical outcome in EOC. Intriguingly, a detailed transcriptomic and proteomic analyses of TAMs isolated from EOC patient ascites showed that TAMs found in the patients with predicted short survival selectively expressed/produced protumorigenic growth factors and cytokines (*CCL18*, *KITLG*, *SEMA6B*, *S100B*) and mediators involved in ECM remodeling (*ADAMTS2*, *CTSB*, *FBLN5*) and angiogenesis (*VEGFB*), whereas TAM associated with longer survival expressed cytokines linked to effector T cell chemoattraction and activation.<sup>100</sup>

### Association between TAMs and the outcomes of immunotherapy

Over the past decade, various ICIs targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4), PD-1, or PD-L1 have been introduced for the treatment of various cancers.<sup>13</sup> However, despite expectations, EOC is one of the few malignancies where ICIs exhibit only modest activity with an objective response rate (ORR) of 8%–9%, and infrequent durable responses.<sup>14 15</sup> The basis of ICI therapy failure in EOC involves multiple mechanisms, including low tumor mutational burden (TMB), abnormal neovascularization, altered metabolism, failure to reverse T cell exhaustion, and robust humoral and cellular immunosuppression in the TME.<sup>16</sup> The abundance and phenotype of different populations of myeloid cells, particularly TAMs, are critical determinants of the primary and adaptive resistance to ICIs in a broad range of solid tumors.<sup>101 102</sup> TAMs confer resistance through direct and indirect effects on T cell effector functions by altering the cytokine/chemokine milieu, as well as by upregulating coinhibitory molecules.<sup>101</sup> Interestingly, TAMs were found to limit the efficacy of ICIs in a mouse model of colon adenocarcinoma by capturing anti-PD-1 mAbs from the surface of PD-1<sup>+</sup> T cells, a process dependent on the interaction between the antibody Fc-domain glycans and FcγRs on TAMs.<sup>103</sup>

Considering TMB and PD-L1 expression as not completely reliable predictors of ICI therapy outcome in EOC,<sup>104</sup> intensive preclinical and clinical research is now focusing on the identification of better immune biomarkers to integrate into common diagnostic assessments and clinical management of EOC patients. For instance, a density of tumor-infiltrating CD8<sup>+</sup> T cells predicts clinical benefit to anti-PD-L1 (avelumab) combined with pegylated liposomal doxorubicin (PLD) in OC patients.<sup>105</sup> Similarly, CXCR5<sup>+</sup> CD8<sup>+</sup> T cells,<sup>106</sup> known as follicular cytotoxic T cells, and CXCL13<sup>+</sup>TIM-3<sup>+</sup>CD103<sup>+</sup> tissue-resident memory CD8<sup>+</sup> T cells<sup>107</sup> can be linked to better response of EOC patients to anti-PD-1 therapy. Recently, HRD and type I IFN signaling pathways were shown to determine a positive response to combined anti-PD-1 and PARPi therapy in patients with EOC. Further spatial single-cell analyses have revealed prominent interactions between exhausted CD8<sup>+</sup> T cells and PD-L1<sup>+</sup> TAMs and/or tumor cells as mechanistic determinants of an improved response. Detailed analyses of

a sample from one extreme responder revealed that the tumor was enriched with PD-L1<sup>high</sup>CD163<sup>+</sup>IBA1<sup>+</sup>CD11b<sup>+</sup> TAMs in a tight cluster with CD8<sup>+</sup> T cells. These findings suggest that the interaction between TAMs and exhausted CD8<sup>+</sup> T cells might be the most relevant cell–cell interaction in PD-1/PD-L1-mediated immunosuppression.<sup>108</sup> In a similar context, M2-like TAMs and a TGF-β signaling pathway signature are correlated with poor responses to ICIs in gynecologic malignancies, including EOC. This may indicate a dynamic interplay between TGF-β and TAMs that suppresses the development of M1-like TAMs and preferentially induces M2-like TAMs thus amplifying the immunosuppression.<sup>109</sup>

Taken together, these results indicate a crucial impact of TAMs on the final response to immunotherapeutic interventions in EOC. However, the detailed mechanisms by which TAMs affect the outcomes of ICI therapy and the potential predictive signatures require further elucidation. Results of future studies will support the development of clinically viable strategies that modulate the TAM phenotype and functions to enhance the sensitivity of EOC to ICI therapy.

