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Targeting Monocytes with TLR7 Ligands as a Novel Opportunity in Immuno-Oncology

Simon S Jensen[1,3]*, Ladan Parhamifar[1,3], Jonas Henriksen[1,2,3] and Thomas L Andresen[1]

[1]Department of Micro and Nanotechnology, Center for Nanomedicine and Theranostics, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark

[2]Department of Chemistry, Center for Nanomedicine and Theranostics, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark

[3]MonTa Biosciences, Produktionstorvet, Kgs. Lyngby, DK-2800, Denmark

*Corresponding author: Simon S Jensen, Technical University of Denmark, Department of Micro- and Nanotechnology, Center for Nanomedicine and Theranostics, Kgs. Lyngby, DK-2800, Denmark, Tel: 4545258121, E-mail: simsko@nanotech.dtu.dk

Abstract

Cancer immunotherapy has for the last decade been one of the fastest developing therapeutic areas in oncology with promising clinical benefits, supporting a role of immunotherapy as a cornerstone in future cancer treatment in combination with existing treatments. Treatment of early stage cancers today is very efficient due to refined surgery, chemotherapy, radiation and use of therapeutic antibodies, but a large group of patients in particular with late stage and metastatic disease have poor prognosis due to lack of efficient treatment options. Thus, there is an urgent need to develop new technologies to provide more efficient treatment of late stage cancers, focused towards cure of these patients. One approach that may lead to better treatment is utilising the immune system of these patients, in order to generate durable anti-tumor memory immune responses. One strategy to boost the immune system of cancer patients has been administration of Toll Like Receptor ligands to overcome systemic and local immune escape mechanisms. In this commentary we discuss a novel delivery technology for systemic targeting of a Toll Like Receptor 7 ligand to monocytes in relation to current knowledge in cancer immunotherapy with perspectives on challenges and opportunities for clinical use of this technology.

Keywords: Immunotherapy; Monocyte; Liposome; TLR; Delivery

Introduction

Immunotherapy has for decades been applied in cancer treatment in combination with chemotherapy or radiotherapy, and novel immunotherapies are expected to be part of future combination treatments. In recent years, it has become clear that both chemotherapy and radiotherapy affect immune responses both systemically and in the tumor microenvironment, supporting immune dependent anti-tumor responses, which opens a window of opportunity to apply immunotherapy with improved therapeutic benefits. Liposomes are able to deliver chemotherapeutic drugs more efficiently to the tumor tissue, but have so far not been extensively used as a direct cancer immunotherapy approach [1]. In our recent work we have explored liposomes for targeting immune cells with an attempt to modify the immune response of monocytes. In the publication by Johansen et al. [2], we have demonstrated specific targeting to monocytes with liposomes consisting of the phospholipids palmitoyl-oleoyl-sn-glycero-3-phosphocholine (POPC) and the cationic lipid dioleoyl-3-trimethylammonium-propane (DOTAP). Formulations with 7.5-10% DOTAP content, corresponding to a zeta-potential between 30-40 mV, showed specific targeting to monocytes when incubated in fresh human whole blood. Liposomes with lower surface charge showed very poor interaction with leukocytes, whereas liposomes with higher surface charge showed interaction with B-cells and at higher charge the liposomes associated with all leukocytes in blood. In order to evaluate if the technology is able to delivery immune stimulating compounds to monocytes and induce their activation, we formulated the immune stimulating Toll like Receptor 7 (TLR7) agonist TMX-202 in liposomes. TMX-202 containing liposomes were able to boost a potent immune activation associated with secretion of IL-12 and IL-6 after whole blood targeting and activation, and showed superior cytokine responses compared to free TLR7 agonist and TLR7 agonist formulated in liposomes without the cationic surface charge. The monocyte targeting formulation was also more potent in inducing a proliferative response in an allogenic mixed leukocyte reaction (MLR), and finally, TMX-202 was able to induce monocyte differentiation into DC-SIGN/CD14 positive dendritic cells (DC), a DC phenotype shown by others to have the ability to migrate into inflamed and cancerous tissue and induce both CD4 and CD8 T-cell responses [3].

