

Tumor-associated macrophages: Role in the pathological process of tumorigenesis and prospective therapeutic use (Review)

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Abstract. The aim of the present study was to evaluate the current body of knowledge regarding tumor-associated macrophages (TAMs) and their potential use in antitumor therapy, based on their role in the pathological process of tumorigenesis. For this purpose, a critical analysis of published data and summarization of the findings available from original studies, focusing on the role of TAMs in the pathological process, and their potential therapeutic application was performed. Promising key avenues of research were identified in this field. The following issues seem the most promising and thus worth further investigation: i) The process of M1/M2 macrophage polarization, macrophage characteristics at intermediate polarization steps and their role in the tumor process; ii) determining the conditions necessary for transitions between the M1 and M2 macrophage phenotypes and the role of signals from the microenvironment in this process; iii) cause-and-effect associations between the quantity and quality of macrophages, and the prognosis and outcome of the pathological process; iv) modulation of macrophages and stimulation of their phagocytic activity with drugs; v) targeted vector-based systems for drug delivery to macrophages; and vi) targeted drug delivery systems with macrophages as carriers, thus potentially combining chemotherapy and immunotherapy.

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1. Introduction

The incidence of cancer is continuously increasing (1-8). Cancer is the second leading cause of death after cardiovascular disease worldwide, accounting for an estimated 9.6 million deaths, or one in six deaths in 2018 (9). A number of highly effective drugs are available for modern pharmacotherapy; however, the results are often unsatisfactory. Cancer treatments are associated with severe side effects, high toxicity, poor pharmacokinetics properties, lack of water solubility, lower therapeutic indices, and development of drug resistance (4,5,10-12). Unfavorable biodistribution is one of the primary factors that reduce drug efficacy and may arise when drug penetration into the desired pathological site is hindered. The drug dose is frequently increased to overcome this hindrance and to improve the treatment efficacy. Hence, a therapeutic effect is often achieved at the cost of higher non-specific toxicity. The problem is particularly pressing in the case of anticancer drugs, the side-effects of which are severe enough to substantially reduce the therapeutic value of the drugs. Novel therapeutic means are continuously sought out in view of this, and immunotherapy is among the most promising avenues. Following the discovery of alternative pathways of macrophage activation, specific attention has been paid to tumor-associated macrophages (TAMs), their roles in the pathological process of tumorigenesis, and the possibility of their use in anticancer therapy. During the writing of this

review, the most recent studies in the field of TAMs were analyzed, and the possibility of using macrophages as a point for therapeutic exposure was considered; in such a structure, a review is presented for the first time, to the best of our knowledge.

A feature of the present review is the systemized presentation of cancer treatment directions aimed at macrophages, the process of their polarization, and their therapeutic use. Three primary strategies are described: i) Drugs that are able to modulate the activity of TAMs; ii) designed carriers for targeted drug delivery to macrophages, TAMs or specific pro-tumor M2-TAMs; and iii) the use of macrophages to target the tumor. At present, research regarding TAMs is largely focused on an increased interest in the search for markers characterizing functionally different subpopulations of macrophages associated with tumor progression and the effectiveness of chemotherapy, which may result in identification of potential targets for treatment (13-18). The simplified dichotomous classification of M1/M2 provides a conceptual basis for describing the polarization of macrophages and the identification of polarizing stimuli (19-24). The high plasticity of macrophages with respect to changes in their polarization under the influence of various microenvironmental conditions opens up prospects for the directed differentiation of macrophages into an antitumor M1 phenotype or blocking of M2 polarization.

The objectives of the present review were to assess the state of TAM research and to evaluate the possible use of TAMs in cancer therapy.

2. TAMs: General characterization

The process of tumorigenesis in the body begins to affect the tumor microenvironment, including macrophages. Blood monocytes penetrate the tumor, and differentiate into macrophages with an anti-inflammatory phenotype in response to signaling molecules produced by the tumor, such as interleukin (IL)-4, IL-10 and transforming growth factor (TGF)- β . These signals suppress antitumor immunity, and stimulate the development of new blood vessels and thus tumor growth and metastasis (17). The role of TAMs in tumor progression are illustrated in Fig. 1.

