Tumor-associated macrophages in breast cancer: Innocent bystander or important player?

Si-Qi Qiua,b,1, Stijn J.H. Waaijera,1, Mieke C. Zwagerc, Elisabeth G.E. de Vriesa, Bert van der Vegtc, Carolien P. Schrödera,⁎

a Department of Medical Oncology, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands
b The Breast Center, Cancer Hospital of Shantou University Medical College, Raoping 7, 515041 Shantou, China
c Department of Pathology, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands

ARTICLE INFO
Keywords:
Breast cancer
Macrophage
Tumor microenvironment
Treatment resistance
Targeted therapy

ABSTRACT
Tumor-associated macrophages (TAMs) are important tumor-promoting cells in the breast tumor microenvironment. Preclinically TAMs stimulate breast tumor progression, including tumor cell growth, invasion and metastasis. TAMs also induce resistance to multiple types of treatment in breast cancer models. The underlying mechanisms include: induction and maintenance of tumor-promoting phenotype in TAMs, inhibition of CD8+ T cell function, degradation of extracellular matrix, stimulation of angiogenesis and inhibition of phagocytosis. Several studies reported that high TAM infiltration of breast tumors is correlated with a worse patient prognosis. Based on these findings, macrophage-targeted treatment strategies have been developed and are currently being evaluated in clinical breast cancer trials. These strategies include: inhibition of macrophage recruitment, re-polarization of TAMs to an antitumor phenotype, and enhancement of macrophage-mediated tumor cell killing or phagocytosis. This review summarizes the functional aspects of TAMs and the rationale and current evidence for TAMs as a therapeutic target in breast cancer.

Introduction
Breast cancer is the most commonly occurring cancer and the leading cause of cancer related death in women worldwide, with an estimated 1.7 million new cases and 521,900 deaths in 2012 [1]. Breast cancer mortality is decreasing but still accounts for 15% of cancer death in females especially due to metastatic disease and resistance to systemic therapy [1].

Initially, research exploring mechanisms involved in metastasis and treatment resistance in breast cancer focused solely on tumor cells themselves. However, in recent years involvement of the tumor microenvironment in inducing distant metastasis and therapeutic resistance has been recognized [2]. Several strategies have been explored to target the non-malignant cells and components in the tumor microenvironment, such as immune cells and extracellular matrix [3]. Tumor-associated macrophages (TAMs) are also part of this tumor microenvironment. TAMs can change their phenotypes, depending on the signals from the surrounding microenvironment, and can either kill tumor cells or promote tumor cell growth and metastasis [4]. Moreover, they can induce resistance to multiple types of treatment in preclinical breast cancer models. Inhibiting the recruitment of macrophages or reprogramming their phenotype improved treatment response in mouse models [5–7]. In a meta-analysis including over 2000 patients with all-stage breast cancers, high TAM infiltrate density in the primary tumor predicted worse patient prognosis [8]. Therefore, TAMs are increasingly considered of interest as a potential therapeutic target in breast cancer. Here, we review the functional aspects of TAMs, as well as the rationale and current evidence for targeting TAMs in breast cancer.

Search strategy
We searched articles published until June 2018 in PubMed using the following terms: “macrophage”, “tumor-associated macrophage”, “breast cancer”, “prognosis”, “molecular imaging”, and “breast tumor” in various combinations. Abstracts of articles in English were reviewed for relevance. We also searched abstracts of annual meetings of the American Society of Clinical Oncology, American Association of Cancer Research and European Society of Medical Oncology, San Antonio Breast Cancer Symposium in 2014–2018 with the same search terms. Reference lists of articles were manually searched for relevant articles.

⁎ Corresponding author.
E-mail address: c.p.schroder@umcg.nl (C.P. Schröder).
† Both authors shared first authorship.
We included in vitro and/or in vivo studies with human breast cancer, mammary tumor cell lines and/or transgenic mammary tumor models. Studies reporting the prognostic value of TAMs in breast cancer with more than 200 patients since 2010 were included. These studies were scored according to REMARK criteria [9] (Table S1). Finally, ClinicalTrials.gov and EudraCT were searched for trials with macrophage-targeted drugs.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ctrv.2018.08.010.

Functional aspects of macrophages in cancer

Under physiological conditions, tissue-resident macrophages are innate immune cells with phagocytic functions. They have extremely heterogeneous characteristics with tissue- and niche-specific functions, thereby playing a role in maintaining tissue homeostasis and hosting defense against pathogens. In many tissues, such as skin, liver, brain, lung, pancreas and kidney, these macrophages originate from both fetal tissue (yolk sac and/or fetal liver) and hematopoietic cells (blood monocytes). An exception is the colon, where resident macrophages are solely derived from blood monocytes under physiological conditions [10].

In cancer, TAMs are involved in tumor biology by mediating tumor growth and progression as well as contributing to therapy resistance [11]. In breast cancer, TAMs can be abundantly present, and may constitute over 50% of the number of cells within the tumor. The breast cancer microenvironment also consists of fibroblasts, adipocytes and several types of leukocytes, such as neutrophils, lymphocytes and dendritic cells [12] (Fig. 1). Resident macrophages and recruitment of circulating monocytes sustain TAM accumulation in breast cancer [13]. Recruited monocytes develop into non-polarized (M0) macrophages by circulating monocytes sustain TAM accumulation in breast cancer [13]. Recruited monocytes develop into non-polarized (M0) macrophages by monocyte colony stimulating factor (M-CSF, also known as CSF1; Fig. 1) [14]. M0 macrophages are highly plastic and can change their phenotypes under influence of environmental signals. The resulting intratumoral macrophage populations can be classified along a functional scale [15,16]. In this classification, M1-like and M2-like macrophages represent two extremes of this functional continuum [15,16]. The M1-like macrophages, also called classically activated macrophages, are stimulated by the type 1 T helper cell (Th1) cytokines such as interferon-γ (IFN-γ) or tumor necrosis factor (TNF). They exhibit antitumor capacity by releasing pro-inflammatory cytokines (such as TNF and interleukin (IL)-2), together with reactive nitrogen and oxygen intermediates [17,18]. In contrast, the M2-like macrophages, also called alternatively activated macrophages, are stimulated by the type 2 T helper cell (Th2) cytokines such as IL-4, IL-10 and IL-13, and show protumor characteristics [18] (Fig. 1). Most TAMs in the tumor microenvironment are closely related to the M2-like phenotype [16]. Next to the binary model of M1-like and M2-like macrophages, attention has been focused on a more spectral polarization model in which a monocyte can develop into different subtypes based on their molecular profile [19].

