

Enhancing cancer immunotherapy with nanomedicine

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Abstract | Therapeutic targeting of the immune system in cancer is now a clinical reality and marked successes have been achieved, most notably through the use of checkpoint blockade antibodies and chimeric antigen receptor T cell therapy. However, efforts to develop new immunotherapy agents or combination treatments to increase the proportion of patients who benefit have met with challenges of limited efficacy and/or significant toxicity. Nanomedicines — therapeutics composed of or formulated in carrier materials typically smaller than 100 nm — were originally developed to increase the uptake of chemotherapy agents by tumours and to reduce their off-target toxicity. Here, we discuss how nanomedicine-based treatment strategies are well suited to immunotherapy on the basis of nanomaterials' ability to direct immunomodulators to tumours and lymphoid organs, to alter the way biologics engage with target immune cells and to accumulate in myeloid cells in tumours and systemic compartments. We also discuss early efforts towards clinical translation of nanomedicine-based immunotherapy.

Beginning approximately 10 years ago, clinical trials showed significant efficacy of checkpoint blockade and chimeric antigen receptor (CAR) T cell therapy in certain cancers, leading to approval by the US Food and Drug Administration (FDA) of multiple therapies and a major change in the role of immunotherapy in oncology broadly. These treatments have induced prolonged complete remissions in a subset of patients, conclusively demonstrating the potential of immunotherapy in cancer^{1–3}. These successes have fuelled a dramatic increase in the number of immunotherapy agents in human testing and an enormous number of clinical trials exploring combination treatments. However, following the initial striking results observed in trials of inhibitors of the programmed cell death 1 (PD1) pathway and CAR T cell therapy for leukaemias and lymphomas, progress has been modest. Combination immunotherapies, such as co-treatment with antibodies that target cytotoxic T lymphocyte antigen 4 (CTLA4) and PD1, have shown moderate increases in efficacy, while eliciting substantial increases in toxicity⁴. CAR T cell therapies that have been effective in haematological cancers have so far generally failed to have a major impact on the treatment of much more prevalent solid epithelial cancers. Thus, additional approaches to safely and effectively drive immune responses against cancer remain an important unmet need.

While efforts aiming to further expand our understanding of human immunology in the context of cancer remain critical, broader success with immunotherapy treatments is not limited by a lack of reasonable

therapeutic targets. A major challenge lies in safely engaging these targets at the right time and place. Cancer drug development has historically focused on the generation of therapeutics that are systemically administered to ensure access to all sites of metastatic disease. This is an effective strategy for drugs designed to specifically act on pathways that are unique to tumour cells (for example, targeted drugs inhibiting mutant tumour kinases). Applying the same paradigm to immunotherapy agents that act on the host immune system can be problematic owing to our inability to site-specifically stimulate tumour-specific immune cells. For example, the approved checkpoint blockade drugs that interfere with inhibitory pathways in adaptive immunity have shown efficacy but also manageable (albeit significant) toxic effects in patients, including gastrointestinal and pulmonary toxicity and autoimmune sequelae⁵. New therapeutics that instead 'turn up' adaptive responses through immunostimulatory pathways have encountered substantial safety issues that have hindered successful therapeutic implementation⁶.

A potential solution is to break with the traditional drug development paradigm and engineer the delivery of immunotherapeutics, focusing their action on target tissues (that is, tumours and tumour-draining lymph nodes) or cell types, to control the timing and location of immunomodulation. To achieve this goal, approaches based on nanomedicine — the formulation of drugs in carrier materials that are smaller than ~100 nm — may offer the means to increase both the safety and the therapeutic efficacy of many immunotherapies. Formulation

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of immunotherapeutics in nanoparticles composed of lipids, polymers or other materials has been used to alter systemic exposure, promote accumulation in tumours, enhance uptake in innate immune compartments and alter signalling at the single-cell level. Such approaches introduce new complexities in drug development, but these are technical hurdles that numerous clinical stage companies have demonstrated to be readily surmountable. In this Review, we discuss the mechanisms by which nanomedicine-based treatments act on the immune system to enhance immunotherapies in pre-clinical models, the challenges facing these approaches and the early stages of clinical translation of these concepts. Protein engineering (such as antibody–cytokine fusions) and generation of tumour-tropic viruses are important alternative strategies to achieve some of the same goals and have been recently reviewed^{7–9}. We focus here on approaches that are complementary to strategies founded in genetic engineering and molecular biology and apply the traditional definition of nanomedicine as biological or small-molecule drugs that are modified through synthetic chemistry or materials science approaches into nanoscale formulations for effective delivery, including chemical conjugation, encapsulation

or physical incorporation within other materials. In the interest of limiting the scope, we also exclude discussions of the use of nanoparticles in vaccines, which has been the subject of recent reviews and has its own large body of relevant literature^{10–13}.

Mechanisms of nanoparticle therapeutics

Nanomedicine-based drug formulations were originally developed to alter the pharmacokinetics and toxicity profiles of chemotherapy agents and to promote their accumulation in tumours (BOX 1). The ability to concentrate drugs within tumour cells and/or the tumour microenvironment (TME) is also of relevance for enhancing immunotherapy. However, nanomaterials also allow novel mechanisms of action for immunotherapy agents, including the ability to display ligands to immune cells, regulate intracellular delivery of cell-impermeable compounds and control the timing of drug release and/or activation.

Promoting immunogenic tumour cell death. Most traditional antitumour drugs aim to kill cancer cells, but not all pathways of cell death are immunologically equivalent. Tumour cell death events that promote an antitumour immune response are referred to as ‘immunogenic cell death’ (ICD)¹⁴. ICD is associated with the extracellular release of damage-associated molecular patterns such as ATP and high mobility group protein B1 (HMGB1) and the surface exposure of calreticulin and heat shock protein 90 (HSP90). These factors promote tumour antigen uptake by antigen-presenting cells and their subsequent activation. Traditional ablative cancer therapies, such as chemotherapy or ionizing radiation, differ in their ability to induce ICD, and their immune potentiation can be offset by toxic effects on responding immune cells. Nanomedicine formulations are an attractive modality to promote ICD because they can concentrate cytotoxic agents in tumour cells. In addition, nanomaterials can be designed to directly interact with external energy sources, allowing amplification of ICD induced by treatments such as radiotherapy and magnetic hyperthermia¹⁵.

Nanoparticle formulation of chemotherapeutics that promote ICD can enhance antitumour immunity by promoting more effective delivery of the drugs to tumour cells (FIG. 1a). For example, Doxil, a clinically approved pegylated liposomal formulation of the anthracycline chemotherapeutic doxorubicin, synergized with checkpoint blockade in the treatment of mouse models of colon adenocarcinoma and fibrosarcoma, leading to stronger antitumour activity compared to treatment with free doxorubicin¹⁶. Doxil induced increased intratumoural CD8⁺ T cell infiltration, decreased the proportion of regulatory T cells (T_{reg} cells) and increased CD80 expression by myeloid cells (including dendritic cells (DCs)) compared with free doxorubicin. Consistent with the importance of adaptive immunity in the effectiveness of the nanoparticle formulation of doxorubicin, efficacy was lost in athymic nude mice. Following these results, combination therapy involving Doxil and checkpoint blockade is now being tested in a phase IIb clinical trial in patients with metastatic breast cancer (TABLE 1).

Box 1 | An overview of nanomedicine

The term ‘nanomedicine’ was popularized in the late 1990s to describe the application of the emerging field of nanotechnology, which seeks to exploit the unique properties and behaviours of structures at the nanometre length scale, to human health^{134,135}. The concept encompasses a broad range of approaches to disease treatment and diagnosis, and the definition of ‘nanomedicine’ is being refined as this discipline matures. In the drug delivery field, nanomedicines are formulations of therapeutics with polymers, lipids, metals or inorganic materials which have at least one physical dimension of ~100 nm or less. Chemical conjugates of protein drugs (such as drug–protein and polymer–protein conjugates) are also sometimes considered nanomedicines due to similarities in their therapeutic goals and pharmacokinetic behaviour. Polymers and nanoparticles became of interest in cancer therapy first for their potential to alter drug pharmacokinetics and later for their capacity to accumulate in tumours through the enhanced permeation and retention (EPR) effect^{136,137}. The EPR paradigm is based on the idea that particles of size 10–100 nm in the bloodstream are too large to escape the vasculature and enter healthy tissues or to be cleared by the kidneys, but can escape from dysfunctional vasculature into tumours if they are not cleared too rapidly by the reticuloendothelial system. Within a tumour, defective lymphatics prevent the particles from being cleared from the tissue, leading to accumulation.