TAMs have attracted substantial attention for the past 30 years (from the time when the concept of a macrophage dichotomy was advanced) (25,26). TAMs are classed as type II-activated macrophages (M2). Stein *et al* (27) first characterized TAMs as alternatively activated macrophages in 1992. Data on TAM markers and TAM-suppressing factors subsequently accumulated in further studies (28-31). The M2 population is highly heterogeneous (32,33). Macrophages with the M2 phenotype serve an important role in the process of tumorigenesis by suppressing the immune response, remodeling the extracellular matrix and stimulating angiogenesis (26). M2 macrophages are characterized by the expression of specific receptors, such as arginase-1, mannose receptor (CD206), CD163, CD209, FIZZ1 and Ym1/2 (22,29).

Macrophages with the M1 phenotype (classically activated macrophages) express bactericide molecules and receptors (34). Macrophages acquire the M1 phenotype in response to endogenous inflammatory stimuli, such as the Th1-associated cytokine interferon- γ , or exogenous stimuli,

such as lipopolysaccharides (23,35). M1 macrophages produce pro-inflammatory cytokines and thereby stimulate the inflammatory response (36). A total of 5,598 publications on TAMs were available on PubMed as of July 10, 2020. The annual number of such publications increased from 51 in 2007 to 660 in 2019.

Macrophages are intricately involved in the immune response, and thus serve a protective role. They participate in the clearance of cellular debris and iron processing, degradation of dead cells and foreign material, response to infection, immunomodulation and modulation of inflammatory processes, angiogenesis, and facilitating wound healing (20,22,23). Furthermore, macrophages serve an important role in organ development, and in tissue turnover and regeneration (37,38). Adverse reactions are also often caused by macrophages and are associated with their M1/M2 polarization. M1 macrophages serve critical roles in innate host defense and in the killing of tumor cells. Therefore, they are considered as antitumor macrophages. M2 macrophages tend to exert an immunosuppressive phenotype, favoring tissue repair, and tumor promotion. Thus, they are considered as pro-tumorigenic macrophages. The expression of inhibitory cytokines in tumor cells or macrophages provides a mechanism of resistance to anticancer therapy. Hence, a therapeutic strategy targeting macrophages or macrophage-derived cytokines may be a promising and effective method for targeting tumorigenesis (39).

TAMs are an important component of the tumor microenvironment, which affects tumor growth, tumor angiogenesis, immunity suppression, metastasis and chemoresistance. TAMs substantially affect the clinical efficacy of these drugs and drug resistance. For example, TAMs release chemoprotective factors, such as cathepsin b and milk-fat globule EGF-VIII, which promotes tumorigenicity of cancer stem cells and induces anticancer drug resistance. Furthermore, drugs targeting TAMs have been shown to exhibit promising results for potential use in anticancer therapy (40). The role that macrophages serve in carcinogenesis has been the focus of several studies, including systematic reviews (41-43).

M1 macrophages promote tumor elimination, whereas M2 macrophages facilitate carcinogenesis (44). As demonstrated over half a century ago, M1 macrophages are capable of killing and eliminating cancer cells in accordance with their primary physiological function, the elimination of foreign and harmful substances (45). M1 cells initiate cytokine production in the tumor microenvironment and facilitate cancer cell destruction by recruiting pro-immunostimulatory leukocytes and phagocytizing tumor cells (46,47). M2 macrophages serve a leading role in tumor spread (48). M2 macrophages have a notable effect on tumor development in both the primary and metastatic foci. Their effects are associated with basement membrane degradation, angiogenesis and general immunosuppression (49,50). Macrophages have been shown to be present not only in the M1 or M2 states in the tumor microenvironment, but also in transitional states, and the role of the transitional states in tumorigenesis remains poorly understood (51). The elimination of all macrophage populations regardless of the polarization state may provide a potentially effective approach to therapy as both primary and metastatic tumorigenesis is reduced as a result (52).

