Cancer nanomedicine for combination cancer immunotherapy

Jutaek Nam[1,2,7], *Sejin Son*[1,2,7], *Kyung Soo Park*[2,3], *Weiping Zou*[4,5], *Lonnie D. Shea*[2,3,6] *and James J. Moon*[1,2,3,5]*

Abstract | Cancer immunotherapy is revolutionizing oncology. However, dose-limiting toxicities and low patient response rates remain major challenges in the clinic. Cancer nanomedicine in combination with immunotherapies offers the possibility to amplify antitumour immune responses and to sensitize tumours to immunotherapies in a safe and effective manner. In this Review, we discuss opportunities for combination immunotherapy based on nanoparticle platforms designed for chemotherapy, photothermal therapy, photodynamic therapy, radiotherapy and gene therapy. We highlight how nanoparticles can be used to reprogramme the immunosuppressive tumour microenvironment and to trigger systemic antitumour immunity, synergizing with immunotherapies against advanced cancer. Finally, we discuss strategies to improve tumour and immune cell targeting while minimizing toxicity and immune-related adverse events, and we explore the potential of theranostic nanoparticles for combination immunotherapy.

[1]*Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI, USA.*

[2]*Biointerfaces Institute, University of Michigan, Ann Arbor, MI, USA.*

[3]*Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA.*

[4]*Department of Surgery, University of Michigan School of Medicine, Ann Arbor, MI, USA.*

[5]*Graduate Program in Immunology, University of Michigan School of Medicine, Ann Arbor, MI, USA.*

[6]*Department of Chemical Engineering, University of Michigan, Ann Arbor, MI, USA.*

[7]*These authors contributed equally: Jutaek Nam, Sejin Son*

**e-mail: moonjj@umich.edu*

https://doi.org/10.1038/s41578-019-0108-1

William B. Coley first reported in 1891 that bacterial toxins could be used as an immunotherapy to treat patients with bone and soft-tissue sarcoma[1]. After a century of research, cancer immunotherapy has revolutionized oncology and offers new treatment options for many types of cancer[2]. Owing to the clinical success of immune checkpoint blockers, immunotherapy has now been established as a new pillar of cancer treatment — a major achievement that was highlighted by the Nobel Prize in Physiology or Medicine 2018, awarded to James Allison and Tasuku Honjo, who discovered immune checkpoints. Conventional cancer treatment modalities, such as surgery, chemotherapy and radiotherapy, have limited efficacy against advanced cancer. By contrast, immune checkpoint blockers can be applied to durably eliminate tumours in a subset of patients with advanced metastatic disease, improving patient survival and reducing side effects[2–4] (BOX 1).

Cancer immunotherapy aims to train the host immune cells in lymphoid tissues and antitumour immune cells in the tumour microenvironment to search for and destroy tumour cells (BOX 2). Antitumour immune responses primed by immunotherapy can promote systemic immune surveillance and eliminate local and disseminated metastatic tumours. Additionally, immunotherapy may establish long-term immune memory and mediate immune protection against tumour recurrence. However, challenges remain to be overcome for cancer immunotherapy to be widely applicable in the clinic. One of the major hurdles is the limited response rate to immune checkpoint blockers (BOX 1). Clinical data suggest that only a fraction (generally 10–30% response rates, depending on the type of cancer) of patients respond to immune checkpoint blockers[5–7]. Patients with non-immunogenic tumours (cold tumours), characterized by a low number of T cells or low expression of programmed cell death 1 ligand 1 (PD-L1), respond poorly to immune checkpoint blockers[8,9]. By contrast, patients with immunogenic tumours (hot tumours) containing a high number of tumour-infiltrating T cells and showing high PD-L1 expression benefit from immune checkpoint blockers with lasting clinical responses[8,9].

Currently, immune checkpoint blockade involves systemic administration of monoclonal antibodies, which can cause off-target side effects by inducing activation of self-antigen-reactive T cells. The combination of multiple immune checkpoint blockers generally improves clinical responses; however, at the expense of a higher number of and more severe immune-related adverse events that result in clinical manifestations of dermatitis, colitis and hepatitis[10–13]. Patients with immune-related adverse events receive delayed administration of immune checkpoint blockers or are treated with corticosteroids or other immunosuppressants, which can diminish antitumour therapeutic efficacy and increase the risk of additional complications and opportunistic infections[11,13]. Thus, to fully realize the potential of cancer immunotherapy, approaches are needed to amplify antitumour T cell immune responses, to convert cold tumours into hot tumours and to sensitize tumours to

Box 1 | **Immune checkpoint blockade therapy**

Immune checkpoints refer to inhibitory signals that regulate T cell responses with the aim of maintaining immune self-tolerance[3]. Therefore, blockade of inhibitory signals can amplify the antitumour activity of antigen-specific T cells and release the brake of the immune system, unleashing the therapeutic potential of endogenous antitumour immune responses. In particular, immune checkpoint blockers that antagonize cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death 1 (PD-1), which downregulate immune responses and promote self-tolerance, have proved effective in the clinic[3,194]. CTLA4 is exclusively expressed on T cells, and upon engaging with CD80 or CD86, which are expressed on activated antigen-presenting cells (APCs), CTLA4 inhibits the early stage of T cell activation. This leads to dampening of effector functions of T cells and an increase in the activity of regulatory T (T_{reg}) cells[195,196]. By contrast, PD-1 is broadly expressed on a wide range of immune cells, including T cells, natural killer cells, B cells, dendritic cells and macrophages, and suppresses both innate and adaptive immunity. PD-1 limits the late-stage effector function of T cells upon binding to its cognate ligands, programmed cell death 1 ligand 1 (PD-L1) and PD-L2, on immune and non-immune cells[197,198]. Importantly, PD-L1 is also expressed on cancer cells and is upregulated in response to inflammatory cytokines secreted by activated dendritic cells and T cells. Therefore, tumours with high expression levels of PD-L1 can acquire immune resistance against T cell-mediated antitumour immunity[199].

Therapeutic antibodies that block CTLA4 (REFS[200,201]) or PD-1 (REFS[202,203]) markedly improve patient survival across multiple cancer types. CTLA4 and PD-1 simultaneously regulate non-overlapping inhibitory pathways, and thus, combination therapy with antibodies against CTLA4 and PD-1 is more effective than monotherapy[10,12]; however, immune-related adverse events associated with dual immune checkpoint blockers remain to be addressed[13]. Several antibodies against CTLA4 and PD-1 or PD-L1 have been approved by the US Food and Drug Administration (FDA), and numerous other immune checkpoint blockers are currently undergoing clinical evaluation, including antibodies against lymphocyte activation gene 3 (LAG3), killer cell immunoglobulin-like receptor (KIR), T cell immunoglobulin 3 (TIM3), T cell immunoglobulin and ITIM domain (TIGIT) and V domain immunoglobulin suppressor of T cell activation (VISTA)[4,204,205].

immunotherapies with minimal off-target toxicity and immune-related adverse events.

A variety of nanoparticle platforms with diverse physicochemical properties have been developed for cancer therapy[14]. Nano-sized materials have the advantage of preferentially accumulating in solid tumours owing to the abnormally leaky vasculature and dysfunctional lymphatic drainage within the tumour microenvironment — a phenomenon termed enhanced permeability and retention (EPR)[15,16]. In addition, synthetic nanoparticles functionalized with tumour-specific affinity ligands can promote active tumour targeting of drugs, including small molecular weight drugs and biomolecules[17,18]. However, failures of nanoparticle-based chemotherapy in clinical trials have raised doubts about the future of cancer nanomedicine[19,20], and in particular, the clinical relevance of the EPR effect is being questioned. Therefore, to improve the efficacy of cancer nanomedicine, active tumour targeting is being explored as a strategy for next-generation cancer nanomedicine[14,21,22].

Specific targeting of nanomedicine can also be exploited to minimize off-target toxicity in immunotherapy and to capitalize on the knowledge of cancer nanomedicine. Nanomedicines delivering immunological agents such as tumour antigens, immuno-adjuvants and cytokines have already shown promising results in clinical trials[14,23]. In this Review, we discuss nano-immunotherapy and highlight the potential and challenges of nanomedicine in combination with cancer immunotherapy (FIG. 1).