Nanomedicines can also be functionalized with targeting molecules such as antibodies, which can increase internalization of drugs into target cells — in this case, drug-to-antibody ratios can be more than 10,000, greatly in excess of ratios for traditional antibody–drug conjugates¹⁵. Currently approximately 50 nanomedicine therapies are approved by the US Food and Drug Administration for cancer and other diseases¹³⁸. Challenges include the fact that current technologies typically deliver only a small percentage of the injected dose into the tumour¹³⁹, and clinical evidence suggests that the efficiency of EPR-based targeting differs significantly among patients¹⁴⁰. However, nanomedicines aimed at enhancing immunotherapy often have a different set of delivery requirements than systems delivering chemotherapies and can act by a number of mechanisms distinct from chemotherapy. Because the immune system acts as a highly dynamic network, effective stimulation of a small number of leukocytes in tumours or lymphoid organs can lead to large changes in the microenvironment driven by a cascade of cell-to-cell communication. Challenges include the need to establish robust manufacturing strategies tailored to the objectives of immunotherapy and the need for better preclinical models to evaluate immune-related toxicity as early as possible in the development pipeline¹⁴¹. Improvements in tools to monitor ongoing immune responses both in preclinical models and in patients undergoing treatment may be key to ensuring the high promise of nanomedicine for cancer immunotherapy is met in the clinic.

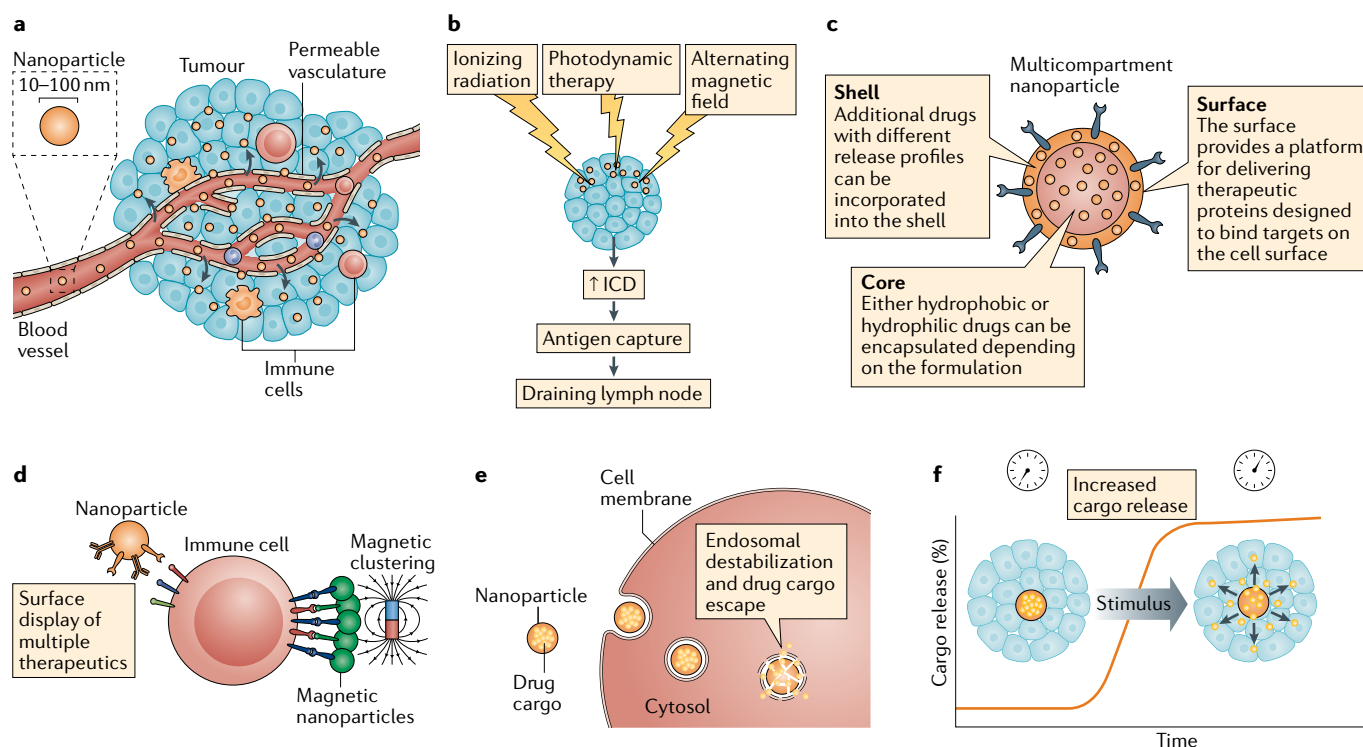


Fig. 1 | Nanomedicines allow unique modes of action in immunotherapy. **a** | Nanomedicines, such as nanoparticles, accumulate within tumours via the enhanced permeation and retention effect, concentrating the drug in tumour sites. **b** | Nanoparticles can be designed to interact with external energy sources, such as ionizing or non-ionizing radiation or magnetic fields to enhance immunogenic cell death (ICD). **c** | Nanomedicines allow combinations of therapeutics, including drugs with very different properties, to be co-delivered to tumour sites. **d** | Multiple ligands can be arrayed on the surfaces of polymers and nanoparticles to enhance engagement of immunostimulatory receptors. **e** | Nanoparticles can be formulated to destabilize endosomal membranes and promote drug delivery into the cytosol. **f** | Nanoparticles allow control of the kinetics of drug release, either preprogrammed through the particle chemistry or through responsiveness to an external stimulus such as light or heat.

A more recent strategy to improve doxorubicin delivery and increase ICD relied on synthetic high-density lipoprotein nanodiscs with the drug covalently tethered to the nanoparticles through an acid-labile linkage. Delivery via nanodiscs upregulated markers of ICD in tumours and synergized with anti-PD1 relative to the free drug in two mouse models of colon cancer¹⁷.

Although capable of inducing ICD, radiotherapy is rarely effective as a monotherapy for promoting sustained antitumour immunity¹⁸. However, numerous clinical studies are currently exploring radiotherapy as a component of combination immunotherapies¹⁹. Radiation kills tumour cells by inducing non-repairable DNA damage, but it can also trigger activation of the cyclic GMP–AMP synthase–stimulator of interferon genes pathway (cGAS–STING pathway) and subsequent production of proinflammatory cytokines, promoting innate and adaptive antitumour immunity²⁰. The radiotherapy dose must be carefully tuned as high radiation doses lead to attenuated STING activation due to DNA exonuclease TREX1-dependent cytosolic DNA degradation²¹. An initial, successful proinflammatory radiotherapy response can also be blunted by the recruitment of immunosuppressive immune cells to the tumour²². One long-standing concern has been that even localized radiotherapy may be detrimental to antitumour immunity due

to damaging or suppressing tumour-infiltrating T cells, but recent findings from preclinical studies suggest that radiotherapy can actually activate tumour-resident T cells, which are comparatively more radioresistant than circulating T cells²³.

Several approaches have used nanoparticles to improve immune priming provoked by radiotherapy. For example, biodegradable polymer nanoparticles designed to bind proteins from the surrounding solution were shown to promote T cell priming following radiotherapy. Intratumoural injection of the nanoparticles following radiotherapy led to capture of protein antigens released from dying cancer cells by the particles, which subsequently passed through lymphatics and were internalized by phagocytic professional antigen-presenting cells in the draining lymph node²⁴. Nanomedicines can also be used as radioenhancers that directly interact with ionizing radiation to upregulate ICD²⁵ (FIG. 1b). Materials containing elements with a high atomic number (*Z*) can both absorb and scatter radiation, and thus high-*Z* nanoparticles that accumulate in tumours can potentiate radiotherapy²⁵. A recent clinical trial demonstrated the ability of intratumourally injected hafnium oxide nanoparticles to double the frequency of pathological complete responses to radiotherapy in patients with sarcomas²⁶, leading to initial clinical approval in Europe.