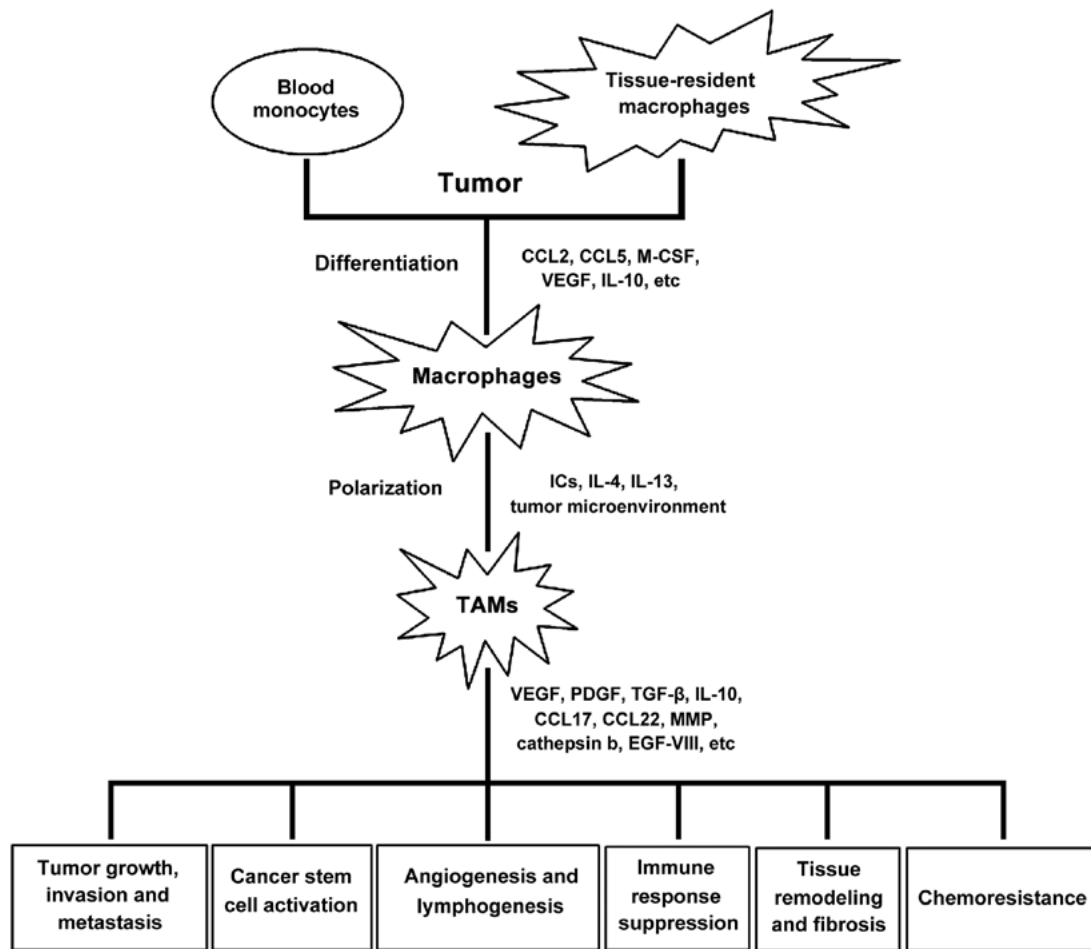


Figure 1. Cellular origin and the role of TAMs in tumor progression. CCL, C-C motif ligand; EGF-VIII, epidermal growth factor; ICs, immunocomplexes; IL, interleukin; M-CSF, macrophage-colony stimulating factor; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor- β ; TAMs, tumor-associated macrophages; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase.