In the tumor microenvironment, cancer cells secrete cytokines to recruit macrophages. M2-like TAMs in return produce high amounts of protumor cytokines to influence tumor progression [16,20,21] (Fig. 1). TAMs inhibit infiltration and function of antitumor CD8+ T-cells (CTLs), stimulate angiogenesis in the tumor, and promote tumor cell proliferation and metastasis [5,22]. Moreover, TAMs induce treatment resistance in breast cancer xenografts in mice [5–7].

Rationale for therapeutic targeting TAMs in breast cancer

Prognostic value of TAMs present in breast cancer tissue

High density of cells expressing macrophage-associated markers in primary breast cancer was associated in general with worse patient prognosis (Table 1) [23–32]. In general, included studies were of high...
Table 1

Studies on the prognostic value of tumor-associated macrophages in patients with primary breast cancer.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Marker</th>
<th>TAMs location</th>
<th>Cut-off point(s) for classifying TAMs</th>
<th>Prognostic value for patient survival</th>
<th>QA</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>562</td>
<td>CD68</td>
<td>Total; Distant and adjacent stromal</td>
<td>&gt; 369 + cells/core</td>
<td>No prognostic value for survival</td>
<td>26</td>
<td>Sousa et al. [20]</td>
</tr>
<tr>
<td>1322</td>
<td>CD68</td>
<td>Intratumoral</td>
<td>≥ 17 + cells/core</td>
<td>Independent prognostic factor for worse DFS</td>
<td>30</td>
<td>Mahmoud et al. [23]</td>
</tr>
<tr>
<td>287</td>
<td>CD68</td>
<td>Tumor stromal</td>
<td>≥ 16 + cells/HPF</td>
<td>No prognostic value for survival</td>
<td>28</td>
<td>Yuan et al. [24]</td>
</tr>
<tr>
<td>278</td>
<td>CD68</td>
<td>Invasive area and surrounding stroma</td>
<td>&gt; 34 + cells/HPF</td>
<td>No prognostic value for survival</td>
<td>23</td>
<td>Tjinen et al. [25]</td>
</tr>
<tr>
<td>222</td>
<td>CD68</td>
<td>Total; Distant and adjacent stromal</td>
<td>&gt; 26 + cells/HPF</td>
<td>No prognostic value for survival</td>
<td>27</td>
<td>Liu et al. [26]</td>
</tr>
<tr>
<td>372</td>
<td>CD68</td>
<td>Intratumoral</td>
<td>&gt; 17 + cells/core</td>
<td>Independent prognostic factor for worse DFS</td>
<td>22</td>
<td>Gwak et al. [27]</td>
</tr>
<tr>
<td>486</td>
<td>CD68</td>
<td>Tumor stromal</td>
<td>&gt; 10% macrophages in tumor stroma</td>
<td>Independent prognostic factor for worse DFS</td>
<td>27</td>
<td>Mohammed et al. [28]</td>
</tr>
<tr>
<td>278</td>
<td>CD163</td>
<td>Total; Distant and adjacent stromal</td>
<td>&gt; 24.2 + cells/HPF</td>
<td>Independent prognostic factor for worse DFS</td>
<td>28</td>
<td>Zhang et al. [29]</td>
</tr>
<tr>
<td>262</td>
<td>CD163</td>
<td>Hot spot area</td>
<td>Upper quartile</td>
<td>Independent prognostic factor for worse DFS</td>
<td>27</td>
<td>Klingens et al. [30]</td>
</tr>
<tr>
<td>10,988</td>
<td>CD68</td>
<td>Total; Distant and adjacent stromal</td>
<td>Mean (not specified)</td>
<td>Independent prognostic factor for worse DFS</td>
<td>36</td>
<td>Ali et al. [31]</td>
</tr>
<tr>
<td>7,270</td>
<td>Proportion immune cell subsets</td>
<td>–</td>
<td>–</td>
<td>Independent prognostic factor for worse DFS</td>
<td>33</td>
<td>Bense et al. [32]</td>
</tr>
</tbody>
</table>

