Magnetic Nanostructures for Cancer Immunotherapy

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Contributor: Stefano Persano

Magnetic nanoparticles (MNPs) represent an attractive class of nanomaterials due to their unique physical and chemical features that allow them to respond specifically to magnetic fields. Among the magnetic class of materials, iron oxide-based nanoparticles are the only inorganic nanomaterials that have been approved by the Food and Drug Administration (FDA) for medical applications. Magnetic nanomaterials are particularly appealing for cancer immunotherapy due to their unique features, which include (i) the traceability of their signal by magnetic resonance imaging (MRI) or by magnetic particle imaging (MPI) techniques ; (ii) their exploitation as carriers to promote the accumulation and the efficient delivery of biotherapeutic compounds, such as genes and peptides, into a specific target cell or tissue; (iii) their ability to mediate the elimination of cancer cells through the production of a local thermo-ablative effect when exposed to an external alternating magnetic field, referred to as magnetic hyperthermia therapy (MHT); and (iv) their intrinsic immunomodulatory properties that can be harnessed to further promote or modulate the immune function.

Keywords: magnetic nanostructures ; surface chemistry ; cancer immunotherapy ; immune therapeutics ; combinatorial immunotherapy ; vaccines ; immunogenic cell death

C1. Introduction

In the past years, a cumulative number of studies have highlighted the critical regulatory role of the immune system in tumour biology [1]. Indeed, it has been proven that the host's immune system interacts with tumour cells throughout the process of cancer formation and progression, shaping the immunogenicity of tumours, either inhibiting or promoting tumour growth and development [2]. These findings have provided the basis for the development of novel cancer therapeutics; however, such complex mechanisms are still a matter of study and pertain to the medical breakthroughs started in the last decade, but which still hold great promise [3].

T cells have been shown to be major players in the generation of protective immunity and, as pointed out by Galon et al., the presence of tumour-infiltrating CD8+ T cells greatly influences the fate of a tumour [4]. Functional analyses of tumour-infiltrating T cells have contributed to a more detailed tumour stratification, which was found to better represent prognostic tools in the treatment of colorectal carcinoma than standard histopathological classifications [4][5][6].

Although the activation of antigen-specific CD8+ T cells is considered a key step for an effective anti-tumour response, it often fails to eradicate cancer cells without a proper activation of the innate immune system [7]. Innate immune cells, such as natural killer (NK) cells, γδ T cells, and macrophages, can recognize and kill tumour cells [8]. Innate immunity is activated in response to a broad variety signals, including pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which are recognized by pattern recognition receptors (PRRs) expressed by immune cells, such as macrophages, dendritic cells, and NK cells. Particularly relevant in tumour control is the role of NK cells, which together with T cells play a complementary function in contrasting tumour growth and propagation [9]. Indeed, NK cells can recognize cells with reduced or absent expression of major histocompatibility complex (MHC) class I molecules, thus ensuring the elimination of cancer cells that evade T cell-mediated killing [9].

Besides exerting its effector activity, innate immune cells have a pivotal role in directing and shaping the type and strength of anti-tumour adaptive immune responses, through the release of pro-inflammatory signalling molecules such as interferon gamma (IFN-γ) and interleukin-12 (IL-12) (Figure 1) [10][11].

Adaptive immunity, involving CD8+, CD4+T cells, and B cells, drives a tumour-specific response aimed at eradicating tumour cells, and contributes to the development of an immunological memory potentially protecting from tumour recurrence.

A series of events are required for the generation of antigen-specific anti-tumour responses, starting with the release of tumour antigens that are taken up by antigen-presenting cells (APCs), such as macrophages (MΦ) and dendritic cells (DCs), and processed into peptides [12][13]. Processed epitopes are loaded onto MHC I or II molecules for cross-presentation and presentation to CD8+ and CD4+ T cells, respectively (Figure 1) In order to induce effective T cell

responses, antigen presentation must be supported by costimulatory signals induced by innate immune cells, such as pro-inflammatory cytokines and costimulatory ligands [14]. Furthermore, tumour antigens can also promote B cell activation by binding B cell receptor (BCR) [15].

Effector T cells must infiltrate tumour tissues where they recognize tumour antigens presented via MHCI, and selectively kill tumour cells. Tumour cell killing further promotes the release of tumour antigens, which can serve to prime additional T cell responses [16]. CD4+ T cells (or T helper cells, Th cells) regulate both cytotoxic cellular immune responses and B cell-dependent antibody production. Th1 cells are characterized by the production and release of IFN-γ, which support tumour cytotoxicity synergistically with TNF-α (Figure 1) [16].