The activation of macrophages is widely regarded as polarization in the direction of the M1 or M2 states. However, the M2 activation state includes heterogeneous and functionally distinct macrophages. Studies on the existence of macrophages of the M2a, M2b, M2c and M2d phenotypes make it possible to specify a number of aspects regarding the nature of the immune response (Table I).

M2a and M2b phenotype macrophages typically exhibit anti-inflammatory activity. Macrophages of the M2c phenotype are very similar to M1 macrophages, with the exception of high (increased) IL-10 expression as opposed to pro-inflammatory cytokines (53). Wang *et al* (54) isolated the M2d phenotype, characterized by decreased secretion of IL-12 and increased secretion of IL-10. M2d macrophages are common in the tumor microenvironment. It is hypothesized that M2d macrophages are induced following stimulation with Toll-like receptor agonists and adenosine, and/or tumor-related factors. Isolation of subtypes of macrophages of the M2 family may facilitate the possibility of their targeted therapeutic use for treatment of tumors.

However, Quail and Joyce (55) demonstrated that the clinical effect of isolation of subtypes of macrophages of the M2 family was limited due to the limitations in the methods of targeted drug delivery to macrophages. However, macrophages preserve their plasticity regardless of polarization and, in particular,

remain capable of switching from one phenotype to another dependent on the stimuli from the microenvironment (55).

The presence of macrophages in primary tumors is associated with a poor prognosis (56-59), with colorectal cancer serving as the only exception (60). M1 and M2 macrophages present in the tumor microenvironment have been the focus of an increasing number of studies (20,28,51). Although the causal associations have not yet been established, the available body of research highlight the possibility of novel therapeutic strategies that are aimed at eliminating macrophages or altering the macrophage phenotype (61).

Since increased TAM infiltration is associated with a poor prognosis and therapeutic failure in cancer, TAM reprogramming toward the anticancer M1 phenotype and TAM suppression may provide promising strategies for the treatment of cancer (62).

3. Prospective use of TAMs for anticancer therapy

Based on the literature search performed for the present review, three macrophage-related strategies of cancer therapy are speculated. These strategies involve drugs that modulate TAM activity; engineered carriers for targeted drug delivery to macrophages, TAMs, or specific pro-tumoral M2-TAMs; and macrophage self-targeting to the tumor.

Table I. Classification of phenotypes of M2 macrophages.

Phenotype of M2 macrophages	Stimulus for polarization	Produced cytokines	Functions
M2a	IL-4, IL-13	IL-10, TGF- β , IL-1 receptor antagonist	Activation of Th2-reactions, eosinophil involvement, connective tissue growth
M2b	IL-1 β , immunocomplex + lipopolysaccharide	IL-1, IL-6, IL-10, TNF- α	Regulation of inflammatory and immune responses, activation of Th2
M2c	IL-10, TGF- β , glucocorticosteroids	IL-10, TGF- β	Remodeling, intercellular matrix synthesis
M2d	IL-6, adenosine	IL-10, IL-12, TNF- α , TGF- β	Tumor metastasis and progression

IL, interleukin; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α .

