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REVIEW

Macrophage and monocyte subsets as new therapeutic targets in cancer immunotherapy

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The introduction of immune checkpoint inhibitors (ICIs) for the treatment of solid cancers dramatically turned the tables in clinical routine. However, therapy success is still limited with up to 70% of non-responders in patients with ICI treatment. Traditionally, most immunotherapy approaches aim at directly stimulating anti-tumor T cell responses. More recently, tumor-associated macrophages have come into focus due to their predominance in solid tumors. Intensive cross-talk with tumor cells and immune as well as stromal cells within the tumor microenvironment can drive either pro- or anti-tumorigenic macrophage phenotypes. In turn, tumor-associated macrophages strongly shape cytokine and metabolite levels in the tumor microenvironment and thus are central players in anti-tumor immunity. Thus, ambivalent macrophage populations exist which raises therapeutic possibilities to either enhance or diminish their functionality. However, molecular signals controlling tumor-associated macrophage polarization are incompletely understood. Gaining in-depth understanding of monocyte/macrophage properties both in circulation and within distinct tumor microenvironments would (i) allow the development of new therapeutic approaches, and (ii) could additionally aid our understanding of underlying mechanisms limiting current therapy with the option of combinatorial therapies to increase efficacy. In this review, we summarize recent data addressing heterogeneity of tumor-associated macrophage populations and we discuss strategies to target macrophages using known molecular pathways with the potential for straight-forward clinical application.

Key words: monocytes, macrophages, tumor-associated macrophages, immunotherapy, immune checkpoint blockade

BLOOD MONOCYTES AND TISSUE MACROPHAGES IN CANCER

Blood monocytes: heterogeneity by redefinition

Circulating human blood monocytes were originally characterized by expression of CD14, a lipopolysaccharide co-receptor.[1] The discovery of CD16 co-expression led to a redefinition of human monocytes in 2010[2] and the classification in classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and non-classical ($CD14^{+}CD16^{++}$) monocytes.[3] These three subsets hold distinct functions and differ in expression levels of additional surface markers, e.g. Fcγ receptors (FcγR), human leukocyte antigens (Figure 1), and chemokine receptors.[4] As already described for other pathological conditions,[3] altered monocyte subset ratios have also been observed in various cancer entities.[5-9] Shifts in monocyte subset frequencies can be due to multiple mechanisms including alterations in monopoiesis and/or direct changes in molecular phenotypes in response to cancer-derived signals. Cytokines regulating monopoiesis, such as colony-stimulating factor (CSF) 1, CSF2, and CSF3, are reported to be altered in the blood of cancer patients which, together with systemic inflammation, reprogram monopoiesis.[10] *In vitro* data suggest that direct contact of monocytes with tumor cells[9,11] or tumor cell supernatant[7,12] trigger direct responses of circulating monocyte subsets to tumor-derived signals. Soluble factors, as well as extracellular vesicles (membrane-enclosed vesicular structures derived from tumor cells), were attributed an active role in inducing pro-tumorigenic phenotypes in monocytes[12,13] and their differentiation into suppressive myeloid cells.[14,15] In summary, monocytes adapt to tumor-derived stimuli, which likely drive their pro-tumorigenic properties.

Tissue macrophages: classification by ontogeny

Like blood monocytes, macrophages are characterized by enormous plasticity and cannot only adapt various

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Subset	Distribution[a]	CD14	CD16 (FcγRIII)	CD64 (FcγRI)	Properties
Classical	85%	++	–	++	Phagocytic
Intermediate	5%	++	+	+	Phagocytic/pro-inflammatory
Non-classical	10%	+	++	+	Pro-inflammatory

[a]In healthy individuals

Figure 1. Monocyte subsets.
Monocyte subset characteristics (based on Wong et al.[3] and Ozanska et al.[4]).

phenotypes upon sensing inflammatory stimuli but are also strongly imprinted by the tissue microenvironment they interact with at steady state.[16-18] In tissues, we can distinguish two main macrophage lineages with specialized function and ontogeny (Figure 2): tissue-resident macrophages (TRMacs) and monocyte-derived macrophages (MoMacs). TRMacs populate tissues before birth, proliferate locally without contribution from the circulating monocyte pool, and have very tissue-specific functions.[19-21] Damage in the TRMac pool results in tissue pathology, as exemplified by decreased lung function due to an insufficient number of alveolar macrophages leading to accumulation of surfactant protein and reduced air exchange in alveoli.[22] In contrast, MoMacs differentiate from blood-derived monocytes and are thus replenished constantly. In homeostasis, MoMacs are dispensable and constitute only a small fraction of total macrophages in many tissues. Nevertheless, MoMacs play a key role in response to inflammatory stimuli and expand dramatically in chronic or acute inflammatory lesions. Taken together, it is now understood that in conditions of chronic inflammation, monocytes are actively recruited into damaged tissue and significantly contribute to pathology upon differentiation into MoMacs.[23] Accordingly, large numbers of MoMacs often accumulate in solid tumors.

TUMOR-ASSOCIATED MACROPHAGES

Tumor-associated macrophages: the in vitro *M1/M2 concept*

Within the solid tumor microenvironment (TME), macrophages are termed tumor-associated macrophages (TAMs), comprising both MoMac and TRMac subsets. TAMs present the most abundant immune cell subset in the TME[24] and are associated with disease outcome across various cancer types.[25] Due to complex signals within the TME, TAMs are polarized and acquire specific molecular signatures that differ from macrophages in healthy tissues. Attempts were made to describe TAM functionality using the 'M1/M2' concept, which was initially introduced to describe macrophage plasticity in response to various stimuli observed *in vitro*.[26] Until recently, TAM characterization was mainly carried out by immunohistochemistry, flow cytometry, and bulk gene expression analysis, mostly using only one or a minimal set of markers. Thus, the full cellular diversity and functional markers of TAMs have been poorly captured. Recent advances in single-cell RNA sequencing have shown that M1 and M2 signature genes are co-expressed across different TAM subsets, and do not clearly identify immune-suppressive or stimulatory TAMs, thus highlighting that *in vivo* macrophage populations do not adhere to the M1/M2 dogma.[27,28]

Tumor-associated macrophages: polarized by the tumor microenvironment

The TME releases a plethora of extracellular mediators which result in distinct tumor-associated phenotypes of MoMacs and TRMacs[29]: tumor-infiltrating MoMacs are characterized by immunosuppressive phenotypes associated with several chronic inflammatory conditions,[30-32] whereas TRMacs reduce the expression of pro-inflammatory mediators and up-regulate tissue remodeling factors.[33] Several recent studies have focused on the role of TRMacs in the TME, demonstrating tissue specificity, which is consistent with our understanding of very strong imprinting of surrounding tissue niches on TRMac identity and function. In lung cancer, immunosuppressive functions have been reported for TRMacs, whereas in breast cancer, TRMacs were shown to directly fuel protective T cell responses.[33-35] It is also becoming clear that TRMacs preferentially localize in the tumor-surrounding stroma and are depleted from tumor islets. In contrast, MoMacs are strongly recruited by the tumor and are localized in tumor nests directly.[33,35,36] Thus, more research is needed to dissect the roles of distinct macrophage populations with respect to the multiple components of the TME. Intriguingly, in these studies, very distinct correlation patterns of two groups of cell types clustering together within patients were described. On the one hand, TRMacs were correlated with multiple memory T cell subsets and B cells; whereas on the other hand several immunosuppressive MoMac populations correlated with regulatory T cells and exhausted/dysfunctional T cells.[36] A causal relationship between these cell types accumulating together has yet to be proven, but it is intriguing that MoMacs are part of a suppressive pro-tumorigenic immune cell context, whereas TRMacs are represented in T helper 1 (Th1) polarized, anti-tumorigenic immune environments.