Cyclic GMP–AMP synthase–stimulator of interferon genes pathway (cGAS–STING pathway). An intracellular signalling pathway that responds to cytosolic double-stranded DNA through the sensor enzyme cGAS to produce the second messenger cyclic GMP–AMP, which subsequently activates STING and can stimulate cells to produce type I interferons and other cytokines.

Table 1 | Clinical translation of cancer immunotherapy nanomedicines

Developer	Concept	Indications	Clinical stage	ClinicalTrials.gov identifiers	Refs
NanoBiotix	Metal nanoparticle radioenhancers in combination with checkpoint blockade	Various solid tumours	Phase I–III	NCT03589339	25–27
Oslo University Hospital/Bristol-Myers Squibb	Pegylated liposomal doxorubicin in combination with checkpoint blockade	Metastatic breast cancer	Phase IIb	NCT03409198	16
Nektar Therapeutics	Reversibly pegylated IL-2 in combination with checkpoint blockade	Various solid tumours	Phase I, phase II	NCT02983045, NCT03138889, NCT03282344, NCT03635983, NCT03785925, NCT03729245, NCT03435640	81–83
Exicure	Intratumoural administration of TLR9 agonist-functionalized nanoparticles in combination with checkpoint blockade	Various solid tumours	Phase Ib/II	NCT03684785	49,50
Torque Therapeutics	Nanoparticle-functionalized antigen-primed T cell therapy	Various solid tumours and lymphomas	Phase I	NCT03815682	113,115,120
Rimo Therapeutics	Metal–organic framework nanoparticles as radioenhancers combined with IDO inhibitors and/or checkpoint blockade	Various solid tumours	Phase I	NCT03444714	29
Coordination Pharma	Nanoscale coordination polymer-based particles	Various solid tumours	Phase I	NCT03781362, NCT03953742	35–37
Moderna Therapeutics	Lipid nanoparticle-delivered mRNA encoding OX40L, IL-23 and IL-36γ with or without checkpoint blockade	Relapsed or refractory solid tumour malignancies or lymphoma	Phase I	NCT03739931	53
Moderna Therapeutics	Lipid nanoparticle-delivered mRNA encoding OX40L	Relapsed or refractory solid tumour malignancies or lymphoma	Phase I	NCT03323398	53
OncoNano	STING-activating polymer micelles	TBD	Phase I projected 2020–2021	NA	48,142
Tidal Therapeutics	Nanoparticles for gene transfer to macrophages and lymphocytes	TBD	Phase I projected 2020–2021	NA	127,128

IDO, indoleamine 2,3-dioxygenase; NA, not applicable; STING, stimulator of interferon genes; TBD, to be determined; TLR9, Toll-like receptor 9.

One of the mechanisms by which radiotherapy promotes antitumour immunity is through the release of DNA into the cytoplasm, which activates the cGAS–STING pathway. In preclinical studies, high-*Z* nanoparticles have been shown to enhance irradiation-induced STING activation in human tumour cells²⁷. In mice, intratumourally administered high-*Z* metal–organic nanoparticles increased the immunogenicity of low-dose radiotherapy when combined with antibodies against programmed cell death 1 ligand 1 (PDL1) as evidenced by shrinkage of both irradiated primary tumours and secondary non-irradiated tumours^{28,29}. This enhanced abscopal response was dependent on both CD8⁺ T cells and CD4⁺ T cells. Such findings have led to nanoparticle radiosensitizers recently entering clinical testing in combination with checkpoint blockade (TABLE 1).

Hyperthermia within tumours can induce ICD and promote an adaptive immune response, but precise heating of tumours without damage to surrounding tissues is often difficult to achieve. However, localized hyperthermia can be realized by use of an externally applied alternating magnetic field to excite paramagnetic iron oxide nanoparticles within the TME. This approach induces tumour ICD and promotes a CD8⁺ T cell-mediated

immune response as demonstrated in preclinical models of glioma, colon adenocarcinoma and melanoma^{30,31}. Nanoparticle-mediated hyperthermia induced the production of a range of proinflammatory cytokines within treated tumours and activated DCs. Combination therapy using magnetic nanoparticle-induced hyperthermia, radiotherapy and a virus-like particle adjuvant was effective in dogs with spontaneously arising oral melanoma³². Motivated by these preclinical findings, the use of magnetic nanoparticles to induce tumour hyperthermia and ICD is being tested clinically. As iron oxide nanoparticles are considered relatively non-toxic, are amenable to functionalization with targeting molecules and can be imaged with magnetic resonance imaging, they have the potential to be clinically successful soon if clear benefits for patient survival are demonstrated.

Nanomaterials have also been used to enhance combination therapies, either via co-encapsulation of multiple drugs in the same particle to guarantee co-delivery to target cells or by combining drugs with modes of action that synergize to produce supra-additive benefits^{33,34} (FIG. 1c). Combinatorial drug loading has been facilitated by the development of chemical methods that allow small-molecule drugs to be directly polymerized

Abscopal response

Immunological response to radiotherapy or other localized therapies whereby the treatment of a malignant lesion results in the regression or stabilization of distant, non-treated lesions.

into a solid particle, and by methods to form nanoparticles with a solid core of one material surrounded by a shell of another material. For example, oxaliplatin was formulated with metal-coordinating groups that allow it to polymerize into a solid particle in the presence of metal ions (for example, zinc). When such nanoparticles were prepared with a phospholipid shell carrying a second reactive oxygen species-producing drug, dihydroartemisinin, the resulting combination therapy-loaded particles elicited pronounced ICD in tumours and synergized with anti-PDL1 therapy in mouse models of colorectal adenocarcinoma³⁵. Such nanoscale coordination polymer formulations carrying undisclosed TME-modulating compounds have recently entered phase I clinical trials (TABLE 1). Nanoparticles have also been designed that both interact with external energy sources and carry immunostimulatory drugs. Inorganic nanoparticles that are 25 nm in diameter coated with a lipid-anchored photosensitizer enhanced calreticulin exposure on the surface of tumour cells and immune cell infiltration in mouse breast tumours when combined with infrared light irradiation compared with the free photosensitizer³⁶. Encapsulation of oxaliplatin in the same nanoparticle allowed combined ICD-inducing effects of photodynamic therapy and chemotherapy, and elicited regression of both irradiated primary tumours and non-irradiated secondary tumours in mouse models of colorectal cancer³⁷.

Ligand presentation to immune cells. Many key immunoregulatory receptor engagements, especially costimulatory signals provided to T cells and natural killer cells, occur at cell–cell junctions. Nanomedicines offer the potential to mimic such an interface by presenting an array of ligands from the particle surface on physiologically relevant size scales (FIG. 1d). For example, agonist anti-CD137 antibodies conjugated to the surfaces of liposomes were shown to activate CD8⁺ T cells approximately tenfold more potently than equivalent concentrations of soluble anti-CD137 antibody³⁸. In the setting of T cell activation, 30-nm-diameter magnetic nanoparticles bearing immobilized peptide–MHC molecules together with nanoparticles bearing anti-CD28 antibody induce minimal T cell triggering because they are individually too small to induce sufficient receptor crosslinking and each nanoparticle presents only a single signal³⁹. However, application of a magnetic field to induce nanoparticle clustering triggered robust T cell activation, proliferation and induction of effector functions. Particles can also present multiple ligands, either to co-engage multiple receptors on target immune cells or to engage multiple cell types simultaneously. Nanoparticles presenting both anti-PD1 and anti-OX40 antibodies elicited enhanced T cell activation and therapeutic efficacy in the treatment of mouse melanoma and breast tumours in comparison with therapy with the same agents administered as a simple drug mixture⁴⁰.

Nanoparticles have also been used to present clusters of death-inducing ligands from the surface of immune cells to circulating tumour cells. Liposomes that are surface conjugated with many copies of recombinant

E-selectin and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) bound to T cells, monocytes and natural killer cells in blood via E-selectin ligand and presented clustered TRAIL to circulating tumour cells, leading to their engagement with the death ligands and tumour cell killing⁴¹. TRAIL-presenting nanoparticles have been shown to inhibit metastasis and prolong survival in mouse and human xenograft tumour models^{42,43}.