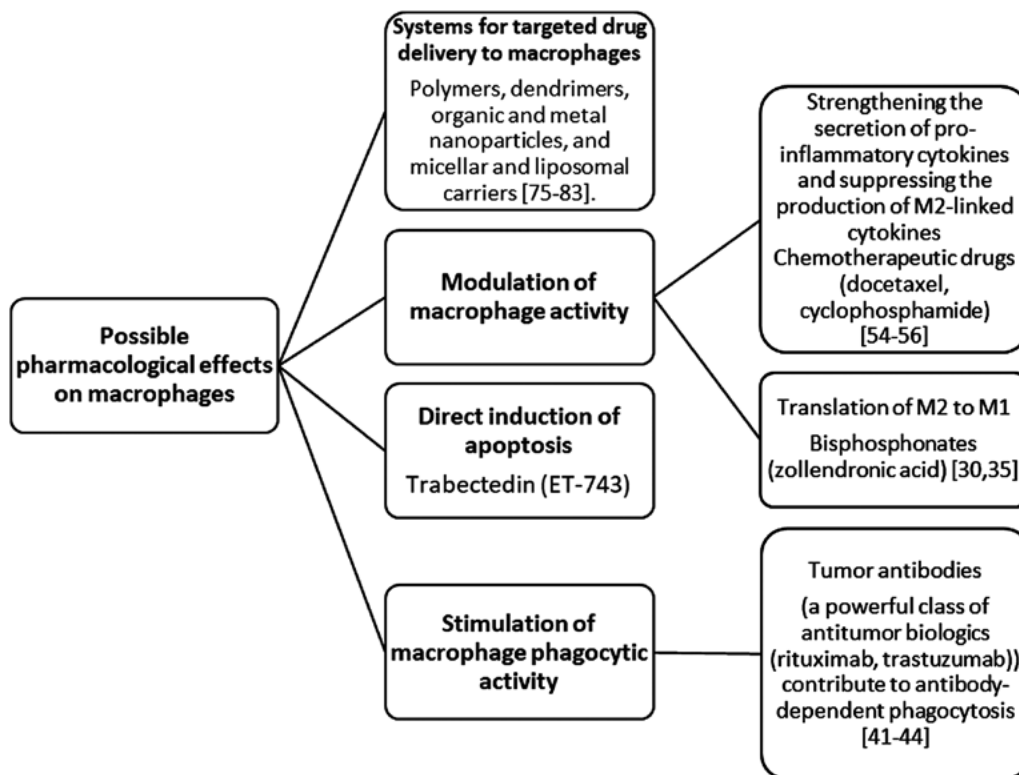


Figure 2. Potential directions of pharmacological influence on macrophages in the treatment of the tumor process.

Drugs modulating macrophage activity. Various drugs that modulate macrophage activity are illustrated in Fig. 2.

Bisphosphonates modulate macrophage activity and are used in the treatment of bone tissue disorders, such as osteoporosis and bone metastases in cancer. A previous preclinical study using a mouse model of breast tumors suggested that an extra skeletal therapeutic effect is additionally exerted by bisphosphonates (63).

Zoledronic acid, which is a medication used in the treatment of cancer, has been shown to revert macrophage polarization from the M2 to the M1 phenotype, thus inhibiting spontaneous breast carcinogenesis (64). Zoledronic acid acts as a potent

inhibitor of farnesyl pyrophosphate synthase, which is a key enzyme of the mevalonate pathway. By inhibiting farnesyl pyrophosphate synthase, zoledronic acid prevents the prenylation of small G-proteins, such as Ras, Rho and Rap1A, which are necessary for cancer cell adhesion, migration and invasion. It has been shown that zoledronic acid binds primarily with microcalcifications present in breast tumors and is then phagocytized by TAMs, leading to apoptosis and M2-to-M1 transformation. It has been demonstrated *in vivo* that zoledronic acid inhibits the production of the proangiogenic factor, matrix metalloproteinase, and triggers the TAM transition from the pro-tumoral M2 phenotype to the antitumor M1 phenotype (65).

In 2013, Rogers (66) examined the antitumor effects of zoledronic acid *in vitro* and *in vivo* by evaluating its effect on macrophages. J774 macrophages were treated with zoledronic acid alone and in combination with doxorubicin *in vitro* and apoptosis and necrosis were evaluated. Following treatment with zoledronic acid, its uptake was estimated by detecting unprenylated Rap1A (uRap1A) in J774 macrophages *in vitro*, and in peritoneal macrophages and macrophage populations from subcutaneous breast cancer xenografts *in vivo*. The treatment of J774 macrophages with 5 μ M zoledronic acid for 24 h significantly increased the uRap1A levels, while apoptotic cell death was induced at higher concentrations or longer exposure times. Doxorubicin (10 nM, 24 h) and zoledronic acid (10 μ M, 24 h) used consecutively increased cell death compared with that observed with the use of either drug alone. Detectable uRap1A levels were observed in peritoneal macrophages and macrophage populations isolated from breast tumor xenografts 24 h after single administration of zoledronic acid at 100 μ g/kg *in vivo* (66). Zoledronic acid concentrations <10 nM were shown to inhibit Rab prenylation in J774 macrophages following long-term exposure in culture. Quantitative mass spectrometry identified 18 different unprenylated Rab proteins and revealed that their accumulation increased at least 7-fold following the treatment of J774 cells with nanomolar concentrations of zoledronic acid (67).