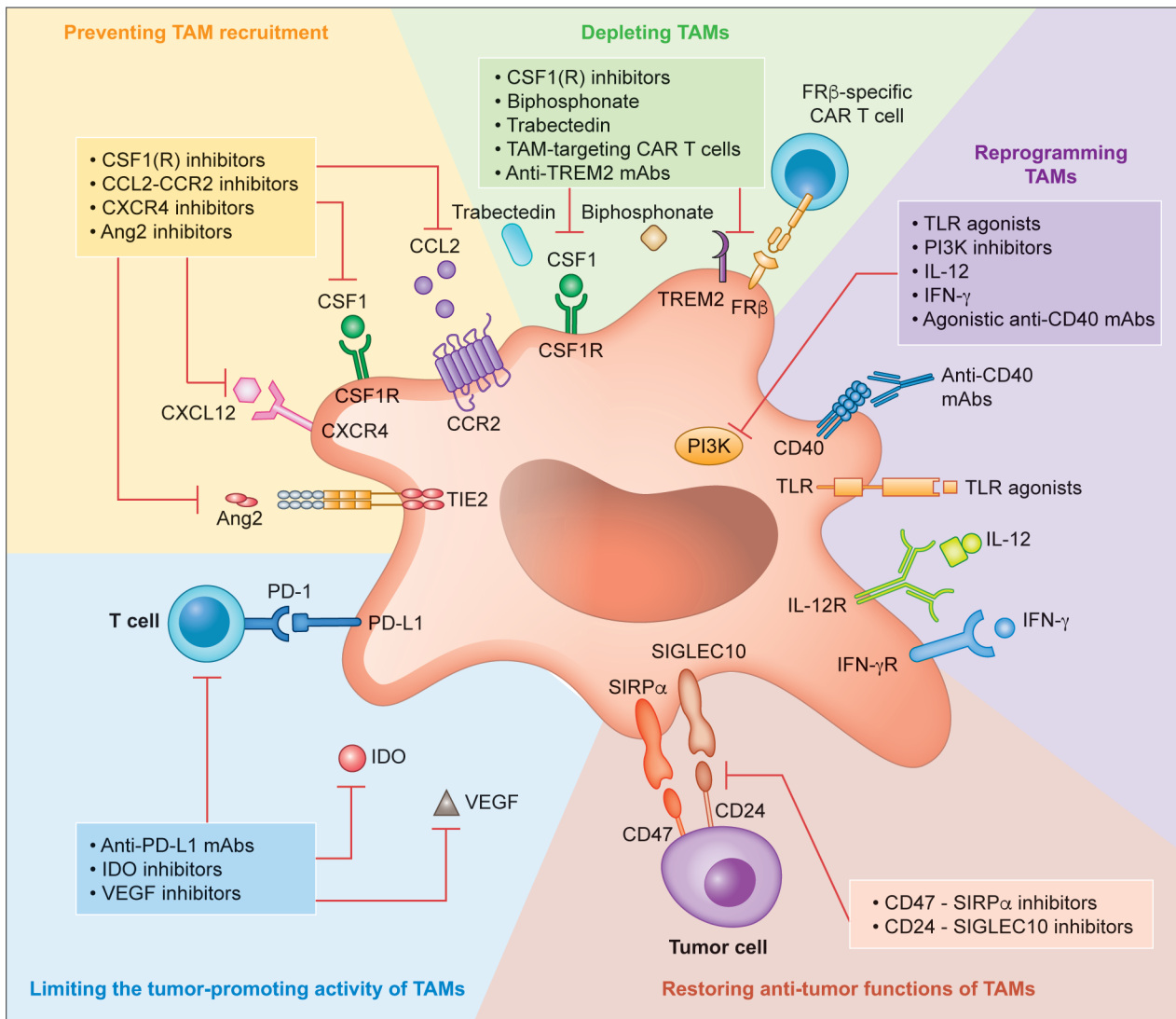
### TARGETING TAMs FOR SUCCESSFUL IMMUNOTHERAPY OF EOC

Accumulating preclinical and clinical studies are improving our understanding of the role of TAMs in tumor progression and resistance to various therapies. Thus, TAMs have been intensively explored as potential targets for cancer therapy and/or combined immunotherapy to improve the clinical outcomes of patients with EOC. The most intensively investigated TAM-targeting strategies include: (1) preventing macrophage recruitment to the TME, (2) depleting TAMs/reducing their survival, (3) TAM reprogramming/repolarization, (4) restoring the antitumor functions of TAMs, and (5) limiting the tumor-promoting activity of TAMs (figure 2).

#### Preventing TAM recruitment

##### CSF1–CSF1R inhibitors

Various chemokines, cytokines, and other factors act as chemoattractants to recruit macrophages into the ovarian TME. Thus, regulating chemoattractants is a promising approach for reducing tumor infiltration of TAMs. Overexpression of CSF1 and its receptor CSF1R is associated with poor prognosis of EOC and thus represent attractive therapeutic targets.<sup>110</sup> Activation of the CSF1–CSF1R signaling pathway promotes the production and proliferation of macrophage precursors and/or their recruitment and retention within inflamed sites, including tumors.<sup>111</sup> Blocking the CSF1–CSF1R axis with mAbs or tyrosine kinase inhibitors can either reduce TAM numbers or alter their tumor-promoting features and thus reduce tumor progression in numerous experimental models (eg, lung carcinoma, glioma and fibrosarcoma).<sup>112–114</sup> Administration of GW2580, a CSF1R inhibitor (CSF1Ri), reduced the infiltration of M2-like TAMs, increased the proportion of M1-like TAMs expressing CCR2, IL-12, and IFN-γ,



**Figure 2 TAM targets for anticancer therapy.** Schematic representation of the most intensively investigated TAM-targeting strategies in EOC. Ang2, angiopoietin-2; CAR, chimeric antigen receptor; CCL2, chemokine (C-C motif) ligand 2; CCR2, chemokine (C-C motif) receptor 2; CSF1, colony-stimulating factor 1; CSF1R, colony-stimulating factor one receptor; CXCL12, C-X-C motif chemokine ligand 10; EOC, epithelial ovarian carcinoma; FR $\beta$ , folate receptor  $\beta$ ; IDO, indoleamine 2,3-dioxygenase; IFN- $\gamma$ , interferon  $\gamma$ ; IFN- $\gamma$ R, interferon  $\gamma$  receptor; IL-12, interleukin 12; IL-12R, interleukin 12 receptor; mAb, monoclonal antibody; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; PI3K, phosphatidylinositol 3-kinase; SIGLEC10, sialic acid-binding Ig-like lectin 10; SIRP $\alpha$ , signal regulatory protein  $\alpha$ ; TAM, tumor-associated macrophage; TLR, toll-like receptor; TREM2, triggering receptor expressed on myeloid cells 2; VEGF, vascular endothelial growth factor

and increased the CD8<sup>+</sup>/CD4<sup>+</sup> T cell ratio accompanied by tumor and ascites regression in a mouse model of advanced EOC.<sup>72</sup>