Polymer nanoparticles that are surface conjugated with an antibody against human epidermal growth factor receptor 2 (HER2) and the ‘eat me’ signal calreticulin in an optimized 1:3 anti-HER2-to-calreticulin ratio slowed HER2⁺ tumour growth by binding to tumour cells and presenting calreticulin to DCs in the TME, promoting phagocytosis. The resulting increased tumour antigen presentation by DCs led to enhanced CD8⁺ T cell infiltration into tumours⁴⁴. In a second example, lipid nanoparticles were used to present signal-regulatory protein- α (SIRP α)-blocking antibodies, reprogramming tumour-associated macrophages (TAMs) from an immunosuppressive phenotype to a tumoricidal phenotype, while simultaneously delivering a small-molecule inhibitor of macrophage colony-stimulating factor 1 receptor (CSF1R) signalling⁴⁵. This approach significantly outperformed simultaneous dosing of both agents in the absence of the nanoparticle formulation as measured by tumour regression and survival. The increased efficacy was attributed to the fact that the antibody acts both as a targeting molecule, localizing the drug-loaded particles on macrophages, and as a direct therapeutic, inhibiting SIRP α signalling. Thus, nanomedicine formulations can alter signalling mediated by immunotherapy drugs that target cell surface receptors in several ways to enhance their net antitumour activity.

Intracellular delivery of danger signals and nucleic acids. One of the major applications of nanomaterials in medicine is promoting cytosolic drug delivery. Hydrophilic and charged compounds such as nucleic acids are generally internalized by cells via endocytosis, but thereafter remain trapped in the endolysosomal pathway. Nanomaterials have been extensively studied as surrogates of natural viruses to promote access of nucleic acids and other drugs to the cytosol (FIG. 1e). In the setting of cancer immunotherapy, this is particularly of interest for delivering compounds to activate cytosolic danger sensor proteins or to deliver RNA or DNA that encodes immunomodulatory proteins. Polymer vesicles (polymersomes) have recently been reported that encapsulate a cyclic dinucleotide agonist of STING for delivery to the cytoplasm⁴⁶. Once internalized by cells, the polymersomes disrupt endosomal membranes in response to endosome acidification, releasing the STING agonist cargo into the cytoplasm. These nanomaterials increased the apparent potency of cyclic dinucleotides by more than 100-fold, and in turn exhibited significantly increased antitumour efficacy in melanoma models following intratumoural administration. Enhanced STING activation has also been reported for liposomal formulations of cyclic dinucleotides incorporating pH-responsive lipids⁴⁷.

Another intriguing approach is the use of synthetic polymers that directly engage danger signal pathways. pH-responsive block copolymer micelles were recently described that disrupt endosomes and directly interact with STING, triggering its activation and downstream signalling⁴⁸. Nanoparticles can also be functionalized with high densities of danger signals such as Toll-like receptor (TLR) agonists, and these immunostimulators are taken up by myeloid cells and antigen-presenting cells more efficiently than free TLR ligands, leading to enhanced immunostimulation^{49,50}. Each of these approaches is moving into clinical testing (TABLE 1).

Delivery of nucleic acid cargos encoding therapeutic proteins can further provide new possibilities for manipulating immunostimulatory or immunosuppressive pathways⁵¹. Systemic administration of a lipid and protamine-based nanoparticle loaded with plasmid DNA encoding a PDL1 trap protein synergized with oxaliplatin chemotherapy to delay tumour growth in models of metastatic colon cancer⁵². Intratumoural administration of lipid nanoparticles delivering mRNA encoding the cytokines IL-23 and IL-36 γ and the T cell costimulator OX40L led to in situ vaccination and CD8⁺ T cell-dependent tumour regression in colon cancer and melanoma models, and this approach is currently being tested in patients⁵³ (TABLE 1).

Temporal control of immunostimulation. The dosing schedule of immunotherapy can have profound effects on treatment efficacy in preclinical models. Timing can be important in terms of both the duration that immune stimuli are present and active in tumours (or tumour-draining lymph nodes) and the ordering of cues delivered in combination therapies⁵⁴. Nanoparticles have long been used to provide sustained release of drugs into tissues or the circulation that otherwise exhibit rapid clearance, and can serve similar roles in immunotherapy⁵⁵ (FIG. 1f). In addition, nanomedicine formulations can be designed to interact with external energy sources such as light or heat to permit precisely controlled timing of drug release. This approach is exemplified by studies of near-infrared light-activatable nanoparticles complexed with TLR9 agonist CpG-containing DNA⁵⁶. In this approach, photolabile DNA strands complementary to CpG DNA were linked to the surface of near-infrared light-sensitive upconversion nanoparticles. Hybridization of CpG oligonucleotides with the nanoparticle-linked DNA complexed the TLR9 agonist to the particles in a 'silent' state. Systemic injection of the nanoparticles into breast tumour-bearing mice led to accumulation in tumours. Subsequent near-infrared light illumination of the tumours cleaved the photolabile DNA anchor, releasing active CpG DNA and triggering localized inflammation in the TME without systemic side effects, driving immune infiltration of tumours and enhanced survival of the mice.

The preceding examples highlight how nanomedicine can allow the more precise application of existing immunotherapy strategies, control timing and location of immunostimulation, and alter signalling from immunostimulatory biologics. In the following sections, we discuss how nanomedicine formulations alter the

outcome of immunotherapies, focusing first on locally applied therapies and then discussing systemic and cell-based modalities.

Enhancing localized immunotherapy

Local injection — intratumourally or peritumourally — of immunotherapy agents to directly modulate the TME or tumour-draining lymph nodes is a treatment strategy in preclinical and clinical development. Localized treatment is based on the expectation that T cell priming in tumour-draining lymph nodes will lead to trafficking of lymphocytes to distal untreated tumours^{57–59}. While such systemic abscopal responses have been anecdotally reported in patients for many years, recent efforts exploring rational combination therapies have begun to show increasing frequencies of systemic antitumour responses^{60–62}. Relative to systemic therapy, local treatment has the potential to increase the safety of immunotherapy by limiting systemic exposure, allow more effective modulation of the TME via increased concentration or persistence of drugs in the TME and may allow the use of combination treatments that would otherwise be too toxic for systemic administration⁵⁹. Two local immunotherapies are currently approved by the FDA for cancer treatment: intravesicular injections of bacille Calmette–Guérin (BCG) bacteria (the live-attenuated tuberculosis vaccine) for treatment of bladder cancer and intratumoural injections of engineered herpes simplex virus type 1 (talimogene laherparepvec) for the treatment of melanoma^{63,64}. Nanoparticle formulations may have an important role to play in this treatment setting by providing a means to promote retention of immunotherapy agents in tumours and to target therapeutics to tumour-draining lymph nodes.

Promoting immunotherapy retention in tumours. The goal of localized immunotherapy is to confine administered drugs to the tumour and/or tumour-draining lymph nodes, maximizing stimulation in the TME, while minimizing toxic effects due to systemic immunostimulation. However, intratumourally injected drugs tend to rapidly disseminate into the systemic circulation, driven in part by the high interstitial fluid pressure in tumours^{38,65–68}. Unlike small molecules or free proteins, nanoparticles can be physically trapped in the TME following injection because the interstitial space in tumours is filled by a collagen-rich extracellular matrix⁶⁹ (FIG. 2). The collagen fibres form a meshwork with irregular spacings ranging from 20 to 130 nm that traps particles of a similar or larger size. This has been experimentally demonstrated in various tumour models, in which nanoparticles approximately 100 nm in diameter or larger diffuse either very slowly or are effectively immobilized in the tumour interstitium^{70–72}. Intratumoural injection of immunostimuli chemically conjugated to liposomes 100–200 nm in diameter leads to their distribution through the local lesion and some accumulation in tumour-draining lymph nodes, but no detectable dissemination into the blood^{38,68}. Such alterations in biodistribution have allowed the safe administration of combination treatments that were lethally toxic as free drugs, with concomitant enhancements in