Another study demonstrated that zoledronic acid combined with ultrasonic treatment was significantly more effective than zoledronic acid alone ($P < 0.01$). The B02 tumor size in mice treated with zoledronic acid and ultrasound was 42% lower ($P < 0.002$) compared with mice treated with bisphosphonate alone (68). Bisphosphonates are administered in liposomes or attached to nanoparticles to improve their pharmacokinetics, reduce the side-effects and to alter their biodistribution (65). Liposomal bisphosphonate forms are capable of inducing the M2-to-M1 phenotypic transition (69).

Thus, studies on bisphosphonates used alone or in combination with anticancer drugs or physicochemical methods for the treatment of tumorigenesis are promising fields of research, and highlight possibility of developing novel therapeutic strategies (70-74).

Drug-dependent stimulation of phagocytic activity to modulate macrophages. The phagocytosis of foreign bodies, apoptotic cells and cancer cells, and the stimulation of adaptive immunity by presenting the antigens of assimilated materials, are two important innate immune functions of macrophages (75). Tumor-specific antibodies are a class of potent biopharmaceuticals, which act by directly inhibiting the transmission of survival signals, mediating antibody-dependent cell cytotoxicity of natural killer cells, inducing complement-dependent cytotoxicity via the activation of the complement cascade, and thus promoting antibody-dependent cell phagocytosis by macrophages (76). Studies have indicated that monoclonal antibodies approved as anticancer drugs, such as rituximab and trastuzumab, exert their therapeutic effects mostly through antibody-dependent cell phagocytosis (77,78). In spite of their potential to stimulate tumor cell invasion, TAMs are capable of tumor cell phagocytosis in the presence of target antibodies (79,80).

Thus, to improve the therapeutic strategy based on stimulating antibody-dependent cell phagocytosis, the Fc

fragments of antibodies should be engineered to increase their interaction with receptors on macrophages (81). Although IgG class antibodies are typically used to design antibody-based therapeutics, the therapeutic potential of other antibody isotypes (IgA and IgE) has been the subject of several preclinical studies, where monocytes/macrophages also serve an important role in affecting the functions of antibody-dependent cell cytotoxicity and antibody-dependent cell phagocytosis (82,83).

Chemotherapeutic drugs are also considered potential means with which to modulate macrophages. A number of anti-cancer chemotherapeutics exert their pharmacological effects on non-tumor cell populations, although additional studies are required in the field, as the current literature is limited to preliminary results from *in vitro* experiments (84-89). In particular, trabectedin and lurbinectedin (a second-generation analog) are efficient in eliminating TAMs (84,85). Trabectedin mechanically interacts with the TRAIL-R2 ligand-receptor, and induces tumor necrosis factor-related apoptosis of mononuclear phagocytes by causing receptor clustering and subsequent caspase 8-dependent apoptosis activation (86).

In addition to exerting cytotoxic effects, certain chemotherapeutics modulate the macrophage response to the tumor (15,87). A previous study using mouse models of fibrosarcoma and breast tumors demonstrated that docetaxel promotes target cell polarization to macrophages with an antitumor M1 phenotype (88). Cyclophosphamide treatment facilitates macrophage infiltration, increases the secretion of proinflammatory cytokines (IL-6 and IL-12) and suppresses the production of pro-tumoral M2-associated cytokines (IL-4, IL-10 and IL-13) (89,90). As a mechanism of self-protection against chemotherapy, chemoresistant cancer cells secrete IL-34, which increases their survival and promotes the polarization of TAMs towards an M2 phenotype to further facilitate an immunosuppressive environment (91). Thus, a combination of chemotherapy and immunotherapy may be more efficient in inducing tumor regression.

