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Review

# Targeting tumor associated macrophages: The new challenge for nanomedicine

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ABSTRACT

The engineering of new nanomedicines with ability to target and kill or re-educate Tumor Associated Macrophages (TAMs) stands up as a promising strategy to induce the effective switching of the tumor-promoting immune suppressive microenvironment, characteristic of tumors rich in macrophages, to one that kills tumor cells, is anti-angiogenic and promotes adaptive immune responses. Alternatively, the loading of monocytes/macrophages in blood circulation with nanomedicines, may be used to profit from the high infiltration ability of myeloid cells and to allow the drug release in the bulk of the tumor. In addition, the development of TAM-targeted imaging nanostructures, can be used to study the macrophage content in solid tumors and, hence, for a better diagnosis and prognosis of cancer disease. The major challenges for the effective targeting of TAM with nanomedicines and their application in the clinic have already been identified. These challenges are associated to the undesirable clearance of nanomedicines by, the mononuclear phagocyte system (macrophages) in competing organs (liver, lung or spleen), upon their intravenous injection; and also to the difficult penetration of nanomedicines across solid tumors due to the abnormal vasculature and the excessive extracellular matrix present in stromal tumors. In this review we describe the recent nanotechnology-base strategies that have been developed to target macrophages in tumors.

## 1. Introduction

In the field of oncology, the use of nanotechnology has provided a broad range of nanostructures designed to improve the delivery of therapeutic compounds towards cancer cells [1]. Different nanostructures have been used to encapsulate pharmacological compounds allowing to overcome common problems of solubility and stability, to reduce their side-effects, to extend their circulating half-time and, in some cases, to enable their controlled release towards the target cell [2–7]. Despite their underlined significant potential to improve the efficacy and to reduce the toxicity of antitumoral drugs, improvements in overall survival of patients are still modest [8–10]. Important hindrance point towards the complexity of the tumor microenvironment (TME), due to its physicochemical and cellular heterogeneity, with a major contribution of the abnormal tumoral stroma and the presence of immunosuppressive cells (majorly tumoral associated macrophages, TAM) [11,12].

In this review article, we provide an overview of the most recent investigations involving the development of nanomedicines for the targeting of macrophages in tumors with therapeutic or diagnostic purposes, and also the results of recent efforts intended to use monocytes/macrophages loaded with nanomedicines as live cell-mediated drug delivery systems (LCDDS) to transport drugs into the bulk of the tumor. Furthermore, we recapitulate the major challenges (i.e. mononuclear phagocytic system and tumor microenvironment) which need to be addressed for the improvement of the actual efficacy of nanomedicines to reach the center of solid tumors and provide an overview of possible solutions and future perspectives in the field of nanomedicine for the treatment of cancer.

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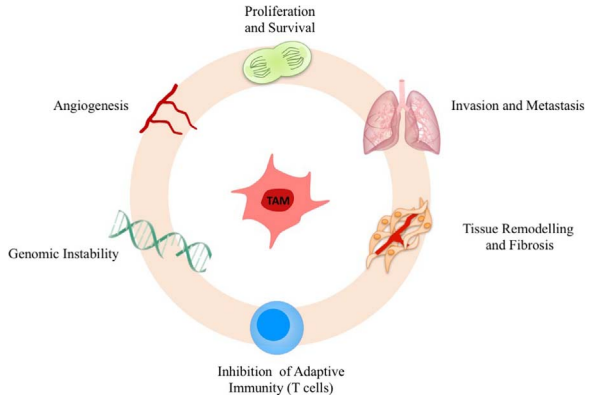


**Fig. 1.** Pro-tumoral functions of Tumor Associated Macrophages (TAM): they induce the proliferation and survival of cancer cells; stimulate the invasion and metastatic properties of cancer cells; enhance abnormal tumor angiogenesis; support tissue remodelling, fibrosis and extracellular matrix deposition in the tumor; contribute to genomic instability; and suppress adaptive immunity, mainly through the inhibition of anti-tumor T cell responses.

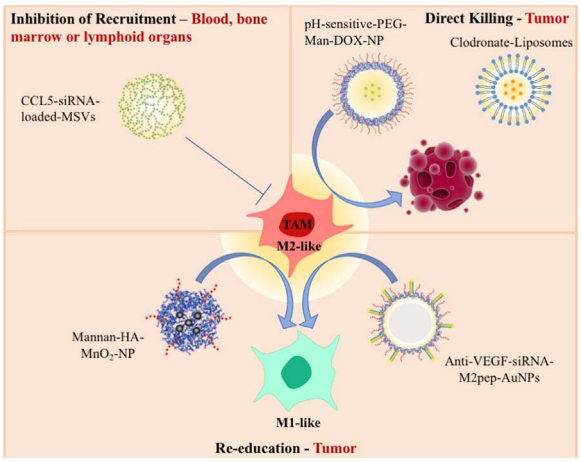


**Fig. 2.** Nanomedicines to target and impact on TAM. Shown on the upper left therapeutic nanostructures to inhibit the recruitment of TAM towards the tumor, on the up right traditional clodronate liposomes and new mannose-targeted-NPs loaded with doxorubicin to kill TAM. In the lower section two NPs engineered to reach and re-educate TAM, from an M2-like pro-tumoral phenotype into M1-like macrophages with antitumor functions, such as direct killing of tumor cells and activation of cytotoxic T cells (see main text for more info).