Heterogeneity of tumor-associated macrophages: lessons learned from single-cell RNA sequencing

Due to the high complexity and plasticity of TAMs, unbiased single-cell RNA sequencing of tumor-associated immune

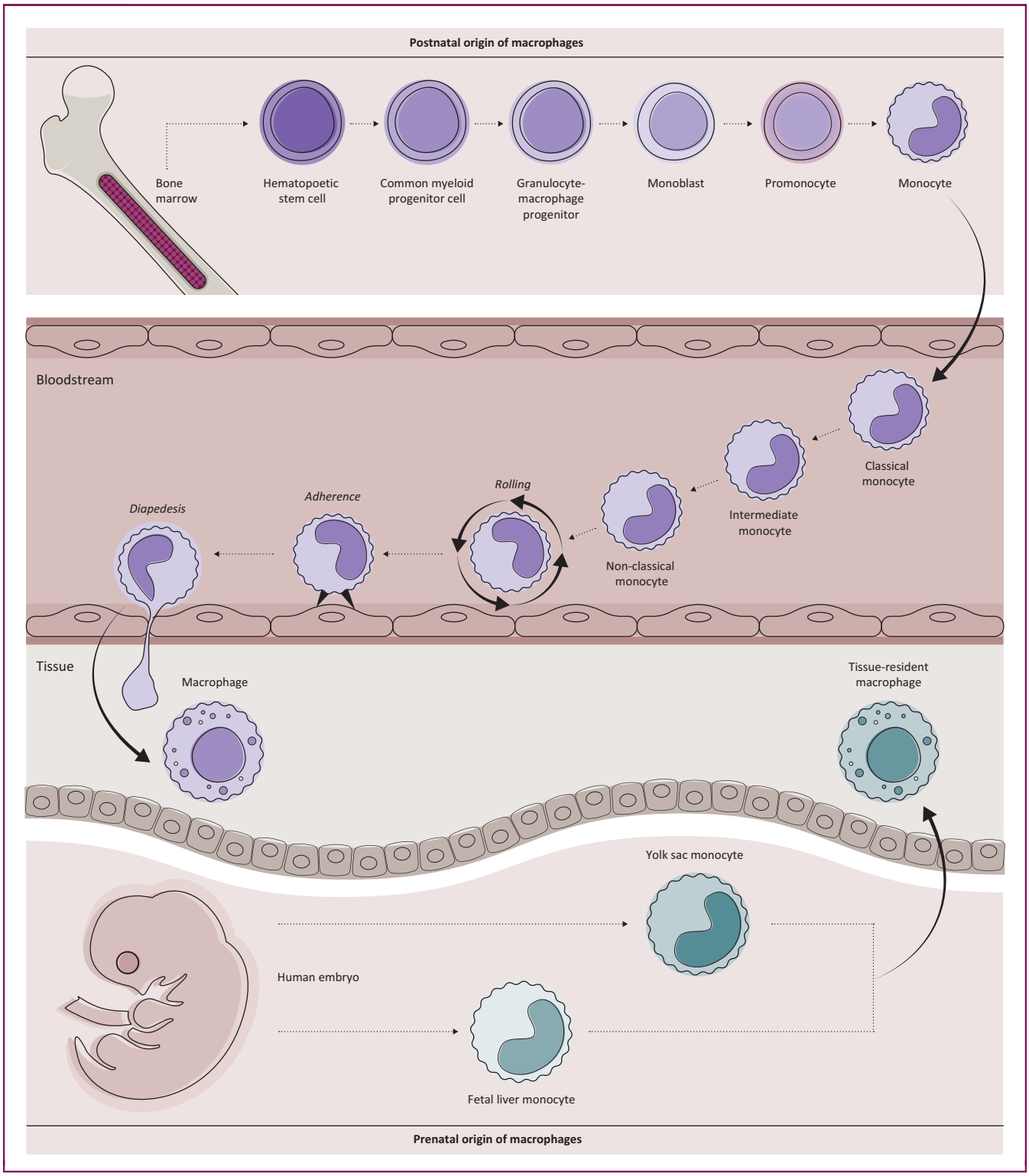


Figure 2. Macrophage ontogeny.
Within tissue, two distinct macrophage types, tissue-resident macrophages and monocyte-derived macrophages, can be discriminated. While tissue-resident macrophages populate tissues before birth (prenatal origin) and proliferate locally, monocyte-derived macrophages are replenished from circulating blood monocytes (postnatal origin). Circulating blood monocytes originate from bone marrow-derived hematopoietic stem cells and give rise to classical monocytes, which then can further differentiate to intermediate and non-classical monocytes on request.

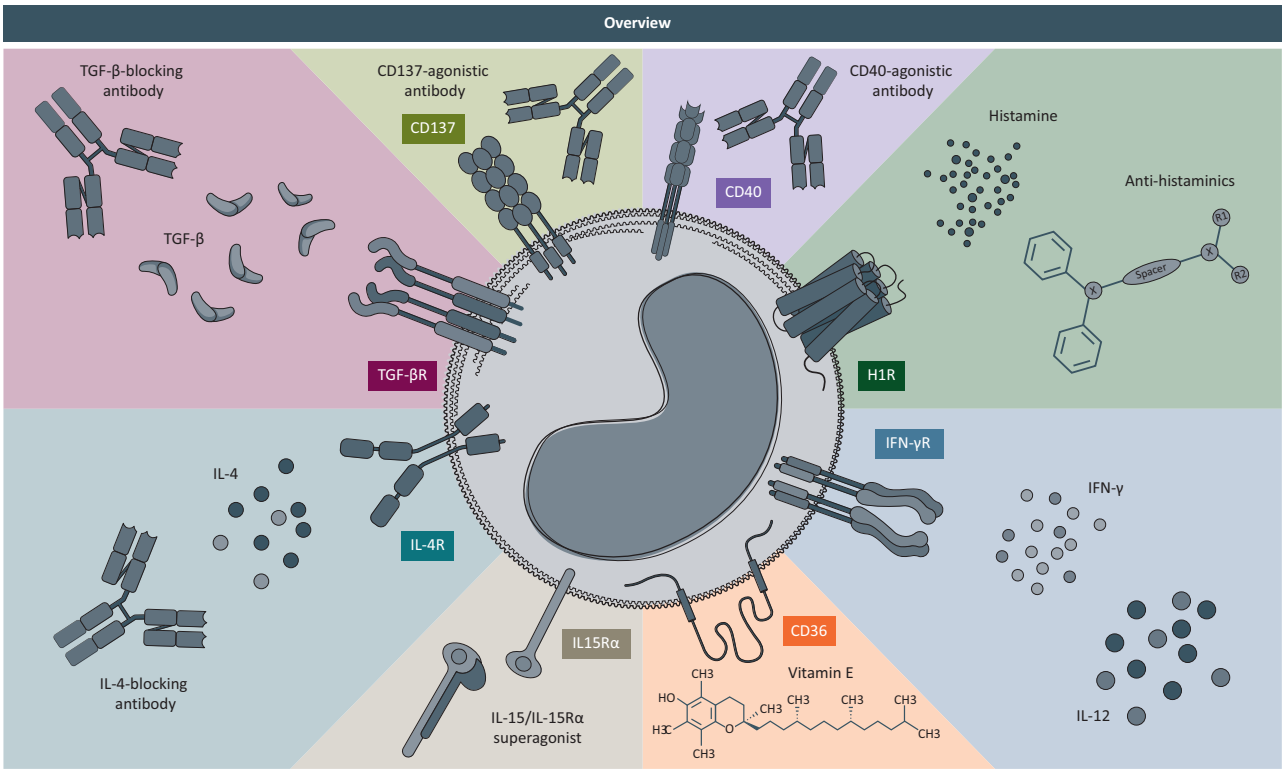
cells provides invaluable insights into TAM function for future translational approaches. Two meta studies recently compiled comprehensive single-cell macrophage maps across cancers: Mulder et al. identified several MoMac populations, including triggering receptor expressed on myeloid cells-2 (TREM2)^{hi} MoMacs and interleukin 4 (IL-4)-induced 1 (IL4I1)^{hi} MoMacs, enriched in tumors across tissues.[37] TREM2^{hi} MoMacs preferentially accumulated in the tumor core, whereas IL4I1^{hi} MoMacs were enriched in the tumor periphery, likely contributing to the differential transcriptional programs of those MoMac subsets.[37] Cheng et al. highlighted a very complex pattern of macrophage transcriptional programs that did not allow consistent classification of macrophages from different tumor types.[28] Nevertheless, a secreted phosphoprotein 1 (SPP1)^{hi} MoMac population was found in 8 out of 15 studied cancer types and was characterized by high expression of genes associated with angiogenesis. In addition, a transmembrane glycoprotein NMB (GPNMB)^{hi} MoMac cluster was identified in some of the studied cancers, which is likely consistent with the TREM2^{hi} MoMacs described in Mulder et al.[37] TRMacs with strong tissue-specific identity were described, including alveolar macrophages and interstitial macrophages across multiple tissues, which were excluded from tumor lesions, confirming previous findings in non-small cell lung cancer (NSCLC).[33,36] Such data integration efforts highlight the enormous complexity of the macrophage compartment and its tissue-specific regulatory patterns, but we are still far from understanding the functional implications of these different macrophage subsets in the TME and identifying universal macrophage targets for immunotherapy.