Several drugs targeting CSF1 or CSF1R have been or are being tested in clinical trials for treatment of EOC. These drugs include antagonistic mAbs (such as emactuzumab, cabiralizumab and LY3022855) and small-molecule inhibitor, pexidartinib (table 2). Due to their limited efficacy as monotherapy, the current studies are evaluating the combinations with standard of care (SoC) chemotherapy and/or ICIs. In a phase I study, emactuzumab alone or in combination with SoC chemotherapy, specifically reduced immunosuppressive TAMs in patients with advanced solid tumors, including EOC. However, this approach did not elicit clinically relevant antitumor

activity.<sup>115</sup> Other clinical trials of CSF1R-targeting agents in EOC have used emactuzumab combined with a CD40 agonist (selicrelumab) (NCT02760797),<sup>116</sup> LY3022855 combined with anti-PD-L1 mAb (durvalumab) or anti-CTLA-4 mAb (tremelimumab) (NCT02718911),<sup>117</sup> and a small-molecule CSF1Ri (pexidartinib, PLX3397) combined with paclitaxel (NCT01525602) or anti-PD-1 mAb (pembrolizumab) (NCT02452424) (table 2). Although, these drugs are well tolerated, the clinical benefits in EOC patients were limited potentially due to indiscriminate ablation of TAMs which might lead to detrimental effects through depletion of proinflammatory TAMs or due to subsequent accumulation of other tumor promoting immune cells such as tumor-associated neutrophils.<sup>118</sup> The results of ongoing clinical trials evaluating

**Table 2** Clinical trials of agents depleting TAMs or preventing their recruitment

Target	Agent	Mechanism of action	Combination partners	Phase	Status	Results	Identifier	Ref.
CSF1R	Emactuzumab	antagonistic anti-CSF1R mAb	Monotherapy; paclitaxel	I	Completed	↓immunosuppressive TAMs; no clinically relevant antitumor activity	NCT01494688	115
			Paclitaxel+anti-VEGF-A mAb bevacizumab	II	Active	–	NCT02923739	–
			Agonistic anti-CD40 mAb selicrelumab	I	Completed	↓CD14 <sup>dim</sup> CD16 <sup>bright</sup> monocytes; ↑activated Ki67 <sup>+</sup> CD8 <sup>+</sup> T cells; limited objective clinical responses	NCT02760797	116
	Cabiralizumab	Antagonistic anti-CSF1R mAb	Anti-PD-1 mAb nivolumab	I	Completed	–	NCT02526017	–
	LY3022855	Antagonistic anti-CSF1R mAb	Anti-PD-L1 mAb durvalumab; anti-CTLA-4 mAb tremelimumab	I	Completed	Limited clinical activity	NCT02718911	117
	Pexidartinib	Inhibitor of CSF1R tyrosine kinase activity	Paclitaxel	I	Completed	–	NCT01525602	–
	(PLX3397)							
CCL2			Pembrolizumab	I/II	Terminated	Insufficient evidence of clinical efficacy	NCT02452424	–
	Carlumab (CNT0888)	Anti-CCL2 mAb neutralizing CCL2-induced chemotaxis	Monotherapy	I	Completed	Evidence of antitumor activity	NCT00537368	123
Ang2			SoC chemotherapy	I	Completed	Limited tumor responses	NCT01204996	122
	MEDI8617	Anti-Ang2 mAb inhibiting Ang2 binding to TIE2	Monotherapy; paclitaxel; paclitaxel+carboplatin; bevacizumab	I	Completed	Limited clinical activity	NCT01248949	124
	Trebananib (AMG-386)	Anti-Ang peptibody inhibiting Ang1/2 binding to TIE2	Paclitaxel+carboplatin	III	Terminated	No improvement in PFS	NCT01493505	125
			Pembrolizumab	I	Active	–	NCT03239145	–
VEGF/Ang2	Vanucizumab (RG7221)	Anti-VEGF/Ang2 bispecific mAb inhibiting neoangiogenesis	Monotherapy; anti-PD-L1 mAb atezolizumab	I	Completed	Reduced tumor vascularity; encouraging antitumor activity	NCT01688206	126
TREM2	PY314	Anti-TREM2 mAb depleting TREM2 <sup>+</sup> TAMs through ADCC and/or ADCP	Monotherapy; pembrolizumab	I	Recruiting	–	NCT04691375	–

ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CCL2, C-C motif chemokine ligand 2; CSF1R, colony-stimulating factor one receptor; CTLA-4, cytotoxic T-lymphocyte antigen 4; mAb, monoclonal antibody; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; SoC, standard of care; TAM, tumor-associated macrophage; TREM2, triggering receptor expressed on myeloid cells 2; VEGF, vascular endothelial growth factor.



emactuzumab combined with SoC chemotherapy and bevacizumab in patients with platinum-resistant EOC (NCT02923739), and cabiralizumab combined with an anti-PD-1 mAb (nivolumab) in patients with advanced solid tumors (NCT02526017) are highly anticipated.