Despite the sophisticated and concerted anti-tumour immune response, the protective immunity of cancer patients often fails, as tumour cells have developed multiple mechanisms to evade immune surveillance [17]. These mechanisms are ascribable to (i) reduced immune recognition, either by the loss of immunogenic tumour antigens or by the downregulation of antigen-presenting molecules; (ii) increased tumour cell resistance to cytotoxic pathways; (iii) induction of an immunosuppressive tumour microenvironment, through the expression of immunoregulatory molecules (programmed death-ligand 1, cytotoxic T-lymphocyte antigen-4); the recruitment of regulatory cells, including myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and tumour-associated macrophages (TAMs), that will secrete immunosuppressive cytokines, such as IL-10 and transforming growth factor-beta (TGF-β).

The identification of the immune-evading mechanisms of tumours is resulting in novel therapeutic strategies aimed at reversing tumour immune evasion. Particular interest is given to the development of strategies that can enhance the recognition of tumour cells by the immune system, such as therapeutic vaccines, adaptive cell therapy, and immunogenic cell death (ICD)-inducing treatments [5][18]. Other approaches are aimed at potentiating anti-tumour responses through the employment of immunotherapeutics, targeting immune checkpoint molecules (i.e., ICBs), and immunomodulators, such as immune adjuvants and cytokines, which, in turn, enhance cytotoxic T cell functions [5][18].

However, standard soluble immunotherapy has often failed to trigger effective cancer immune responses. This lack of effectiveness is due to an inadequate delivery of immunomodulators, as a consequence of their rapid degradation and elimination as free molecules. Likewise, DCs inappropriately uptake soluble vaccine antigens and adjuvants, resulting in an impaired antigen presentation and priming of anti-tumour immune responses [19][20][21].

To overcome the delivery limitations of soluble immunotherapies, nanoparticles have emerged as versatile vectors for the encapsulation, protection, and spatial–temporal-controlled delivery of antigens, adjuvants, and immunomodulators, while allowing, by controlling the structural parameters of the nanoparticles, to increase the uptake efficiency to targeted cells [21][22].

Magnetic nanoparticles (MNPs) represent an attractive class of nanomaterials due to their unique physical and chemical features that allow them to respond specifically to magnetic fields [23]. Among the magnetic class of materials, iron oxide-based nanoparticles are the only nanomaterials that have been approved by the Food and Drug Administration (FDA) for medical applications [24]. Magnetic nanomaterials are particular appealing for cancer immunotherapy due to their unique features, which include (i) the traceability of their signal by magnetic resonance imaging (MRI) or by magnetic particle imaging (MPI) techniques [25]; (ii) their exploitation as carriers to promote the accumulation and the efficient delivery of biotherapeutic compounds, such as genes and peptides, into a specific target cell or tissue; (iii) their ability to mediate the destruction of cancer cells through the production of a local thermo-ablative effect when exposed to an external alternating magnetic field, referred to as magnetic hyperthermia therapy (MHT)

Progress on the synthesis and functionalization procedures in the last few decades have enabled to obtain MNPs with very-well-controlled physicochemical features, including size, shape, crystallinity, charge, magnetic properties, and surface functionalities [23][26]. Furthermore, compared to nanoformulations conventionally applied for cancer immunotherapy, such as polymeric and lipid nanoparticles, MNPs can be easily synthesized with inexpensive procedures suitable for large-scale production [27].

All these features render MNPs a particularly appealing platform for the development of combinatorial immunotherapies with enhanced therapeutic efficacy, by simultaneously tackling different tumour immune-escape mechanisms [23][26][28].

This review provides an overview of the recent advances in the use of MNP-based nanostructures to enhance the effectiveness of cancer immunotherapy. We highlight the impact of the physicochemical features and surface engineering of magnetic delivery platforms on their therapeutic effect, and describe the use of magnetic nanosystems to enable the development of combinatorial therapeutic approaches for improving the efficacy of cancer immunotherapies.

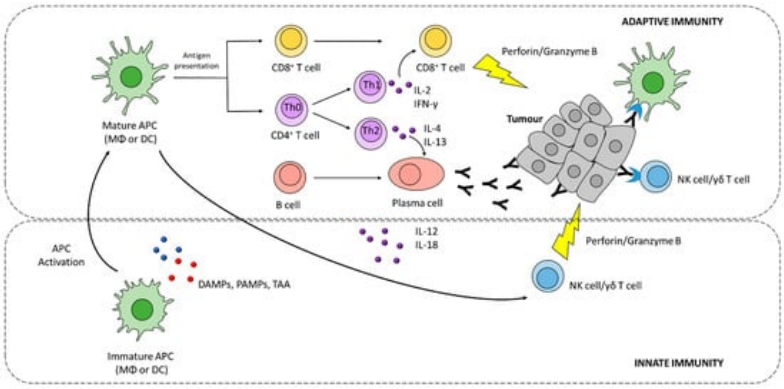

Figure 1. Overview of innate and adaptive anti-tumour immunity. Activated NK cells and γδ T cells can directly recognize and kill tumour cells through the release of perforin and granzyme B. Antigen-presenting cells (APCs), such as macrophages and dendritic cells, represent the main link between innate and adaptive immunity. Resting APCs can be activated by DAMPs and PAMPs, and then migrate to the secondary lymphoid organs where they present antigens and activate lymphocytes ($CD8^+$ and $CD4^+$ T cells, B-cells). $CD4^+$ T cells primarily provide help for B lymphocytes and $CD8^+$ T cells, whereas most $CD8^+$ T cells exhibit cytotoxicity toward tumour cells. On the other hand, B cells are the source of antibodies directed against the tumour, which contribute to tumour recognition and antibody-dependent cell cytotoxicity (ADCC).