Nanomaterials for immunotherapy

Nanoparticle-based delivery systems have several advantages for applications in cancer immunotherapy compared with conventional nanomedicine[24]. Conventional cancer nanomedicine generally aims to deliver cytotoxic agents directly to cancer cells; however, immunotherapy often targets non-tumour cells, including resident immune cells within secondary lymphoid tissues or immune and stromal cells in the tumour microenvironment (BOX 2) — cells and tissues that could readily be targeted by nanoparticles. In addition, the subsequent interaction between nanoparticles and cells or organs can be regulated by functionalizing nanoparticles and by modifying their surface[25–30]. The physicochemical properties of nanoparticles can also be tuned to promote their interaction with and stimulation of innate immune cells, such as dendritic cells and macrophages[31,32]. Nanoparticle-based delivery can further improve the pharmacological properties of drugs, including their solubility, in vivo stability and pharmacokinetic profile, and protect biologic drugs from premature release and degradation[33–37]. Nanoparticles can be designed to traffic to intracellular compartments and can be programmed to release agents in response to biochemical changes in the target microenvironments (for example, pH, redox potential and enzymes) or external stimuli (for example, light and electrical and magnetic fields)[38–41]. Controlled release can increase the therapeutic index of drugs and enable dose titration. Nanoparticle-based targeted delivery can also reduce off-target toxicity and immune-related adverse events, which is particularly important for potent immunotherapies that can induce severe dose-limiting toxicity, such as a cytokine storm[42–44]. Importantly, targeted delivery of nanoparticles, combined with controlled and localized drug release, may allow for dose sparing of immune checkpoint blockers or activation of immunotherapies only at the intended sites of action, thus alleviating safety issues associated with nonspecific systemic distribution of immunotherapies. Finally, the intrinsic optical, magnetic and electrical properties of nanoparticles can be used for cancer therapy[45–50], especially in combination with immunotherapies[51–53]. For example, gold nanoparticles can be applied for photothermal therapy by exploiting their strong light absorption upon excitation of surface plasmon oscillations and efficient heat generation by subsequent thermal relaxation[45,47,54]. Inorganic nanoparticles composed of heavy metal elements can potentiate radiotherapy by enhancing radiation scattering and improving the photoelectric effect[46,55]. Thus, compared with single molecule-based agents, inorganic nanoparticles with intrinsic optical, magnetic and electrical properties can be targeted to the tumour and serve as effective platforms for cancer therapy.

Conventional immunotherapeutic interventions generally fail to convert cold tumours into hot tumours, especially for advanced tumours with a myriad of immunosuppressive mechanisms that impair T cells[7] (FIG. 2). Chimeric antigen receptor (CAR) T cell therapy has been approved by the US Food and Drug Administration (FDA) for the treatment of haematological malignancies; however, promoting infiltration of CAR T cells into solid

tumours remains challenging[56]. Cancer nanomedicines offer the possibility to induce tumour-directed cytotoxic effects and convert cold into hot tumours by debulking cold tumours and simultaneously removing biological barriers to T cell infiltration.

The direct killing of tumour cells by chemotherapy, photodynamic therapy and radiotherapy can initiate antitumour immune responses owing to immunogenic cell death, which is characterized by the release of damage-associated molecular patterns and antigens from dying tumour cells, leading to the stimulation of innate and adaptive immune responses against tumour cells[57,58] (FIG. 3). Biomaterial-based strategies can be applied to exploit the immunogenic cell death-inducing properties of traditional chemotherapeutic agents to not only kill cancer cells but also initiate in situ vaccination against a broad repertoire of tumour-associated antigens[40,59–62]. Alternatively, cancer vaccination can be used to expand the number and functionality of tumour-specific T cells to achieve potent antitumour efficacy in combination with immune checkpoint blockade[63–65] (BOX 1). This is particularly exciting because hot tumours feature T cells against tumour-specific mutant antigens resulting from tumour-specific DNA or RNA alterations (that is, neoantigens)[66–68]. Neoantigen vaccines in combination with immune checkpoint blockers have shown promise in seminal clinical trials[63,65], and a variety of biomaterials are being explored for the design of precision nanomedicines for combination immunotherapy[69–71].

Combination immunotherapies generally aim to prime the tumour microenvironment and to modulate immune cells by applying nanomedicines that have

Box 2 | **Important immune cells in cancer immunotherapy**

Effector T cells

Naive T cells are primed to become effector cells through interaction with professional antigen-presenting cells (APCs) that present major histocompatibility complex (MHC)–antigen complexes and immunogenic signals[206]. Subsequently, $CD8^+$ T effector cells migrate to the tumour, bind to cancer cells by recognizing their cognate antigen presented on MHC class I molecules and kill their target cancer cells[206]. $CD4^+$ T helper (T_H) cells (T_H1, T_H2 and T_H17 cells) support $CD8^+$ T effector cells by producing cytokines[207]. A small portion of T effector cells differentiate into long-lived memory cells that proliferate in response to immune checkpoint blockers, improving their clinical response[208–210].

Regulatory T cells

Activated regulatory T cells (T_{reg} cells) directly suppress T cells by producing interleukin-10 (IL-10) and transforming growth factor-β (TGFβ), and they inhibit the expression of co-stimulatory ligands of dendritic cells[211]. Indoleamine 2,3-dioxygenase (IDO) activates the phosphatase and tensin homologue (PTEN) pathway, providing long-term maintenance and stability of immunosuppressive T_{reg} cells[212]. Deletion of the PTEN pathway in T_{reg} cells in the tumour microenvironment can be used to restore antitumour responses. Tumour-infiltrating T_{reg} cells upregulate immunosuppressive markers, including T lymphocyte antigen 4 (CTLA4), glucocorticoid-induced tumour necrosis factor (TNF) receptor (GITR), inducible T cell co-stimulator (ICOS), lymphocyte activation gene 3 (LAG3), and CC-chemokine receptor 4 (CCR4) and CCR8 (REF.[213]), which could be targets for cancer immunotherapy.

Dendritic cells

Dendritic cells are potent APCs with an essential role in immune activation at the interface between innate and adaptive immunity[214]. Dendritic cells phagocytose antigens at the tumour site and migrate to secondary lymphoid organs, where they present antigens to T cells, inducing their proliferation and differentiation. Dendritic cells in the tumour microenvironment promote immunosuppression through secretion of IDO and IL-10, leading to T cell tolerance and anergy[215]. Targeting dendritic cells may promote antigen processing and presentation. Dendritic cells can be targeted by DEC205, CLEC9A (also known as DNGR-1), CLEC12A and dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN)[216,217].

Natural killer cells

Natural killer cells can recognize and kill tumour cells without exposure to tumour antigens[218]. Instead, the behaviour of natural killer cells is determined by the balance of activating and inhibitory signals expressed by normal and abnormal cells[219]. Upon recognition of tumours by engagement of an activation receptor such as NKG2 (REF.[220]), natural killer cells proliferate and kill tumour cells through granzyme B-mediated and perforin-mediated apoptosis or by expression of death receptor ligands.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells are a heterogeneous and immature population of cells that can secrete immunosuppressive agents, including nitric oxide, arginase and reactive oxygen in tumours[221]. They suppress T cell proliferation and cytotoxicity, inhibit natural killer cells and expand T_{reg} cells. High numbers of myeloid-derived suppressor cells can be correlated with a poor response to immune checkpoint blockade therapy and poor prognosis[222].

Tumour-associated macrophages

Tumour-associated macrophages can be classified into inflammatory, antitumoural M1-like macrophages and pro-tumoural M2-like macrophages, which together constitute more than 50% of tumour-infiltrating cells in certain cancer types[223]. Usually exhibiting the M2 phenotype, they are key modulators of tumour invasion and metastasis, and they release TGFβ, IL-10 and arginase 1, which inhibit natural killer cells and cytotoxic T cells. They can also capture and degrade immune checkpoint blockers within the tumour microenvironment, reducing their efficacy[224]. Tumour-associated macrophages are potential biomarkers for diagnosis and prognosis of cancers and attractive therapeutic targets[223]. CD206 (mannose receptor), haemoglobin receptor (CD163) and colony-stimulating factor 1 receptor (CSF1R) can be targeted for elimination or reprogramming of M2 macrophages[225,226].