Response to immune checkpoint blockade: do tumor-associated macrophages play a role?

TAMs have also been shown to contribute to response to ICIs in *in vivo* models of colon adenocarcinoma, mammary carcinoma, melanoma, and osteosarcoma. In these studies, programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) blockade drives an immune-stimulatory phenotype in macrophages that contributes to effector T cell responses and tumor control.[38-40] In patient cohorts of urothelial cancer[41] and NSCLC,[42] the presence of inflammatory macrophages predicts response to ICI therapy. However, the clinical monitoring is most often too shallow to get sufficient information on specific macrophage subsets. Usually, CD68 is used as a pan-macrophage marker in histology. Nevertheless, the use of this marker is limited due to its expression on other cell types including granulocytes, dendritic cells (DCs), fibroblasts, endothelial cells, and lymphoid subsets and its inability to distinguish between macrophage subsets.[43] Additionally, the spatial distribution should be considered during analysis as macrophage subsets reside within different parts of the tumor and its TME.[44] Spatial transcriptomics is a novel technique that enables the determination of gene expression throughout the tissue space,[45] which would allow to get a global picture of formalin-fixed tissue slides allowing unbiased immune profiling. This approach might allow for future prediction of response to ICI therapy on the basis of not only macrophage but multiple immune cell subsets. However, one caveat is that current spatial transcriptomics approaches do not achieve single-cell resolution. Method development strategies are tackling this problem, thus holding great promise for future applications.

Tumor-associated macrophages: involvement in hyperprogression

With the advent of ICI therapy, an unconventional response pattern termed hyperprogression has been observed in up to 30% of patients receiving PD-1/PD-L1-targeting ICIs.[46] In contrast to pseudoprogression, where an increase in the tumor burden is mediated by infiltration of immune cells, patients with hyperprogression show an accelerated tumor growth and an immense deterioration of the patients' general health condition. Despite increasing case reports,[47-53] the existence of hyperprogression is still questioned: alternatively, this patient subpopulation might suffer from an extremely aggressive cancer type for which chemotherapy might be more efficient than immunotherapy to slow down tumor growth acceleration especially in the first weeks of treatment.[54] Some studies suggest an involvement of monocytes in the promotion of hyperprogression.[55] Besides well-known immunomodulatory factors influencing monocytes such as cytokines, chemokines, and extracellular vesicles, external factors, such as therapeutic antibodies directed against immune checkpoints, are also hypothesized to play a specific, immunomodulatory role in monocyte modulation, especially with regard to hyperprogression. Despite modifications of ICI antibodies in their Fc part, these antibodies still show a high affinity to FcγRI (CD64) and IIb (CD32B),[56] whereas the latter is considered to have inhibitory effects and might therefore limit therapeutic efficacy and promote drug resistance, especially considering FcγRIIB expression on tumor cells.[57] Romano et al. reported the *ex vivo* binding of ipilimumab to non-classical monocytes via FcγRIIIA (CD16A) triggered antibody-dependent cellular cytotoxicity-mediated lysis of regulatory T cells (T_{regs}). This effect was only observed for CD16-expressing non-classical monocytes.[58] Using an *in vivo* mouse model, Arlauckas et al. showed that PD-1^{-} TAMs can heist T cell-bound anti-PD-1 antibodies, which can be prevented by blockage of Fcγ receptors on TAMs, resulting in enhanced tumor regression.[59] These findings highlight the participation of Fc/FcγR interactions in the context of therapy efficacy. For the first time, Lo Russo et al. provided proof for the active role of therapeutic antibodies in hyperprogression in a xenograft model: hyperprogression was abrogated in all mice when treating them with the anti-PD-1 $F(ab)_2$ fragment compared to treatment with the whole antibody nivolumab, which correlated with suppressive macrophage infiltration.[50]



Target	Biological function	Interference	Effects on macrophages/monocytes	References
CD40	T cell activation and differentiation	Agonistic antibody	Pro-inflammatory cytokine/chemokine secretion CD80/CD86 expression ↑ Inflammatory phenotype induction	70,72,74,75,77,78
CD137	T cell activation and differentiation	Agonistic antibody	Pro-inflammatory cytokine release IL-10 secretion ↓ Monocyte survival and migration ↑ Differentiation to DC Suppressive phenotype induction Osteoclast differentiation	89–92,94,95
IFN-γ	MHC class I and II upregulation on APCs	IFN-γ or IL-12	Suppressive phenotype reversion	97–99
IL-4	Mϕ polarization to tissue repair phenotype DC stimulatory potential ↓ Cancer cell proliferation ↑; resistance to apoptosis ↑	Blocking antibody	IL-10 release ↓ Suppressive Mϕ generation ↓	109–112,114–116
IL-15Rα	T and NK cell effector function and proliferation	IL-15/IL-15Rα superagonist	Suppressive phenotype reversion	123–125
TGF-β	T_{reg} expansion T and NK cell inhibition Monocyte chemotaxis Polarization to immunosuppressive Mϕ phenotype	Blocking antibody	Suppressive phenotype reversion	140,142
H1R	Ambiguous	Antihistamine treatment	Reversion of Mϕ-mediated immunosuppression	148,151–153
CD36	Cancer cell apoptosis	Vitamin E	TNF-α release Inhibition of IL-6 and NO production Prostaglandin E2 synthesis ↓	159–161,163–165,167

These data highlight the rationale to develop Fc-optimized antibodies or antibody fragments for clinical use.

TUMOR-ASSOCIATED MACROPHAGES AS A THERAPEUTIC TARGET IN SOLID CANCERS

Since it was recognized that TAMs play a key role in supporting tumor immune evasion and metastasis,[60] a strong focus has been put on targeting TAMs. So far, the main strategies for targeting TAMs include macrophage depletion, inhibition of recruitment, and repolarization.[61] However, tumor-specific molecular targets are thus far lacking and still need to be explored in (pre-)clinical settings.

Targeting tumor-associated macrophages: the long shot

Ongoing research has already revealed various therapeutic TAM-related targets with new ones constantly emerging. *TREM2* is one of the genes selectively and highly expressed on tumor-infiltrating MoMacs and has recently emerged as a cancer immunotherapy target.[30,62,63] Multiple studies have shown a role for TREM2-expressing MoMacs in anti-tumor immunity and response to ICI therapy. In patients, TREM2 expression correlates with poor outcomes, poor therapy response, and metastasis.[64-66] Mice deficient for *TREM2*, or treated with a TREM2-blocking antibody, showed reduced tumor burden as well as improved response to ICIs in various tumor models.[30,62,63] It is clear that the loss of *TREM2* remodels the TAM compartment. However, the precise mechanisms of how TREM2 modulates macrophage identity, and by which mechanisms TREM2-expressing MoMacs regulate anti-tumor immunity is unknown. Other MoMac targets are less clear. Immune checkpoint ligands expressed on MoMacs include PD-L1, PD-L2, T cell immunoglobulin and mucin-domain containing (TIM-)3, TIM-4, indoleamine-pyrrole 2,3-dioxygenase, and IL4I1, some of which might be controlled by upstream interferon or CD40 signaling.[36,37] Targeting those factors increased anti-tumor immunity in preclinical models or in clinical settings.[67] However, whether the observed effects are mediated via macrophages is unclear and it is likely that other cell types such as DCs play equally important roles. This underscores the need for in-depth research to identify and study new targets on various immune cell populations for future therapies.

Targeting tumor-associated macrophages: possibilities within reach of clinical practice

In this section, we will discuss selected potential targets on TAMs that could be adapted for clinical use in the near future (Figure 3).