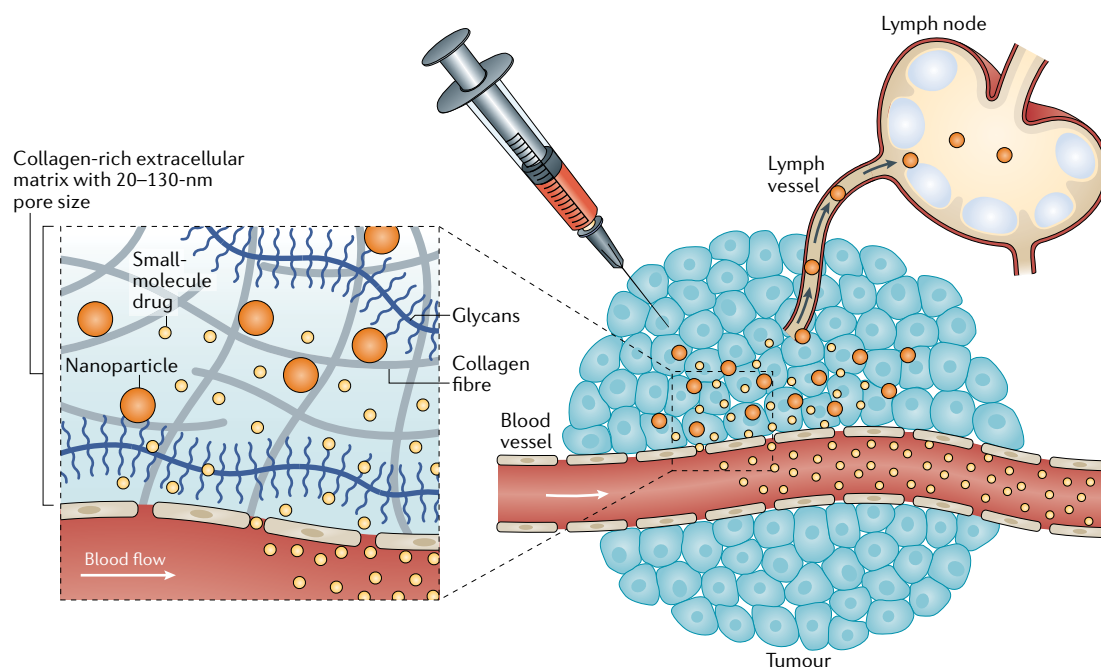


Fig. 2 | Nanomedicines improve tumour retention and lymph node trafficking. When nanomedicines are administered locally, the tumour's dense extracellular matrix, composed of a collagen-rich hydrogel with a pore size of 20–130 nm, preferentially retains nanomedicines and promotes their trafficking to the lymph node, whereas small-molecule drugs are rapidly cleared into the systemic circulation due to their small size and the high interstitial fluid pressure in tumours.

antitumour activity. On the basis of similar principles, recombinant plant virus nanoparticles with a diameter of 30 nm when injected intratumourally or administered by lung inhalation act through undefined innate immunostimulatory pathways to promote antitumour immunity and cancer regression⁷³. In a related approach in which the tumour is surgically removed and thus does not provide a defined site for administration, a nanoparticle encapsulating anti-CD47 suspended in a fibrin gel matrix to promote retention was sprayed on a tumour resection site to provide localized and sustained release of the antibody and thus block the 'do not eat me' signal provided by residual tumour cell CD47 binding to SIRPα on macrophages⁷⁴.

Targeting lymphoid tissues. Lymphoid organs can also be sites of interest for immunomodulation in cancer. Tumour-draining lymph nodes accumulate tumour antigens, and tumour antigen-specific T cells accumulate in these sites⁷⁵. However, tumours release cytokines and other factors to suppress the draining lymph nodes⁷⁶, T_{reg} cells preferentially migrate to these sites⁷⁷ and tumour-draining lymph nodes are a preferential site for tumour cell metastasis — all factors that promote tolerance rather than immunity. Thus, delivery of immunostimulatory compounds to tumour-draining lymph nodes has been pursued to reverse this immunosuppression and exploit the presence of tumour antigen and pre-existing antigen-specific T cells at these sites. Particles with sizes ranging from 5 to 50 nm injected into tissues are too large to efficiently enter the blood vasculature, and instead preferentially enter lymphatic vessels, and are thus targeted to downstream lymph nodes⁷⁸.

On the basis of this principle, TLR agonists conjugated to 30-nm-diameter polymer nanoparticles injected intradermally near melanomas led to an accumulation of the TLR agonists in tumour-draining lymph nodes, activation of DCs in these sites and induction of T cell priming that slowed tumour progression and enhanced survival^{75,79}. Nanoparticles can also be targeted to DCs and myeloid cells in tumours and the spleen, as discussed in the following section.

Localized immunotherapy is attractive for its potential to allow very potent combinations of immunostimulatory agents to be safely used to dramatically reprogramme the TME. The effectiveness of this strategy in the clinic hinges on the capacity of systemic immunity being produced from such local stimulation, and will likely require support from systemic checkpoint blockade to be fully effective, to support T cells infiltrating distal untreated tumour sites. The alternative is to pursue approaches to systemically concentrate immunotherapies in disseminated tumours, as discussed in the next section.

Systemic targeting of tumours

Altering pharmacokinetics of immunotherapy agents for enhanced safety. One challenge facing immunotherapy agents, especially immune agonists such as drugs that engage costimulatory receptors, is the high level of toxicity observed when such drugs are administered systemically and stimulate circulating lymphocytes. Nanomedicine formulations are being developed to promote greater immune activation within tumours, while limiting extratumoural stimulation (FIG. 3a). For example, lethal side effects from systemic administration

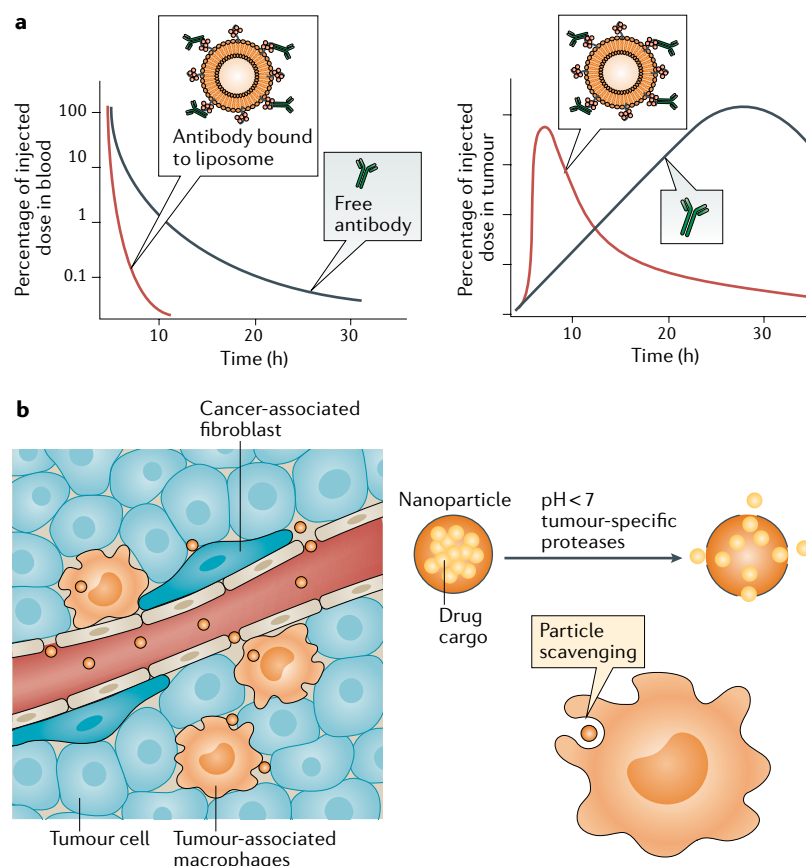


Fig. 3 | Systemic targeting of tumours by intravenously administered nanomedicines. a | Nanoparticles alter the pharmacokinetics of immunomodulatory drugs in a manner that can increase safety and efficacy. Illustrated is the case of an antibody therapeutic, which as a free drug will exhibit a long half-life in the blood and slowly accumulate in tumours. The same drug conjugated to a nanoparticle scaffold can be delivered to similar levels into the tumour in a shorter time window, accompanied by more rapid clearance from the systemic circulation, providing similar stimulation in the tumour microenvironment but substantially lowering systemic exposure. **b** | In addition to permeable blood vessels to promote nanoparticle localization via the enhanced permeation and retention effect, the tumour microenvironment has unique attributes that can be leveraged to effect drug targeting. For example, phagocytic myeloid cells, such as tumour-associated macrophages, readily scavenge nanoparticles and they can act as depots for drugs that act directly on tumours, such as chemotherapeutics, or can themselves be the targets for immunomodulatory drugs designed to decrease immunosuppression. In addition, tumour-specific properties, such as a slightly acidic pH or the presence of higher than normal levels of certain enzymes, can act as triggers for drug release from nanoparticles, and such systems have been used to both directly target cancer cells and modulate stromal cells, such as cancer-associated fibroblasts or immunosuppressive immune cells.

of an anti-CD137 agonist antibody and IL-2 were eliminated by conjugating these proteins to the surfaces of liposomal carriers⁸⁰. Liposome conjugation substantially reduced the half-life of the drugs in the bloodstream, while maintaining their accumulation to equivalent levels in tumours via the enhanced permeation and retention effect (BOX 1), allowing robust stimulation of antitumour T cell responses, while ablating the major toxicity observed with the free drugs.