BCSS: breast cancer specific survival; DFS: disease-free survival; HPF: high power field; OS: overall survival; QA: quality assessment according to REMARK checklist (score ranges from 0 to 40 for each study, for details please see Supplementary Table 1); TAM: tumor-associated macrophage; +: positive; †: associated with improved survival; ‡: associated with worse survival; §: intratumoral TAMs; patients with † ER-; † ER+; † HER2+; † ER-/HER2+; † ER+ /HER2+; ‡ ER- /PR-/HER2+ tumors. The prognostic value of TAMs in this table is for all breast cancers, irrespective of subtype otherwise specified.
CD68, a glycoprotein mainly localized in the endosomal compartment, has been widely used as a human pan-macrophage marker [33]. CD68 + macrophage infiltration was associated with poor prognostic breast cancer characteristics: larger tumor size, higher tumor grade, lymph node metastasis, vascular invasion, hormone receptor negativity, human epidermal growth factor receptor 2 (HER2) expression and basal phenotype [23,25]. Moreover, high in


derived VEGF-A promotes tumor cell extravasation. **Metastatic tumor cell growth**: VEGF-A promotes breast tumor cell seeding and persistent growth after seeding through activation of the VEGFR1-Focal adhesion kinase (FAK1)-CSF1-receptor (CSF1R), epidermal growth factor (EGF) and EGF receptor (EGFR), colony stimulating factor 1 (CSF1) and CSF1 receptor (CSF1R). **Intravasation**: Vascular endothelial growth factor A (VEGF-A) is secreted from macrophages in the tumor microenvironment of metastasis (TMEM) structure, which consists of the direct contact of a TIE2-expressing TAM, a mammalian enabled overexpressing tumor cell and an endothelial cell. TMEM-derived VEGF-A promotes tumor cell intravasation. **Extravasation**: In the metastatic sites, macrophages contribute to premetastatic niche establishment. The metastasis-associated macrophages (MAMS)
TAMs and demonstrated the predictive value in patients with breast cancer. These prognostic and predictive values of TAMs, generated from gene expression profile analysis using the CIBERSORT algorithm, were demonstrated in a breast cancer subtype dependent manner (Table 1). In ER- tumors, a higher fraction M2 TAMs was strongly associated with a lack of pathologically complete response (pCR) to neoadjuvant chemotherapy and a poorer outcome [31]. In ER+/HER2- tumors, a higher fraction of M0 TAMs was associated with poorer outcome [31,32], while a higher fraction of M1 TAMs was associated with a higher pCR rate and better patient prognosis [32].

Taken together, in general, high infiltration of TAMs is associated with unfavourable clinicopathological features and survival in patients with primary invasive breast cancer. Their polarization, localization and the relative amount related to other immune type fractions in a tumor lesion may be more important than their mere presence. For instance, it is conceivable that M1/M2 ratio affects outcome in breast cancer, as has been shown in ovarian cancer [38]. Besides aspects regarding TAMs, tumor aspects such as breast cancer molecular subtype could be taken into account for determining the prognostic and/or predictive role of TAMs.

**Preclinical evidence for role of TAMs in breast tumor growth and metastasis**

**Tumor growth**

Proturn TAMs were required for primary invasive mammary tumor formation in a transplantable p53-null mouse model studied for early progression [39]. Targeting TAMs with either selective monocyte targeting chemotherapeutic agent trabetectin, or CSF1 inhibitors, decreased TAM infiltration, reduced tumor growth and metastasis formation, while prolonging survival in a breast cancer xenograft mouse model [40,41].

Overexpression of cyclooxygenase-2 (COX-2) in macrophages by adenoaviral COX-2 transfection maintained the protumor M2-like phenotype [42]. In human peripheral blood mononuclear cell culture experiments, epinephrine-induced COX-2 expression increased IL-10 and indoleamine 2,3-dioxygenase (IDO) levels, which inhibited CTL proliferation and IFN-γ production. This CTL suppression could be reversed in *in vivo* and *ex vivo* breast tumor cultures by means of COX-2 inhibitor celecoxib [43]. Moreover, COX-2+ TAMs enhanced MCF-7 and MDA-MB-231 proliferation, by activating phosphoindostide 3-kinase (PI3K)-Akt signaling as well as apoptosis inhibition through increased Bcl-2 and decreased Bax expression [42] (Fig. 2). Blocking PI3K-Akt signaling with adenoviral siRNA Akt1 transfection suppressed this [42].

**Metastasis**

In animal models, TAMs regulated all metastatic processes, including local invasion, blood vessel invasatation, extravasation at distant sites and metastatic cell growth promotion [2] (Fig. 2). Local invasion largely depends on extracellular matrix (ECM) characteristics. TAM production of matrix metalloproteinases (MMPs), cysteine cathepsins and serine proteases, allowed ECM disruption and subsequent tumor cell invasion into the surrounding tissue [44]. Also secretion of secreted protein acidic and rich in cysteine (SPARC) [45], chemokine (C-C motif) ligand 18 (CCL18) [46] and epidermal growth factor (EGF) [47] by TAMs had protumor effects (Fig. 2). These factors mediated tumor cell adherence to fibronectin [46], increased tumor infiltration by regulatory T cells [48], and destabilized ECM by activating E2F3 signaling in TAMs [49]. Interfering with these processes reduced tumor cell invasiveness and metastasis in *in vitro* and *in vivo* breast cancer models [45–47].

A subset of TAMs, the perivascular TIE2-expressing TAMs, promoted invasation by expressing vascular endothelial growth factor A (VEGF-A) [50] (Fig. 2). Inhibition of TIE2 kinase or blocking TIE2 ligand angiopoietin-2 (Ang2), inhibited invasation and metastasis in the PyMT mammary tumor model [51,52]. In the same model, macrophages induced epithelial mesenchymal transition and early intravasation in pre-malignant lesions, thereby fueling late metastasis [53].