### CCL2–CCR2 inhibitors

The accumulation of macrophages in tumors is also mediated by the CCL2–CCR2 signaling pathway. CCR2 expressed on circulating inflammatory monocytes binds to CCL2 produced by malignant and stromal cells promoting monocyte differentiation into TAMs on extravasation into the tumor stroma.<sup>119</sup> Inhibition of CCL2–CCR2 signaling limits the accumulation of inflammatory monocytes in tumors and delays tumor progression and metastasis in multiple mouse experimental models (eg, breast, prostate and colorectal cancer).<sup>119,120</sup> Because EOC cells can release CCL2<sup>45</sup> and some paclitaxel-resistant EOC cell lines exhibit high CCL2 expression,<sup>121</sup> targeting CCL2–CCR2 axis might represent a promising strategy to deplete TAMs in EOC. Two phase I trials have since investigated the effects of an anti-CCL2 mAb (carlumab, CNTO888) as monotherapy or combined with SoC chemotherapy in patients with solid tumors, including advanced EOC. Despite a favorable safety profile, the objective clinical responses were limited (NCT00537368 and NCT01204996)<sup>122,123</sup> (table 2).

### Ang2–TIE2 inhibitors

Besides chemokines and other soluble factors directly involved in TAM infiltration into the TME, TIE2<sup>+</sup> monocytes are recruited into the tumor via the growth factor Ang2, which further enhances the proangiogenic activities of TAMs in EOC.<sup>73</sup> However, in clinical studies, a mAb targeting Ang2 (MEDI3617) (NCT01248949)<sup>124</sup> or an anti-angiopoietin peptibody (trebananib, AMG-386) (NCT01493505)<sup>125</sup> in combination with bevacizumab and/or SoC chemotherapy did not improve the disease outcomes in patients with advanced EOC. Nevertheless, a bispecific mAb targeting both VEGF and Ang2 (vanucizumab, RG7221/RO5520985) reduced tumor vascularity and displayed encouraging anticancer activity in an early phase clinical trial in patients with advanced solid tumors, including EOC (NCT01688206)<sup>126</sup> (table 2). These findings provide a rationale for simultaneous targeting of Ang2 and VEGF in EOC.

### Depleting TAMs

Beyond mAbs and tyrosine kinase inhibitors targeting CSF1 or CSF1R, TAMs can be directly depleted by pharmacological agents, including bisphosphonates (eg, clodronate and alendronic acid) and trabectedin. Besides their direct antitumor effects,<sup>127</sup> these agents also modulate the immune contexture in the TME. For instance, clodronate reduces the numbers of TAMs by inhibiting the secretion of proangiogenic cytokines by ECs in syngeneic murine models of EOC<sup>128</sup> and trabectedin has the capacity to promote caspase-8-dependent apoptosis in mononuclear

phagocytes.<sup>129</sup> However, these pan-macrophage therapeutic approaches may limit critical proinflammatory responses and/or cause systemic toxicities. Thus, it is necessary to develop more specific agents that re-educate the TME by restraining tumor-promoting M2-like TAMs while promoting antitumor M1-like TAMs for clinical testing. Recent technological advances have aided the generation of chimeric antigen receptor (CAR) T cells targeting specific immunosuppressive TAM subsets. For instance, CAR T cells specific for folate receptor  $\beta$  (FR $\beta$ ) can recognize and lyse FR $\beta$ <sup>+</sup> M2-like TAMs, as demonstrated in solid tumors, including EOC. Importantly, this approach reprograms the TME by promoting endogenous antitumor T cell-mediated immunity and tumor regression in murine tumor models.<sup>130</sup>

Recently, transmembrane protein triggering receptor expressed on myeloid cells 2 (TREM2) has emerged as an ideal candidate for promoting M2-like TAM depletion in EOC.<sup>131,132</sup> TREM2<sup>+</sup> TAMs display enhanced capacity to produce clinically relevant immunosuppressive factors in EOC patients. In addition, TREM2 expression is linked to immune cell exhaustion and resistance to anti-PD-1 therapies in murine models of ovarian, breast and colon cancer.<sup>131,132</sup> Consistent with this notion, anti-TREM2 mAbs were shown to deplete TAMs, potentiate the activation of intratumoral CD8<sup>+</sup> T cells, and reverse anti-PD-1 therapy resistance in the above-mentioned experimental models.<sup>131,132</sup> Based on promising preclinical findings, humanized anti-TREM2 mAb (PY314) is currently being tested in a phase I clinical trial in patients with advanced solid tumors, including EOC (NCT04691375) (table 2).

### Reprogramming TAMs

#### TLR agonists

Reprogramming immunosuppressive TAMs into immunostimulatory TAMs is rapidly emerging as a new therapeutic approach. One such strategy involves activating TLRs expressed by macrophages and other myeloid cells by TLR agonists to trigger potent inflammatory reactions, including the generation of TAMs with M1-like phenotypes, which could protect the host against infections and promote antitumor immunity.<sup>133</sup> TLR7 (852A, NCT00319748) and TLR8 (motolimod (VTX-2337), NCT01294293 and NCT01666444) agonists have since been evaluated in clinical trials in patients with solid cancers, including advanced EOC (table 3). TLR agonists demonstrated considerable potency in patients with bladder, superficial basal cell carcinoma, and cervical cancer, which led to their approval by the United States Food and Drug Administration as anticancer agents.<sup>134</sup> However, 852A and motolimod displayed limited clinical activity in EOC in two clinical trials (NCT00319748 and NCT01666444, respectively).<sup>135,136</sup> Nevertheless, motolimod induced clinically relevant immunomodulation of the TME in patients with injection site reactions (NCT01666444).<sup>136</sup> In addition, vidutolimod (CMP-001), a virus-like particle-encapsulated TLR9 agonist, is currently being tested in combination with an anti-PD-L1