A second strategy that is now in clinical development manipulates the bioactivity of immunostimulatory cytokines by administering them as an inactive prodrug that is masked with polyethylene glycol (PEG) chains.

NKTR-214 is a recombinant human IL-2 cytokine that is engineered to include on average six cleavable PEG chains and becomes active only when the majority of the PEG chains are hydrolysed^{81,82}. This pegylation approach provides not only sustained dosing but also selectively decreases the affinity of IL-2 for the heterotrimeric IL-2 receptor complex (IL-2R $\alpha\beta\gamma$), which is abundantly expressed by T_{reg} cells, relative to the heterodimeric IL-2 receptor complex (IL-2R $\beta\gamma$), which is preferentially expressed on CD8⁺ T cells, and thus favours the activation of CD8⁺ T cells over T_{reg} cells⁸¹. Phase I and phase II clinical trial results for NKTR-214 have been promising in terms of toxicity and signs of efficacy⁸³. NKTR-214 blurs the distinction between a biological drug and a nanoparticle, as at the core of each 'particle' is a single protein. However, it is useful to examine NKTR-214 through the lens of nanomedicine because the synthetic PEG polymer constitutes approximately 88% of the mass of the conjugate, and this labile PEG coating profoundly modulates the pharmacokinetics, pharmacodynamics and receptor selectivity of the drug.

Nanomedicine formulations are also being pursued as a means to allow safe systemic administration of innate immune stimulators such as TLR agonists, STING agonists and ligands for other danger sensors. STING agonists have been formulated within nanoparticles designed to both target tumours via the enhanced permeation and retention effect and increase drug potency by promoting endosomal escape into the cytosol, where the STING receptor resides. Whereas STING agonists are generally ineffective when given systemically, this approach has allowed intravenous administration to potentiate immunotherapy in mouse models of melanoma^{46,84} and breast cancer⁴⁷. An important issue that remains to be studied more deeply is whether such approaches are safe when treatment is with the doses of STING agonist that are needed for treatment of large tumours, as nanoparticle clearance by macrophages and DCs of the spleen and liver or other tissue sites may be a source of off-tumour, on-target toxicity.

Targeting myeloid cells. Systemic targeting of myeloid cells using nanomedicine is a clinically attractive approach because myeloid cells often have a suppressive role as myeloid-derived suppressor cells or TAMs, and because they are logical targets due to their propensity to phagocytose nanoparticles and microparticles⁸⁵ (FIG. 3b). In murine tumour models, intravenously administered nanoparticles readily accumulate in TAMs^{86,87}, which can serve as cellular reservoirs for antitumour therapeutics⁸⁸. Radiotherapy can increase TAM numbers, induce macrophage-mediated changes in vascular leakage and thereby further enhance nanoparticle accumulation in TAMs⁸⁹. Notably, myeloid cells have been reported to take up tenfold more nanoparticles than tumour cells even when the particles are conjugated with a ligand intended to promote tumour cell uptake⁹⁰. Recent preclinical studies have sought to exploit this effect to deplete suppressive myeloid populations in cancer. Polymeric nanoparticles or micelles 20–30 nm in diameter administered intradermally rapidly reach the systemic circulation by passing through lymphatics to the

thoracic duct, and subsequently accumulate in macrophages and myeloid cells in the spleen and tumours^{91,92}. Formulation of the chemotherapy agent 6-thioguanine in these particles led to depletion of myeloid-derived suppressor cells in both sites, enhancing adoptive T cell therapy⁹¹, a result that has also been reported for subcutaneously administered lipid nanoparticles carrying a hydrophobic gemcitabine derivative⁹³.

An alternative to depleting suppressive myeloid cells is to reprogramme them to a phenotype that promotes antitumour immunity. Innate immunostimulants that mimic danger signals produced by pathogens induce proinflammatory cytokine production and polarize macrophages from an immunosuppressive state towards a tumoricidal phenotype⁹⁴. Repetitive systemic administration of cyclodextrin nanoparticles loaded with the TLR7 and TLR8 agonist R848 led to preferential nanoparticle accumulation in TAMs in mouse tumours and promoted both macrophage re-education and a CD8⁺ T cell-dependent immune response, with a subset of mice completely rejecting the initial tumour and subsequent rechallenge⁹⁵. Several recent studies have also used nanoparticles to target small interfering RNAs or microRNAs to tumour-associated myeloid cells to promote reprogramming of the TME and antitumour immunity, exploiting the capacity of nanoparticles to both target myeloid cells and promote transfection^{96–98}. The FDA-approved iron supplement ferumoxytol, which is composed of dextran-coated iron oxide nanoparticles, was recently found to reprogramme macrophages towards an antitumour phenotype. When administered systemically before intravenous administration of tumour cells, ferumoxytol prevented the seeding of liver tumours⁹⁹. Understanding the specific attributes of these iron oxide nanoparticles that lead to alterations in macrophage function may suggest new approaches to myeloid cell reprogramming.

An intriguing alternative to relying on spontaneous phagocytic uptake of particles by myeloid cells is to preferentially attract these cells to engulf the nanoparticle by using a chemokine-releasing formulation. Nanocapsules that slowly release the monocyte–macrophage attractant CC-chemokine ligand 2 were shown to promote chemotaxis of myeloid cells *in vitro*¹⁰⁰. Once internalized, the same nanocapsule then released co-encapsulated small interfering RNA targeting *Cebpb*, which encodes a transcription factor driving immunosuppression. *Cebpb* knockdown by these particles was demonstrated in both the spleen and tumours following systemic administration in tumour-bearing mice.

Targeting non-immune stromal cells. Stromal cells within the TME have important roles in suppressing immune responses and supporting tumour growth¹⁰¹, and have thus also been investigated as nanomedicine drug targets to potentiate immunotherapy. The proliferation and extracellular matrix production of cancer-associated fibroblasts (CAFs) impede the immune response by compressing tumour blood vessels and inducing hypoxia, but the use of small-molecule drugs, such as angiotensin receptor blockers, to alter CAF activity is challenging due to systemic side effects.

Polymer nanoparticles designed to dissolve rapidly under the slightly acidic conditions found in tumours allowed selective delivery of angiotensin receptor blockers to CAFs within the TME as measured by the levels of active drug in the tumour in comparison with treatment with the free drug¹⁰². This nanomedicine therapy, when combined with checkpoint blockade, increased survival and reduced metastasis following primary tumour resection in multiple breast tumour models. A second strategy is to use nanoparticles to concentrate chemotherapy agents in CAFs for selective depletion of these suppressive cells. Acetylated carboxymethylcellulose nanoparticles were discovered to preferentially accumulate in α -smooth muscle actin-positive CAFs in breast and pancreatic tumours, mediated in part by opsonization of the particles with albumin and subsequent interaction with the albumin receptor SPARC (secreted protein acidic and rich in cysteine) expressed by these cells¹⁰³. Delivery of docetaxel to CAFs using these particles led to enhanced survival in models of breast and pancreatic cancer^{104,105}.

In summary, nanoparticles have the potential to substantially alter the pharmacokinetics as well as tissue-level and cell-level biodistribution of therapeutics. These properties can be exploited to increase the safety of drugs that would otherwise be too toxic due to non-specific stimulation of cells in the circulation or healthy tissues, and also to enhance efficacy due to concentrated uptake in key cellular targets such as tumour-associated myeloid cells.