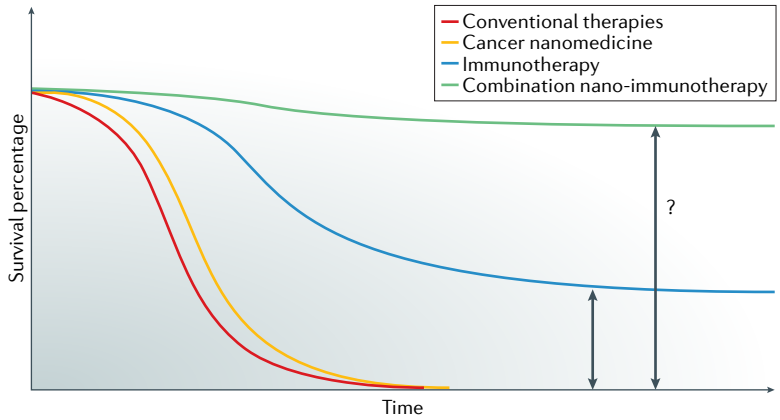

Fig. 1 | **Potential clinical benefits of combination nano-immunotherapy.** Compared with cancer nanomedicine, immunotherapy substantially improves patient median overall survival by eliciting robust antitumour immunity with long-term memory responses. However, only a fraction of patients responds to the current cancer immunotherapies. Nano-immunotherapy may achieve improved median survival with long-term responses. Adapted with permission from REF.[4], Elsevier.

been traditionally examined for non-immunological cancer treatment, such as chemotherapy, photothermal therapy, photodynamic therapy, radiotherapy and gene therapy (TABLE 1). The synergy of these nanomedicines with immunotherapies offers a promising strategy to address current limitations faced by the field of cancer immunotherapy (FIG. 1).

Chemotherapy and immunotherapy

Chemotherapy has long served as the standard-of-care cancer treatment, and therefore, there is a strong interest in combining chemotherapy and immunotherapy. Recent phase III clinical trials have shown that, compared with the standard of care, programmed cell death 1 (PD-1)/PD-L1 immune checkpoint blockers combined with the chemotherapeutic paclitaxel delivered by albumin nanoparticles can prolong progression-free survival and overall survival in patients with non-small-cell lung cancer[72,73] and patients with triple-negative breast cancer[74,75]. There are further ongoing phase III trials examining chemo-immunotherapy in urothelial carcinoma[76,77]. In particular, a subset of patients with non-small-cell lung cancer, who were stratified as poor responders to immune checkpoint blockade and showed low PD-L1 expression in tumours, also responded to chemo-immunotherapy, potentially broadening the applicability of immune checkpoint blockers[78,79]. However, a significant fraction of patients treated with chemo-immunotherapy exhibited immune-related adverse events of grade 3 (moderate-to-severe symptoms) or higher (life-threatening symptoms), with a 10–20% rate of treatment discontinuation owing to severe adverse events associated with chemo-immunotherapy[72–75]. This highlights the problem of off-target toxicity and the need to develop targeted and localized delivery systems.

Some chemotherapeutic agents (for example, doxorubicin (DOX), mitoxantrone and oxaliplatin) not only kill tumour cells but also trigger immunogenic cell death and systemic immune activation[57,58]. During the immunogenic cell death process, chemotherapy-treated dying tumour cells release a variety of soluble danger signals, such as calreticulin (CRT), ATP, CXC-chemokine ligand 10 (CXCL10) and high-mobility group box 1 (HMGB1)[57]. These danger signals recruit and prime dendritic cells, which in turn phagocytose dying tumour cells and present tumour antigens to activate T cell responses[57] (BOX 2). Thus, in contrast to cancer vaccines, which rely on a defined set of tumour antigens, immunogenic cell death-inducing chemotherapeutic agents can elicit antitumour immune responses against a broad repertoire of tumour antigens found on dying tumour cells, providing a promising platform for combination immunotherapy. Tumour-targeted delivery of these chemotherapeutic agents has been extensively examined using a wide range of nanoparticle platforms, including liposomes, polymer micelles and polymer–drug conjugates[16]. Importantly, nanoparticle-mediated delivery can reduce off-target toxicity of chemotherapy and extend the therapeutic index, thus providing an advantage in terms of safety, especially for combination therapy using potent immunotherapeutic agents with a high incidence of immune-related adverse events.

Preclinical studies on the combination of nanoparticle-mediated chemotherapy and immune checkpoint blockers have shown promising results (TABLE 1). For example, synthetic high-density lipoprotein (sHDL) nanodiscs can be used as nanocarriers for DOX, exhibiting increased blood circulation time and tumour accumulation compared with the free drug or liposomal formulations. Delivery of the drug by sHDL nanodiscs triggers immunogenic cell death of cancer cells and sensitizes tumours to immune checkpoint blockade[40]. Notably, the combination of sHDL–DOX therapy with anti-PD-1 immune checkpoint blockers results in a sevenfold increase in the number of interferon-γ (IFNγ)$^+$CD8$^+$ T cells compared with that seen in free DOX treatment and eventually in the complete regression of established CT26 colon carcinoma tumours in 80–88% of animals as well as the prevention of tumour recurrence and metastasis to the liver[40]. Importantly, there was no apparent cardiotoxicity in the treated animals, whereas animals treated with free DOX exhibited cardiac tissue damage[40].

Chemotherapy combined with immune adjuvants may also offer a practical approach to increase immunity. For example, dendrimers carrying DOX and CpG (a Toll-like receptor 9 (TLR9) agonist) can be modified with an aptamer against prostate-specific membrane antigen (PSMA) in 22RV1 prostate tumour-bearing mice[25]. DOX, which is a chemotherapeutic agent, forms a stable complex with CpG, which is a potent immune-stimulating agent. Paclitaxel (PTX) is also an attractive chemotherapeutic drug for combination immunotherapy because PTX can induce TLR4-mediated dendritic cell maturation and promote CD8$^+$ T cell responses[80,81] (BOX 2). Intratumoural administration of poly(γ-glutamic acid) (γ-PGA) microparticles carrying PTX and the TLR7 agonist imiquimod leads to local and systemic antitumour immunity and inhibition of distant tumour growth in B16F10 tumour-bearing mice[34]. Alternatively, administration of poly(lactic-co-glycolic acid) (PLGA) nanoparticles co-loaded with PTX and detoxified