2. Magnetic Nanomaterials for Cancer Immunotherapy: Synthesis and Properties

MNPs, thanks to their response to a magnetic field of a different nature, show unique advantages compared to other types of nanocarriers, which make them also promising for the field of cancer immunotherapy [23][26]. In particular, their unique capability as contrast agents in non-invasive molecular imaging techniques, such as MRI and MPI, can assist in the monitoring of the accumulation of magnetic nanoformulations at the target site [29]. Likewise, the utilization of MNPs as heat mediators in magnetic hyperthermia enable tumour ablation and the priming of anti-tumour immunity [30][31]. As the efficiency of MNPs as contrast agents as well as heat mediators depends on their physicochemical properties, the optimization of these properties is required for the synthesis of high-quality MNPs with a tunable size, shape, and composition.

MNPs usually have an overall hydrodynamic size smaller than 100 nm with a typical magnetic core size below 30 nm. Their magnetic properties can be tuned by the choice of size, shape, crystalline structure, and composition, among which iron oxides, such as magnetite (Fe3O4) and maghemite (γ-Fe2O3), or other mixed ferrites, such as zinc-ferrite (ZnFe2O4) or manganese-ferrite (MnFe2O4), are the most relevant for immune applications given the minimized toxicity of the Fe, Zn, and Mn ions of which these ferrites are made. Moreover, the magnetic properties can be fairly modulated by varying other physicochemical features related to surface structure and colloidal stability, or in other words, to the aggregation state of individual MNPs [23][26]. Indeed, the magnetic properties of iron oxide nanoparticles can be further redesigned by clustering a controlled number of individual superparamagnetic nanoparticles into superparamagnetic nanoparticle clusters, often termed magnetic nanobeads [32].

A wide range of methods have been reported for the preparation of high-quality MNPs, including wet chemical techniques (co-precipitation, solvothermal, thermal decomposition, sol-gel synthesis, microemulsion, and chemical redox), physical processes (gas-phase deposition and electron beam lithography), and bacterial and microorganism-based synthesis (Figure 2) [33][34][35]. Among these methods, co-precipitation, solvothermal, and thermal decomposition are the most commonly employed manufacturing processes. Usually, wet chemical methods, such as the thermal decomposition method, involve the decomposition of precursors into liquid media, such as 1-octadecence, at a high temperature and in the presence of capping agents and surfactants, such as oleic acid [34]. During the synthesis, the reaction conditions, including temperature and pressure, play important roles in determining the morphology and size of the MNPs, and consequently their magnetic properties [34].

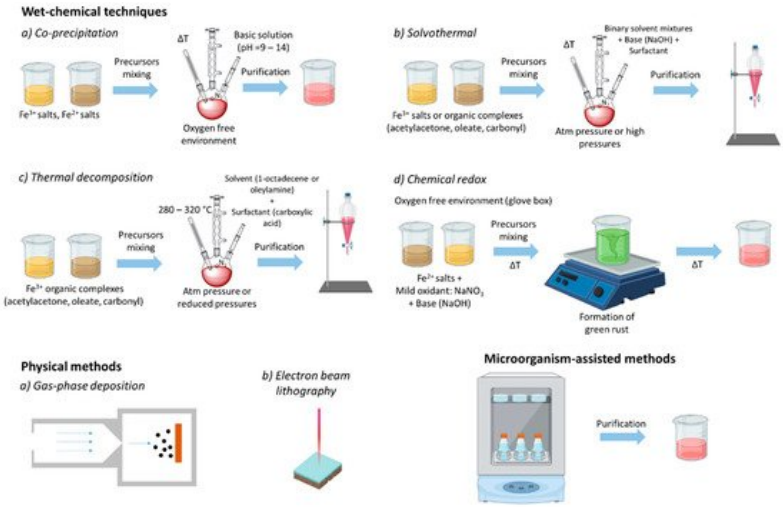

Figure 2. Different methods proposed for the synthesis of magnetic nanoparticles: wet chemical techniques (co-precipitation, solvothermal, thermal decomposition, chemical redox, etc.), physical methods (gas-phase deposition and electron beam lithography), and microorganism-assisted methods.