Macrophages played a major role in tumor cell extravasation, by establishing the pre-metastatic niche at distant metastatic sites [54]. The CCL2-CCR2 signaling pathway promoted the early recruitment of inflammatory monocytes to the pre-metastatic niche. Here the recruited monocytes developed into metastasis-associated macrophages (MAMs). TAM recruitment was mediated by FMS-like tyrosine kinase 1 (FLT1, also known as VEGFR1)-local adhesion kinase (FAK1)-CSF1 and CSF1-C-ets-2-microRNAs signaling pathways in macrophages [58,59] (Fig. 2). In addition, the Ang2-TIE2 pathway contributed to post-seeding metastatic growth. Blocking these pathways dramatically reduced metastases outgrowth in mouse models [52,58,59]. Also pattern recognition scavenger receptor MARCO, co-expressed with M2-like markers on TAMs, played a role in promoting breast cancer metastasis [35]. MARCO antibody treatment of mice bearing 4 T1 mammary carcinoma repolarized M2-like to M1-like TAMs, thus inhibiting metastasis. Additionally, it increased germinal center formation and CD4+ /CD8+ T cell ratio in the draining lymph nodes thereby improving tumor immunogenicity [35]. The granulo-cyte-macrophage colony stimulating factor (GM-CSF) and CCL18 feedback loop also contributed to macrophage stimulated metastasis. In a humanized mouse model bearing a human breast cancer xenograft, GM-CSF activated TAMs, which induced epithelial-mesenchymal transition and metastasis through CCL18. Inhibition of GM-CSF or CCL18 with antibodies broke the feedback loop and reduced metastasis formation [21].

Together, these results show that several signaling pathways in macrophages are likely to be involved in tumor progression, including tumor growth and all steps in tumor metastasis (Fig. 2). Reduction of macrophage infiltration, inhibition of involved signaling pathways, or interruption of the interaction between TAMs and tumor cells could thus be potential targets in breast cancer therapy.

**Preclinical evidence for a role of TAMs in breast cancer treatment resistance**

In multiple cancer types including breast cancer, TAMs profoundly influence therapy efficacy of conventional treatments such as chemotherapy and radiotherapy, but also targeted drugs and immunotherapy, including checkpoint blockade [60].

**Chemotherapy**

In mouse tumor models and breast cancer tissue of patients, paclitaxel treated tumors showed higher infiltration of TAMs compared to non-treated tumors [6,61]. Preclinically, TAM infiltration was mediated by elevated CSF1 mRNA expression in tumor cells following exposure to paclitaxel [6]. The recruited TAMs suppressed paclitaxel-induced mitotic arrest and promoted earlier mitotic slippage in breast cancer cells [62]. Inhibiting TAM recruitment by blocking CSF1-CSF1 receptor (CSF1R) signaling, enhanced paclitaxel effect and prolonged survival of the mice [6,62]. This was accompanied by enhanced CTL infiltration, and decreased vascular density through reducing VEGF mRNA expression [6]. CTLs were required for the improved paclitaxel effect, since
### Table 2

Drugs targeting tumor-associated macrophages in clinical trials for breast cancer patients.

<table>
<thead>
<tr>
<th>Target</th>
<th>Drugs</th>
<th>Clinical Trials identifier</th>
<th>Phase</th>
<th>Indication</th>
<th>Subtype</th>
<th>Drug combined with</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1-CSF1R inhibition</td>
<td>Pexidartinib</td>
<td>NCT01596751 (Active not recruiting)</td>
<td>I/II</td>
<td>B</td>
<td>All/TN</td>
<td>Erlinib</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT01525602 (Completed)</td>
<td>Ib</td>
<td>S - B</td>
<td>All</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT01042379 (Recruiting; arm closed for pexidartinib)</td>
<td>II</td>
<td>B</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Emactuzumab</td>
<td>NCT02323191 (Recruiting)</td>
<td>I</td>
<td>S - B</td>
<td>TN</td>
<td>Atezolizumab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT02760797 (Completed)</td>
<td>I</td>
<td>S - B</td>
<td>TN</td>
<td>Selicrelumab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT01494668 (Completed)</td>
<td>I</td>
<td>S - B</td>
<td>All/TN</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LY3022855 (Completed)</td>
<td>I</td>
<td>B - P</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT02718911 (Recruiting)</td>
<td>I</td>
<td>S - B</td>
<td>All</td>
<td>Durvalumab, tremelimumab</td>
</tr>
<tr>
<td></td>
<td>ARY-382</td>
<td>NCT01316822 (Completed)</td>
<td>I</td>
<td>S - B</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT02880371 (Recruiting)</td>
<td>I/II</td>
<td>S - B</td>
<td>TN</td>
<td>Pembrolizumab</td>
</tr>
<tr>
<td></td>
<td>Lacnotuzumab</td>
<td>NCT02435680/EUCTR 2015-00179-29</td>
<td>II</td>
<td>B</td>
<td>TN</td>
<td>Carbopepilin, gemicitabine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT02807844/EUCTR 2016–000210-29</td>
<td>I</td>
<td>B</td>
<td>HR+/HER2-</td>
<td>Doxorubicin, cyclophosphamide, paclitaxel</td>
</tr>
</tbody>
</table>

**Ang2: angiopoietin-2; B: breast cancer; BRCA mut: BRCA1/2 germline mutation carriers; CCL2: chemokine (C-C motif) ligand 2; CCR2: CCR2 receptor; COX-2: cyclooxygenase-2; CR3: complement receptor 3; CSF1(R): colony stimulating factor 1 (receptor); HER2: human epidermal growth factor receptor 2; HR: hormone receptor; HRR: homologous recombination repair deficient solid tumors; M: melanoma; na: not applicable; NRP1: neuropilin-1; P: prostate cancer; S: solid tumors; SIRPα: signal-regulatory protein alpha; TLR7: toll-like receptor 7; TN: triple-negative; Δ: liposomal doxorubicin, gemicitabine, paclitaxel and carboplatin, docetaxel; △: paclitaxel and trastuzumab, capecitabine and lapatinib.**