These experiments indicate that this technology has the potential to selectively boost monocyte activation *in vivo*. Activation of monocytes leads to differentiation of the monocytes into monocyte derived antigen presenting cells (APCs) like inflammatory dendritic cells (iDC) and activated macrophages as outlined in Figure 1. When combined with existing treatments like radiotherapy (RT), certain types of chemotherapy, monoclonal antibodies or immune checkpoint inhibitors (ICI), tumor cells undergoing apoptosis or immunogenic cell death (ICD) are prone to phagocytosis. The activated APCs phagocytose tumor antigens and migrate to lymph nodes to activate CD4 Th1-helper cells and cytotoxic CD8 memory cells that are able to patrol, recognize and eliminate tumors and metastasis in the patient (Figure 1).
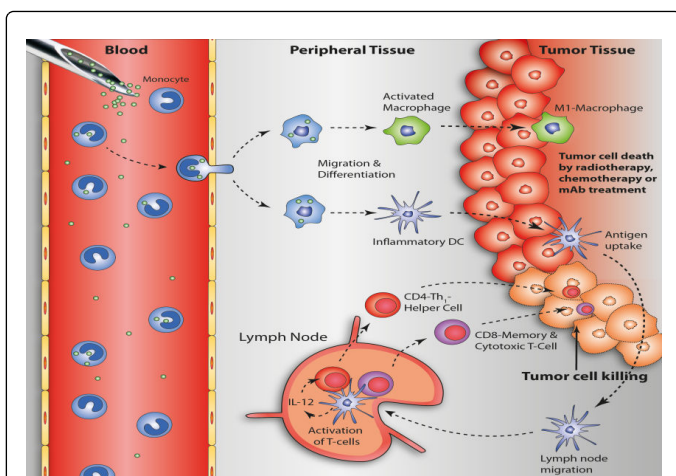
Figure 1: Overview of the monocyte targeting technology as a novel cancer immunotherapeutic approach. Liposomes loaded with TLR7 agonist target monocytes in the blood to produce antitumor cytokines e.g. IL-12 and activate the monocytes. We further hypothesize that activated monocytes migrate into peripheral tissue like sites of tumor growth and start differentiation into antigen presenting cells like activated macrophages and inflammatory dendritic cells. Tumor cells that have undergone immunogenic cell death induced by radiation or chemotherapy are phagocytosed by the activated APCs that take up tumor antigens and subsequently migrate to lymph nodes to activate and present antigen to CD4 and CD8 T-cells to boost Th1 and CD8 cytotoxic memory responses, that ultimately lead to recognition and potentially elimination of metastases in the patient.

This hypothesis outlined in figure 1 is supported by a number of publications. First, it was shown by Steinman and colleagues, that TLR-activated monocytes in the blood migrate into tissue and present tumor antigens to activate antigen specific T-cell responses [3]. This finding is supported by the fact that the TLR7/8 agonist R848 is able to stimulate monocyte and lymphocyte migration into peripheral tissue in both mice [4] and non-human primates [5]. Once the activated APCs have migrated into tumor sites, tumor cells undergoing ICD by e.g. radiotherapy are phagocytosed and tumor antigens are presented by the APCs in draining lymph nodes [6]. The combination of radiotherapy with immunotherapy has demonstrated promising preclinical results, Dovedi et al. have shown that R848 administered systemically, is able to induce complete remission in tumor bearing mice in a CD8-T-lymphocyte dependent manner, and further demonstrate that rechallenge of long-term surviving mice with identical tumor cells, have an anti-tumor long-lasting memory response which is able to rescue 75% of the rechallenged mice without further treatment [7]. The monocyte targeting immunotherapy may also be combined with chemotherapeutic treatment using other inducers of ICD, like oxaliplatin, doxorubicin, cyclophosphamide and mitoxantrone, of which several are used frequently in the clinic as part of the FOLFOX regimen for treatment of colon and colorectal cancer, for breast cancer treatment and for lymphoma treatment as part of the R-CHOP regimen [8].

TLR7/8 mediated leukocyte activation has also demonstrated an enhanced therapeutic benefit in combination with monoclonal antibody therapy. TLR7/8 treated monocytes or leukocytes, have showed an enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) towards tumor cells treated with rituximab (anti CD20) and trastuzumab (anti Her2), through upregulation of Fcγ receptors on monocytes and NK-cells, leading to enhanced tumor cell phagocytosis and anti-tumor activity in mouse tumor models [9,10].