For instance, thermal decomposition and the solvothermal method can deliver MNPs soluble in non-aqueous media, as they are coated by alkylic surfactant molecules (such as oleic acid). On the contrary, MNPs prepared by the co-precipitation method are directly soluble in aqueous media, being coated by tiny polar molecules (such as sodium citrate). In both cases, the MNPs can be stabilized and functionalized by adding/replacing an outer layer of the shell coating, which can have multiple roles. Third, it can also introduce chemical groups feasible for the further functionalization of the MNPs with different biomolecules [26].

To optimize the effectiveness of MNP-based immunotherapy, key structural design considerations must be taken into account.

Early studies focused on nanoparticle delivery to tumours exploiting a mechanism known as the enhanced permeability and retention (EPR) effect [36]. In particular, a size of less than 100 nm has been identified as optimal to ensure higher accumulation of iron oxide nanoparticles to tumours [36]. While these delivery strategies of nanoparticles directly to the tumour are becoming an increasingly appealing option for reshaping the tumour microenvironment, the design of novel nanosystems for cancer immunotherapy is also aimed to trigger tumour-specific responses by harnessing the natural tropism of nanoparticles towards secondary lymphoid organs (including spleen and lymph nodes), where T cell priming occurs. For instance, lipidoid-stabilized iron oxide nanoparticles with a 30 nm core size had approximately 20-fold higher capacity to carry biomolecules such as antigens and adjuvants to lymph nodes via lymphatic drainage compared to smaller (10 nm) or larger (100 nm) nanoparticles [37].

Delivery platforms with a large size (>500 nm) lead to a prolonged retention at the injection site and are mostly taken up by local DCs, which after nanoparticle internalization will migrate to the draining lymph nodes [38]. Interestingly, a biodistribution study shows that transport through the lymphatic system results in an ~1000-fold increase in accumulation into local draining lymph nodes, which can substantially reduce off-target side effects and improve T cell priming [39]; thus, nanoparticles with a size around 30 nm may be preferred for lymph node targeting.

Among the various morphologies of iron oxide nanoparticles, octapod- and plate-shaped nanoparticles with a similar aspect ratio and surface charge showed a higher immunomodulatory potential by inducing inflammasome activation [40]. However, spherical nanoparticles diffuse less efficiently through the vascular wall than rod- and bar-shaped nanoparticles with a similar size range [41]. It has been reported that reshaping iron oxide nanoparticles from spheres to cubes markedly increases their heating performance [42]. In addition, controlled clustering of iron oxide nanocubes into nanoparticle assemblies that are anisotropic in their shape can preferentially increase the MNPs' heating power [43].

Generally, local administration of positively charged MNPs promote a stronger immune response than nanoparticles having a net negative or neutral surface charge [44][45]. Though, cationic nanoparticles display reduced tissue penetration, probably due to the interaction with the negatively charged components of the ECM [46]. Consequently, positively charged nanoparticles are usually retained at the injection site, where they can be more easily taken up by local DCs, compared to neutral and anionic nanoparticles [47]. Contrarily, slightly negatively-charged nanoparticles or neutral nanoparticles possess a superior circulation time and therefore may achieve enhanced tumour accumulation when systemically injected.

Besides surface charge, other surface physicochemical properties can affect tremendously the behaviour of MNPs in biological conditions, thus improving targeting efficiency, biocompatibility, therapeutic efficacy, stability, loading capacity, and efficiency [48]. After synthesis, most of the MNPs prepared by non-hydrolytic methods are capped by long hydrophobic chains that act as stabilizing agents, making them soluble in organic solvents. Consequently, surface modification of these nanoparticles is firstly required to enable their water solubilisation, making them ready for any further modification. For nanoparticles produced by hydrolytic methods, charged capping molecules, such as citrate molecules, are usually exchanged with other spacer ligands, such as polyethylene glycol derivatives or dextran shells, which help to improve long-term colloidal stability in biological environments [33][34].

Surface modification has been also exploited to facilitate the loading of immunomodulators that can activate and/or boost the immune responses in patients [49][50]. The most common surface modification strategies, such as ligand exchange, porous silica, phospholipid, and polymer coating, have been extensively explored to facilitate loading of various immunotherapeutics, including TLR agonists and monoclonal antibodies onto MNPs through non-covalent or covalent interactions, taking advantage of the properties associated with the coating material (e.g., large pore size of the porous silica shell and large number of reactive functional groups of polymers) (Figure 3) [49][50][51][52].

A range of surface chemistry strategies has also been explored to facilitate multiple-drug loading. In this regard, the highly porous structures of mesoporous silica-coated ferumoxytol nanoparticles were capable to load both a checkpoint inhibitor (anti-PD-L1 antibody) and chemotherapeutic drug (cabazitaxel) for achieving an anti-tumoural synergistic effect against prostate cancer [49]. Likewise, surface modification of MNPs with a lipid shell enabled the co-encapsulation of the α-helix-antigen fusogenic peptide (α-AP) with indocyanine green (ICG), an imaging agent, leading to the development of a theragnostic nanoplatform (α-AP-fmNP) [50]. In the context of DC-based vaccines, α-AP-fmNP-loaded DCs were revealed to possess antigen presentation capability and their in vivo migration toward lymph nodes, as confirmed by imaging techniques, was dramatically enhanced upon application of magnetic force, thus preventing anergy and resulting in a significantly improved anti-tumour efficacy [50].