**Drugs: ALX148: SIRPα fusion protein; AMG780: anti-Ang-1/2 mAb; ARRY-382: anti-CSF1R TKI; BI 836880: TLR7 agonist; BTH1677: 1,3-1,6 β-glucan; carlumab: anti-CCL2 mAb; celecoxib: selective COX-2 inhibitor; selicrelumab: CD40 agonistic mAb; emactuzumab: anti-CSF1R monoclonal antibody (mAb); imiquimod: TLR7 agonist; Ly3022855: anti-CSF1R mAb; nesvacumab: anti-Ang2 mAb; nivolumab: PD-L1 inhibitor; olaparib: anti-PARP-1 inhibitor; pembrolizumab: anti-PD1 mAb; pexidartinib: anti-CSF1R tyrosine kinase inhibitor (TKI); rebastinib: anti-TIE2 TKI; Ti-061: anti-COX4 mAb; trabectedin: DNA minor groove binder; trebananib: anti-Ang1/2 bispecific peptide; TTI-621: SIRPα-Fc fusion protein; vanucizumab: anti-Ang2-vascular endothelial growth factor A (VEGF-A) bispecific mAb; vescemucab: anti-NRP1 mAb; zoledronic acid: osteoclast-mediated bone resorption inhibitor; 852A: TLR7 agonist.**

**Drugs combined with:** atezolizumab: anti-programmed death ligand 1 (PD-L1) mAb; bevacizumab: anti-VEGF-A mAb; durvalumab: anti-PDL1 mAb; exemestane: aromatase inhibitor; olaparib: poly (ADP-ribose) polymerase inhibitor; spartalizumab: anti-PD1 mAb; pembrolizumab: anti-programmed death 1 (PD1) mAb; selicrelumab: CD40 agonist mAb; temsirolimus: mammalian target of rapamycin inhibitor; trastuzumab: anti-human epidermal growth factor receptor 2 mAb; tremelimumab: anti-cytotoxic T-lymphocyte-associated protein 4 mAb.
and CTL depletion diminished the effect of the anti-CSF1R–paclitaxel treatment [6]. Macrophages also inhibited the antitumor effect of other chemotherapeutic agents, such as doxorubicin, etoposide, gemcitabine and CMF regimen (cyclophosphamide, methotrexate, 5-fluorouracil), in in vitro or in vivo studies [62,63].

However, TAM recruitment was only partially blocked by CSF1-CSF1R inhibition, leaving a population of perivascular TAMs unaffected [6]. Although the phenotype of remaining TAMs has not been identified, at least a proportion of them were perivascular TIE2-expressing TAMs [22], which were an essential source of VEGF-A [50]. Together, these data indicate that other mechanisms, besides VEGF-A secretion, may contribute to TAM-mediated chemoresistance in breast cancer. One of those mechanisms might involve TAM-derived cathepsins, specifically cathepsin B and cathepsin S, which protected murine mammary tumor cells from paclitaxel-, etoposide- or doxorubicin induced cell death in ex vivo co-cultures [61]. Although the downstream signaling pathways were ill-defined, this protective effect was abrogated by a cathepsin inhibitor both in vivo and ex vivo [61]. Another chemoprotective effect resulted from TAM-derived IL-10. An IL-10 antibody reversed IL-10 mediated paclitaxel resistance of human breast cancer cells in ex vivo co-culture studies [64]. Possibly, IL-10-mediated drug resistance is associated with up-regulation of signal transducer and activator of transcription 3 (STAT3) signaling and elevation of antiapoptotic bel-2 gene expression in tumor cells [64]. The importance of TAM-derived factors such as IL-10 in chemoresistance, suggests that repolarization to a more M1-like phenotype is a potential strategy to enhance chemotherapy efficacy. This was already shown for selective class IIa histone deacetylase (HDACIIa) inhibitor TMP195. This drug modulated TAMs into the M1-like phenotype, and decreased tumor burden in MMTV-PyMT mice, particularly when combined with paclitaxel [65].

Taken together, TAM-targeted therapy could be a potential strategy to reverse chemoresistance and improve chemotherapeutic efficacy in breast cancer.

Radiotherapy

In MMTV-PyMT mice, radiation induced tumor CSF1 expression dose dependently [6]. TAM depletion by CSF1R blockade enhanced the effect of radiotherapy for mammary tumors in the same mouse model [7]. CSF1R blockade increased CTL infiltration and reduced presence of CD4 + T cells in the tumors. Interestingly, depleting CD4 + T cells had the same effect as CSF1R blockade when combined with radiotherapy, highlighting the interaction of macrophages with other immune cells [7]. MMP14 expression may also account for TAM-induced radiotherapy resistance. In a 4T1 tumor bearing mouse model, MMP14 blockade repolarized M2-like to M1-like TAMs. Moreover, MMP14 blockade inhibited angiogenesis, increased vascular perfusion and enhanced the effect of radiotherapy [66]. Topical application of the cream containing 1,6 beta-glucan, which increased direct killing of antibody-targeted tumor cells in vitro, increased γ-irradiation in the tumor [65]. Furthermore, TAMs expressed PD-1 and PD-L1 [22,77]. PD-1 expression on TAMs appeared to be dependent of their phenotype.

In summary, TAM depletion or repolarization could be a potential strategy to enhance radiotherapeutic efficacy in breast cancer.

Anti-HER2 targeted therapy

Trastuzumab has antitumor activity by interference with HER2 oncogenic signaling and the activation of antibody dependent cellular cytotoxicity (ADCC) [68]. The adaptive immune system also plays a role in the antitumor efficacy of trastuzumab [69]. In HER2+ TUBO mammary tumor bearing mice, CTLs were essential for the therapeutic effect of anti-HER2 antibody treatment. CTL infiltration in the tumor increased after antibody treatment, accompanied with tumor regression. However, rapid tumor regrowth was seen after CTL depletion by an anti-CD8-depleting antibody [69], suggesting a T cell dependent mechanism for HER2 antibody treatment resistance. This may be mediated by TAMs, as they inhibited CTL infiltration in TUBO tumor bearing mouse model [5]. TAM depletion as well as repolarizing M2-like to M1-like TAMs, dramatically increased the therapeutic effect of a HER2 antibody. Also CTL infiltration and IFN-γ production in the tumor increased [5]. However, merely increasing the tumor infiltrating CTLs without removal of TAMs failed to reverse anti-HER2 resistance [70].