CD40. Signaling of CD40 via its ligand CD40L is the major co-stimulatory signal necessary for T cell activation and is involved in various immune functions such as coagulation, infection control, and immune system regulation.[68,69] CD40/CD40L signaling leads to DC activation with up-regulation of co-stimulatory molecules[68-70] as well as their migration to the lymph node[71] and consequently to T cell activation and differentiation.[70] In monocytes, CD40 activation induces pro-inflammatory cytokine and chemokine secretion, as well as up-regulation of CD80 and CD86 expression.[68,72,73] Thus, T cell activation is enhanced. In malignant cells, CD40 signaling is able to induce apoptosis both *in vitro* and *in vivo*.[68] Therefore, agonist-mediated induction of CD40 signaling might promote anti-tumor immunity.[70] In various cancer models, CD40 agonists were shown to induce a tumor-specific immune response by triggering the cancer-immunity cycle.[73,74] In TAMs, CD40 agonists have been reported to induce an anti-tumorigenic phenotype *in vitro*.[75,76] Long et al. described that these CD40 agonist-redirected macrophages were able to degrade fibrotic tissue in pancreatic carcinoma and thereby enhance chemotherapy efficacy.[77] In this context, several reports highlight the importance of macrophages, which can boost an anti-tumor immune response independent of T cells.[78,79] Preclinical studies with CD40 agonist monotherapy have shown efficacy; however, mainly immunologically active and immunotherapy-responsive tumor models were used.[80] Additionally, CD40 agonist treatment was linked to increased PD-L1 expression on monocytes[81] and DCs[82] highlighting the potential need for combination with ICIs. Combinatorial therapy with anti-PD-1 has already shown promising results in mouse models.[73] Despite these positive effects, the use of CD40 agonists is linked to toxicities including cytokine release syndrome and hepatotoxicity.[74] Therefore, there are already attempts of modifying CD40 agonists by bispecific antibodies to ensure specific targeting, e.g. by fibroblast activation protein being expressed to a higher degree in the tumor stroma.[74,83] Moreover, $F(ab)_2$ fragments of CD40 agonists (CDX-1140) did not induce cytokine release *in vitro* with a good safety profile in animal models.[84] Additionally, fusions of CD40 and blocking PD-L1 single-chain variable Fcs have been reported.[85] Clinical trials showed a certain degree of toxicity upon systemic administration[80] and have been mainly tested in combination with other therapies. In pancreatic ductal adenocarcinoma, combination with chemotherapy was well tolerated and associated with anti-tumor activity.[79,86] Various clinical trials, including combinations with ICIs and/or chemotherapy, are ongoing (Table 1).[73]

CD137. CD137 (also known as 4-1BB), a member of the tumor necrosis factor receptor superfamily 9, acts as a

Figure 3. New potential therapeutic targets on tumor-associated macrophages.
Potential therapeutic targets currently under investigation are summarized, and their mode of action and the effect on monocytes and/or macrophages (Mϕ) are indicated.
APC, antigen-presenting cell; DC, dendritic cell; H1R, histamine receptor H1; IL, interleukin; INF-γ, interferon-γ; MHC, major histocompatibility complex; Mϕ, macrophage; NK, natural killer; NO, nitric oxide; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; T_{reg}, regulatory T cell.

Table 1. Selected overview of running clinical trials

Reference[a]	Phase	Status[b]	Intervention[c]	Cancer type
			CD40	
NCT03424005	1b/2	ANR	Selicrelumab[#1], atezolizumab, bevacizumab	Breast cancer
NCT03555149	1b/2	ANR	Selicrelumab[#1], bevacizumab, atezolizumab	Colorectal cancer
NCT03193190	1b/2	R	Nab-paclitaxel, gemcitabine, oxaliplatin, leucovorin, fluorouracil, atezolizumab, cobimetinib, PEGPH2O, BL-8040, selicrelumab[#1], bevacizumab, RO6874281, AB928, tiragolumab, tocilizumab	Pancreatic ductal adenocarcinoma
NCT04364230	1/2	R	Peptide vaccine, polyICLC, CDX-1140[#2]	Melanoma
NCT04536077	2	R	CDX-1140[#2]; CCX-301, CDX-1140[#2]	Pancreatic cancer
NCT04616248	1	NYR	CDX-1140[#2], CCX-301, radiotherapy, poly-ICLC	Breast cancer
NCT04491084	1/2	R	CDX-1140[#2], CCX-301, stereotactic body radiation therapy	NSCLC
NCT04520711	1/1b	R	TCR-transduced T cells, CDX-1140[#2], pembrolizumab	Epithelial cancer
NCT03502330	1	ANR	APX005M[#3], cabiralizumab; APX005M[#3], cabiralizumab, nivolumab	Melanoma NSCLC Renal cell carcinoma
NCT03719430	2	R	APX005M[#3], doxorubicin	Soft-tissue carcinoma
NCT03165994	2	ANR	APX005M[#3], radiation, paclitaxel, carboplatin, surgical resection	Esophageal cancer
NCT02706353	2	R	APX005M[#3], pembrolizumab	Melanoma
NCT03389802	1	R	APX005M[#3]	Pediatric CNS tumors
NCT04337931	2	ANR	APX005M[#3]	Melanoma
NCT04130854	2	ANR	mFOLFOX, radiation, APX005M[#3]	Rectal adenocarcinoma
NCT04495257	1	R	APX005M[#3], nivolumab, ipilimumab	Melanoma Renal cell carcinoma
NCT02600949	1	R	Imiquimod, pembrolizumab, sotigalimab[#3], peptide vaccine	Colorectal adenocarcinoma Pancreatic ductal adenocarcinoma
NCT02376699	1	ANR	SEA-CD40[#4]; SEA-CD40[#4], pembrolizumab SEA-CD40[#4], pembrolizumab, gemcitabine, nab-paclitaxel	B Cell lymphoma Follicular lymphoma Hodgkin lymphoma Large B Cell, diffuse lymphoma Non-Hodgkin lymphoma NSCLC Melanoma Squamous cell carcinoma Pancreatic ductal adenocarcinoma
			CD137	
JapicCTI-205153	1	R	STA551[#5]; STA551[#5], atezolizumab	Advanced/metastatic solid tumors
NCT04121676	1	R	AGEN2373[#6]; AGEN2373[#6], botensilimab	Advanced/metastatic solid tumors
NCT04903873	1/2	R	EU101[#7]	Colorectal cancer NSCLC
NCT04501276	1	R	ADG116, ADG106[#8]	Advanced/metastatic solid tumors
NCT03792724	1/2	NYR	Urelumab[#9], nivolumab	PD-1/PD-L1 sensitive tumors
NCT02845323	2	ANR	Nivolumab, urelumab[#9]	Urothelial cancer
NCT03431948	1	ANR	Nivolumab, urelumab[#9], radiation	Advanced solid tumors
NCT02451982	2	R	Cyclophosphamide, pancreatic tumor vaccine, nivolumab, urelumab[#9]	Pancreatic cancer
NCT02652455	1	ANR	Nivolumab, urelumab[#9], cyclophosphamide, fludarabine, aldesleukin, autologous tumor-infiltrating lymphocytes	Melanoma
NCT02658981	1	ANR	Urelumab[#9]; Nivolumab, urelumab[#9]; Anti-LAG-3 monoclonal antibody BMS 986016, nivolumab, urelumab[#9]	Glioblastoma Gliosarcoma Recurrent brain neoplasm
NCT03318900	1	ANR	Aldesleukin, CD8-positive T-lymphocytes, cyclophosphamide, utomilumab[#10]	Ovarian cancer
NCT03414658	2	R	Vinorelbine, trastuzumab, avelumab, utomilumab[#10]; Trastuzumab, avelumab, utomilumab[#10]	Breast cancer
NCT03290937	1	ANR	Cetuximab, irinotecan hydrochloride, utomilumab[#10]	Colorectal cancer
NCT03217747	1/2	ANR	Avelumab, utomilumab[#10]; Avelumab, ivuxolimab, utomilumab[#10]; Avelumab, utomilumab[#10], radiation; Avelumab, ivuxolimab, utomilumab[#10], radiation	Castration-resistant prostate cancer