**Table 3** Clinical trials of TAM reprogrammingagents in EOC

Target	Agent	Mechanism of action	Combination partners	Phase	Status	Results	Identifier	Ref.
TLR7	852A	TLR7 agonist inducing the activation of innate immune cell populations	Monotherapy	II	Completed	↑immune cell activation - ↑CXCL10↑IL-1ra; modest clinical benefit	NCT00319748	135
TLR8	Motolimod	TLR8 agonist inducing the activation of innate immune cell populations	PLD; paclitaxel	I	Completed	-	NCT01294293	-
TLR9	Vidutolimod (CMP-001)	VLP-encapsulated TLR9 agonist	PLD	II	Completed	Improved OS in patients with ISRs	NCT01666444	136
			Anti-PD-L1 mAb avelumab; avelumab+anti-4-1BB mAb utomilumab; avelumab+anti-OX40 mAb	II	Active	-	NCT02554812	-
Pl3K $\gamma$	Eganelisib (IP-549)	Selective Pl3K $\gamma$ inhibitor reprogramming key immune suppressive cells	Monotherapy; nivolumab	I	Active	-	NCT02637531	-
			Dual adenosine receptor antagonist etrumadenant (AB928)+PLD	I	Completed	-	NCT03719326	-
CD40	Selicrelumab	Agonistic anti-CD40 mAb potentiating APC functions	Paclitaxel+carboplatin	I	Completed	Evidence of antitumor activity with 20% of patients displaying PRs	NCT00607048	146
			Bevacizumab; bevacizumab+vanucizumab	I	Completed	-	NCT02665416	-
	CDX-1140	Agonistic anti-CD40 mAb potentiating APC functions	Monotherapy; gemcitabine+paclitaxel; pembrolizumab or FLT3L	I	Active	-	NCT03329950	-
			Pembrolizumab+bevacizumab	II	Not yet recruiting	-	NCT05231122	-
IL-12	GEN-1	IL-12 plasmid/lipopolymer complex stimulating the antitumor immune responses	Neoadjuvant paclitaxel+carboplatin	I	Completed	↑IL-12, IFN- $\gamma$ ; ↑CD8 <sup>+</sup> T cells and ↓immunosuppression in the TME; preliminary clinical activity	NCT02480374	155
			Neoadjuvant paclitaxel+carboplatin	II	Recruiting	-	NCT03393884	-
HER2	CT-0508	CAR macrophages targeting HER2 <sup>+</sup> tumor cells	Monotherapy	I	Recruiting	-	NCT04660929	-
CD47	AO-176	Antagonistic anti-CD47 mAb promoting phagocytosis and direct tumor cell killing	Monotherapy; paclitaxel; pembrolizumab	I/II	Recruiting	-	NCT03834948	-
	Magrolimab (Hu5F9-G4)	Antagonistic anti-CD47 mAb promoting phagocytosis and direct tumor cell killing	Monotherapy	I	Completed	PRs in 2 out of 13 OC patients; ↓CA125	NCT02216409	167
			Avelumab	I	Completed	-	NCT03558139	-
			Anti-EGFR mAb cetuximab	I/II	Completed	↑TAM infiltration; disease stabilization in some patients	NCT02953782	166
	TTI-622	Fusion protein blocking CD47	PLD	I/II	Recruiting	-	NCT05261490	-
	SL-172154	Fusion protein targeting CD47 on tumor cells and CD40 on APCs		I	Recruiting	-	NCT04406623	-
CD47/mesothelin	NI-1801	Anti-CD47/mesothelin bispecific mAb	Monotherapy	I	Recruiting	-	NCT05403554	-
CD47/PDL1	PF-07257876	Anti-CD47/PD-L1 bispecific mAb	Monotherapy	I	Recruiting	-	NCT04881045	-
SIRP $\alpha$	BI-765063	antagonistic anti-SIRP $\alpha$ mAb	Anti-PD-1 mAb ezabenlimab	I	Recruiting	-	NCT03990233	-

Continued

**Table 3** Continued

Target	Agent	Mechanism of action	Combination partners	Phase	Status	Results	Identifier	Ref.
IDO	Epacadostat	IDO inhibitor restoring the activation of immune cells	Monotherapy	II	Terminated	Lack of evidence of superiority	NCT01685255	176
			CRS-207 (Listeria-based vaccine expressing human mesothelin); CRS-207+pembrolizumab	I/II	Terminated	Lack of clinical activity	NCT02575807	-
			Pembrolizumab	II	Terminated	-	NCT03602586	-
			Monotherapy	I	Active	-	NCT02042430	-
			Nivolumab; nivolumab+chemotherapy	I/II	Completed	-	NCT02327078	-

APC, antigen-presenting cell; CAR, chimeric antigen receptor; CXCL10, C-X-C motif chemokine ligand 10; EGFR, epidermal growth factor receptor; FLT3L, FMS-like tyrosine kinase three ligand; HER2, human epidermal growth factor receptor 2; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; ISR, injection site reaction; mAb, monoclonal antibody; OC, ovarian cancer; OS, overall survival; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; PI3K $\gamma$ , phosphatidylinositol 3-Kinase  $\gamma$ ; PLD, pegylated liposomal doxorubicin; PR, partial response; SIRP $\alpha$ , signal regulatory protein  $\alpha$ ; TAM, tumor-associated macrophage; TLR, toll-like receptor; TME, tumor microenvironment; VLP, virus-like particle.

mAb (avelumab) in patients with advanced or metastatic solid tumors, including platinum-resistant EOC, in a phase II clinical trial (NCT02554812) (table 3). To improve the pharmacokinetic profiles and reduce the risk of severe side effects, ongoing preclinical studies are focusing on specific and targeted delivery of TLR agonists.<sup>137</sup>