Also, blocking the interaction between CD47 and signal-regulatory protein alpha (SIRPs) may be a macrophage-mediated way to improve trastuzumab efficacy. Blocking CD47, the ‘don’t eat me’ signal expressed by tumor cells, increased phagocytosis of breast cancer cells in vitro. Furthermore, CD47 antibody inhibited growth of a human breast cancer xenograft [71]. However, targeting SIRPs with high-affinity monomers did not increase direct macrophage phagocytosis. But combined with trastuzumab, the monomers increased macrophage-mediated antibody dependent cellular phagocytosis (ADCP) by lowering the ADCP threshold. In a breast cancer xenograft, the combination showed synergistic antitumor effect [72]. The ADCP capacity of macrophages appeared to be dependent of their phenotype. In vivo, M1-like macrophages in the presence of trastuzumab were more potent in phagocytosis compared to M2-like macrophages [73]. Moreover the combination of CD47 blockage and trastuzumab enhanced neutrophil-mediated ADCC [74]. Additionally, blocking the CD47-SIRPs axis increased DNA sensing in dendritic cells, which improved the antitumor immunity with an enhanced CTLs response [75].

Together, these data provide a new paradigm of potential combination therapeutic strategy with TAM-targeted treatment for breast cancer patients receiving anti-HER2 treatment. The anti-HER2/TAM targeting combination in clinical trials is summarized in Table 2.

Immunotherapy

The programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) axis, which induces immune tolerance of activated T cells, has become a target in cancer immunotherapy. Intravitral imaging of a MC-38 colon cancer allograft illustrated that macrophages mediated PD-1 therapy resistance through capturing the PD-1 antibody by the Fcγ receptor, thereby preventing T cell drug exposure [76]. Furthermore, TAMs expressed PD-1 and PD-L1 [22,77]. PD-1 expression on TAMs correlated negatively with their phagocytic capacity both in vitro and in vivo [77]. This has raised interest in the combination of macrophage-targeted therapy and immune checkpoint modulation in breast cancer. Proof of concept was demonstrated by combining CSF1R blockade with PD-1 and CTLA4 inhibitors in a mouse model bearing a mouse pancreatic tumor. The combination potently elicited tumor regression, while PD-1 and CTLA4 inhibitors as single agents showed limited efficacy [78]. The HDACIIa inhibitor TMP195 changed macrophage function and rescued the inhibitory tumor microenvironment by activating CTLs in MMTV-PyMT mice [65]. Combining TMP195 with PD-1 antibody resulted in tumor shrinkage, which the PD-1 inhibitor alone did not. This suggests that the immune suppressive environment created by TAMs induces anti-PD-1 resistance in this model.

Stimulating macrophages via the co-stimulatory CD40 molecule by agonistic antibodies, resulted in macrophage-mediated tumor regression in a pancreatic cancer bearing mouse model [79]. Moreover, CD40 stimulation accompanied upregulation of PD-L1 expression on TAMs [80]. Combining CD40 stimulation and PD-L1 inhibition had synergistic antitumor effects in mice bearing EMT-6 mammary tumors [80]. This combination showed also synergistic antitumor effects accompanied by increased infiltration of dendritic, monocyotic and T cells in the HER2/neu-expressing mammary tumor allograft [81]. Innate immune cells, such as macrophages, can also be stimulated by pathogen-associated molecular patterns (PAMPs). An example is BTH1677, a fungal-derived 1,3–1,6 beta-glucan, which increased direct killing of antibody-targeted tumor cells by macrophages in vitro, through Fcγ receptors and complement receptor 3 (CR3) [82]. BTH1677 also repolarized M2-like to M1-like TAMs in vitro and enhanced CD4 T cell proliferation and IFN-γ production [83]. Furthermore, BTH1677 demonstrated synergistic antitumor effects with anti-PD-1 and PD-L1 antibodies in a 4T1 tumor
Several small molecules and antibodies have been developed to target the CSF1-CSF1R axis, and are or have been evaluated in clinical trials for solid tumors including breast cancer (Fig. 3; Table 2). These drugs were well tolerated in phase I trials, also when combined with paclitaxel [85,86]. Moreover, emactuzumab, a CSF1R-antibody, decreased CD163+ TAMs infiltration in serially collected tumor biopsies of patients with various solid tumors, including breast cancer [86].

### CD47-SIRPa inhibition

Several drugs targeting the CD47-SIRPa axis are in early clinical development (Fig. 3; Table 2). In a phase I trial, intratumoral injection of TTI-621, a SIRPa-Fc fusion protein, showed tolerability and some antitumor efficacy in patients with cutaneous T cell lymphoma [87]. In addition, intravenous administration of fusion protein ALX148 that binds CD47 is studied in combination with trastuzumab or the PD-1 antibody pembrolizumab (NCT03013218).