Continued

Table 1. Continued

CD137				
NCT02554812	1/2	ANR	Avelumab, utomilumab[#10]; Avelumab, utomilumab[#10], PF-04518600; Avelumab, utomilumab[#10]; Avelumab, utomilumab[#10], vidutolimod	Bladder cancer Gastric cancer Head and neck squamous cell carcinoma Melanoma NSCLC Ovarian cancer
NCT03971409	2	R	Avelumab, utomilumab[#10]	Breast cancer
NCT05059522	3	R	Avelumab, utomilumab[#10], vidutolimod, PF04518600	NSCLC Ovarian cancer Solid tumors Urothelial cancer
Interferon-γ/interleukin 12				
NCT03112590	1/2	ANR	INF-γ, paclitaxel, tastuzumab, pertuzumab, post-therapy surgery	Breast cancer
NCT03132675	2	R	Tavokinogene telseplasmid[#11], pembrolizumab, immunopulse	Melanoma
NCT03567720	2	R	Tavokinogene telseplasmid[#11], pembrolizumab, immunopulse Tavokinogene telseplasmid[#11], pembrolizumab, immunopulse, nab-paclitaxel	Breast cancer
NCT04526730	2	R	Tavokinogene telseplasmid[#11], nivolumab, immunopulse	Melanoma
NCT02555397	1	ANR	Ad5-yCD/mutTKSR39rep-hIL12[#12]	Prostate cancer
NCT04911166	1	R	ADV/IL-12 gene therapy[#13], atezolizumab	NSCLC
NCT03030378	1	R	Edodekin alfa[#14], pembrolizumab	Metastatic solid tumors
NCT01468896	1/2	ANR	Cetruximab, edodekin alfa[#14]	Head and neck squamous cell carcinoma
NCT04235777	1	1	M7824[#30], M9241[#15]; M7824[#30], M9241[#15], stereotactic body radiation therapy	Urogenital cancer Urothelial cancer
NCT05361798	2	R	M9241[#15], stereotactic body radiation therapy	Prostate cancer
NCT04633252	1/2	R	Androgen deprivation therapy, prednisone, M7824[#30], docetaxel, M9241[#15]	Prostate cancer
NCT05286814	2	R	Floxuridine, 5-fluorouracil, irinotecan, oxaliplatin, leucovorin, M9241[#15], gemcitabine, dexamethasone	Colorectal cancer Intrahepatic cholangiocarcinoma
NCT04708470	1/2	R	Bintrafusp alfa[#30], NHS-IL12[#15], entinostat	Anal cancer Cervical cancer Colon cancer Neck cancer Oropharyngeal cancer Penile cancer Vaginal cancer Vulvar cancer
NCT04303117	1/2	R	NHS-IL12[#15], M7824[#30]	Kaposi sarcoma
NCT04491955	2	R	CV301, MSB0011359C[#30], N-803[#29]; CV301, MSB0011359C[#30], N-803[#29], NHS-IL12[#15]	Colorectal cancers Small bowel cancers
NCT04287868	1/2	R	PDS0101, M7824[#30], NHS-IL12[#15]	Anal cancer Cervical cancer HPV cancers Oropharyngeal cancer Penile cancer Rectal cancer Vaginal cancer Vulvar cancer
NCT02498912	1	ANR	4H11-28z/fIL-12/EGFRt^{+} genetically modified T cells[#16]	Solid tumors
NCT04613492	1	R	MEDI9253[#17], durvalumab	Advanced/metastatic solid tumors
NCT03439085	2	ANR	MEDI0457[#18], durvalumab	HPV cancer
NCT04471987	1	R	IL12-L19L19[#19]	Advanced/metastatic solid tumors
NCT03393884	1/2	R	GEN-1[#20], carboplatin, paclitaxel	Ovarian cancer Fallopian tube cancer Peritoneal cancer
NCT05352750	1	R	SON-1010[#21]	Advanced solid tumors
NCT04388033	1/2	R	Dendritic cell/tumor fusion vaccine, IL-12, temozolomide	Glioblastoma
NCT05077033	1	R	phIL12 GET[#22]	Basal cell carcinoma
NCT05095441	1	NYR	C5252[#23]	Glioblastoma

Table 1. Continued

Interleukin 4				
NCT05013450	1/2	R	Dupilumab[#24], PD-1/PD-L1 blockade	NSCLC
Interleukin 15/interleukin 15 receptor				
NCT04491955	—	—	Multiple target combination—details on clinical trial see section **Interferon-γ/interleukin 12**	—
NCT04261439	1	R	NIZ985[#25], spartalizumab; NIZ985[#25], spartalizumab, tislelizumab	Advanced solid tumors Melanoma NSCLC
NCT04234113	1	R	SO-C101[#26]; SO-C101[#26], pembrolizumab	Anal cancer Thyroid cancer Bladder cancer Biliary tract cancer Cervical cancer Cutaneous squamous cell carcinoma Gastric cancer Head and neck squamous cell carcinoma Hepatocellular carcinoma Melanoma Mesothelioma Merkel cell carcinoma Microsatellite instability high NSCLC Ovarian cancer Renal cell carcinoma Small-cell lung cancer Thymic cancer Triple-negative breast cancer
NCT05619172	2	NYR	SOT101[#26], cetuximab	Colorectal cancer
NCT05256381	2	R	SOT101[#26], pembrolizumab	Castration-resistant prostate cancer Colorectal cancer Cutaneous squamous cell carcinoma Hepatocellular carcinoma NSCLC Ovarian cancer
NCT04250155	1	R	XmAb24306[#27]; Atezolizumab+ XmAb24306[#27]	Advanced/metastatic/recurrent solid tumors
NCT04616196	1/2	R	NKTR-255[#28], cetuximab	Anal squamous cell carcinoma Cervical cancer Colorectal cancer Cutaneous squamous cell carcinoma Head and neck squamous cell carcinoma
NCT05327530	2	R	Avelumab+NKTR-255[#28]	Urothelial cancer
NCT05445882	2	NYR	N-803[#29]; N-803[#29], BN-Brachyury; N-803[#29], bintrafusp alfa[#30]	Castration-resistant prostate cancer
NCT04847466	2	R	N-803[#29], pembrolizumab, PD-L1 t-haNK	Gastroesophageal junction cancer Head and neck squamous cell carcinoma
NCT03022825	2/3	R	N-803[#29], intravesical Bacillus Calmette—Guerin	Bladder cancer
NCT05096663	2/3	R	N-803[#29], pembrolizumab	NSCLC
NCT02138734	1/2	R	N-803[#29], intravesical Bacillus Calmette—Guerin	Bladder cancer
NCT04247282	1/2	ANR	M7824[#30], N803[#29]+TriAd vaccine	Head and neck cancer
NCT03493945	1/2	R	M7824[#30], N803[#29]; M7824[#30], N803[#29], MVA-BN-Brachyury, FPV-Brachyury; M7824[#30], N803[#29], MVA-BN-Brachyury, FPV-Brachyury, epacadostat	Advanced/metastatic solid tumors Prostate cancer
NCT03520686	3	ANR	N-803[#29], pembrolizumab; N-803[#29], carboplatin, nab-paclitaxel, pembrolizumab; N-803[#29], cisplatin or carboplatin, pembrolizumab, pemetrexed	NSCLC
NCT04927884	1/2	ANR	N-803[#29], PD-L1 t-haNK, sacituzumab Govitecan-Hziy, cyclophosphamide	Triple-negative breast cancer

Continued

Table 1. Continued

Interleukin 15/interleukin 15 receptor				
NCT03228667	2	ANR	N-803[#29], pembrolizumab; N-803[#29], nivolumab; N-803[#29], atezolizumab; N-803[#29], avelumab; N-803[#29], durvalumab; N-803[#29], pembrolizumab, PD-L1 t-haNK; N-803[#29], nivolumab, PD-L1 t-haNK; N-803[#29], atezolizumab, PD-L1 t-haNK; N-803[#29], avelumab, PD-L1 t-haNK; N-803[#29], durvalumab, PD-L1 t-haNK	Cervical cancer Colorectal cancer NSCLC Gastric cancer Head and neck squamous cell carcinoma Hepatocellular carcinoma Melanoma Merkel cell carcinoma Microsatellite instability Mismatch repair deficiency Small-cell lung cancer Renal cell carcinoma Urothelial cancer
Transforming growth factor beta				
NCT04247282	—	—	Multiple target combination—details on clinical trial see section **Interleukin 15/interleukin 15 receptor**	—
NCT03493945	—	—	Multiple target combination—details on clinical trial see section **Interleukin 15/interleukin 15 receptor**	—
NCT04491955	—	—	Multiple target combination—details on clinical trial see section **Interferon-γ/interleukin 12**	—
NCT04303117	—	—	Multiple target combination—details on clinical trial see section **Interferon-γ/interleukin 12**	—
NCT04235777	—	—	Multiple target combination—details on clinical trial see section **Interferon-γ/interleukin 12**	—
NCT04287868	—	—	Multiple target combination—details on clinical trial see section **Interferon-γ/interleukin 12**	—
NCT04633252	—	—	Multiple target combination—details on clinical trial see section **Interferon-γ/interleukin 12**	—
NCT04708470	—	—	Multiple target combination—details on clinical trial see section **Interferon-γ/interleukin 12**	—
NCT05445882	—	—	Multiple target combination—details on clinical trial see section **Interleukin 15/interleukin 15 receptor**	—
NCT04574583	1/2	ANR	SX-682, M7824[#30], MVA-BN-CV301, FPV-CV301	Advanced/metastatic cancer Head and neck squamous cell carcinoma Triple-negative breast cancer
NCT04432597	1/2	R	PRGN-2009, M7824[#30]	Anal cancer Cervical cancer HPV-positive solid cancer Oropharyngeal cancer Penile cancer Rectal cancer Vaginal cancer Vulvar cancer
NCT04297748	1/2	R	89Zirconium-M7824, M7824[#30]	NSCLC
NCT04417660	2	R	M7824[#30]	Thymic cancer Recurrent thymoma
NCT03436563	1/2	ANR	M7824[#30]	Colorectal adenocarcinoma
NCT04835896	1/2	NYR	M7824[#30], paclitaxel	Gastric cancer
NCT03427411	2	ANR	M7824[#30]	Anal cancer Cervical cancer HPV-positive solid tumors Oropharyngeal cancer Penile cancer Vaginal cancer
NCT03631706	3	ANR	M7824[#30]	NSCLC
NCT05005429	2	R	M7824[#30]	Mesothelioma
NCT03620201	1	ANR	M7824[#30]	Breast cancer
NCT03554473	1/2	R	M7824[#30], temozolomide; M7824[#30], topotecan	Small-cell lung cancer
NCT03840902	2	ANR	M7824[#30], etoposide, pemetrexed, carboplatin, paclitaxel, cisplatin, intensity-modulated radiation therapy	NSCLC
NCT04066491	2/3	ANR	M7824[#30], gemcitabine, cisplatin	Biliary tract cancer
NCT05145569	1	NYR	Carboplatin AUC 5 and paclitaxel, M7824[#30]	Ovarian cancer
NCT03315871	2	R	PROSTVAC-V, PROSTVAC-F, M7824[#30], CV301	Prostate cancer
NCT04551950	1	ANR	M7824[#30], carboplatin, paclitaxel, bevacizumab, cisplatin; M7824[#30], carboplatin, paclitaxel, cisplatin; M7824[#30], cisplatin, radiotherapy	Cervical cancer
NCT04481256	n.a.	R	Radiatin, bintrafusp alfa[#30], paclitaxel, carboplatin	Squamous cell carcinoma
NCT04246489	2	ANR	Bintrafusp alfa[#30]	Cervical cancer
NCT05061823	3	R	Bintrafusp alfa[#30]	Cancer
NCT05012098	2	R	Bintrafusp alfa[#30]	Esthesioneuroblastoma