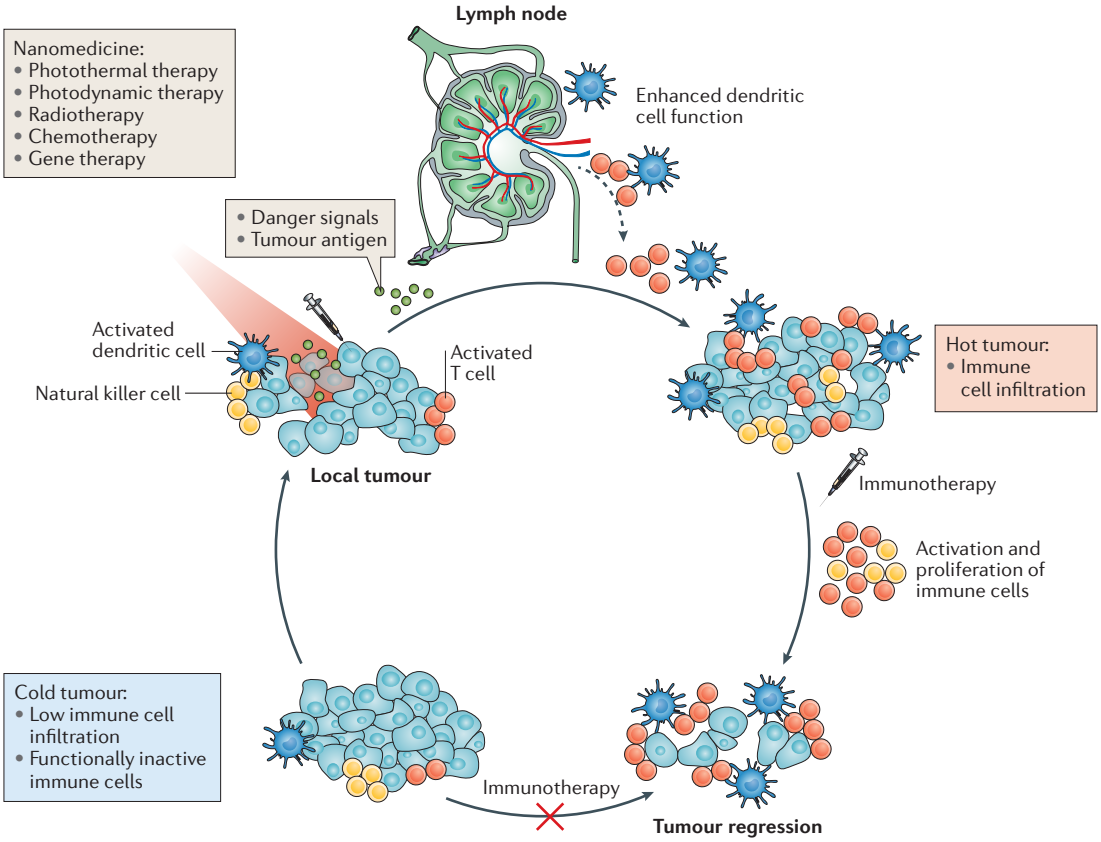

Fig. 2 | **Nanomedicine approaches for combination cancer immunotherapy.** Non-immunogenic, cold tumours exhibit various immune evasion mechanisms, including limited T cell infiltration and immunosuppressive pathways, and therefore are resistant to current forms of immunotherapy. Nanomedicines traditionally designed for photothermal therapy, photodynamic therapy, radiotherapy, chemotherapy or gene therapy can be used to convert cold tumours into immunogenic, hot tumours. Nanomedicines can have cytotoxic effects against tumour cells in the immunosuppressive tumour microenvironment, which leads to debulking of the tumour mass, release of tumour antigens and danger signals and dendritic cell-mediated antitumour immunity.

bacterial lipopolysaccharide (LPS; a TLR4 agonist) causes an increase in the number of T helper 1 (T_H1) cells (BOX 2) and thus antitumour efficacy compared with PTX treatment alone in a B16F10 mouse model[82]. Sequential delivery of PLGA nanoparticles carrying PTX, CpG and small interfering RNA (siRNA) against interleukin-10 (IL-10) promotes IL-10-specific gene knockdown, immunogenic cell death of tumour cells and suppression of the T_H2 cell immune response (BOX 2), leading to extended survival of mice bearing B16F10-OVA melanoma[83]. Cisplatin–adjuvant combination therapies can also be combined with immunotherapy because cisplatin can induce immunogenic cell death[84]. Liposomes loaded with cisplatin and CpG can be intratumourally administered, which leads to an increase in tumour cell apoptosis, a decrease in the number of immunosuppressive myeloid-derived suppressor cells and regulatory T (T_{reg}) cells (BOX 2) in the spleen and in the tumour microenvironment, proliferation of $CD8^+$ T cells and inhibition of tumour growth in B16F10 tumour-bearing mice compared with administration of liposomes delivering only cisplatin or CpG[84].

The synergy between multiple components has been tested in preclinical studies combining immunogenic cell death-inducing chemotherapy, adjuvant molecules and immune checkpoint blockers (TABLE 1). For example, dying tumour cells undergo immunogenic cell death upon exposure to mitoxantrone, which can be explored for whole tumour cell-based vaccination[60]. Dying tumour cells can be surface-modified with CpG-loaded nanoparticles and administered in combination with immune checkpoint blockers, which leads to complete tumour regression in ~78% of tumour-bearing mice and the establishment of long-term immunity against tumour recurrence[60]. Alternatively, in situ immunization with PLGA microparticles co-delivering DOX and CpG induces immunogenic cell death and leads to the activation of dendritic cells and T cells, which is further amplified by coadministration of anti-OX40 and/or cytotoxic T lymphocyte antigen 4 (CTLA4) immune checkpoint blockers[85] (BOX 1). This triple combination causes a reduction in primary and distant tumour burden in multiple murine models of EL4 and A20 lymphomas and B16 melanoma[85], suggesting potent immune activation by combination immunotherapy.

Finally, immune-stimulatory cytokines can be combined with chemotherapy to augment antitumour immune responses. For example, chitosan-based nanogels loaded with PTX and IL-2 can be further modified with an erythrocyte membrane to extend

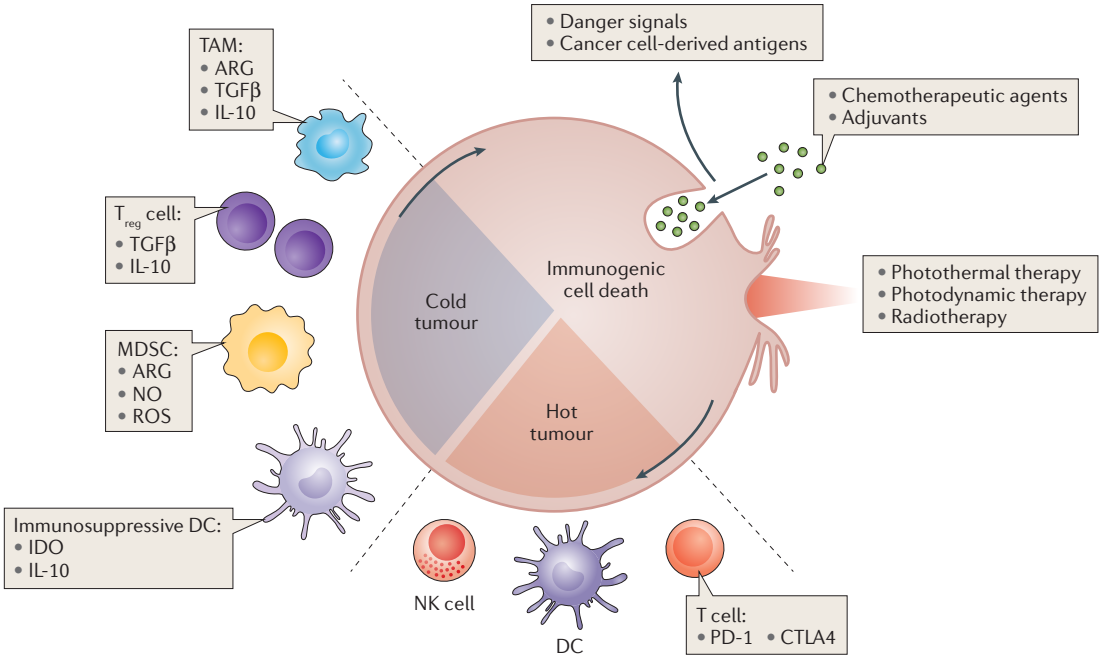

Fig. 3 | **Immune cells in the tumour microenvironment as potential targets for nano-immunotherapy.** Cold tumours are populated by immunosuppressive immune cells, including regulatory T (T_{reg}) cells, myeloid-derived suppressor cells (MDSCs), tumour-associated macrophages (TAMs) and dendritic cells (DCs), which suppress the cytotoxic functions of natural killer (NK) cells and T cells by secreting immunosuppressive factors, including transforming growth factor-β (TGFβ), indoleamine 2,3-dioxygenase (IDO), interleukin-10 (IL-10), arginase (ARG), NO and reactive oxygen species (ROS). Photothermal therapy, photodynamic therapy and radiotherapy can be applied to target and modulate the functions of immunosuppressive cells in the tumour microenvironment to amplify the antitumour efficacy of combination immunotherapy. CTLA4, cytotoxic T lymphocyte antigen 4; PD-1, programmed cell death 1.

their blood circulation time[43]. Compared with PTX or IL-2 monotherapy, the dual combination is more effective in promoting CRT exposure on tumour cells and in the activation of dendritic cells, leading to induction of $CD8^+$ T cell responses and a reduction in the number of T_{reg} cells in B16F10 tumour-bearing mice[43]. Thermosensitive sponge-like nanoparticles co-delivering DOX and IFNγ also significantly improve therapeutic outcomes, extending the survival of B16F10 melanoma-bearing mice[86]. Similarly, biodegradable, lipid-coated mesoporous silica nanoparticles can be applied for the co-delivery of DOX, IL-2 and all-*trans* retinoic acid (ATRA) for chemo-immunotherapy[44]. ATRA increases tumour cell sensitivity to $CD8^+$ T cell-mediated and natural killer cell-mediated lysis[87], and thus, compared with the soluble mixture of the drugs or with nanoparticles delivering the individual agents, the combination therapy leads to an increase in the number of tumour-infiltrating $CD8^+$ T and natural killer cells as well as inhibition of tumour growth and metastasis in B16F10 tumour-bearing mice.

