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Review

Nanoparticles as a promising method to enhance the abscopal effect in the era of new targeted therapies☆

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ABSTRACT

Over the last decade, immunotherapy has emerged as a hopeful alternative in cancer therapy. Different drugs are used to stimulate the immune system and block negative immune regulatory pathways, known as "immune checkpoint inhibitors (ICI)". Although clinical studies have reported efficacy and safety with the use of ICI, only a small group of patients have obtained a clinical benefit. Because of this, immunomodulation based on immunogenic cell death produced by radiotherapy (RT) has been well positioned as an alternative to increase the clinical effect on the primary neoplasm, but also in distant tumours, a phenomenon known as the "abscopal effect". Early clinical outcomes with RT-ICI combination are promising, but the rate of abscopal responses remains low. These developments have opened a path to evaluate the use of nanotechnology as antigen-capturing nanoparticles (AC-NPs) for improving clinical outcomes in metastatic disease treated with RT-ICI. In this review, we aim to highlight the basic characteristics of nanoparticles and its application in oncology, focusing on their potential to enhance abscopal responses.

1. Introduction

In recent years, novel therapeutics based on immune modulation have continued to expand targeted options for patients with early-stage and advanced cancer. The use of immune checkpoints inhibitors (ICI), such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein PD-1, and its ligand PD-L1, has shown to be safe and effective.[1,2] To date, ICI has been approved in Hodgkin's lymphoma, non-small cell lung cancer, melanoma, renal cell cancer, bladder transitional cell carcinoma and squamous cell head and neck cancer.[3] However, existing research estimates that only 10–20% of patients obtain long-lasting benefit from ICI.[4] It is proposed that long-term remission rates might be improved by combining ICI with radiotherapy (RT), based on the induction of immunogenic cell death produced by RT. Data from pre-clinical and clinical studies suggests that RT-ICI combination induces any grade of abscopal responses, and might improve overall survival.[2,5] However, published data from clinical trials evaluating abscopal responses using RT-ICI remains limited. Considering this, other methods to enhance the abscopal response by exposure of immune cells

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to cancer-specific antigens after RT are under investigation. Recently, nanomaterials have been studied as "antigen capturing nanoparticles" (AC-NP) formulations to improve immunotherapy effectiveness and increasing abscopal responses.[6]

In this review, we aim to describe the general characteristics and properties of nanoparticles, their use in oncology and the potential effect for improving abscopal responses in combined therapies such as RT-ICI.

2. Nanoparticles: intrinsic mechanisms

Nanoparticles (NPs) are defined as materials with overall dimensions of less than 100 nm, usually prepared as nanospheres or nanocapsules. In recent years, they have emerged as an important component in medicine, with applications ranging from fluorescent biological labels,[7] contrast agents,[8] detection of pathogens and proteins,[9,10] to drug and gene delivery,[11,12] among other uses. There are several kinds of NPs, varying in terms of materials and processes used for their preparation, as shown in Fig. 1.

In oncology, NPs have had a remarkable impact in the treatment of various types of neoplasms, since their use helps overcome 3 essential obstacles involved in the delivery of therapeutic agents: (i) physiological barriers that confer drug resistance to tumoural tissues, (ii) cellular mechanisms involved in drug resistance, and (iii) distribution, biotransformation and clearance of drugs.

As an effort to improve treatment response in cancer, NPs have been associated with therapeutic agents as a potential enhancer of their effects, taking advantage of their capability to act as a drug vehicle able to target tumour tissues or cells, while protecting the drug from premature inactivation during its delivery. Relying on convection across the leaky tumoural vasculature, an accumulation of intravenously injected NPs takes place.[13] This uptake can also result from the recognition of ligand-associated NPs; a technique denominated "active targeting".[14] Since lymphatic clearance is usually heavily compromised in tumoural tissues, an accumulation of NPs in the neoplastic interstitium is also promoted, a concept known as enhanced permeability and retention effect.

A well-known example of the advantages provided by NPs is Abraxane®, a nanoparticle loaded form of paclitaxel that has shown improved transport of the drug to the tumour site, allowing higher dose usage while also decreasing secondary effects.[15]

3. Nanoparticles and radiotherapy

RT is an essential pillar of cancer treatment, being beneficial in about 50% of all cancer patients.[17] This treatment is based on the administration of energy to tumoural cells, usually by irradiation with photons or ions, strong enough to damage neoplastic cells or their vasculature, thus inducing tumoural death.