### CD40 stimulation

CD40 agonistic antibodies are studied in early clinical trials, some of which also include breast cancer patients (Table 2). Two phase I trials with selicrelumab, a fully human CD40 agonist monoclonal antibody, showed tolerability. Partial tumor responses were observed in four and stable disease in seven of 29 patients in one trial and stable disease was the best response in the other trial [88,89]. Interestingly, a patient with advanced pancreatic ductal adenocarcinoma showed a partial response, whereas trabectedin has also shown to cause TAMs depletion in tumor microenvironment. Other macrophage-targeted therapies in breast cancer include angiopoietin 2 (Ang2)-TIE2 axis inhibition, cyclooxygenase-2 (COX-2) inhibition and bisphosphonates. The Ang2-TIE2 signaling mediates angiogenesis and metastasis. Expression of COX-2 in TAMs is essential to promote tumor cell proliferation. Bisphosphonates have been widely used in breast cancer. Only preclinical evidence suggests that bisphosphonates cause TAM apoptosis. This figure was prepared using a template on the Servier medical art website (http://www.servier.fr/servier-medical-art).

**Fig. 3.** Macrophage-targeted therapies in breast cancer. Macrophage-targeted therapies are aimed at activating macrophages’ tumor killing activity, or inhibiting their recruitment and tumor-promoting functions. Activation of macrophages’ antitumor activity can be achieved by stimulating the co-stimulatory receptor CD40, complement receptor 3 (CR3) and Toll-like receptor 7 (TLR7). These treatment strategies have been demonstrated to repolarize the tumor-promoting M2-like tumor-associated macrophages (TAMs) to an antitumor M1-like phenotype. In addition, blocking the interaction between CD47 and signal-regulatory protein alpha (SIRPa), a ‘don’t eat me’ signal, can enhance macrophages’ phagocytic function and thereby improve their antitumor activity. Inhibition of macrophage accumulation within the breast tumor microenvironment has been demonstrated to reduce tumor growth and metastasis in preclinical studies. This treatment strategy includes inhibition of colony stimulating factor 1 (CSF1)-CSF1 receptor (CSF1R) axis or chemokine (C-C motif) ligand 2 (CCL2)-CCL2 receptor (CCR2) axis. Besides, caspase-8 dependent TRAIL receptor-mediated monocye apoptosis induced by a DNA-binding marine alkaloid trabectedin has also shown to cause TAMs depletion in tumor microenvironment. Other macrophage-targeted therapies in breast cancer include angiopoietin 2 (Ang2)-TIE2 axis inhibition, cyclooxygenase-2 (COX-2) inhibition and bisphosphonates. The Ang2-TIE2 signaling mediates angiogenesis and metastasis. Expression of COX-2 in TAMs is essential to promote tumor cell proliferation. Bisphosphonates have been widely used in breast cancer. Overall, macrophage-targeted therapy can augment immune checkpoint inhibition efficacy in preclinical breast cancer models. Table 2 summarizes ongoing studies with this combination in patients with breast cancer.

### Current evidence for therapeutic targeting of TAMs in patients with breast cancer

Based on the tumor-promoting functions of TAMs, several drug interventions are employed in clinical trials. These drugs mainly focus on repolarizing or depleting TAMs, but also on stimulating anti-tumoral macrophages.

#### CSF1-CSF1R inhibition

Several small molecules and antibodies have been developed to target the CSF1-CSF1R axis, and are or have been evaluated in clinical trials for solid tumors including breast cancer (Fig. 3; Table 2). These drugs were well tolerated in phase I trials, also when combined with paclitaxel [85,86]. Moreover, emactuzumab, a CSF1R-antibody, decreased CD163+ TAMs infiltration in serially collected tumor biopsies of patients with various solid tumors, including breast cancer [86].
pembrolizumab treatment [91].

**TLR7 stimulation**

Imiquimod, a cream for topical administration to treat basal cell carcinomas, was studied in a prospective phase II trial in 10 patients with breast cancer skin metastases [92]. Two patients showed a partial response, which was defined as residual disease less than 50% of original tumor size. In one partial responder, T-cell infiltration increased. In the other responder, the immunosuppressive environment was reversed, with lower levels of IL-6 and IL-10 in the tumor supernatant. The lower cytokine levels suggest macrophage repolarization, but this was not studied directly.

In a phase I trial, 10 patients received single imiquimod application on one skin metastasis and a combination with radiotherapy on another skin metastasis. Complete response was observed in one-, and partial response in four of nine patients who received imiquimod only. For the combination, complete and partial responses were observed in three and five out of the nine patients, respectively. Imiquimod was tolerated well, with mostly low grade adverse effects such as dermatitis and pain [93].

Another TLR7 stimulant 852A, was administrated subcutaneously in a phase II trial in heavily pretreated patients with recurrent ovarian (n = 10), breast (n = 3) and cervical (n = 2) cancers [94]. Best response was stable disease in two patients. Moreover, unanticipated toxicities such as myocardial infarction and infection occurred.

**CCL2-CCR2 inhibition**

Halting CCL2 neutralization accelerated breast cancer metastasis in a preclinical study [95]. Development of the monoclonal antibody carlumab against CCL2 in breast cancer was discontinued because of the lack of clinical efficacy [96]. Other drugs targeting the CCL2-CCR2 axis, like small molecules CCX872-b and BMS-81360 are currently in phase I-II trials, but they are not including patients with breast cancer. The lower cytokine levels suggest macrophage repolarization, but this was not studied directly.

**Ang2-TIE2 inhibition**

Several drugs have been designed to target the Ang2-TIE2 axis and studied in patients with breast cancer (Fig. 3; Table 2). In a randomized study 228 patients received paclitaxel 90 mg/m² once weekly (3-weeks-on/1-week-off) and were randomly assigned 1:1:1:1 to also receive blinded bevacizumab 10 mg/kg once every 2 weeks plus either trebananib 110 mg/kg once weekly (Arm A) or 3 mg/kg once weekly (Arm B), or placebo (Arm C); or open-label trebananib 10 mg/kg once a week (Arm D). The primary endpoint progression-free survival did not differ between the treatment arms [97].