Photothermal therapy and immunotherapy

Hyperthermia — treatment of disease by heat administration — is effective at ablating established, local tumours owing to limited heat dissipation in tumour tissues with abnormal vasculature[88]. Tumour cells can be destroyed at temperatures of 40–44 °C, which cause DNA damage, protein denaturation and disruption of the cellular membrane, resulting in eradication of tumour tissues[89]. In addition, febrile temperature can induce immune responses by various mechanisms, including the expression of heat shock proteins and an increase in the migration of lymphocytes to the tissues with elevated temperature[90].

Photothermal therapy is a minimally invasive treatment method, in which photon energy is converted into thermal energy to induce hyperthermia[54]. Selective tumour ablation can be achieved by directional control of focused incident irradiation following administration of photoactive molecules (photosensitizers), which induce localized heat transfer to the surrounding environment by non-radiative relaxation of the incident photon energy[91]. Near-infrared (NIR) light exhibits minimal absorption and scattering by tissue components, including skin, blood and biomolecules, and therefore achieves deep tissue penetration[92]. Conventional organic molecule-based photosensitizers are limited by photobleaching, low absorption cross section and poor NIR photothermal conversion efficiency; by contrast, inorganic nanoparticle photosensitizers offer several key advantages, including a high molar extinction coefficient, resistance to photodegradation and strong NIR responsiveness[47,93].

Nanoparticle-based photothermal therapy can also promote antitumour immune activation through the release of tumour antigens and immune-stimulatory molecules by ablated tumour cells (FIG. 2). For example, NIR-based photothermal therapy using gold nanoshells stimulates the expression of pro-inflammatory cytokines

(IL-6, tumour necrosis factor (TNF), IL-1β, IL-12, p70, granulocyte–macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF)) and chemokines (CXCL1, CC-chemokine ligand 2 (CCL2) and CCL4) and induces dendritic cell maturation in tumour-draining lymph nodes[51] (FIG. 2). However, efficient control of distal tumours or metastases cannot be achieved by photothermal therapy alone owing to suboptimal immune activation, photothermal therapy-induced expansion and tumour accumulation of immunosuppressive myeloid-derived suppressor cells[51] (BOX 2). In addition, immune responses induced by photothermal therapy can be dampened if tumours are heated to >45 °C, presumably because of temperature-dependent adverse effects on cytokines, chemokines and the vasculature and heat-induced adverse stress in tumour cells and stromal cells. Moreover, small tumours release fewer tumour antigens and endogenous immune-stimulatory damage-associated signals[52]. However, photothermal therapy requires high temperatures for rapid and complete cell death and thus can achieve only limited immune activation. Small tumours are more likely to be completely ablated with photothermal therapy; by contrast, large tumours are prone to tumour relapse caused by residual cancer cells and therefore are often resistant to photothermal therapy.

These limitations can be addressed by combining photothermal therapy with immune-stimulating agents and nanoparticles to synergistically induce antitumour immunity for the treatment of large established tumours and distant metastases[94,95] (TABLE 1). For example, photothermal therapy using chitosan-coated hollow copper sulfide nanoparticles carrying CpG results in the ablation of primary, treated tumours and in the activation of plasmacytoid dendritic cells, which trigger an immune response against untreated, distant tumours[38]. Upon photothermal therapy, these nanoparticles disintegrate into small particles, which are then rapidly cleared by renal excretion, minimizing long-term toxicity.

Photothermal therapy combined with conventional immune checkpoint blockers offers a promising approach to reverse photothermal therapy-induced immunosuppression within the tumour microenvironment. For example, photothermal ablation of primary tumours using single-walled carbon nanotubes promotes infiltration of T_{reg} cells into tumours[96]. Coadministration of anti-CTLA4 antibodies effectively removes T_{reg} cells in untreated secondary tumours, which enables the treatment of distal tumours and lung metastases in mice. Similarly, anti-PD-L1 immune checkpoint blockers (BOX 1) combined with plasmonic gold nanostar-mediated photothermal therapy leads to an increase in the number of T cells and B cells and a decrease in the number of immunosuppressive myeloid-derived suppressor cells, enabling the synergistic control of primary tumours and untreated, distal tumours[97]. Alternatively, photothermal therapy can be combined with adoptive T cell therapy to overcome immunosuppression of in situ-primed T cells by myeloid-derived suppressor cells and to promote the antitumour effect of the therapy[51].

The combination of photothermal and chemotherapy has been traditionally studied using human tumour xenograft models or syngeneic murine tumours and single tumours, thus mainly focusing on the heat-mediated direct killing of tumour cells[98]. Using late-stage murine tumour models, we recently demonstrated that combining photothermal therapy using polydopamine-coated spiky gold nanoparticles with a subtherapeutic dose of DOX can trigger robust systemic antitumour immunity against local and disseminated tumours[53]. This combination treatment leads to the elimination of residual tumour cells from locally treated tumours and to an abscopal effect, that is, the regression of untreated distant tumours following local treatment, which significantly extends animal survival compared with photothermal therapy or chemotherapy alone in advanced models of bilateral CT26 colon carcinoma and TC-1 submucosa lung metastasis[53]. Interestingly, $CD8^+$ T cells are necessary and sufficient to achieve tumour regression and to prevent tumour relapse in local primary tumours, whereas regression of untreated, distant tumours requires both $CD8^+$ T cells and natural killer cells, providing insight into the roles of the major effector cells in combination photothermal therapy and chemotherapy.

These results indicate that, although immune responses induced by local photothermal therapy are generally weak and dominated by immunosuppressive mechanisms, the combination with immunotherapeutic components can substantially enhance immune stimulation and overcome immunosuppression within the tumour microenvironment. In particular, photothermal immunotherapy might be effective against advanced cancer owing to the potential to ablate large, bulky tumours while eliciting systemic antitumour immunity against metastatic tumours.

Photodynamic therapy and immunotherapy

In photodynamic therapy, diseased cells and tissues are destroyed by a combination of light and photosensitizers, which generate cytotoxic reactive oxygen species (ROS), such as singlet oxygen, hydrogen peroxide and hydroxyl and superoxide anion radicals[99]. Photodynamic therapy damages subcellular organelles and plasma membranes; in addition, dying tumour cells release tumour antigens and cytosolic components that provoke inflammation and stimulate immune responses (FIG. 2). In particular, photodynamic therapy induces the accumulation of neutrophils at the treatment site within minutes after irradiation[100]. Neutrophils destroy tumour cells by releasing toxic substances and lysosomal enzymes, and they trigger the subsequent invasion of monocytes and macrophages, which help to eliminate remaining tumour cells and secrete inflammatory cytokines and chemokines to stimulate immune responses[100] (FIG. 3). Photodynamic therapy also increases the expression of heat shock proteins and other stress-induced proteins, leading to dendritic cell activation and thus presentation of tumour antigens to T cells[101].