Monocyte targeting using liposomes has previously been reported in the literature aimed towards auto-immune disorders, infectious diseases or cancer. Targeting approaches include surface modifications utilising anionic lipids, peptides, antibodies and lectin based targeting, in addition to conjugation of proteins or polysaccharides, whereas cationic liposomes mainly have been used for gene or plasmid related delivery approaches [11]. Other targeting technologies have focused on antibody conjugated particles targeted to dendritic cell receptors like DC-SIGN, DEC-205, DCIR, BDCA2 and CD32 with encouraging results [12].

Imiquimod is currently the only TLR7 agonist approved for clinical use, for treatment of superficial skin cancer in the Aldara^{TM} cream, but a number of clinical trials are being conducted with TLR7/8 agonists mainly as topical or subcutaneous administration for skin or cutaneous cancers as monotherapy, or as adjuvants in combination with tumor antigens [13]. A promising example was a recent study from Rook et al., where topical treatment with R848 was shown to have a beneficial effect in treatment of cutaneous T-cell lymphoma [14].

Although treatment with TLR7 agonists in topical cancer show good anti-cancer effects, it has been challenging to administer these agonists by oral or systemic routes. In a clinical phase II study in hepatitis C virus infected patients, R848 was administered by the oral route and showed therapeutic effects on plasma hepatitis virus titers, but with dose-limiting toxicity [15]. In another study the TLR7 agonist 852A was tested in a phase II study in patients with metastatic melanoma with three weekly intravenous doses. The study showed prolonged disease stabilization in some patients, increased serum IFNα and IP-10, and dose-limiting toxicity in two patients [16]. These studies indicate that systemic use of TLR7 agonists in patients may be a challenge due to a narrow therapeutic window, and calls for alternative technologies to provide a targeted approach to enhance TLR7 mediated immune activation of TLR7 expressing cells, while limiting effects on other cells and organs. Our preclinical results suggest that targeting of monocytes with TLR7 agonists and subsequent activation may lead to enhanced anti-tumor activity. In addition, formulation of TLR7 agonists in liposomes, change the pharmacokinetic profile and may reduce toxic effects, either through lower dosing or reduced toxicity on healthy cells [17].

To translate this monocyte targeting approach into therapeutic use in patients, three major issues are relevant to consider with the knowledge we have gained through earlier preclinical studies and clinical trials using TLR7 agonists, first, systemic administration of R848 and 852A in clinical trials showed a narrow therapeutic window, with dose-limiting toxicity [15,16]. Major toxicities observed (>25% of patients) were nausea, chills, pyrexia, myalgia, pain in extremities, headache, shivering, lymphopenia and backpain [15,16], most of which are influenza like symptoms as seen for administration of therapeutic cytokines like IL-2 [18]. In comparison to cytokine based cancer immunotherapy, TLR7 based immunotherapy is expected to lead to systemic cytokine elevation, however, the cytokines produced after TLR7 ligation consists of a mix of pro-inflammatory and anti-tumorigenic cytokines like IFNα, IFNγ, IL-12, IL-1RA, IP-10, IL-6 and TNFα [5,7,9,15,16]. In contrast to administration of therapeutic cytokines, TLR7 based targeted immunotherapy will lead to strong

activation of leukocytes. Activated leukocytes and in particularly APCs, will migrate into the tissue and promote tumor infiltration and production of anti-tumor cytokines locally, which will contribute to reprogramme the tumor microenvironment. Consequently, systemic use of TLR7 agonists administered as a free drug have shown considerable toxicity, which should be considered for future clinical trials. Another important point is the balance between productions of pro-inflammatory cytokines in the periphery compared to local production from APCs migrated into tumor and peripheral tissues.

Second, lessons learned from earlier preclinical and clinical studies with TLR7 agonists administered two or three times weekly, have showed that such dosing schedule may be too frequent for this type of immunotherapy since preclinical studies have demonstrated a tolerance induction of the TLR7 receptor after TLR7 ligand treatment *in vivo* [19,20]. It was shown that dosing schedules in mice were optimal with 5-10 days interval, compared to 3 days interval, since the frequent dosing showed lack of repeated IFN-secretion from plasmacytoid DCs. This finding correlated with reduced anti-tumor activity with frequent dosing compared to dosing every 5-7 days [19,20]. If these mouse studies can be translated to a similar TLR7-tolerance induction in humans, future clinical trials with TLR7 agonists should preferably be conducted with at least a five day dosing schedule. An additional intriguing consideration regarding toxicities seen after frequent dosing of R848 and 852A in humans is the possibility that cytokines potentially related to toxicity like IL-6 and TNFα are not necessarily associated with TLR7 tolerance as seen for IFNα. In restrospect, dosing 2-3 times a week may mean that IFNα was mainly produced with the initial dose in previous human trials, with subsequent administrations leading to repeated inductions of inflammatory cytokines like IL-6 and TNFα, which may contribute to the dose-limiting toxicity observed rather than an anti-tumor activity involving IFNα-induction. Thus, a less frequent dosing schedule likely reduces toxicity, and may improve anti-tumor activity through a potent IFNα secretion every 7-10 days. For the monocyte targeting TLR7-based immunotherapy approach, it will be important to set a dosing-schedule and level, which will allow production of anti-tumor cytokines for each administration, and lead to activation and migration of APCs into the peripheral tissue.