Continued

Table 1. Continued

Transforming growth factor beta				
NCT04595149	2	R	Bintrafusp alfa[#30]	Esophageal cancer
NCT04349280	1	ANR	Bintrafusp alfa[#30]	Urothelial cancer
NCT04396535	2	ANR	Bintrafusp alfa[#30], docetaxel	NSCLC
NCT04708067	1	R	Bintrafusp alfa[#30], hypofractionated radiation therapy	Intrahepatic cholangiocarcinoma
NCT04874311	2	R	Bintrafusp alfa[#30], doxorubicin	Soft-tissue sarcoma
NCT04396886	2	ANR	Bintrafusp alfa[#30]	Nasopharyngeal carcinoma Non-keratinizing carcinoma
NCT04789668	1/2	ANR	Bintrafusp alfa[#30], pimasertib	Breast cancer Hematopoietic and lymphoid cell neoplasm Melanoma Neoplasm in the brain NSCLC
NCT04878250	2	NYR	Bintrafusp alfa[#30]	Urothelial cancer
NCT04457778	1	R	M6223, bintrafusp alfa[#30]	Metastatic solid tumors
NCT02723955	1	ANR	Feladilimab, bintrafusp alfa[#30]	Colorectal cancer Deficient mismatch repair tumor Esophageal cancer Epstein—Barr positive tumor Head and neck carcinoma HPV-positive tumor Melanoma Mesothelioma Microsatellite instability-high tumor NSCLC Prostate cancer Squamous cell carcinoma Urothelial cancer
NCT03834662	1	ANR	AVID200[#31]	Advanced/metastatic solid tumors
NCT05537051	1	NYR	PM1021, PM8001[#32]	Advanced solid tumors
NCT05028556	1	R	Y101D[#33]	Metastatic/advanced solid tumors
NCT05381935	1	NYR	ES014[#34]	Advanced solid tumors
NCT04862767	1	R	TASO-001[#35], aldesleukin	Solid tumors
Histamine				
NCT04165096	2	ANR	Pembrolizumab, MK-5890, diphenhydramine[#36], acetaminophen	NSCLC
NCT04863950	2	R	Lomustine, imipramine hydrochloride[#37]	Glioblastoma
NCT03253289	1	R	Meclizine[#38]	Hepatocellular carcinoma
2022-001284-27	2	ANR	Fexofenadine hydrochloride[#39], pembrolizumab	NSCLC
Vitamin E				
NCT04245865	2	R	Fluorouracil, calcium folinate, oxaliplatin, bevacizumab, capecitabine, tocotrienol	Colorectal cancer
NCT02705300	2	ANR	FOLFOXIRI, tocotrienol	Colorectal cancer
NCT04175470	2	R	Bevacizumab, tocotrienol	Ovarian cancer

CNS, central nervous system; EGFR, epidermal growth factor receptor; HPV, human papillomavirus; IL, interleukin; NSCLC, non-small-cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

[a]NCT identifiers refer to trials registered at www.clinicaltrials.gov, https://rctportal.niph.go.jp, or https://www.clinicaltrialsregister.eu/ctr-search/search/.

[b]Status: R, recruiting; ANR, active, not recruiting; NYR, not yet recruiting.

#Intervention.

#1 Selicrelumab—fully human IgG2 agonistic monoclonal antibody (mAb) to CD40.

#2 CDX-1140—fully human IgG2 agonistic mAb to CD40.

#3 APX005M (alias sotigalimab)—is a humanized rabbit IgG1 agonistic mAb to CD40.

#4 SEA-CD40 (alias dacetuzumab)—is a humanized IgG1, non-fucosylated agonistic mAb to CD40.

#5 STA551—human IgG1/lambda agonist switch antibody to CD137 with binding only in the presence of ATP.

#6 AGEN2373—fully human IgG1 agonistic mAb to CD137.

#7 EU101—fully humanized IgG1 agonistic mAb to CD137.

#8 ADG106—fully human IgG4 agonistic mAb to CD137.

#9 Urelumab—fully human IgG4 agonistic mAb to CD137.

#10 Utomilumab—fully human IgG2 agonistic mAb to CD137.

#11 Tavokinogene telseplasmid—intratumoral injected plasmid-encoding IL-12 by electroporation using the immunopulse.

#12 Ad5-yCD/mutTKSR39rep-hIL12—replication-competent oncolytic adenovirus encoding IL-12 gene, a yeast cytosine deaminase (yCD) and a mutant form of herpes simplex virus type 1 thymidine kinase (HSV-1 TKSR39).

#13 ADV/IL-12 gene therapy—adenoviral-mediated interleukin-12.

#14 Edodekin alfa—recombinant IL-12.

#15 M9241 (alias NHS-IL12)—two IL-12 molecules fused to a human IgG1-recognizing DNA/histone complexes.

#16 4H11-28z/fIL-12/EGFRt+ genetically modified T cells—genetically modified autologous T cells transduced with a retroviral vector expressing a chimeric antigen receptor targeting the human tumor-associated antigen MUC16ecto and encoding IL-12 fused to the signaling domain of the zeta chain of the TCR/CD3 complex (28z) and a truncated form of the human epidermal growth factor receptor (EGFRt).

#17 MEDI9253—oncolytic viral agent containing the oncolytic, live-attenuated, replication-competent strain of the avian paramyxovirus Newcastle disease virus (NDV) engineered to include a transgene encoding IL-12.

#18 MEDI0457—DNA Plasmid-encoding interleukin-12/HPV DNA plasmids therapeutic vaccine.

#19 IL12-L19L19—fusion protein of recombinant IL-12 with human mAb L19 specific for the extra-domain B of fibronectin in tandem diabody format.

#20 GEN1—IL-12 plasmid with PEG-PEI-cholesterol lipopolymer.