In RT, one of the main issues when prescribing a treatment is achieving a favourable ratio of treatment efficacy to side effects, also known as the "therapeutic index". The main ways to accomplish this include the following steps: better conformation of the administered dose to the tumour volume, enhancement of radio-resistance in healthy tissue, increasing radio-sensitisation in the neoplasm and reversing radiation resistance in the tumoural tissue.[18]

Among these materials, gold NPs have become a popular choice because of the advantages provided, such as an improved lifetime in the bloodstream, relatively easy synthesis in a varied size range, straightforward attachment of ligands that enable the targeting of tumoural cells and good biocompatibility.[19–21] One of the advantages provided by gold NPs in increasing RT efficacy has been shown by an increase of dose absorption in tissue associated with NPs.[22] Another benefit reported is the lower dose reported after having traversed a region containing these materials, thus increasing the therapeutic index.[23] It has also been reported that free radical production is increased in irradiated tissue exposed to NPs, performing as a means of radio-sensitisation for tumours.[24]

An example of these mechanisms in action was reported in a study by Guo et al.,[25] where they analysed the radiation enhancement effects in liver cancer cell lines by using 14.4 and 30.5 nm polyethylene glycol (PEG)-coated gold NPs. Their results indicated that PEG-coated gold NPs were stable in blood. The in vitro bio-distribution assay showed PEG-coated gold NPs had higher distribution in cancer cells, with about 10^3 NPs found per cell. Electronic microscope direct observation illustrated that PEG-coated gold NPs (GNPs) hybridised with blood proteins and formed a 30–50 nm Au-protein corona. Also, these gold NPs had low toxicity at the concentration of

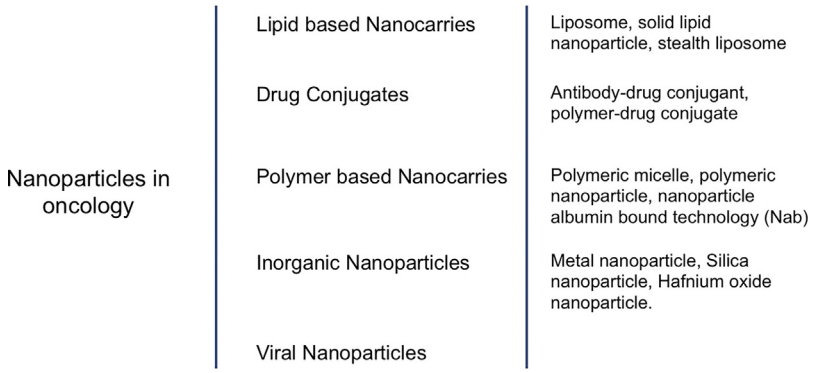

Fig. 1 – Overview of the nanoparticles under investigation in oncology.

10^{-4} M. In vitro RT showed gold NPs significantly enhanced the irradiation effect and decreased the survival of two types of liver cancer cells. With these findings, the authors conclude PEG-coated GNPs can be considered as a potential agent in liver cancer radiation therapy.

4. Enhancing the abscopal effect by NPs-mediated immunotherapy

There are limited case reports describing tumour regression outside the radiation treatment field. This occurrence is called the "abscopal effect" and has emerged from a rare to a relevant event with the advent of new targeted therapies, predominantly with the use of concomitant ICI and RT.[16] Recently, a systematic review reported an abscopal response rate of 26.5% in metastatic melanoma patients treated with a combination of ipilimumab (monoclonal antibody against CTLA-4) and RT. In addition, a benefit in overall survival (OS) of 8 months compared to baseline (ipilimumab alone) was found.[2]

Several undergoing prospective studies are evaluating different results regarding therapies combining RT and ICI. Among the first published outcomes, Tang *et al.* reported in a phase I trial involving 35 patients with miscellaneous neoplasms treated with ipilimumab and stereotactic body radiation therapy (SBRT), a 32% of abscopal response.[26] Although preliminary results are promising, the response rate of abscopal effect remains low, and new strategies, as nanoparticles, are being developed and evaluated as a means to enhance the abscopal effect in patients treated with ICI and RT.

4.1. *Why do ICI increase the chance to unleash an abscopal response in combination with RT?*

ICI drugs targeting the CTLA-4 and PD-1/PD-L1 checkpoint pathway disrupt inhibitory signalling allowing for increased anti-tumour immune responses. Based on preclinical studies, it has been posited that the tumour irradiation induces an immunogenic cell death (i.e. the release tumour-associated antigens and immune stimulatory molecules), enhances antigen presentation and promotes antigen diversity, thus, potentially enhancing the generation of anti-tumoural immunity.[27] RT may therefore allow an increased clinical activity of ICI by promoting the priming of the antitumour immune response.