### PI3K inhibitors

Blocking the PI3K signaling pathway represents another therapeutic approach for repolarizing TAMs and inhibiting tumor progression in EOC. Triptolide, an inhibitor of the PI3K–Akt–nuclear factor- $\kappa$ B axis, promoted M1 TAMs polarization and reduced the proliferation, migration, and invasion of a cisplatin-resistant human EOC cell line and lowered the tumor burden in mice.<sup>138</sup> However, the effects of triptolide are relatively unspecific due to broad, not only antitumor but also anti-inflammatory and immunosuppressive activities.<sup>139</sup> Thus, specific targeting of PI3K pathway could be a better approach to avoid potential side effects. For instance, administration of eganelisib (IPI-549), which selectively inactivates the PI3K $\gamma$  isoform expressed by macrophages that is associated with immunosuppression and tumor growth,<sup>140</sup> restored the immunostimulatory transcriptional program, resulting in CTL activation. It also synergized with ICI therapy to promote tumor regression and increased survival in murine models of melanoma, breast and colon cancer.<sup>140 141</sup> Eganelisib is currently being tested as monotherapy and in combination with nivolumab (MARIO-1, NCT02637531) or in combination with a dual adenosine receptor antagonist (etrumadenant, AB928) plus PLD in patients with advanced solid tumors, including EOC (NCT03719326) (table 3).

### CD40 agonists

Among the TAM repolarization strategies, agonistic mAbs targeting the co-stimulatory molecule CD40 have also been a focus of clinical investigation in EOC. CD40 signaling in macrophages and dendritic cells (DCs) stimulates IL-12 production and promotes T<sub>H1</sub> cell-dependent immunity, which includes IFN- $\gamma$  production by lymphocytes and upregulation of MHC class I molecules, features typically associated with antitumor activity.<sup>142</sup> In several preclinical models (eg, pancreatic carcinoma), anti-CD40 mAbs potentiated a macrophage-driven shift in the tumor immune landscape, and had considerable anticancer effects as monotherapy<sup>143 144</sup> or in combination with other agents, including ICIs.<sup>145</sup> Consistent with this notion, the anti-CD40 mAb selicrelumab combined with SoC chemotherapy was well tolerated and a partial response was observed in 20% of patients with advanced solid tumors, including EOC (NCT00607048).<sup>146</sup> Other potential therapeutic partners, such as bevacizumab and/or vanucizumab (NCT02665416), are under clinical investigation. In addition, phase I and II trials assessing the therapeutic potential of another anti-CD40 mAb (CDX-1140) with pembrolizumab or chemotherapy (NCT03329950) or with pembrolizumab plus bevacizumab (NCT05231122)

in patients with EOC are ongoing (table 3). The results of these studies will provide the first insight into whether anti-CD40 mAb-mediated TAM repolarization can skew the immune landscape in the ovarian TME toward improved responsiveness of ICI therapy. To avoid activating macrophages outside malignant tissue, which might result in immunotherapy-related adverse events, novel agents are currently under development, including bispecific antibodies with conditional activity dependent on binding to a tumor-specific antigen (eg, ABBV-428 targeting human CD40 and mesothelin),<sup>147</sup> CAR T cells engineered to constitutively express CD40L,<sup>148</sup> or intratumorally delivered oncolytic viruses armed with the *CD40L* gene (eg, LOAd703, a designed adenovirus armed with trimerized *CD40L* and *4-1BBL*).<sup>149</sup>

### IL-12 and IFN- $\gamma$

Polarization of TAMs in the TME is largely impacted by cytokines, including IL-12 and IFN- $\gamma$ , which typically promote their differentiation toward the M1-like phenotype.<sup>89</sup> Thus, on IFN- $\gamma$  exposure, TAMs purified from EOC ascites retrieved the M1 phenotype, down-regulated the secretion of tumor-promoting mediators (eg, CCL18, VEGF and MMP-9), and potentiated the adaptive antitumor immune responses.<sup>150</sup> Despite expectations, the success of recombinant IL-12 and IFN- $\gamma$  in cancer immunotherapy trials was limited, mainly due to their short half-life and toxicity-related side effects. Thus, there has been some progress toward the development of alternative delivery methods using appropriate carriers with the purpose of achieving greater therapeutic outcomes accompanied with reduced toxicity.<sup>151–152</sup> Initial phase I trials of an IL-12-plasmid/lipopolymer complex (termed GEN-1) administered intraperitoneally in patients with advanced EOC demonstrated favorable safety profiles.<sup>153–154</sup> This complex also showed potential synergy with SoC chemotherapy, in terms of reduced intratumoral immunosuppression with preliminary evidence of clinical activity (NCT02480374).<sup>155</sup> GEN-1 is now being tested in a phase 2 clinical trial (OVATION 2, NCT03393884) (table 3). Several other strategies aimed at enhancing local delivery and controlling the release of IFN- $\gamma$ , including liposomes, biodegradable microspheres, gene therapy or nanoparticles, have been investigated but their success was limited due to pharmacologic and pharmacodynamic obstacles.<sup>151</sup>

Moreover, recent preclinical studies have focused on the generation of genetically engineered T cells and/or myeloid cells with stable expression of TAM-reprogramming cytokines in the TME. T cells engineered to release IFN- $\gamma$  and granulocyte-macrophage CSF were shown to activate TAM precursors leading to IL-12 production and tumor rejection in a murine model of OC.<sup>156</sup> Similarly, CAR T cells engineered to release inducible IL-12 on CAR engagement in the tumor lesion helped eliminate neoplastic cells, which was accompanied by the accumulation of activated TAMs in a syngeneic mouse model of glioblastoma and melanoma.<sup>157</sup>