Photosensitizers for photodynamic therapy are usually composed of hydrophobic aromatic repeating units, such as tetrapyrrole and phenothiazinium, and they can

Table 1 | **Preclinical studies of nanomedicine-based combination cancer immunotherapy**

Immunotherapeutic agents or gene	Delivery platform or modality	Model	Type	Refs
Combination chemotherapy				
DOX + anti-PD-1 Ab	Synthetic high-density lipoprotein nanodisc	Mouse colon carcinoma	CT26 and MC38	40
DOX + CpG	Poly(amidoamine) dendrimer	Human prostate cancer xenograft in athymic Balb/c mice	22RV1	25
PTX + imiquimod	γ-PGA microparticle	Mouse melanoma	B16F10	34
PTX + LPS	PLGA nanoparticle	Mouse melanoma	B16F10	82
PTX + CpG + IL-10 siRNA	PLGA nanoparticle	Mouse melanoma	B16F10-OVA	83
CpG + cisplatin	Liposome	Mouse melanoma	B16F10	84
Mitoxantrone-treated cells + CpG + anti-PD-1 Ab	Hyaluronic acid-cationic lipid nanoparticle	Mouse melanoma and colon carcinoma	B16F10-OVA and CT26	60
DOX + CpG + anti-OX40 Ab + anti-CTLA4 Ab	PLGA microparticle	Mouse lymphoma and mouse melanoma	EL4, A20 and B16fLuc	85
PTX + IL-2	RBCm-coated chitosan-based nanogel	Mouse melanoma	B16F10	43
DOX + IFNγ	Thermosensitive nanoparticle	Mouse melanoma	B16F10	86
ATRA + DOX + IL-2	Lipid-coated hollow mesoporous silica nanoparticle	Mouse local and metastatic models	B16F10	44
Combination photothermal therapy				
CpG	Chitosan-coated hollow copper sulfide nanoparticle	Mouse breast cancer	EMT6-OVA and EMT6	38
Anti-CTLA4 Ab	Single-walled carbon nanotube	Mouse breast cancer	4T1	96
Anti-PD-L1 Ab	Gold nanostar	Mouse bladder cancer	MB49	97
Adoptive T cell therapy	Gold nanoshell	Mouse melanoma	B16F10	51
DOX	Polydopamine-coated spiky gold nanoparticle	Mouse colon carcinoma and mouse submucosa lung tumour	CT26 and TC-1/luc	53
Combination photodynamic therapy				
Oxaliplatin + anti-PD-L1 Ab	NCP@pyrolipid	Advanced murine colorectal tumour	CT26 and MC38	113
Anti-PD-L1 Ab	ZnP@pyrolipid	Mouse metastatic TNBC	4T1 and TUBO	59
Anti-PD-L1 siRNA	Micelle	Mouse melanoma	B16F10	39
Imiquimod + anti-CTLA4 Ab	Up-conversion nanoparticle	Mouse colon carcinoma	CT26	115
IDO inhibitor	Chlorin-based nanoscale metal–organic framework	Mouse colon carcinoma	CT26 and MC38	116
Combination radiotherapy				
Cowpea mosaic virus	Cowpea mosaic virus nanoparticle	Mouse ovarian carcinoma	ID8	137
Anti-CD40 Ab	Gold nanoparticle	Mouse pancreatic cancer	Panc02	152
IDO inhibitor	Hf-based nanoparticle	Mouse head and neck, prostate, colon and breast cancers and glioblastoma	SQ20B, U87MG, PC-3, CT26 and TUBO	153
Combination RNAi therapy				
PD-L1	PEI-PEG-FA	In vitro human epithelial ovarian cancer	SKOV-3-Luc	36
IDO	DC vaccine	Mouse breast cancer	4T1	164
SOCS1	PLGA nanoparticle	–	–	171
STAT3	PLGA nanoparticle	Mouse T cell lymphoma	EG7-OVA	171
IL-10	PLGA microparticle	Mouse B cell lymphoma	A20	35
TGFβ	Liposome-protamine-hyaluronic acid nanoparticle	Mouse melanoma	B16F10	172
PD-L1	Micelle nanocomplex	Mouse melanoma	B16F10	39
IL-6	Radiofrequency thermal ablation	Mouse breast adenocarcinoma	R3230 and MATBIII	173
VEGF	M2pep-modified gold nanoparticle	Mouse human lung adenocarcinoma xenograft model	A549-luciferase-C8	26

Table 1 (cont.) | **Preclinical studies of nanomedicine-based combination cancer immunotherapy**

Immunotherapeutic agents or gene	Delivery platform or modality	Model	Type	Refs
Combination mRNA vaccine				
OVA	Two-component mRNA vaccine complex	Mouse T cell lymphoma	EG7-OVA	178
MUC1	Mannose-modified liposome	Mouse TNBC	4T1	29
OVA	Two-component mRNA vaccine complex	Mouse T cell lymphoma and mouse lung carcinoma	EG7-OVA and LLC	179
IL-2 and IL-12	Liposome-mediated pDNA	Mouse head and neck squamous cell carcinoma	SCC VII	175
IP-10	Chitosan-FA-PEG nanoparticle	Mouse melanoma and mouse human hepatocellular carcinoma xenograft	B16	176

Ab, antibody; ATRA, all-*trans* retinoic acid; CpG, immunostimulatory oligodeoxynucleotide containing unmethylated CpG motifs; CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOX, doxorubicin; DTX, docetaxel; FA, folic acid; Hf, hafnium; IDO, indoleamine 2,3-dioxygenase; IFNγ, interferon-γ; IL, interleukin; IP-10, interferon-γ-inducible protein 10; LPS, lipopolysaccharide; M2pep, M2 macrophage-targeting peptide; MUC1, mucin 1; NCP, nanoscale coordination polymer; OVA, ovalbumin; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; pDNA, plasmid DNA; PEG, polyethylene glycol; PEI, polyethylenimine; PGA, poly(glycolic acid); PLGA, poly(lactic-co-glycolic acid); PTX, paclitaxel; RBCm, red blood cell membrane; RNAi, RNA interference; siRNA, small interfering RNA; SOCS1, suppressor of cytokine signalling 1; STAT3, signal transducer and activator of transcription 3; TAM, tumour-associated macrophage; TGFβ, transforming growth factor-β; TNBC, triple-negative breast cancer; TRP2, tyrosinase-related protein 2; VEGF, vascular endothelial growth factor.; ZnP, zinc pyrophosphate

selectively localize in specific intracellular organelles, such as the Golgi apparatus, endoplasmic reticulum, mitochondria and lysosomes[102]. Subsequent photodynamic therapy then causes organelle-confined damage owing to the short diffusion distance (<0.1 μm) of cytotoxic oxygen species, which subsequently collapse, limiting their impact on other organelles[103]. Mitochondrial damage through disruption of ATP generation is one of the primary mechanisms by which photodynamic therapy kills cells[104]. However, the use of hydrophobic photosensitizers can lead to loss of photoactivity owing to undesired physicochemical properties of these compounds in biological media, which can cause poor solubility and aggregation[105]. Nanoparticle-based delivery approaches can improve the performance of photodynamic therapy by increasing the colloidal stability and photoresponsiveness of photosensitizers and by improving tumour accumulation in tissues and specific subcellular compartments[106–108]. For example, mitochondria-targeted nanoparticles can achieve an increase in photodynamic therapy-induced apoptosis and antitumour immune activation by dendritic cells and $CD8^+$ T cells[106] compared with non-targeted nanoparticles. Targeting tumour cell surface receptors can also be effective for inducing tumour cell killing and immune activation, regardless of the subcellular localization of nanoparticles[107,108].

Photodynamic therapy can also be used to target and ablate non-tumour cells within the tumour microenvironment. For example, carcinoma-associated fibroblasts (CAFs), which constitute a major fraction of the tumour stromal cell population in various tumours, including colorectal, breast, ovarian, bladder and lung carcinoma, can promote proliferation of tumour cells, induce immune suppression and prevent T cell migration into the tumour microenvironment[109,110]. Photodynamic therapy can target CAFs to increase the number and enhance the cytotoxic effector function of tumour-infiltrating $CD8^+$ T cells and to inhibit the growth of murine 4T1 breast cancer[28]. Similarly, photodynamic therapy-mediated selective depletion of tumour-resident immunosuppressive lymphocytes, such as T_{reg} cells, causes tumour-specific, systemic antitumour effects through manipulation of the balance between effector and suppressor immune cells[27].