4.2. *How could NPs be used as a booster for the abscopal effect?*

Recently, a limited number of studies have evaluated the use of NPs to improve treatment response to immunotherapy and the induction of the abscopal effect by

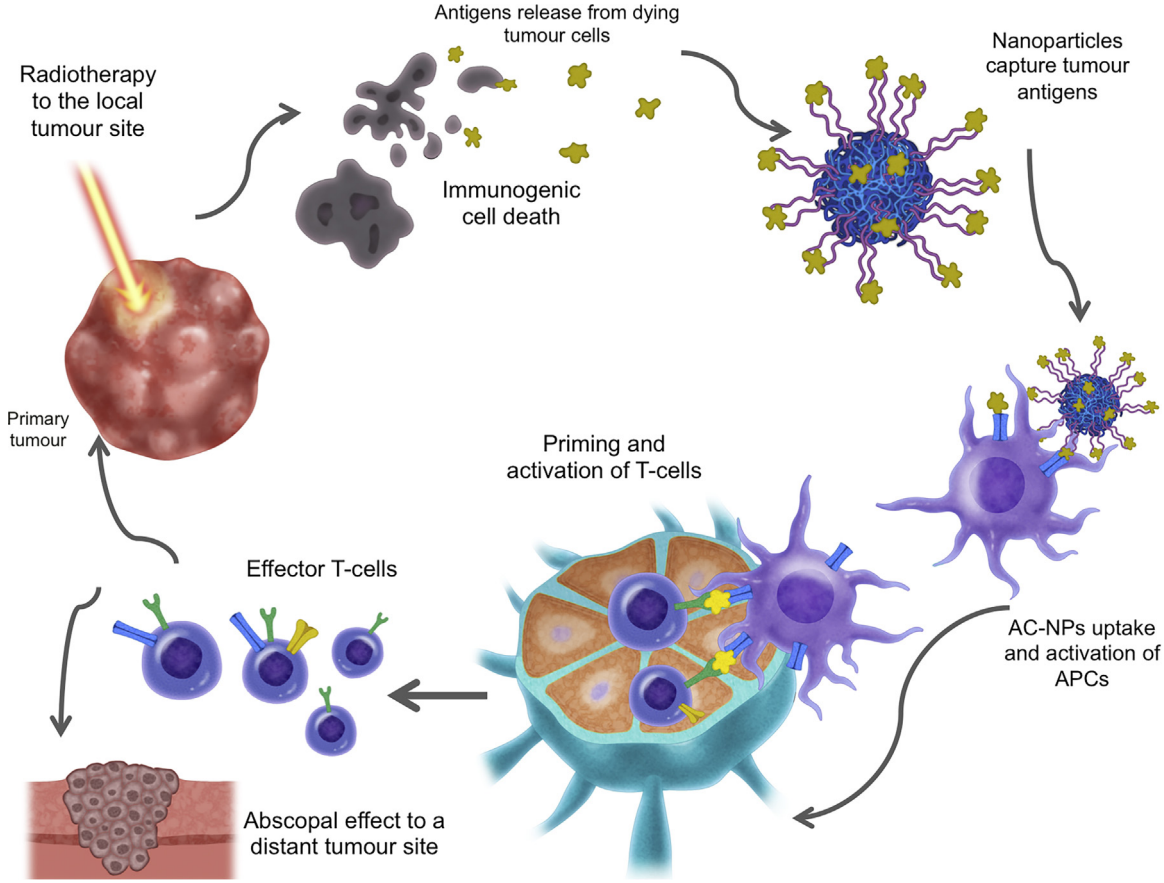

Fig. 2 – Effects of antigen-capturing nanoparticles (AC-NPs) in relation to the cancer immune cycle. Radiotherapy induces immunogenic cell death releasing new antigens for its recognition by the immune system. AC-NPs bind to tumour antigens and improve their presentation to APCs. Finally, the activated effector T-Cells is directed to the primary site and distant tumours (metastases).

capturing tumour-derived protein antigens (TDPA) released during RT, transporting them to antigen presenting cells (APCs) and, thus, promoting cancer immunity as shown in Fig. 2.[28–33]