In a pioneer study, iron oxide nanoparticles coated by carboxy-dextran were proven to activate the NF-κB pathway in macrophages, which plays important roles in inflammatory responses and immune activation/regulation, promoting M1 macrophage polarization [53]. Thus, it appears clear that the coating materials of the iron oxide nanoparticles have a significant influence on mediating the iron oxide nanoparticle's immunomodulatory properties. Mulens et al., in this regard, reported that the polyethyleneimine (PEI)-coated superparamagnetic iron oxide nanoparticles induced Toll-like receptor 4 (TLR4) activation in both murine and human macrophages, with consequent upregulation of IL-12 production and surface expression of maturation markers such as CD40, CD80, and CD86, indicating M1 polarization [54]. Contrarily, treatment with different iron-based formulations (Venofer, Ferinject, and Ferrlecit) reduced the differentiation of monocytes into M1 macrophages and myeloid DCs [55], suggesting that the M1-polarization induced by PEI-iron oxide nanoparticles observed in the earlier studies could be influenced by the coating material

Besides the coating material, the chemical composition of the MNP core is another factor that influences the immunomodulatory properties of these nanoparticles. For instance, it has been shown that oppositely to hematite phase (Fe_2O_3) nanoparticles, magnetite (Fe_3O_4) iron oxide nanoparticles display a great capacity in promoting macrophage polarization from a pro-tumoural M2 into an anti-tumoural M1 profile [53][56].

3. Combinatorial Approaches to Potentiate Cancer Immunotherapy

The clinical successes achieved with the use of cancer immunotherapy mostly based on checkpoint inhibitors have profoundly changed the treatment of several malignancies [57]. However, there are still many challenges that need to be addressed in order to exploit the full potential of immunotherapy and improve the overall response rates in patients, as tumour cells develop multiple mechanisms to escape immune recognition and immune cell killing. As such, combination immunotherapy is emerging as a strategy to treat cancer; yet, effective synergism with enhanced safety is still under investigation. Indeed, the combination of multiple therapeutics frequently appears to induce stronger toxicity, potentially limiting their clinical implementation.

Several MNP-based platforms have been reported to facilitate the development of combinatorial treatments aiming at merging multiple immunotherapeutic approaches together (combinatorial immunotherapy) or to combine cancer immunotherapy with standard-of-care therapies, including chemotherapy or hyperthermal therapy (multimodal treatments), that are being evaluated in preclinical settings and have displayed promising results in enhancing the therapeutic effect of single-agent immunotherapy and potentially reducing the toxicity of combinatorial immunotherapies (**Table 1**).

Table 1. Overview of the different magnetic nanostructure-based combinatorial immunotherapy approaches.

Magnetic Nanostructure	Surface Chemistry	Immunotherapeutic Drug	Therapeutic Approach	Remarks	Re
Iron nanoparticles (nano-aAPC)	Dextran functionalized with both MHC-Ig dimer and anti-CD28 antibody	MHC-Ig dimer, anti-CD28 antibody	Adoptive immunotherapy	Application of an external magnetic field induced nano-aAPC aggregation on naive cells, enhancing T cell proliferation in vitro and following adoptive transfer in vivo.	[52
Iron oxide nanoclusters (Magnetosome)	Cancer cell-derived plasma membrane functionalized with anti-CD205 antibody	TAAs, CpG ODN	Vaccine/Immune adjuvant	Cancer cell membranes serve as a reservoir of TAAs and their co-delivery with TLR9-agonist lead to a great proliferation of T-cells with superior cytotoxic activity. The application of an external magnet enhanced lymph node retention and anti-CD205-mediated $CD8^+$ DCs targeting of nanoparticles.	[51
Iron oxide nanoclusters (IO-LPMONs)	Mesoporous organosilica shell having large pore size	OVA antigen	Vaccine/TAMs repolarization	Simultaneous T cell activation and TAMs repolarization induced strong inhibition of tumour growth.	[58
Iron oxide nanospheres ($IO@FuDex^3$)	Fucoidan and dextran functionalized with multiple antibodies	Anti-PD-L1, anti-CD3 and anti-CD28 antibodies	T cell activation/Immune checkpoint inhibitor	$IO@FuDex^3$ can directly induce T-cell activation and block the immunosuppressive PD-L1 pathways via intravenous administration. The combination of $IO@FuDex^3$ and magnetic navigation demonstrated a highly improved therapeutic efficacy.	[59
Iron oxide nanoparticle-loaded micelles (poly(I:C)–Pt(IV)–IONP micelles)	DSPE-PEG(2000)-Pt(IV) prodrug functionalized with poly(I:C)	Poly(I:C)	Immune adjuvant/Chemotherapy	Pt(IV) prodrug synergized with TLR3-agonist inducing a more potent activation of DCs than cisplatin and poly(I:C).	[60
Iron oxide superparticles (Fe_3O_4-R837 SPs)	Poly(ethylene glycol)-block-poly(lactic-co-glycolic acid) copolymer	R837, anti-PD-L1 antibody	ICD/Immune adjuvant/Immune checkpoint inhibitor	Photothermal therapy promotes cancer cells killing, with consequent release of TAAs, and triggers the release of R837 immune adjuvant for a more effective vaccination strategy. Fe_3O_4-R837 SPs efficiently synergize with PD-L1 antibody to eliminate the primary tumours and prevent tumour metastasis to lungs/liver.	[61
Core-shell ferrite nanoparticles ($CoFe_2O_4@MnFe_2O_4$ nanoparticles)	Dimercaptosuccinic acid molecule	Anti-PD-L1 antibody	ICD/Immune checkpoint inhibitor	Magnetic hyperthermia induced TAAs release eliciting a systemic immune response affecting distant metastatic tumours. The combined MHT and checkpoint inhibitor demonstrate the great potentials in inhibiting the growth of both primary and metastatic tumours.	[62