Third, in contrast to systemic IL-2 treatment, where patients experience increased leukocyte blood levels [18], treatment with R848 induces transient blood monocyte and lymphocyte depletion, with migration into peripheral tissue for the first 3 days after treatment, with the dominant remaining blood leukocytes being neutrophils. After 3 days and onwards, monocytes and lymphocytes are recruited to the blood compartment, but are not fully normalized until 2 weeks after treatment [5]. In contrast to IL-2 treatment, targeted delivery of TLR7 containing liposomes to monocytes is expected to lead to higher levels of migration of activated APCs and lymphocytes into peripheral tissue, including tumor tissue, and lead to generation of antigen specific memory T-cells [3,7,21].

Based on the above mentioned preclinical and clinical data and experience gained during clinical trials, combinations using the TLR7 targeted delivery technology may be considered. The strong synergistic effect seen in combining radiotherapy with immunotherapy is an attractive clinical opportunity that is currently being explored in a number of clinical trials [22]. Most of these trials are conducted with RT in combination with ICI, other monoclonal antibodies or therapeutic cytokines, and a single study with topical TLR7 agonist treatment. These trials are very interesting to follow, to learn first of all

if therapeutic benefits are reached, but also to learn if these combinations may lead to potential changes in T-cell effector ratios in the tumor microenvironment. A rational combination of RT with the TLR7 targeted immunotherapy may be conducted in patients receiving fractionated RT as curative monotherapy, to prevent chemotherapy induced leukopenia and subsequent challenges with insufficient monocyte targeting and activation. Relevant patient inclusion in such trial could be patients with inoperable metastatic lung or head and neck cancer [6,22].

Another interesting group of patients to target in a clinical trial could be combinations of the TLR7 targeted immunotherapy with therapeutic antibody therapy that is highly dependent on the ADCC effect mediated by monocytes and NK cells like rituximab, trastuzumab or cetuximab [9,10]. Relevant patient groups may be breast cancer patients treated with trastuzumab as monotherapy or lymphoma patients treated with rituximab as part of the R-CHOP regimen.

A third interesting combination may be patients treated with ICD inducing chemotherapy like doxorubicin, oxaliplatin, mitoxantrone or cyclophosphamide [8]. Relevant patient groups may be breast cancer patients or lymphoma patients where doxorubicin is part of the R-CHOP regimen, where a beneficial synergistic activity may relate to demonstrated benefits from combining TLR7-activation with both rituximab, doxorubicin and cyclophosphamide [8,9].

Finally, the combination of TLR7-targeted immunotherapy with ICIs may be a promising approach, since ICIs are able to block immunosuppressive tumor escape mechanisms, but not to promote anti-tumor immunogenic responses with increased APC activity and production of anti-tumor cytokines like IL-12, IFNα and IFNγ. Interesting therapeutic combinations could be metastatic melanoma patients treated with ICIs, since these patients are known to be immune responsive [21].

In summary, a targeted delivery technology able to deliver TLR7 agonists directly to monocytes have promising perspectives, and additional preclinical data are warranted to explore therapeutic potential in more detail. It will be interesting to explore anti-tumor activity in combination with RT, chemotherapy, mAbs or ICIs in mouse anti-tumor models, to explore relations to TLR7 tolerance induction and effects on leukocyte dynamics, potential toxicity and how the tumor microenvironment is affected in relation to effector and suppressor T-cell populations. It will also be important to explore plasma cytokine profiles after administration compared to a non-targeting technology, as well as effects on the myeloid derived suppressor cells, which are known to develop into M1-macrophages upon TLR7 treatment [22].

Acknowledgement

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