Furthermore, macrophages can be also manipulated to secrete IL-12<sup>158</sup> or express specific cell surface receptors. As an example, primary human monocyte-derived macrophages were modified using an adenoviral vector with a CAR targeting human epidermal growth factor receptor 2 (HER2) to recognize and eliminate HER2<sup>+</sup> tumor cells. A single infusion of CAR macrophages decreased tumor burden and delayed the progression of solid tumor xenografts, including HER2<sup>+</sup> EOC, in mice. Interestingly, it was observed that, regardless of CAR expression, these engineered macrophages displayed a proinflammatory M1 phenotype, which resisted the effects of immunosuppressive cytokines, thus facilitating antitumor immune response.<sup>159</sup> This approach is currently being evaluated in a phase I clinical trial in patients with HER2<sup>+</sup> solid tumors, including EOC (NCT04660929) (table 3). Given these promising results, genetically engineered macrophages represent an exciting future direction for targeting the immunosuppressive microenvironment in solid tumors. However, the heterogeneity and plasticity of macrophage subsets need to be carefully analyzed to optimize the clinical responses in a tumor-specific manner.

### Restoring the antitumor functions of TAMs

The phagocytosis of malignant cells and the presentation of tumor antigens to T cells by APCs, such as macrophages and DCs, are crucial for activating antitumor immunity. Phagocytosis is triggered by ‘eat me’ signals displayed on target non-self or modified-self cells that engage phagocytic receptors on macrophages. Conversely, healthy cells display ‘don’t eat me’ signals (eg, CD47 and CD24), which bind to cognate inhibitory receptors, such as signal regulatory protein- $\alpha$  (SIRP $\alpha$ ) and sialic acid-binding Ig-like lectin 10, representing important innate immune checkpoints for preventing autoreactivity.<sup>160</sup> These interactions are also employed by malignant cells to escape phagocytosis. Supporting this notion, high expression of CD47 and CD24 in EOC cells is correlated with poor clinical outcomes.<sup>161–164</sup> Importantly, blocking these pathways with neutralizing anti-CD47 and anti-CD24 mAbs enhanced non-specific macrophage-mediated phagocytosis, thus inhibiting tumor growth in murine EOC models.<sup>163–165</sup> Anti-CD47 mAbs AO-176 (NCT03834948) and magrolimab (Hu5F9-G4) (NCT02216409, NCT03558139 and NCT02953782) and anti-SIRP $\alpha$  mAb (BI-765063) (NCT03990233) have been or are currently being investigated in multiple clinical trials in various tumors, including EOC (table 3). Although antagonistic anti-CD47 mAbs, alone or in combination, showed promising clinical activity,<sup>166–167</sup> the ubiquitous expression of CD47 can result in off-target and adverse side effects, particularly platelet aggregation and hemagglutination. Thus, several new strategies have been developed to limit the risk of side effects, such as anti-CD47/tumor antigen or immune checkpoint molecule bispecific antibodies (NI-1801 or PF-07257876, respectively) and CD47-targeting fusion proteins (TTI-622 and SL-172154), which are now under clinical investigation (table 3).

## Limiting the tumor-promoting activity of TAMs

### IDO inhibitors

An alternative strategy to resolve TAM-mediated immunosuppression involves direct inhibition of their pro-tumoral functions, including the production of IDO, which is implicated in the suppression of CTL proliferation<sup>168 169</sup> and linked to peritoneal dissemination and poor survival outcomes in patients with EOC.<sup>170 171</sup> Indeed, competitive inhibition of IDO with 1-methyl-D-tryptophan was shown to reactivate immune cells, including CD8<sup>+</sup> T and NK cells, and reduced tumor cell dissemination and invasion in preclinical OC models.<sup>171 172</sup> Despite promising results in early phase clinical trials,<sup>173–175</sup> an IDO inhibitor (epacadostat) failed to demonstrate sufficient clinical activity in patients with EOC in phase II clinical trials (NCT01685255, NCT02575807)<sup>176</sup> (table 3). This lack of efficacy might be explained by metabolic adaptation involving a switch from tryptophan catabolism toward the serotonin pathway, resulting in elevated nicotinamide adenine dinucleotide (NAD<sup>+</sup>) further reducing T cell effector functions. This adaptive resistance might be overcome by combining epacadostat with A2a/A2b purinergic receptor antagonists, as demonstrated in a preclinical IDO-overexpressing experimental EOC model.<sup>177</sup>

### Immune checkpoint (PD-L1) inhibitors

Myelomonocytic cells are part of tumor-extrinsic pathways of primary and adaptive resistance to ICIs by expressing several immunosuppressive molecules, including checkpoint ligands, such as PD-L1, PD-L2.<sup>66 178</sup> Besides the indisputable impact of PD-1–PD-L1 blockade on the functional capacity of CTLs,<sup>179–181</sup> this approach also affects the survival, proliferation, and activation of human and murine immunostimulatory TAMs leading to TAM-mediated anticancer activity, and reduced tumor growth *in vivo*.<sup>182</sup> Several mAbs targeting PD-L1 (eg, avelumab, atezolizumab and durvalumab) have been tested in numerous clinical studies in patients with EOC. However, the preclinical impact of anti-PD-L1 monotherapy or combination with other ICIs were not confirmed in the early phase clinical trials in patients with advanced EOC.<sup>14 183</sup> These findings have prompted subsequent studies to focus on new combinatorial approaches, in which PARPi and anti-angiogenic drugs are the most promising candidates for enhancing the clinical effectiveness of ICIs. PARPi could potentiate ICI activity by multiple mechanisms, which are mainly induced by DNA damage. PARPi-mediated DDR failure promotes the accumulation of double-stranded DNA in cytosol, activating the well-characterized cyclic guanosine monophosphate-adenine monophosphate synthase/stimulator of interferon genes pathway, resulting in the production of type I IFNs and enhanced antitumor immune responses.<sup>184</sup> Defects in DDR pathway are also often associated with an increased TMB, which can be further amplified in the presence of HRDs like *BRCA1/2* mutations and correlates with tumor-infiltrating lymphocyte (TIL) accumulation in the TME.<sup>185 186</sup> In addition, PARPi upregulate PD-L1