Magnetic Nanostructure	Surface Chemistry	Immunotherapeutic Drug	Therapeutic Approach	Remarks	Re
FePt/MoS_2-FA nanocomposites (FPMF NCs)	FePt capped by dimercaptosuccinic acid, MoS_2 modified by thiol-polyethylene glycol-folate	CpG ODN, anti-CTLA-4 antibody	ICD/Immune checkpoint inhibitor	PDT act as ICD inducer and its ability to inhibit primary tumours and prevent metastasis was significantly improved when combined with chemotherapy drug/immunotherapeutics.	[63]
Janus nanobullets integrating chlorine e6 (Ce6) loaded, disulfide-bridged mesoporous organosilica bodies with magnetic heads(M-MONs@Ce6)	Asymmetric mesoporous silica growth, coated with cancer cell membrane	Anti-CTLA-4 antibody	ICD/Immune checkpoint inhibitor	The combination of PDT and magnetic hyperthermia elicits ICD, resulting in tumour-specific immune responses. When combined with anti-CTLA-4 antibody, synergistically enables the eradication of primary and deeply metastatic tumours.	[64]
Iron nanoparticles (FeNPs)	Poly(acrylic acid) (PAA) co-grafted with dopamine (DA) and amine-terminated PEG (5 kDa)	R837	ICD/Immune adjuvant/Immune checkpoint inhibitor	The combination of MNP-based MHT with local injection of nanoformulated TLR7-agonist and systemic injection of anti-CTLA4 antibody resulted in systemic immune responses that inhibited tumour metastasis and recurrence.	[65]

3.1. Combinatorial Immunotherapies

A promising approach to enhance the effectiveness of cancer immunotherapy involves the combination of treatments addressed to generate or expand antigen-specific cytotoxic immune responses, for instance through cancer vaccines, with therapeutic approaches designed to balance the immune suppressive TME. The combined effects of these approaches can be further improved using magnetic nanocarriers, enabling the co-loading of antigens and adjuvants and boosting the tumour or lymph node targeting selectivity. For example, the stimulation of T cell response through the delivery of an antigen (OVA), with the simultaneous repolarization of tumour-associated macrophages (TAMs), was achieved using magnetic core–shell nanospheres (IO-LPMONs) composed of an iron oxide (IO) core and a mesoporous organosilica shell with large pores (6.3 nm diameter), which allowed a high encapsulation efficiency of OVA and its delivery to DCs [58]. The formulation was proven to stimulate the maturation of DCs and consequently the expansion of both $CD4^+$ and $CD8^+$ OVA-specific T cells, which resulted in a strong T cell immunity against tumours. In addition, it was also demonstrated in this work that the repolarization of TAMs from an immunosuppressive M2 phenotype to tumour suppressing M1 phenotype was achieved due to the intrinsic adjuvant property of iron oxide nanoclusters. The synergistic effects of T cell activation together with macrophage repolarization demonstrated an enhanced therapeutic efficacy, inhibiting tumour growth.