Enhancing cellular immunotherapy

Alongside checkpoint blockade therapeutics, adoptive cell therapy (ACT) of cancer using CAR T cells is the second major class of immunotherapy to have made a major clinical impact to date. CAR T cells generated by virally transducing autologous patient T cells with a synthetic antigen receptor targeting CD19 have been approved for the treatment of acute lymphoblastic leukaemia and refractory large B cell lymphoma¹⁰⁶. Recent promising data from a phase I clinical trial have also indicated promise for CAR T cells in treating multiple myeloma¹⁰⁷. In addition, numerous small trials have reported positive responses in the treatment of patients with T cells transduced with tumour-specific T cell receptors (TCRs) or using natural T cell products prepared from tumour-infiltrating lymphocytes or sorted from peripheral blood¹⁰⁸. These promising results have led to a major translational effort including the launch of numerous cell therapy companies and both academic and company-sponsored clinical trials. However, ACTs have had more modest success in treating common epithelial cancers, motivating efforts to enhance the ability of T cells to overcome the immunosuppressive microenvironment of solid tumours. Strategies to genetically engineer new functions into immune cells will play an important role in the future of ACT¹⁰⁶. However, a number of nanomedicine-based approaches are also being explored to enhance ACT, and these provide complementary avenues to enhance the longevity, phenotype and function of cell therapy products.

Linking therapeutics to immune cells. Many signalling pathways in T cells have been identified that can regulate lymphocyte function, metabolism and/or differentiation in ways that would be beneficial to antitumour immunity. However, targeting these pathways with drugs remains a challenge. Genetically engineering T cells to express supporting factors (such as cytokines) can be challenging because it requires avoiding therapeutic levels that are too low, too high or too prolonged^{109–111}, and systemic administration of drugs that modulate T cell function often faces dose-limiting toxicity. An alternative is to link supporting drugs to adoptively transferred cells¹¹².

In this approach, supporting drugs are encapsulated in or otherwise formulated into nanoparticles that are chemically attached to the plasma membrane of the donor cells (FIG. 4a). These nanoparticle ‘backpacks’ are designed to release the drug at a prescribed rate or under selected microenvironmental conditions, such that the donor cell is stimulated in an engineered autocrine manner, limiting bystander stimulation of other cells and healthy tissues¹¹³. In preclinical models, this approach has allowed large doses of supporting cytokines such as IL-2 or IL-15 that are lethal when administered as free systemic drugs to be used without toxicity, substantially

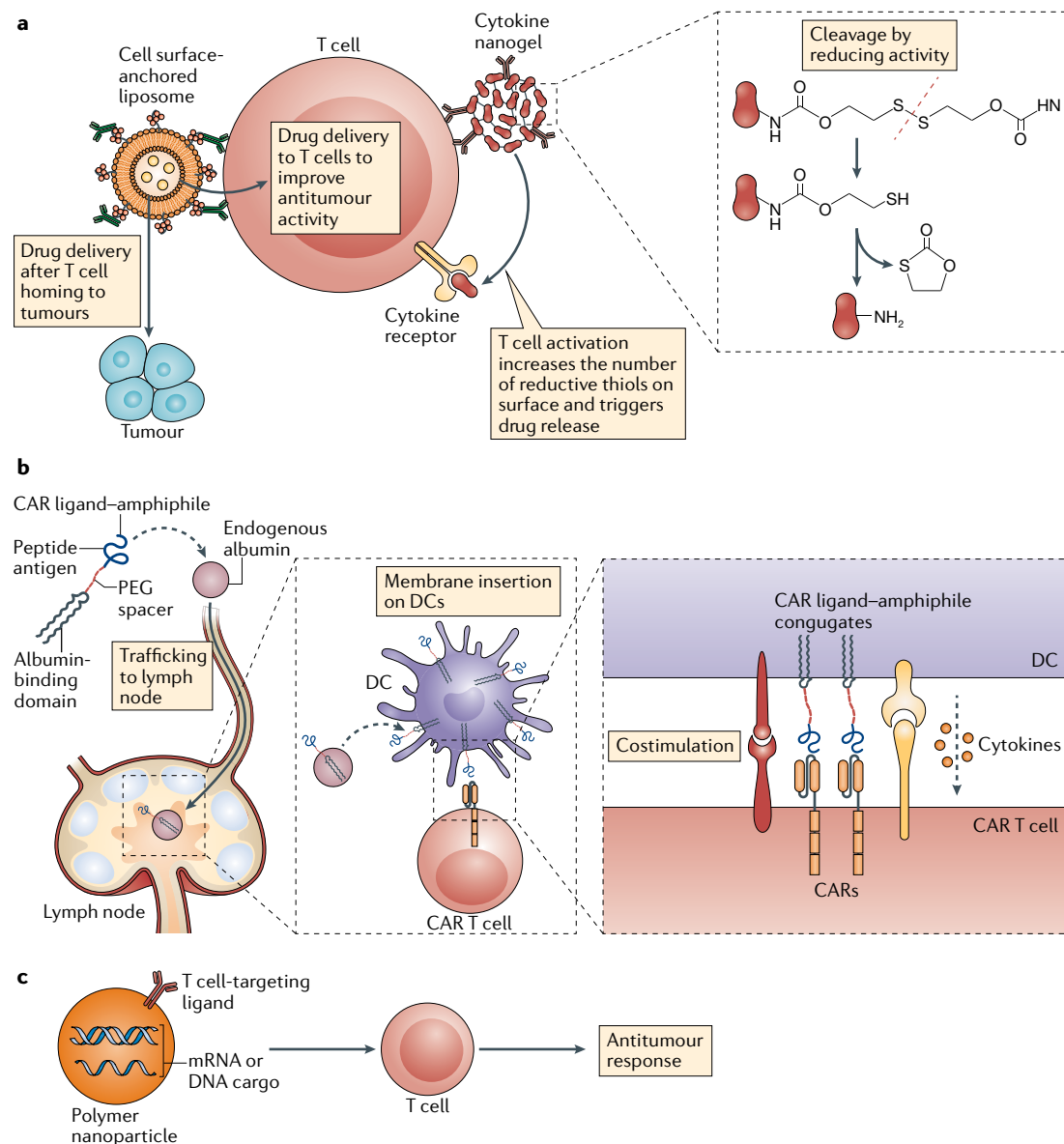


Fig. 4 | Enhancing cellular immunity of cancer. **a** | Conjugation of drug-releasing nanoparticles to the plasma membrane of T cells has been used to deliver drugs to tumours or tumour-infiltrating immune cells or to provide a continuous, autocrine supply of cytokines (such as IL-15 and IL-2) to the carrier cell, promoting T cell expansion and effector functions. **b** | Polymer amphiphiles conjugated to a chimeric antigen receptor (CAR) ligand bind endogenous albumin and pass to lymph nodes, where they are displayed on dendritic cells (DCs) and provide stimulation to antitumour CAR T cells, effectively acting as a boosting vaccination. **c** | Polymer nanoparticles carrying either mRNA or plasmid DNA and displaying a T cell-targeting ligand genetically reprogramme endogenous lymphocytes to promote antitumour immune responses. PEG, polyethylene glycol.

widening the therapeutic window for supporting cytokine-based ACT^{113–115}.

Cell-conjugated nanoparticles have also been used to backpack lymphocytes with small molecule-supporting drugs^{116–118}. Notably, conjugation of nanocarriers to cell surface proteins causes the nanoparticles to follow the cell surface localization of their protein anchors: particles preferentially conjugated to CD45, α L integrin (also known as LFA1) and CD2 were found to traffic to the immune synapse during the early stages of T cell activation¹¹⁸, mirroring the transport of these receptors during T cell priming¹¹⁹. The timing of cell stimulation can be controlled by linking release of the encapsulated drug to local changes in the environment. For example, nanoparticles were designed to release IL-15 in response to the upregulation of cell surface reducing conditions as T cells become triggered through the TCR, amplifying T cell function in tumours and tumour-draining lymph nodes, while minimizing systemic stimulation¹¹⁵. This approach of backpacking T cells with cytokines has recently entered clinical trials for a variety of solid tumour types (TABLE 1).