expression in tumor cells.<sup>187</sup> Durvalumab combined with a PARPi (olaparib) was tested in three phase I/II clinical studies in patients with recurrent EOC. In one study (MEDIOLA), the 12-week disease control rate (DCR) was 81% (NCT02734004)<sup>188</sup> and the other studies reported a DCR of 53% (durvalumab plus olaparib or VEGFR1-3 inhibitor (cediranib), NCT02484404),<sup>189</sup> and enhanced IFN- $\gamma$ /TNF- $\alpha$  production, an increase in the numbers of TILs and a DCR of 71% (NCT02484404).<sup>190</sup> However, further research is needed to identify potential predictors of the therapeutic response. Moreover, there is evidence that blocking the VEGF–VEGFR pathway is necessary to improve the efficacy of combined anti-PD-L1 and PARPi therapy. In this respect, an ongoing phase III trial (DUO-O, NCT03737643) is investigating the benefit of durvalumab combined with chemotherapy and bevacizumab, followed by durvalumab, bevacizumab, and olaparib in the maintenance setting in patients with newly diagnosed advanced HGSOE after cytoreductive surgery. The results of these trials are urgently awaited.

### VEGF–VEGFR inhibitors

The anti-angiogenic drug bevacizumab has now been employed for first-line management of advanced EOC for more than 7 years.<sup>9</sup> Based on preclinical findings from experimental models of EOC, bevacizumab can synergize with ICIs.<sup>191</sup> Thus, the combination of bevacizumab and nivolumab has been associated with improved ORR (28.9%) and PFS (median 9.4 months) in patients with relapsed EOC.<sup>192</sup> However, most patients develop resistance to bevacizumab treatment, which seems to be largely mediated by TAMs.<sup>193 194</sup> Consistent with this, bevacizumab-resistant EOC exhibited a restored response after treatment with a TAM-depleting anti-CSF1 mAb in a murine experimental model.<sup>195</sup> Specifically, VEGF–VEGFR blockade enhanced tumor hypoxia resulting in chemoattraction of proangiogenic TAM subsets to restore neoangiogenesis.<sup>196</sup> This effect underlies the limitations of therapeutically targeting a single mediator, such as VEGF, due to the redundancy within the TME. Thus, combinatorial approaches targeting neoangiogenesis are needed. For instance, blocking the Ang2–TIE2 pathway or simultaneous inhibition of VEGF–VEGFR and Ang2–TIE2 axis by mAbs or other agents have been investigated clinically in patients with advanced EOC, as described above. Using mAbs to target molecules specifically expressed by proangiogenic TAM subsets might represent a more durable therapeutic strategy. This could be achieved by identifying the surface markers selectively expressed on TAM subsets coexpressing VEGF and/or TIE2 to achieve selective depletion without inhibiting anticancer TAM subsets.

## CONCLUSIONS

EOC is a highly lethal malignancy with limited responses to the current immunotherapy approaches, primarily due to indolent anticancer immunity and robust humoral and cellular immunosuppression.<sup>16 102</sup> TAMs constitute

the most abundant infiltrating immune cell population in human EOC and ascites, and are associated with disease progression, therapy resistance, and poor clinical outcomes.<sup>31 36 89 90</sup> Thus, TAMs represent attractive targets for developing novel anticancer regimens aiming to reverse the strong immunosuppression that exists within the TME.

Current TAM-targeting strategies mainly seek to deplete M2-like TAMs and/or favor their repolarization toward an inflammatory M1-like phenotype. However, the clinical implementation of these approaches has been limited to date, mostly due to the high heterogeneity and plasticity of TAMs.<sup>19 133</sup> Because TAMs display high potential for adopting distinct phenotypes and functions in response to the local microenvironment, various TAM states exist in different tumor types and locations within the same tumor.<sup>18</sup> To design and develop effective anticancer agents targeting TAMs, it is crucial to expand our knowledge of the biology and behavior of these cells, and to identify markers that can distinguish tumor-promoting TAMs from anticancer TAMs in order to rationally define which TAM states need to be suppressed or enhanced. Moreover, there are several questions regarding the development of TAM-targeting agents that remain to be addressed. First, how does the TAM landscape evolve during cancer progression and in response to SoC therapy and/or immunotherapy? Second, what is the optimal treatment schedule in clinical practice? Most combinatorial regimens are developed based on coadministration paradigms that are not necessarily the most efficient approach in the clinic. Third, what is the best strategy for targeting the key interactions between TAMs and other immune/non-immune cell compartments that promote tumor progression in immunologically ‘indolent’ tumors such as EOC?

In conclusion, better understanding of the temporal and spatial evolution of the TAM compartment in EOC on therapeutic intervention will enhance our insight into this extremely complex immune cell component. In this context, exploiting various functional, epigenetic, and metabolic pathways intrinsic to macrophages to target TAM-mediated immunosuppression and tumor progression in combination with other immunotherapeutic agents might enhance the effectiveness of anticancer therapies, and thus lead to the development of superior combinatorial regimens for clinical care of patients with EOC in the future.

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