In a phase Ib study trebananib (10 mg/kg or 30 mg/kg) was combined with paclitaxel and trastuzumab in patients (n = 20 for each trebananib dose group) with HER2+ recurrent or metastatic breast cancer. This combination was tolerable and three out of 17 achieved complete responses with 30 mg/kg compared to none out of 20 at the 10 mg/kg dose [98]. So far, Ang2-TIE2 inhibition shows limited clinical efficacy in patients with breast cancer.

**Trabectedin**

In TAMs and tumor cells derived from ascitic fluid of ovarian cancer patients, ex vivo trabectedin treatment reduced TAM viability and inflammatory mediators CCL2 and IL-6 production by TAMs and tumor cells [99]. Furthermore, seven out of nine trabectedin treated patients with ovarian cancer, showed reduced peripheral monocyte counts [91]. Trabectedin was studied in several phase II trials in patients with metastatic breast cancer. The drug was tolerable with transient and manageable adverse events. Trabectedin 1.3 mg/m² intravenous infusion every 3 weeks resulted in objective responses in three out of 25 patients and a progression free survival (PFS) of 3.1 months at a median follow-up of 7 months [100]. Another phase II trial in patients with HER2+ (n = 37) or triple negative (n = 50) metastatic breast cancer showed only partial responses in four out of 34 evaluable HER2+ patients with median PFS of 3.8 months [101].

**Commonly used drugs in oncology that may affect macrophages**

**Bisphosphonates**

Bisphosphonates such as zoledronic acid are commonly used in clinical practice for breast cancer. Accumulating evidence suggests that macrophages contribute to the antitumor effect of bisphosphonates. Preclinically bisphosphonates caused apoptosis in macrophage in vitro [102]. However, the precise effect of bisphosphonates on TAMs in patients with breast cancer has not yet been studied.

**COX-2 inhibition**

Selective COX-2 inhibitor celecoxib showed changes in RNA expression in example proliferation related genes in pre- and post-treatment primary tumor material of patients with breast cancer [103]. Interestingly, M1-like macrophage marker HLA-DRα was upregulated in tumors after treatment with celecoxib, suggesting increased presence of M1-like macrophages [103]. Antitumor activity of celecoxib in patients with breast cancer however is disappointing [104]. In a window of opportunity trial, tumor/stroma response to preoperative celecoxib will be studied by determining CD68 and CD163 expression in tumor biopsies before and after celecoxib treatment in patients with primary invasive breast cancer (NCT03185871).

**Other drugs**

Despite the preclinical support for a TAM mediated protrumor role of GM-CSF [21], in the clinical setting no evidence was found for a detrimental effect of this- or other commonly used growth factors such as granulocyte colony-stimulating factor.

Taken together, data from early clinical trials in breast cancer patients are now becoming available. So far, evidence in general shows limited clinical efficacy.

**Conclusions and future perspectives**

Collectively, many preclinical studies illustrated the protumor function of TAMs in breast cancer. TAMs play a role in tumor growth, progression, treatment resistance and immune suppression. However, the clinical efficacy of targeting TAMs in breast cancer so far has been limited. Potential options to improve this include combination strategies. Particularly in view of the immunosuppressive role of TAMs in the breast cancer microenvironment, results of clinical trials combining TAM targeting and checkpoint inhibition are eagerly awaited. First results of anti-CSF1R antibody cabiralizumab and anti-PD-1 antibody nivolumab combination showed a tolerable safety profile and four partial responses in 31 patients with advanced pancreatic cancer [105]. Data on clinical efficacy of TAM-targeted therapies in patients with breast cancer is limited. A careful approach in targeting the total population monocytes or macrophages is needed, as for example classical CD14+CD16+CD33+HLA-DR+ monocytes may be beneficial to obtain a response to immunotherapy [106]. Also strategies combining TAM-targeted agents with chemotherapy, radiotherapy or HER2 targeted drugs may induce synergistic therapeutic effects. Additional macrophage-targeted agents, are currently being evaluated in other cancer types (Table S2).

To improve targeting TAMs, also a number of challenges need to be addressed. For some targets such as CD47, the effect is probably not solely mediated by TAMs. Some drugs such as CSF1R tyrosine kinase inhibitor pexidartinib target more tyrosine kinases, which makes it difficult to study the contribution of targeting TAMs on its antitumor
effect [6]. Improving insight in these interactions can potentially improve these intervention strategies. This is of particular importance when considering for instance resistance to macrophage-targeted therapy involving cross talk between TAMs and other cells. This was described in a recent study, demonstrating that tumor-associated fibroblasts impaired the antitumor effects of a CSF1R inhibitor [107]. Furthermore, the timing of the anti-TAM treatment may influence results of TAM targeting treatments, especially regarding combination strategies. For instance, the increasing awareness of macrophage activation syndrome after T cell-engaging therapies, which is characterized by severe immune activation and immune mediated multiple organ failure, may call for upfront macrophage-directed therapies in this setting, such as IL-6 blockade [108].

To improve TAM directed therapy, monitoring whole body TAM dynamics and phenotype upon TAM targeting therapy is crucial. Techniques such as molecular imaging might provide whole body insight in macrophages populations, heterogeneity (between primary and metastatic tumors), and pharmacodynamics. This approach has been tested preclinically using imaging modalities such as a radiolabeled nanobody PET tracer targeting M2 marker CD206 [109]. Clinically, the FDA and EMA approved imaging agent Lymphoseek (99mTc-tilmanocept) targeting CD206 has been used for lymphatic mapping in sentinel lymph node biopsy in multiple cancer types, including breast cancer [110].

Acknowledgments

We would like to thank Karin de Visser (NKI) for her helpful advice. This work was supported by The Abel Tasman Talent Program (ATTP).

Conflicts of interest

None.

References