#21 SON-1010—single-chain human IL12 linked to a single-chain variable region antibody fragment.
#22 phIL12 GET—intratumoral gene transfer of plasmid coding for IL-12.
#23 C5252—genetically engineered oncolytic herpes simplex virus type 1 (oHSV-1) expressing the IL-12 and an antibody directed against PD-1.
#24 Dupilumab—fully human IgG4 antagonistic dimeric mAb to IL-4 receptor α/γc and IL-4 receptor α/IL-13 receptor α.
#25 NIZ985—recombinant IL-15/IL-15Rα heterodimer.
#26 SO-C101 (alias SOT101)—fusion protein of recombinant IL-15 linked to IL-15Rα sushi (cytokine-binding) domain.
#27 XmAb24306—recombinant IL-15/IL-15Rα cytokine fusion complex.
#28 NKTR-255—polyethylene glycol-conjugated recombinant human IL-15 agonist.
#29 N-803—mutated form of the cytokine IL-15 (IL-15N72D) and a soluble, dimeric IL-15 receptor alpha (IL-15Ra) Fc fusion protein (IL-15Ra-Fc) (IL-15N72D/IL-15Ra-Fc).
#30 M7824 (alias bintrafusp alfa, MSB0011359C)—bifunctional fusion protein of human IgG1 mAb against PD-L1 fused with two extracellular domains of TGF-β receptor type II.
#31 AVID200—receptor ectodomain trap.
#32 PM8001—bifunctional protein composed of the extracellular domain of the TGF-β receptor type II receptor fused to a humanized anti-PD-L1 IgG1 single-domain antibody.
#33 Y101D—recombinant anti-PD-L1 and TGF-β bispecific antibody.
#34 ES014—anti-CD39 and TGF-β bispecific antibody.
#35 TASO-001—TGF-β2 targeting antisense oligonucleotide.
#36 Diphenhydramine—H1-antihistamine antagonist.
#37 Imipramine hydrochloride—H1-antihistamine antagonist.
#38 Meclizine—H1-antihistamine antagonist.
#39 Fexofenadine hydrochloride—H1-antihistamine antagonist.
[c]https://www.cancer.gov/publications/dictionaries/cancer-drug.

co-stimulatory molecule by engaging to its ligand (CD137L), predominantly expressed on activated antigen-presenting cells (APCs).[87] Especially, the effects on T cell function have made CD137 a target of interest with promising agonistic anti-tumorigenic effects including long-term activation and survival of T cells in multiple tumor models.[87,88] In monocytes, the effect of CD137 agonists can be double-edged[89]: CD137 agonists are able to create an anti-tumorigenic milieu by inducing pro-inflammatory cytokine secretion while reducing interleukin 10 (IL-10) release and enhancing monocyte survival and migration. Furthermore, monocytes proliferate upon CD137 agonist signaling and differentiate into monocyte-derived DCs, thereby enhancing T cell proliferation.[90] Gauttier et al. demonstrated a substantial role for macrophages in the agonistic targeting of CD137. In this study, complete tumor regression was observed in 40%-60% of animals in an *in situ* model for hepatocellular carcinoma; and therapy efficacy was associated with macrophage recruitment within the tumor nodules.[91] Nevertheless, there are conflicting reports indicating pro-tumorigenic effects of CD137 agonistic therapy on macrophages. Geng et al. reported a CD137 agonist-mediated differentiation to a pro-tumorigenic phenotype[92] and Jiang et al. showed a promotion of bone metastasis by transformation of monocytes/macrophages into osteoclasts via CD137 activation.[93] These reported pro-tumorigenic properties of monocytes/macrophages *in vitro* and in animal models should at least be considered as potential factors limiting the applicability of CD137 agonists. Despite promising reports in various cancer models, clinical trials have not met expectations.[94] Monotherapy studies reported dose-dependent hepatotoxicity with urelumab with limited clinical activity (BMS-663513; NCT00309023, NCT00612664, NCT01471210).[95] Another promising candidate, utomilumab (PF-05082566), showed at least a better safety profile (NCT01307267).[96] Due to the high frequency of hepatotoxicity, there are novel attempts to improve anti-CD137 agonists as described by Kamata-Sakurai et al. who demonstrated an anti-CD137 switch antibody (STA551) with potent anti-tumor efficacy *in vitro* and in animal models and now tested in a phase I trial (Table 1) by exerting its agonistic activity only in the presence of extracellular ATP which is highly elevated in solid tumors.[94]

Interferon gamma. Interferon-gamma (INF-γ) is predominantly secreted by activated T and natural killer (NK) cells and to a lesser extent by APCs and B cells. Its production is up-regulated by inflammatory or immune stimuli including mitogens and cytokines such as interleukin 12 (IL-12) and interleukin (IL-15).[97] Via a positive feedback loop, INF-γ triggers the up-regulation of major histocompatibility complex (MHC) class I and II antigen processing and presentation on macrophages via INF-γ receptor 1/2, thereby in turn promoting T cell activation. As a key cytokine-mediating Th1 immunity, INF-γ has a major contribution in cancer beyond its action on macrophages: INF-γ induces tumor cell cycle arrest, tumor cell death (apoptosis, necroptosis), inhibition of angiogenesis, and induces T and NK cell trafficking into the tumor.[98] In contrast, INF-γ is attributed with pro-tumorigenic properties including promotion of angiogenesis and epithelial—mesenchymal transition, enhancing the immunosuppressive properties of myeloid cells, and induction of indolamine-2,3-dioxygenase synthesis. In macrophages, INF-γ is shown to re-educate TAMs from a pro- to an anti-tumorigenic phenotype, enhances MHC class I and II expression, and induces IL-12 expression, which in turn activates T and NK cells.[99] Mouse models showed that immunotherapy with ICIs was linked with INF-γ production in present T cells[97] and Manguso et al. attributed resistance to immunotherapy to defects in INF-γ signaling.[100] This observation was in line with patient data: in patients with NSCLC and urothelial cancer under anti-PD-L1 treatment with durvalumab, Higgs et al. described a higher overall response rate and longer median progression-free survival for patients with increased INF-γ gene signature.[101] Furthermore, in melanoma patients not responding to anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapy, 75% were associated with genomic defects in INF-γ signaling genes in the tumor.[102] These findings are in line with our understanding of IFN-γ as a major T cell effector cytokine and Th1 immune mediator. Nevertheless, clinical trials in various cancer entities had no positive effect on the treatment outcome.[97,99] Furthermore, endogenous administration of INF-γ led to systemic toxicity and serious side effects which limits the use INF-γ as well. Therefore, development of alternative

delivery routes (liposomes, polymers, nanoparticles, gene therapy) are ongoing.[97,103] Additionally, administration of IL-12 in various set-ups (e.g. engineered T cells, mRNA, or fusion proteins) is considered to increase INF-γ production[104] (Table 1). Daud et al. proofed the concept of gene transfer utilizing *in vivo* DNA electroporation for IL-12 delivery[105] and showed a 41% overall response rate with 36% complete response in melanoma patients in combination with pembrolizumab.[106]

Interleukin 4. IL-4 is a classical type II cytokine that polarizes macrophages into a specialized phenotype promoting tissue repair mechanisms, which is required for anti-parasite immunity, and contributes to fibrosis and allergy pathology.[107] In addition to its effects on macrophages, IL-4 dampens T cell stimulatory mechanisms in DCs,[108] and drives cancer cell proliferation and resistance to apoptosis.[109-112] High post-operative IL-4 levels after resection of NSCLC lesions correlated with tumor recurrence, which indicates a potential role of IL-4 in metastasis.[113] In preclinical models of lung adenocarcinoma, colon, and mammary carcinoma, IL-4 blockade reduced tumor growth and enhanced anti-tumor immunity. Mechanistically, IL-4 blockade increased immunostimulatory activity of DCs in lung cancer,[108] and decreased suppressive IL-10 in TAMs, while also increasing IL-12 and INF-γ levels measured in whole tissue.[114] In addition, inhibition of STAT6, which is the downstream signaling target of IL-4, led to a reduction of lung metastases in a mammary carcinoma model.[115] A novel strategy allows to target IL-4 signaling specifically in macrophages by the administration of extracellular vesicles carrying STAT6-silencing antisense nucleotides leading to tumor shrinkage in models of colorectal and hepatocellular carcinoma.[116] In a mouse model of STAT6 deficiency in CD11b+ myeloid cells, lung cancer growth was significantly inhibited, and the mobilization of suppressive myeloid cells was reduced.[117] However, the local effects of IL-4 on different processes of the TME have to be carefully considered. In a breast cancer mouse model, IL-4 had a protective effect by normalizing tumor vasculature and inducing tumor cell death,[118] even though human breast cancer progression is also driven by direct IL-4 signaling on cancer cells.[109] Thus, in cancers that are contained by a functional Th2 immune response driven by $CD4^+$ T cells, IL-4 signaling plays a controversial role and might not be an ideal target. In contrast, cancers that are dominated by suppressive myeloid cells, $CD8^+$ T cells, and Th1-polarized $CD4^+$ T cells could profit from IL-4 blockade. Currently, in one clinical trial, blocking of IL-4 is being tested in patients with NSCLC using dupilumab (NCT05013450; Table 1) which was initially approved for atopic dermatitis, asthma, and nasal polyps with chronic sinusitis.