In another strategy, iron oxide nanoparticles were modified with a checkpoint inhibitor (anti-PD-L1 antibody) and anti-CD3/CD28 antibodies providing activating signals to T cells, with the aim to overcome the immunosuppressive tumour microenvironment and promote the anti-tumoural activity of tumour-infiltrating lymphocytes [59]. Anti-CD3, anti-CD28, and anti-PD-L1 antibodies were conjugated onto the surface of fucoidan dextran-coated iron oxide nanoparticles (IO@FuDex) by using a reductive amination. The obtained multifunctional magnetic nanoparticles (IO@$FuDex^3$) were intravenously administrated into 4T1 mammary carcinoma-bearing mice. To minimize the undesired off-target accumulation of the antibodies and to achieve in situ expansion of tumour-infiltrating T cells, an external neodymium magnet of 0.22 T was applied at the tumour site for 4 h for three consecutive days (4 h/day). The authors showed that the field gradient of more than 10 T/m at the distance of 2 cm from the applied magnet significantly favoured the accumulation of the magnetic nanoformulations at the tumour site, thus reducing any off-target effect on the tumour-surrounding healthy tissues. After treatment, the growth of 4T1 primary tumours was extensively suppressed by simultaneously promoting the activation of cytotoxic T cells and blocking the immunosuppressive PD-L1 pathway at the tumour microenvironment using the multifunctional IO@$FuDex^3$ under magnetic navigation. Additionally, the IO@$FuDex^3$ formulation showed to be also efficient for the treatment of CT26 colon cancer and lung metastasis in a 4T1 breast tumour model. The antibodies' conjugation onto IO@FuDex3 and magnetic navigation minimized the observed adverse events, and notably, were effective at extending the survival of treated mice with a dose more than 100 times inferior to soluble anti-PD-L1.

Therapeutic approaches like the one described could potentially improve the therapeutic index of antibody-based immunotherapies, also allowing the development of combination therapies with reduced toxicities.

3.2. Immunotherapy in Combination with Other Cancer Therapies

Multimodal therapeutic strategies based on the combination of immunotherapy and other cancer therapies (e.g., chemotherapy and magnetic hyperthermia) have displayed synergistic effects that potentiate its efficacy, compared to single-based therapy, and have recently gained increased attention.

Chemotherapy is the most commonly utilized therapeutic modality to treat cancer in the clinic. Thus, the combination of immunotherapy and chemotherapy has been considered to develop strong collective anti-tumour effects. To integrate these two therapies into a single magnetic nanosystem, Hernández-Gil and co-workers reported magnetic micelles of phospholipids containing iron oxide nanoparticles to co-deliver anticancer platinum(IV) prodrug to induce tumour cell death, and TLR3 ligand poly(I:C) as an immunostimulant to activate DCs to promote protective anti-tumour immune responses [60]. The inert platinum(IV) prodrug became active as cisplatin in the highly reducing environment of the tumour site, exerting a cytotoxic effect against tumour cells. On the other side, poly(I:C) stimulated DCs by inducing TLR 3 signalling with a consequent increase in the production of IL-12. The secreted IL-12 mediated the activation of NK cells and T cells, increasing their cytotoxic activity against malignant cells. The cytotoxic effect of cisplatin combined with the induction of both innate and adaptive immunity by poly(I:C) prevented tumour growth.

Photothermal therapy is a minimally invasive and promising therapeutic approach relying on the activation of photosensitizing agents by laser irradiation at near-infrared (NIR) to generate heat for the thermal ablation of tumours [66]. Photothermal therapy is shown to be effective at generating immunogenic cell death (ICD) and has been recently exploited in combination with immunotherapies in preclinical studies to overcome tumour resistance mechanisms [66]. Besides plasmonic materials, iron oxide nanoparticles absorb and efficiently convert heat infrared radiation at 800 nm into heat, making them interesting for photothermal applications [67]. For example, magnetically targeted photothermal immunotherapy of 4T1 triple-negative breast tumours was realized using nanoclusters of spherical iron oxide of 150 nm diameter with a high photothermal conversion efficiency of 68.2%. These magnetic nanoclusters contained self-assembled ultrasmall superparamagnetic iron oxide nanoparticles that act as photothermal agents under a laser excitation of 808 nm, and the synthetic TLR7-agonist imiquimod (R837) loaded into an amphiphilic polymer matrix of mPEG-PLGA [61]. The heating to 50 °C generated upon light irradiation with an NIR-laser of power density 0.33 W/cm^2 for 30 min triggered a rapid release of the encapsulated R837 molecules at the tumour site and concomitantly promoted tumour cell elimination. The release of the TLR7 agonist together with the release of tumour-associated antigens (TAAs) by dying cancer cells led to DC maturation and the secretion of various cytokines (e.g., TNF-α and IL-6). Although the antitumor responses induced in the treated mice successfully inhibited the growth of primary orthotopic 4T1 tumours, they failed to protect from the spontaneous growth of the metastatic nodules in the lung and liver. To further improve the anti-tumour effect and, particularly, to treat/prevent metastatic disease, the authors combined the photothermal/R837-based treatment with intravenous injection of the PD-L1 antibody, resulting not only in primary tumour elimination but also in the prevention of the spontaneous growth of metastatic nodules in lungs and liver. This synergistic therapy also displayed abscopal effects that led to complete tumour growth inhibition of untreated distant tumours through the triggering of immune cell infiltration into the TME. Despite the induction of a strong systemic anti-tumour immune response, the combination treatment did not display signs of toxicity in the treated mice, thus indicating that strategies like the one proposed may be suitable for the development of safe and effective combinatorial immunotherapies.