Although photodynamic therapy triggers antitumour immunity, its effect is generally too weak to control established tumours with an immunosuppressive tumour microenvironment[111]. In addition, photodynamic therapy inherently induces immune tolerance or suppression through the extensive release of self-antigens owing to collateral damage to healthy cells, oxidative modification of danger signals that promote tolerance and the release of immunosuppressive cytokines[112] (FIG. 3). Thus, to counteract the immunosuppressive effects of monophotodynamic therapy, it can be combined with immune checkpoint blockade. Nanoscale coordination polymer core-shell nanoparticles combined with photodynamic therapy, chemotherapy and immune checkpoint blockade promote pro-inflammatory cytokine release and synergistic activation of dendritic cells and $CD8^+$ T cells, enabling control of local and distant tumour growth in an advanced model of murine colorectal cancer[113]. Coordination polymer core-shell nanoparticle-mediated photodynamic therapy further improves the therapeutic efficacy of anti-PD-L1 immune checkpoint blockers in a murine model of metastatic triple-negative breast cancer[59]. Anti-PD-L1 immune checkpoint blockade leads to an increase in the number of tumour-infiltrating natural killer cells and T cells (BOX 1), and photodynamic therapy causes infiltration of B cells into distant tumours, indicating complementary immune responses induced by the combination therapy.

Up-conversion nanoparticles can convert NIR light into visible light, by which most conventional photodynamic therapy photosensitizers are strongly excited[105]. Therefore, the resonance energy transfer of up-converting nanoparticles enables NIR-based photodynamic therapy for deep tissue applications, including cancer immunotherapy[114]. For example, up-conversion

nanoparticles loaded with a chlorin e6 (Ce6) photosensitizer and a TLR7 agonist induce robust immune stimulation following NIR-based photodynamic therapy compared with free TLR7 agonist, and coadministration of anti-CTLA4 antibody leads to depletion of intratumoural T_{reg} cells and allows control of metastatic CT26 colorectal cancer in mice[115]. A nanoscale metal–organic framework carrying the photosensitizer Ce6 and indoleamine 2,3-dioxygenase (IDO) inhibitors can be used for combination photodynamic therapy and immune checkpoint blockade[116]. IDO is an immunoregulatory enzyme that is highly expressed in tumours and catalyses the oxidative catabolism of tryptophan, preventing the clonal expansion of T cells and promoting T cell anergy and apoptosis[117] (BOX 2). The synergistic action of photodynamic therapy and IDO blockade generates systemic antitumour immunity and inhibits the growth of local and metastatic tumours.

Photodynamic therapy is typically dominated by a type II photoreaction that is dependent on oxygen concentration and thus requires synchronized interactions between light, photosensitizers and oxygen[104]. Therefore, the efficacy of photodynamic therapy diminishes in hypoxic tumours, especially in solid tumours with limited oxygen supply and abnormal tumour vasculature[118]. Fractional photodynamic therapy or innovations in the design of nanoparticle photosensitizers, for example, hybrid protein oxygen nanocarriers, are being explored to overcome this limitation[41,119,120]. By contrast, photothermal therapy is not affected by oxygen deficiency and thus can be effective in the treatment of highly hypoxic solid tumours[121]. Despite progress in this field, the activation mechanisms of innate and adaptive immune responses and the differential contribution of photothermal and photodynamic therapy to local and systemic antitumour efficacy remain elusive thus far; therefore, mechanistic studies are required to facilitate clinical translation of these therapies for cancer immunotherapy.

Radiotherapy and immunotherapy

Radiotherapy is widely used for cancer therapy, with >50% of patients with cancer receiving radiation over the course of their disease[122]. In radiotherapy, high-energy ionizing radiation (X-rays, γ-rays or fast-moving charged particles, such as ions, electrons and protons) are used to generate free radicals and damage DNA and cellular components to promote tumour cell death[123]. Tumour cells are more susceptible to radiotherapy than most healthy cells because tumour cells often have defects in the DNA repair machinery, making them more vulnerable to radiotherapy-mediated DNA damage. Radiotherapy also induces immunogenic cell death in irradiated tumour cells[124] and causes an increase in the intracellular peptide pool in dying tumour cells through radical-induced degradation of proteins and expression of radiation-responsive proteins, such as those related to DNA repair and protein breakdown[125]. Therefore, more antigen peptides are presented on the surface of dying tumour cells, sensitizing them for recognition and killing by $CD8^+$ T cells[124,125]. Radiotherapy further promotes activation of dendritic cells through stimulator of interferon genes (STING)-mediated cytosolic DNA detection, type I interferon[126,127] and other pro-inflammatory cytokines and chemokines, such as IL-1β, TNF and CXCL16 (REF.[128]). These complementary immune-stimulatory events induce an abscopal effect and generate systemic antitumour immunity against untreated distant tumours[129,130].

Although antitumour immunity is induced by radiotherapy, immunosuppression within the tumour microenvironment can dampen the abscopal effect[131]. In fact, radio-monotherapy can promote the recruitment and expansion of immunosuppressive cell types, such as T_{reg} cells, that are less radiosensitive than other lymphocytes[132,133]. Furthermore, it can cause an increase in the expression of immune-inhibitory proteins, such as PD-L1 and transforming growth factor-β (TGFβ), within the tumour microenvironment[134] (FIG. 3). The combination of radiotherapy with immunotherapy can be applied to overcome immunosuppression[135], and clinical trials are ongoing examining conventional radiotherapy combined with immune checkpoint blockers[136].

Radiotherapy can be combined with cowpea mosaic virus nanoparticle in situ vaccination[137]. Compared with single agent radiotherapy or virus particles alone, their combination triggers an increase in the number of tumour-infiltrating T cells and significantly delays tumour growth in a mouse model of ID8 ovarian carcinoma, suggesting that the combination treatment can turn an immunologically cold tumour into a hot tumour (FIG. 2). The combination of radiotherapy with immune checkpoint blockers can further improve the efficacy of radiotherapy and augment systemic antitumour immunity even in poorly immunogenic murine tumours, including a triple-negative breast cancer 4T1 tumour and a TUBO spontaneous mammary tumour[138,139]. Moreover, the triple combination of radiotherapy with anti-CTLA4 and anti-PD-L1 antibodies (BOX 1) improves the local and abscopal responses against B16F10 melanoma, TSA breast cancer and pancreatic ductal adenocarcinoma 4662 compared with a combination of radiotherapy with a single immune checkpoint blocker, indicating non-redundant mechanisms of action and synergy[134,140]. Notably, in phase I and II clinical trials, patients with low-grade B cell lymphoma and cutaneous T cell lymphoma treated with radiotherapy and CpG exhibited systemic immune stimulation with a reduced T_{reg} cell population compared with pretreatment specimens, yielding objective response rates of 27% and 36%, respectively[141,142].

Nevertheless, it remains unclear how the radiation dose and treatment regimen of radiotherapy influence immune-mediated effects. For example, high-dose irradiation is often needed to kill tumour cells and release a sufficient amount of tumour antigens and danger signals[143] (FIG. 2); however, high-dose irradiation can also increase the number of T_{reg} cells and pro-tumour M2-phenotype macrophages[144,145] (BOX 2). Low-dose irradiation induces the differentiation of tumour-associated macrophages into macrophages with an antitumour M1 phenotype[144,145]. Interestingly, fractionated radiotherapy, in which low radiation doses are administered multiple times to achieve high total radiation, minimizes side

effects and improves therapeutic efficacy of radiotherapy by promoting repair of healthy cells and by amplifying abscopal effects[128,146,147].

Inorganic nanoparticles composed of heavy atoms, such as gold, titanium dioxide and iron oxide, are excellent agents for scattering and absorption of γ-ray and X-ray radiation, and they can sensitize tumours to low-dose radiation[46,48–50]. Radiation of inorganic nanoparticles leads to the production of distinct short-range Auger electrons owing to the photoelectric effect. Auger electrons then generate ROS, which can eliminate radio-resistant hypoxic tumours[148]. Inorganic nanoparticles also increase the deposition of radiation energy within tumours and promote radiation-induced damage in cancer cells through multiple biochemical pathways, including oxidative stress, cell cycle arrest, DNA repair inhibition, autophagy, mitochondrial damage and endoplasmic reticulum stress[149–151]. Moreover, inorganic nanoparticles can promote the immunogenicity of tumour cells by inflicting ionization-induced mutation of tumour cell DNA[135]. For example, in a murine model of pancreatic adenocarcinoma, gold nanoparticle-aided radiotherapy combined with anti-CD40 antibodies leads to complete regression of treated subcutaneous tumours in 60% of animals and reduces the mass of untreated orthotopic tumours by 74%[152]. By contrast, anti-CD40 treatment alone leads to only 40% tumour regression and 34% tumour reduction, demonstrating the efficacy of the combination of radiotherapy and immunotherapy for the treatment of local and distant tumours. Hafnium-based nanoparticles carrying an inhibitor against immunosuppressive IDO can be directly injected into local tumours in mouse models of breast and colorectal cancer. Subsequent low-dose X-ray irradiation leads to regression of local tumours and abscopal responses against untreated distal tumours[153].