Attaching nanoparticle formulations to immune cells substantially alters the biodistribution of the nanomedicine — for example, providing greatly enhanced accumulation of particles in tumours when attached to tumour-reactive T cells¹¹³. Thus, this approach can also be used to enhance the delivery of drugs that are meant for other cells in the TME. For example, attachment of lipid nanoparticles carrying a potent but poorly soluble camptothecin chemotherapy agent to T cells led to pronounced enhancements of drug accumulation in tumours in a lymphoma model, allowing increases in survival, whereas the systemic administration of the free drug had no impact on tumour progression¹²⁰.

Targeting drugs to circulating lymphocytes. Using nanomaterials as a strategy to associate drug reservoirs with donor cells in ACT provides the opportunity for exquisite control over cell stimulation but it is a single-time-point intervention; the temporal window for therapeutic intervention is limited to the timing of cargo release from the drug carrier. An alternative is to use nanomaterials to directly target drugs to lymphocytes *in vivo* through chemically conjugated antibodies or other targeting moieties that will bind to target cell surface receptors. This strategy can be used to target either endogenous immune cells or adoptively transferred cells, but it may be particularly efficient in the case of ACT, in which the donor cells serve as an enriched tumour-specific target population. For example, liposomes targeted to T cells using surface-conjugated IL-2 or antibody fragments against a congenic cell surface receptor allowed more than 95% of circulating T cells delivered by ACT to be labelled following a single injection, leading to significant enhancements in expansion of transferred T cells in a melanoma model^{117,121}. By comparison, targeting of endogenous T cells using polymeric nanoparticles conjugated with anti-PD1 antibody allowed approximately 5% of circulating PD1⁺ T cells to take up nanoparticles within 1 h of injection¹²². Despite this modest particle uptake, administration of anti-PD1-targeted particles

carrying small-molecule inhibitors of transforming growth factor- β or a TLR7 agonist led to pronounced therapeutic activity that was absent from particle formulations lacking the targeting moiety or equivalent doses of the free drug.

An alternative to delivering nanomaterials to cells in the blood is to target compounds to lymph nodes, with the goal of engaging lymphocytes as they recirculate through these secondary lymphoid organs. As described already, nanoparticles can efficiently pass from parenteral injection sites into lymphatic vessels, and hence to lymph nodes. Lymphatic targeting can also be achieved by use of endogenous lymph-transported proteins as chaperones: this has been demonstrated using polymer conjugates that bind to albumin^{123,124}. Recently, an approach to target stimulation to CAR T cells was demonstrated, whereby an albumin-binding lipid tail was linked via a PEG spacer to a small-molecule or peptide ligand for a CAR. This lipid-PEG-CAR ligand construct was efficiently shuttled to lymph nodes following parenteral injection and decorated the surfaces of macrophages and DCs through insertion of the lipid tail into antigen-presenting cell plasma membranes (FIG. 4b). Boosting of CAR T cells by DCs coated with the CAR ligand led to enhanced CAR T cell expansion, increased effector functions and enhanced tumour rejection in several syngeneic mouse tumour models¹²⁵.

Gene delivery to lymphocytes *in vivo*. Ex vivo genetic manipulation or expansion in ACT is laborious and costly¹²⁶. Nanoparticle formulations designed to target specific lymphocyte subpopulations *in vitro* or *in vivo* and promote their transfection with encapsulated nucleic acids thus represent a third major application of nanomedicine to cellular immunotherapy. Viral vectors are the primary means of producing clinical cell products transduced with CARs or TCRs, but viral production that adheres to good manufacturing practice is a significant financial cost and viral vectors can be limited by the size of transgene that can be introduced and often do not provide control over the cell types that are transduced. By contrast, synthetic nanoparticle formulations can be designed to specifically transduce target cell subpopulations through antibody-directed or ligand-directed cell binding, can carry larger gene cargos and can be produced at scale by simple chemical synthesis. To this end, it was recently demonstrated that polymeric nanoparticles can be used to perform targeted gene expression or gene editing in primary T cells through *in vitro* addition of antibody-targeted particles carrying mRNA¹²⁷.

Going a step further, strategies to generate CAR-transduced or TCR-transduced T cells directly *in vivo* would represent a major breakthrough to allow the treatment of a larger population of patients more rapidly at lower cost. Synthetic nanoparticles are not prone to the same issues of pre-existing or treatment-induced antivector immunity or off-target toxicity that can be of concern with viral vectors, but they suffer from lower efficiencies in cellular transduction compared with their biological counterparts. However, this latter drawback may be less of a concern in the setting of *in vivo* CAR or TCR delivery, because transduced T cells are intrinsically

self-amplifying as they encounter tumour antigen. This feature was observed in preclinical studies using a polymeric nanoparticle formulation to target transposon-based DNA carrying a CAR to circulating T cells in a lymphoma model, where transduction of a small founder population of T cells led to substantial T cell expansion over the course of 1–2 weeks and substantial antitumour activity¹²⁸ (FIG. 4c). This nanomedicine-based approach to deliver CARs in vivo is now moving to clinical testing (TABLE 1). Lipid nanoparticles capable of delivering mRNA into DCs have also recently been reported¹²⁹, and have moved into early clinical testing for vaccines, suggesting multiple avenues for systemically targeting immune cell populations for gene delivery.

Altogether, nanomedicine approaches appear promising both as agents for ex vivo engineering of therapeutic cells and as a platform for directly modifying cells in vivo. The potential of synthetic formulations to achieve delivery of nucleic acids to target cells in vivo without induction of strong antivector immunity could open new opportunities for cellular immunotherapies, while also removing many of the challenging logistics associated with patient-specific cell manufacturing.

Conclusion and future perspectives

As demonstrated by the many examples discussed here, nanomedicine has the potential to enhance cancer immunotherapies in diverse ways, and preclinical evidence provides clear motivation for clinical testing of many concepts. Initial human trials are now under way for several of these approaches, and the next few years should provide substantial insight into the clinical potential of these technologies.

The field faces several immediate challenges, where solutions could have significant impact on clinical translation of immunotherapy. Safe methods to systemically target potent innate immune stimulators such as STING or TLR agonists to tumours remain to be developed. Although these agents demonstrate potent antitumour activity when administered intratumorally in preclinical models and are currently being evaluated for intratumoural administration in early clinical trials,

intratumoural administration does not guarantee an avoidance of systemic distribution and subsequent toxicity from such small-molecule compounds, and precludes direct action on disseminated disease. The tropism of nanoparticles for myeloid cells in the circulation, spleen and liver makes it unclear if nanomedicine-formulated innate stimulators will be a safe solution. A second major challenge is the robust delivery of genetic material encoding CARs, TCRs or supporting proteins to lymphocytes in vivo. As noted earlier, the first attempts to use polymeric materials to target RNA or DNA into T cells have recently been reported, but the efficiency of transfection in vivo remains very low and it is unclear if short-lived mRNAs or randomly integrating transposon DNAs are the answer. However, the potential impact of a safe and effective means for gene delivery to lymphocytes or other immune cells in vivo cannot be underestimated.

Emerging areas of immunology and immunotherapy also present new opportunities for the future. Recently, the phenomenon of long-lived reprogramming of myeloid cells following exposure to certain innate immune stimuli has begun to be understood at the molecular level and the genetic level. This process, known as trained immunity, is characterized by hyper-responsiveness of myeloid cells to innate stimuli such as TLR agonists and is induced through epigenetic and metabolic reprogramming of myeloid cells and/or their precursors by stimuli such as β -glucan or systemic BCG vaccination¹³⁰. Such myeloid training has been shown to mediate heterotypic protection against bacterial infections^{131,132}. Therapeutically inducing trained immunity has been proposed as a strategy that may be relevant in cancer, and the ability of nanoparticles to efficiently target the bone marrow and myeloid cells makes nanomedicine-based approaches to this task appear attractive¹³³. Such approaches that specifically amplify innate immune attack against tumours could be an important complement to current therapies that predominantly focus on adaptive immunity.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

D.J.I. and E.L.D. are co-inventors on patents related to nanoparticle delivery of innate immune stimulators assigned to Massachusetts Institute of Technology (MIT). D.J.I. is an inventor on patents related to nanomedicine-based immunotherapy assigned to MIT that have been licensed to Torque Therapeutics, Elicio Therapeutics and Strand Therapeutics, of which D.J.I. is a co-founder.

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