Similarly, exploiting the heat generated by MNPs under radiofrequency excitation in the so called MHT has also shown a synergistic effect with cancer immunotherapies in preclinical studies. MHT may be particularly suitable for increasing the responsivity to checkpoint blockade therapies, as it has the potential to promote immune cell trafficking into tumours and, by inducing ICD, to expand the pool of antigen-specific T cells [62]. Its clinical implementation also may be favoured given that the accumulated MNPs into the tumour tissue generate heat locally, without affecting the adjacent healthy tissues and, importantly, in contrast to photothermal therapy, it particularly enables the treatment of deep-seated tumours, owing to the unlimited tissue penetration ability of the alternating magnetic field in MHT. Pan et al. recently reported magnetic hyperthermia therapy using dimercaptosuccinic acid-modified $CoFe_2O_4$@$MnFe_2O_4$ core–shell nanoparticles as a heat mediator with an SAR of 110 W/g at a condition of 577 kHz and 1.7 mT, in combination with checkpoint blockade immunotherapy for the elimination of both primary and metastatic tumours [62]. The authors demonstrated the excellent biocompatibility of the superparamagnetic nanoparticles up to a nanoparticle concentration of 400 µg/mL against both tumour and non-malignant cells. Although the conditions of the external alternating magnetic field used in this study are far from the clinical conditions of MHT (frequency, f, of 110 kHz and maximum field intensity, H, of 30 mT), this proof-of-principle study provided insights into the double therapeutic action of magnetic field-triggered heat. The heat generated by core–shell MNPs at the primary tumour site not only promoted direct tumour cell killing, but also induced a T cell-mediated anti-tumoural immune response that prevented the growth of distant tumours. Importantly this anti-tumour effect was

enhanced when combined with anti-PD-L1 therapy to potentiate T-cell killing activity against tumour cells. Indeed, the therapeutic effect achieved by the combination treatment was superior in preventing primary and metastatic tumour growth compared to other therapeutic approaches, such as surgical resection alone or in combination with anti-PD-L1 treatment. Future work may require conducting these studies with MNPs that enable heating by applying an external magnetic field that does not exceed the biological limit of $H \times f \leq 5 \times 10^9$ $Am^{-1}s^{-1}$, thus not raising concerns about the safety and clinical translatability of this strategy.

Recently, Wang et al. prepared multifunctional nanoparticles to combine photodynamic therapy (PDT) with magnetic hyperthermia (MHT) to synergistically improve the immunogenic capacity of dying cancer cells to elicit anti-tumour immune responses [64]. The authors developed bullet-shaped Janus magnetic mesoporous organosilica nanoparticles (M-MONs@Ce6) composed of iron oxide nanoparticles placed on the head of the Janus particle used as a heat mediator for MHT and having the body of the disulphide-bridged mesoporous organosilica, into which the most commonly used photosensitizer for PDT, chlorine e6 (Ce6), was incorporated. Next, in order to improve the colloidal stability in physiological environments and to attain the homologous tumour targeted accumulation of M-MONs@Ce6, each nanoparticle was further entirely coated with breast cancer cell-derived membrane. The biodegradability of the nanostructures made them highly responsive to redox/pH variations, thus ensuring the precise release of the photosensitizer over time in the acidic and reductive conditions of TME. Furthermore, the application of an alternating current magnetic field (32.5mT, 262 kHz) for 20 min prior to PDT not only destroyed the tumour cells but also improved the tumour oxygenation via promoting blood vessel damage, which is more beneficial for PDT in the hypoxic regions of tumours. Therefore, under irradiation of 606 nm light (0.15 W/cm^2, 10 min), the released chlorine e6 at the tumour had a high capability in the enhancement of intracellular reactive oxygen species, which was sufficient to eradicate cancer cells. Consequently, after the combined application of the treatments, primary breast tumour growth was strongly inhibited and this outcome correlated with profound changes in the TME, including increased number of cytotoxic T cells and decreased frequency of Tregs [64]. This immune response was further amplified by combination with anti-CTLA-4 antibody, thus suppressing the growth of both primary and metastatic tumours [64].

4. Conclusions

To conclude, magnetic nanosystems hold tremendous potential for the development of safe, more effective, and personalized cancer treatments, allowing a localized delivery of payload drugs and facilitating the rational design of novel combinatorial therapies based on immunotherapeutic treatments, exploiting the adaptive and/or the innate immune system. Although in the initial phase of its development, magnetic-guided immunotherapy represents an additional tool that could also help to advance the field of cancer immunotherapy. Future studies aimed to overcome the current technical limitations of magnetic field-generating equipment and to improve the magnetic properties of magnetic nanomediators could help expand the use and clinical implementation of magnetic-responsive nanosystems.

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