Interleukin 15/Interleukin 15 receptor. IL-15 exerts its effect by first binding to the IL-15 receptor alpha (IL-15Rα) on the surface of IL-15R-expressing cells and is then trans-presented and bound to a receptor complex composed of the IL-2 receptor β and common γ chains presented mainly on lymphocytes[119,120] inducing T and NK cell proliferation, immunoglobulin synthesis in B cells, survival of memory T and NK cells, and triggering of NK cell effector function.[121-123] Based on the stimulatory role of IL-15 on lymphocytes, it has become a target of interest for cancer therapy. The short *in vivo* half-life of IL-15[121] led to the creation of IL-15/IL-15Rα complexes, often called 'super-agonists', to (i) increase their half-life and (ii) avoid the need for trans-presentation and cell—cell contact.[119] This approach led to increased effector function in cancer models[121] with increased T cell and NK cell-mediated cytotoxicity, recruitment of DCs, and reversed transforming growth factor beta (TGF-β)-mediated NK cell paralysis.[120,124] Mattiola et al. described an anti-tumor feedforward loop between NK cells and macrophages.[122] In this study, IL-15Rα trans-presented by inflammatory macrophages, enhanced NK cell cytotoxicity, which in turn re-educated suppressive macrophages boosting their anti-tumorigenic activity. Despite the improved action of IL-15/IL-15Rα complexes, monotherapy efficacy in cancer models was limited. The combination with immunotherapy, in contrast, enhanced the anti-tumor efficacy resulting in improved survival, decreased tumor growth, and enhanced cytotoxicity.[120,125,126] In clinical trials, IL-15/IL-15Rα was reported to be well tolerated in patients with advanced solid cancers[127] and showed an objective response rate in 6 of 21 patients.[128] Various trials in patients with advanced or metastatic solid tumors in combination with ICIs are ongoing (Table 1).

Transforming Growth Factor Beta. TGF-β is a pleiotropic cytokine involved in both activating and dampening the immune response and thereby promoting immune tolerance and homeostasis.[129] This double-sided behavior can also be observed in cancer, where TGF-β acts as a tumor suppressor in the early phase while with disease progression it switches into a tumor promoter.[130-134] In the initial phase of carcinogenesis, TGF-β inhibits tumor cell growth and proliferation, promotes apoptosis, and blocks growth factor, cytokine, and chemokine production.[132,133,135] With disease progression, TGF-β promotes the expansion of T_{regs} while inhibiting T and NK cell proliferation and effector function.[133,135] Furthermore, TGF-β induces monocyte chemotaxis[133] and promotes macrophage differentiation toward an immunosuppressive phenotype,[135] which can contribute to tumor metastasis.[136] Based on this dual role, TGF-β-blocking antibodies were developed which showed efficacy in cancer models.[131,133,134,137] The consequences of TGF-β blocking on macrophages are still understudied, but potentially lead to a reversion of their immunosuppressive phenotype.[138,139] Combinations of irradiation and TGF-β-blocking antibodies enhanced the anti-tumor response in mice, which could even be boosted by additionally using an anti-PD-1 blocking antibody.[133] Feun et al. reported a significant correlation between the poor outcomes of patients with advanced hepatocellular carcinoma treated with pembrolizumab with high baseline plasma TGF-β levels.[140] Therefore, a concurrent TGF-β blockade might strengthen

the efficacy of ICIs.[132] First clinical trials showed that TGF-β-targeting antibodies both in mono- and combination therapies were safe and well tolerated (NCT00356460, NCT00923169, NCT01112293, NCT01401062, NCT01646203)[141] with many clinical studies in various phases still ongoing (Table 1).

Histamine. Histamine, a well-known biogenic amine, is involved in various physiological and pathophysiological processes, and increasing evidence suggests an active role of histamine and its receptor antagonists in cancer.[142] The effect of histamine on tumor cells, such as their growth, is still controversial; both pro- and anti-tumorigenic effects have been described and seem to be dependent on cell/cancer type, histamine concentration, and involved histamine receptors.[143] In monocytes and macrophages, histamine receptor expression is also controversial.[144] However, *in vitro* data indicate that histamine receptor expression changes with the process of macrophage differentiation.[145-148] In line with tumor cells, the effect of histamine on monocytes and macrophages and their contribution to tumorigenesis is still ambiguous.[146,149,150] Remarkably, Li et al. showed that histamine polarizes monocytes via histamine receptor H1 to pro-tumorigenic macrophages, which goes in line with an increased expression of the immune checkpoint V-domain Ig suppressor of T cell activation (VISTA).[151] However, knockout of histamine receptor H1 or antihistamine treatment was able to revert both macrophage-mediated immunosuppression and revitalized T cell cytotoxic function. So far, less studies focused on the effect of histamine on therapy response, especially with ICIs. *In vitro* data support the synergistic effect of a histamine receptor H1-targeted antihistamines with platinum drugs in lung and pancreatic cancer cell lines.[152] In another study, antihistamine treatment sensitized cells to chemotherapy and reverted multidrug resistance in *in vitro* models for NSCLC, breast, and prostate cancer.[153] Li et al. reported an enhanced efficacy of both anti-PD-1 and anti-CTLA-4 antibodies in combination with a histamine receptor H1 inhibition by fexofenadine hydrochloride, a histamine receptor H1 antagonist, in mouse models.[151] Retrospective analysis of cancer patients with antihistamine consumption during ICI therapy showed a significant improvement in survival. Low plasma histamine levels were linked to a tripled objective response rate to anti-PD-1 treatment in line with Faustino-Rocha et al. who reported higher histamine levels linked with faster metastasis formation.[142] Grauers Wiktorin et al., in contrast, reported an improved anti-tumor efficacy of anti-PD-1/anti-PD-L1 treatment in combination with histamine dihydrochloride in a colorectal mouse model highlighting the controversies in this topic.[154] Currently, the only running trial with antihistamines in cancer patients receiving ICIs uses diphenhydramine (a histamine receptor H1 antagonist) in combination with pembrolizumab, acetaminophen, and MK-5890 (an anti-CD27 antibody) or MK-4830 (an antibody targeting the myeloid-specific anti—immunoglobulin-like transcript 4, ILT4) to assess objective response rate (Table 1).

Vitamin E. The vitamin E group encompasses eight structurally related compounds (α-, β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol), which possess antioxidant properties[155] and are thus implicated in reduced risk for cardiovascular diseases, immunomodulation, anti-allergic effects, and neurological and hepatic health.[156] γ-tocopherol and γ-tocotrienol were described as inducers of apoptosis in mouse and human *in vitro* models for breast[157-159] and pancreatic cancer.[160] Vitamin E might also mediate tumor-associated properties in monocytes and macrophages, as it was reported to induce tumor necrosis factor alpha (TNF-α)-producing macrophages,[161] inhibit interleukin 6 (IL-6) and nitric oxide (NO) production,[162] and reduce prostaglandin E2 synthesis,[163] which can lead to enhanced tumorigenesis, angiogenesis, and metastasis.[155] However, Yuan et al. proposed a vitamin E-mediated increase in ICI efficacy, which mainly depended on monocyte-derived DCs.[164] The anti-tumorigenic effects were consistent with *in vivo* studies.[165] In contrast, results from clinical trials studying vitamin E for cancer prevention[166-169] or as a therapy supplement[170-173] were inconclusive. A limited number of clinical trials in the context of improved efficacy are ongoing, with only two trials including monoclonal antibody therapies (NCT04245865, NCT04175470; Table 1).

CONCLUSIONS

In this review, we discuss the various subsets of monocytes and macrophages, their origin, their properties, and their role in cancer and response to immunotherapy. Both monocytes and macrophages are influenced and primed by their microenvironment, which turns them into either pro- or anti-tumorigenic modulators. Therefore, monocytes and macrophages can contribute actively to cancer progression. Nevertheless, their properties have not been fully understood, especially in the context of immunotherapy success. Monocytes and macrophages present various promising new targets, thus providing novel potential therapy approaches. Furthermore, their contribution to the efficacy of current and future therapies needs to be (re-)evaluated for potential therapy advances. We believe that a thorough molecular understanding of monocyte/macrophage populations is a pre-requisite to unlock their potential as a key target in immunotherapy to improve therapy outcome in combinatorial therapies.

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DISCLOSURE

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