## 2. Opportunities offered by macrophages for the application of nanomedicine in cancer

### *2.1. Nanomedicines to target tumor associated macrophages*

Tumor Associated Macrophages (TAMs) are a key component of the tumor microenvironment. They may represent up to 50% of the tumor mass and their prominent role in the evasion from immune surveillance has been established [13,14]. TAM infiltration in tumor tissues has been shown to support tumor growth, angiogenesis, invasion and metastasis, and their high density in tumors is correlated with tumor progression and resistance to therapies (Fig. 1) [11]. These findings point out TAMs as promising targets for novel antitumoral therapeutic strategies. These strategies can be divided into three main groups: i) inhibition of TAM recruitment to the tumor, ii) direct killing of TAMs, iii) re-education of TAM from their M2-like protumoral phenotype into a M1-like antitumoral phenotype [14–16]. Thus, the development of new nanomedicines to target and impact on TAM stands as a promising opportunity to switch the tumor-promoting immune suppressive microenvironment, characteristic of tumors rich in macrophages, to one that kills tumor cells, is anti-angiogenic and promotes adaptive immune responses (Fig. 2). Several nanotechnological approaches with this purpose have been reported.

#### *2.1.1. Inhibition of TAM recruitment to the tumor*

Monocytes/macrophages are recruited from the blood and infiltrate the tumor as an immune reaction to a damage event (see Section 2.2). Thus, it is feasible to impair the generation of TAM by targeting and impacting the monocytes, either in the blood, in the bone marrow or in the lymphoid organs. In 2011 siRNA-loaded lipid NPs were proposed as a way to reduce the expression of the chemokine receptor CCR2, which is required for the recruitment of monocytes to the tumor. The intravenous injection of these NPs led to their accumulation in the spleen and bone marrow, where they delivered the associated siRNA into the $Ly6C^{high}$ monocytes (TAM precursors) resulting in reduced tumor growth in two different xenograft tumor models [17]. A similar approach has been recently presented by Ban et al. through the targeting of the CCL5-CCR5 axis. They developed bone marrow-targeted biodegradable mesoporous silicon nanoparticles (MSVs) loaded with liposomal CCL5-siRNA and decorated on their surface with a thioaptamer for E-selectin, expressed on the bone marrow endothelium (Fig. 2). The CCL5-siRNA-loaded-MSVs intravenously injected into 4T1 bearing mice resulted in the re-programming of immunosuppressive myeloid cells in the bone marrow (evaluated as defective expression of CCR5), and this result was translated into a significant reduction of tumor growth and increased $CD8^+$ T-cell infiltration in the tumor. These antitumor immune responses were significantly enhanced by the combination of the CCL5-siRNA-loaded-MSVs with the CCR5 inhibitor Maraviroc® [18]. Another target of interest to inhibit TAM recruitment is the colony-stimulating factor receptor (CSF-1R). Several CSF-1R inhibitors have demonstrated promising antitumoral effects in different murine tumor models, and some of them are now progressing in clinical trials [19–21]. Despite these positive results, the targeting of CSF-1R using NPs has not been explored yet.

#### *2.1.2. Kill TAM in the tumor*

A few anticancer nanomedicines have demonstrated ability to kill TAM. Interestingly, more than 20 years ago, nanotechnology approaches were developed to deliver bisphosphonates, such as clodronate or zoledronate, to tumors resulting in depletion of TAM, better antitumoral effect, impaired angiogenesis and decreased metastasis (Fig. 2) [22,23]. The intratumoral injection of alendronate conjugated with glucomannan into sarcoma-bearing mice, was used to target the mannose receptors in TAM, achieving their effective depletion [24]. The development of folate-decorated liposomes loaded with zoledronic acid was also applied to target TAM in tumors, however, this composition did not result in a reduction of tumor growth when applied to tumor-bearing mice: KB (human nasopharyngeal) and C-26 (mouse colon adenocarcinoma) [25,26]. Despite of this, nowadays, the use of clodronate-liposomes is still a common approach used by biomedical researchers to deplete macrophages in a non-located manner, thus room to improve the specific delivery of bisphosphonates towards TAM in specific diseased-tissues (i.e. solid tumor), by means of new nanocarriers, is still available.

Several nanocarriers, including liposomes and PLGA nanoparticles, have been decorated with mannose for the specific targeting of the mannose receptor (MR or CD206), highly expressed in TAM (M2-like macrophages) [27,28]. In order to favour the uptake of the mannosylated-nanomedicines by TAM in the tumor, and prevent their uptake by macrophages in other locations (i.e. MPS see Section 3), Zhu et al. engineered mannosylated-PLGA NPs shielded with pH-sensitive-PEG moieties for the delivery of doxorubicin (DOX) [28,29] or siRNA into TAM [30] (Fig. 2). Several peptides, proteins and aptamers were also investigated to target TAM specifically. For example, PEGylated liposomes conjugated with the peptide LyP-1 and loaded with DOX were shown to reach TAM in metastatic lymph nodes, causing the inhibition