Firstly, the physicochemical properties of NPs are fundamental for their performance. These include size, shape, and surface charge, among other characteristics. Size is one of the main features involved in immune stimulation by NPs. This can be related to the fact that these NPs fall within the same size range of pathogens (i.e. viruses and small bacteria).[34] A size of below 100 nm enables NPs to utilise the augmented permeability and retention effect that is characteristic of solid tumours.[35] The shape of NPs can affect immune activation (i.e. spherical NPs have a higher macrophage uptake).[36] Also, the circulation time of NPs in the bloodstream is a critical variable. A recent study based on lipid nanoparticles (LNPs) shows that the longest circulation time is obtained using LNPs that better mimic natural membranes. This study reported that surface modification of LNPs by using a "self" peptide segment of CD47 resulted in a diminished blood clearance by macrophages and, hence, a longer circulation time.[37,38] Innate immune activation can also be influenced by the texture of microparticles. For example, polystyrene-block-poly (ethylene oxide) microparticles having a budding texture that could be more easily phagocytised and induced a faster neutrophil recruitment to the injection site than smooth particles.[39]

Secondly, NPs delivery can significantly boost immunogenicity of tumour antigens by co-delivery of antigens and adjuvants to the same location.[30] Thus, NPs-mediated immunogenic response includes:[6]

- Capability of delivering TDPAs to APCs
- Increase intratumoural $CD8^+T/T_{reg}$ and $CD4^+T/T_{reg}$ ratios
- Active targeting of designed NPs through molecular recognition for delivering antigens to specific APCs.

In relation to APCs activation, all targeted NPs have shown higher efficacy than non-targeted NPs in stimulating antigen specific $CD8^+$ T cell responses after administration on mice.[40] Dudziak et al.[41] reported that targeting different APCs populations could induce distinct immune responses. For example, the use of chimeric monoclonal antibodies to target antigens to the two major types of APCs found in the spleen, delivery of antigen to $CD8^+DEC205^+$ APCs led to a preferential antigen presentation by MHC class I molecules, whereas targeting to $CD8^-33D1^+$ APCs led to the presentation by MHC class II molecules. Thus, in immunotherapy treatments, the key to successful APCs targeting is to select an appropriate APC surface target and the appropriate payload, be it antigen or adjuvant to elicit a protective and long-term immune response.

NPs also act in targeting the tumour microenvironment (tumour-associated macrophages (TAM) and T_{reg}), which play a critical role in enhancing tumour cell invasion and metastasis. A study using NPs composed of poly (ethylene glycol)-block-poly (D,L-lactide) (PEG-PLA) that encapsulated CTLA-4 siRNA (siCTLA-4) showed a direct cell activation both in vitro and in vivo. These cationic lipid-assisted PEG-PLA-based NPs efficiently delivered siRNA into T cell in vitro and significantly increased the percentage of anti-tumour $CD8^+$ T cells, while also decreasing the ratio of $CD4^+FOXP3^+T_{reg}$ among tumour infiltrating lymphocytes (TILs), resulting in the inhibition of tumour growth and prolonged survival time.[42,43]

Finally, tumours can develop phenotypes that are less immunogenic.

For example, the secretion of anti-inflammatory cytokines by the tumour, suppresses the ability of resident immune cells to act against tumour cells. This phenomenon can be minimised using NPs. Xu et al.[44] used a combination of NPs that included NPs against TGB-β, and NPs encapsulated with tumour antigen. This NPs combination reduced the TGB-β levels, producing an increased ratio of $CD8^+$ T-lymphocytes/T_{regs}.

To date, few studies are available to assess NPs and RT-ICI. Min et al.[6] reported that biodegradable and biocompatible antigen-capturing nanoparticles (AC-NPs) could improve cancer immunotherapy and induce the abscopal effect. Other study combined polymer NPs treatment with PD-1 checkpoint blockade therapy in irradiated tumours, inducing regression of primary tumours and distant tumours in bilateral syngeneic mouse tumour models.[45]

Conclusion and future directions

The use of immunotherapy has shown to be safe and effective. However, its efficacy has been limited by tumour cell heterogeneity. The development of nanotechnology, specifically Ac-NPs, has allowed to expose the immune system to a wide variety of antigens, enhancing the immune response directly on the primary tumour and distant tumours (abscopal effect). This type of treatment combinations is promising, but further studies are necessary to design preclinical and clinical trials to evaluate its feasibility in oncology treatments.

Conflicts of interest

None declared.

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