4. Systems for targeted drug delivery to TAMs

Systems for targeted drug delivery to TAMs are associated with the second strategy of the macrophage-related therapy of cancer. After establishing the positive effects of a drug, the next focus of research should be to determine strategies with which to selectively deliver the drugs to TAMs with minimal side-effects on healthy cells (92-97).

Phagocytic activity is extremely high in macrophages. Microparticles and nanoparticles are efficiently phagocytized by macrophages. However, the rate of phagocytosis is influenced by certain properties of micro- and nanoparticles, such as the shape, size, contact angle and surface charge (98-100). Liposomes are captured by macrophages more rapidly and in greater quantities when their size is increased to >100 nm, particularly when 1-3 μ m in size. A decrease in liposome size to <100 nm similarly increases their capture by macrophages (101). The composition and structure of particles also affects their capture by macrophages (102,103).

Particles with highly positive or highly negative ζ potentials are captured with improved efficiency by macrophages compared with particles having a nearly neutral ζ

potential. Spherical particles are captured more efficiently than cylindrical particles (104).

There is still no universal method available to ensure specific molecular targeting to TAMs. In 2013, Cieslewicz *et al* (105) reported the discovery of the so-called M2pep peptide sequence. The M2pep preferentially binds to mouse M2 cells, including TAMs, and exhibits a low affinity for other cells. Confocal visualization revealed that M2pep accumulated in TAMs *in vivo* after being injected into the tail vein of mice. The injection of M2pep with a pro-apoptotic peptide into the tail vein increased mouse mortality, and selectively reduced the M2-like TAM population. The study by Cieslewicz *et al* (105) was among the first to describe a molecular targeting construct for mouse TAMs, supporting the targeted approach to cancer therapy.

Cancer immunotherapy aimed at selectively modulating M2-like TAMs and enabling the reversal of the M2-to-M1 ratio is a promising therapeutic strategy. In 2017, Ngambenjawong and Pun (106) reported the construction of a high-avidity macrophage-selective drug delivery platform on the basis of M2 macrophage-targeting peptides (M2pep) grafted on poly(N-(2-hydroxypropyl)methacrylamide). Furthermore, polymer-grafted M2pep exhibited increased serum stability in addition to increased M2 macrophage-selective toxicity (106).

A targeted system was constructed using a copolymer of hyaluronic acid with poly(lactic acid) and poly(glycolic acid). The copolymer was assembled together with the anticancer drug, SN38, in an aqueous phase, and the nanoparticle surface was then coated with methoxypoly(ethylene glycol)-b-poly(histamine methacrylamide) via hydrophobic association to improve the colloid stability both *in vitro* and *in vivo*. When the nanoparticles arrived into the tumor microenvironment, which is acidic, the coating was detached from the nanoparticle surface as a result of the charge transition of the poly(histamine methacrylamide) blocks from a neutral hydrophobic to a positively charged hydrophilic state through an acid-acid state, which was induced by the protonation of the imidazole groups in the tumor microenvironment (an acidic medium). The exposure of the nanoparticle shell led to an increased uptake of nanoparticles by CD44-expressing tumor cells, including cancer cells and TAMs (107).

Nanosystems can rationally be designed to attain multivalent states and, when necessary, multifunctional entities with multiplex and/or enhanced biological activity. Nanosystems engineered to contain macrophage-specific targeting fragments and loaded or associated with drugs are promising options for modulating or even eliminating pro-tumoral macrophages *in vivo* (108). Engineered nanosystems include polymers, dendrimers, organic and metal nanoparticles, and micellar and liposomal carriers (109-117).

5. Macrophages as carriers of anticancer drugs: Integration of chemotherapy and immunotherapy to target tumorigenesis

Taking advantage of the ability of macrophages to target and migrate to the tumor is the third promising strategy involving the use of macrophages. Macrophages have attracted substantial interest as carriers for drug delivery. This is due to their ability to target the tumor, their high phagocytic activity toward

drug-loaded nanoparticles and their capability to directly kill cancer cells (118). For example, peritoneal macrophages can be loaded with drugs, typically included in nanoparticles or liposomes, and can then be transferred back to animals or patients (119,120). Another approach that takes advantage of macrophage self-targeting to the tumor is the *in vivo* administration of nanoparticles of a suitable size with a specific ligand to allow nanoparticle uptake by macrophages or TAMs, and subsequent prolonged release (121-123). The long-term survival of the macrophage host is limited by the toxicity of the drug. It is thus advisable to use systems for drug delivery to decrease the acute toxicity to the macrophage carrier. When the drug is not toxic to macrophages, a proper formulation ensures the prolonged release of the loaded drug from macrophages for at least two weeks, as demonstrated by Dou *et al* (124,125) for indinavir associated with nanoparticles.

With the appropriate strategy for nanoparticle encapsulation to ensure intracellular stability, biological preparations, such as proteins, can be loaded in macrophages (126,127). Chang *et al* (101) demonstrated that the size of nanoparticles internalized in macrophages may substantially affect their macrophage uptake. Smaller nanoparticles (30-50 nm) exhibited increased macrophage uptake compared with larger nanoparticles (100-500 nm), but this reduced the macrophage migration velocity at the same time. Nanoparticles with a size of 100 nm were shown to provide a good balance between efficient drug loading and macrophage migration (101).

To investigate the pharmacological activity of carriers captured by macrophages, macrophages have been loaded with temperature-sensitive liposomes for inducible release (121), nanosized silica-gold nanoshells for photothermal therapy (120) and iron oxide nanoparticles (119,128,129).

In 2015, Miller *et al* (122) described the use of polymeric nanoparticles, that included a platinum (IV) prodrug and a clinically tested carrier based on a copolymer of poly(lactic acid) and poly(glycolic acid) with polyethylene. The nanoparticles were shown to facilitate the long-term circulation of the drug and its uptake in TAMs. The simultaneous visualization of the carrier and its useful load with the drug revealed that TAMs serve as a drug depot and accumulate substantial quantities of the carrier, which gradually releases platinum to damage DNA in the neighboring cancer cells (122).

The reduction of TAM survival is generally considered to improve the therapeutic effects of anticancer therapy. The direct induction of apoptosis with chemical or synthetic substances provides an efficient strategy with which to eliminate TAMs. Trabectedin (ET-743) is an anticancer drug used in the treatment of platinum-sensitive soft-tissue sarcomas. The drug causes selective TAM depletion in cancer patients by activating the extrinsic apoptotic pathway through TRAIL receptors. As trabectedin directly affects monocyte/macrophage-mediated host defense in addition to targeting TAMs (85), designing TAM-specific agents may result in a reduction of side-effects.

6. Conclusions

Reviewing TAM-related literature revealed that the number of publications in this field has increased in number over the past three years, highlighting novel possibilities for the use of a combination of immunotherapy and chemotherapy to

treat cancer. However, there are several issues which warrant further investigation. These issues include: Attaining a deeper understanding of the process of M1/M2 macrophage polarization, macrophage characteristics at intermediate polarization steps, and their role in tumorigenesis; the conditions that are necessary for transitions between the M1 and M2 macrophage phenotypes and the signals that this process is dependent on from the microenvironment; the cause-and-effect relationships between the quantity and quality of macrophages, and the prognosis and outcome of the pathological process; modulation of macrophages and stimulation of their phagocytic activity with drugs; development of suitable and safe targeted vector-based systems for drug delivery to macrophages; and the development of targeted drug delivery systems with macrophages as carriers, thus potentially combining chemotherapy and immunotherapy.

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Authors' contributions

OVZ, IVM, TFK, EVA and SAR were all involved in drafting and revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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