Inorganic nanoparticles are promising platforms for maximizing the immunogenic effect of radiotherapy while minimizing the radiation dose and thus the potential toxicity of the treatment, which is particularly important for combination radiotherapy–immunotherapy, which is limited by toxicity and immune-related adverse events[154]. Clinical studies are underway to investigate and improve the toxicity profiles of combination radiotherapy–immunotherapy[155], and nanomaterials could greatly contribute to the efficacy and safety of the treatment (FIG. 1). For example, nanoparticles that can scavenge free radicals and exert antioxidative functions in normal tissues could reduce the risk of radiation-induced toxicity. Inorganic nanoparticles composed of gadolinium or hafnium have already entered clinical trials to sensitize tumours to radiotherapy[151]. Nanoparticle platforms could achieve dose sparing of fractionated radiotherapy and maximize the abscopal effect of radiotherapy through radiosensitization and radioprotection, improving cancer immunotherapy.

Gene therapy and immunotherapy

RNA interference (RNAi) therapeutics, such as siRNA, microRNA (miRNA) and short hairpin RNA (shRNA), can be used to target and knock down specific cellular signalling molecules, including cytokines and chemokines. RNAi therapeutics exploit the endogenous cellular machinery, referred to as RNA-induced silencing complex (RISC), which guides complementary mRNA binding and subsequent mRNA degradation to control gene expression[156]. Therefore, RNAi therapeutics can achieve highly sequence-specific gene silencing for cancer therapy[157,158].

The clinical translation of RNAi-based therapeutics has been hampered by the inherent instability of RNAs, rapid degradation of RNA in vivo and poor cellular uptake owing to the strong anionic charge of RNA. Nanoparticles can be designed for RNAi therapeutic delivery to address these limitations and improve the efficacy of gene therapy[159] (TABLE 1), in particular, for the targeting of immune checkpoints, including the PD-1–PD-L1 pathway[160,161] (BOX 1). For example, folic acid-modified polyethylenimine nanoparticle-mediated siRNA delivery for the silencing of PD-L1 enables sensitizing of epithelial ovarian cancer cells to T cell-mediated killing[36]. Silencing of PD-L1 can also be achieved by siRNA-mediated gene knockdown in tumour-specific human $CD4^+$ and $CD8^+$ T cells (BOX 2), improving their effector functions and antigen-specific cytotoxicity[162]. Alternatively, RNAi therapeutics can be applied to silence other immunosuppressive proteins, such as IDO, signal transducer and activator of transcription 3 (STAT3) and suppressor of cytokine signalling 1 (SOCS1), that are upregulated in immune cells and stromal cells within the tumour microenvironment[163]. For example, tumour antigen-loaded dendritic cells transfected with siRNA against IDO can be injected into 4T1 tumour-bearing mice, which leads to an increase in the number of $CD8^+$ T cells and a decrease in the number of T_{reg} cells[164]. Liposomes conjugated with mannose and loaded with siRNA against IDO can be administered to target mannose receptors on dendritic cells in the lymph nodes, decreasing apoptosis of $CD4^+$ and $CD8^+$ T cells and improving antitumour efficacy in a murine model of melanoma. Tolerogenic dendritic cells can be further inhibited by STAT3 siRNA[165,166]. Alternatively, STAT3 siRNA can be delivered by PLGA nanoparticles to restore dendritic cell maturation and functionality[33]. STAT3 siRNA conjugated to CpG allows TLR9-mediated uptake of STAT3 siRNA and knockdown of STAT3 in myeloid-derived suppressor cells (BOX 2) in a murine prostate cancer model[167], providing robust antitumour efficacy. In addition, siRNA-mediated knockdown of SOCS1 improves antigen presentation by dendritic cells and generates robust antitumour immune responses[168–170].

RNAi-based therapeutics can be synergistically used with other treatment modalities designed to promote immune stimulation (TABLE 1). For example, delivery of siRNA against SOCS1 together with PLGA nanoparticle vaccines significantly increases the expression of pro-inflammatory cytokines in dendritic cells, including TNF, IL-2, IL-6 and IL-12, and enhances the antigen-specific $CD8^+$ T cell response compared with the same formulation without SOCS1 siRNA[171]. PLGA nanoparticles can also be loaded with STAT3 siRNA, imiquimod (a TLR7 agonist) and the model antigen ovalbumin (OVA) for dendritic cell activation.

This triple combination significantly inhibits tumour growth in an EG7-OVA tumour model compared with the same formulation without STAT3 siRNA[171]. Dual delivery of CpG and siRNA against IL-10 by PLGA microparticles promotes T_H1 cell:T_H2 cell-balanced antitumour immune responses (BOX 2) in a murine model of B cell lymphoma[35]. The delivery of vaccine nanoparticles carrying the tyrosinase-related protein 2 (TRP2) peptide antigen and CpG in combination with hyaluronic acid liposomes loaded with siRNA against TGFβ significantly increases the level of tumour-infiltrating $CD8^+$ T cells and inhibits the growth of late-stage murine B16F10 tumours when combined with TGFβ siRNA[172].

RNAi therapies can also be combined with traditional therapeutics designed to debulk tumours or to remove tumour-associated cells. For example, intravenous administration of cationic micelles carrying siRNA against PD-L1 leads to a 55% decrease in PD-L1 expression in a murine B16F10 tumour model; co-treatment with photodynamic therapy further provides strong antitumour efficacy[39]. Radiofrequency thermal ablation combined with PD-L1 siRNA treatment by micelle-like nanoparticles causes elimination of residual tumours[173]. The tumour vasculature also provides a facile target for RNAi therapeutics. For example, tumour-associated macrophages (BOX 2) induce tumour vascularization and overexpression of angiogenic vascular endothelial growth factor (VEGF) while promoting immunosuppression within the tumour microenvironment. The macrophages can be targeted by gold nanoparticles modified with the tumour-associated macrophage-specific M2 macrophage-targeting peptide (M2pep) and anti-VEGF siRNA, which leads to complete regression of human lung adenocarcinoma in a murine xenograft model[26].

Alternatively to RNAi-mediated gene knockdown, gene expression can be promoted for reversing immunosuppression or for stimulating immune responses. For example, PD-L1 can be neutralized using a PD-L1 trap. Lipid-protamine nanoparticles can be used for the systemic delivery of plasma DNA encoding a PD-L1 trap fusion protein that binds to and blocks the PD-1/PD-L1 pathway[174] (BOX 1). Interestingly, intravenous administration of the nanoparticles results in transient and local expression of the PD-L1 trap in orthotopic CT26-FL3 colorectal cancer cells. Combination with oxaliplatin, which is an immunogenic cell death-inducing agent, significantly improves tumour infiltration of $CD4^+$ and $CD8^+$ T cells, reduces PD-L1 expression and leads to potent antitumour efficacy in multiple models, including CT26-FL3, B16F10 and 4T1 tumours[174]. Compared with coadministration of anti-PD-L1 monoclonal antibodies and oxaliplatin, the PD-L1 trap and oxaliplatin combination therapy leads to a decrease in the number of T_H17 cells (BOX 2). T_H17 cells are implicated in autoimmune disease and associated with immune-related adverse events, suggesting a favourable safety profile of this strategy[174]. Lipid-protamine-DNA nanoparticles encoding traps for immunosuppressive IL-10 and CXCL12 can reverse immunosuppression and extend animal survival in murine orthotopic models of 4T1 breast cancer and KPC pancreatic cancer[174].

Alternatively, DNA-based gene therapy can be used to improve the delivery and expression of immunostimulatory proteins for combination immunotherapy. Liposome-mediated delivery of plasmid DNA encoding IL-2 and IL-12, used in conjunction with radiotherapy, leads to the activation of $CD8^+$ T cells, natural killer cells and macrophages in the tumour and in the circulation as well as favourable therapeutic outcomes in a murine head and neck squamous cell carcinoma model[175]. The combination of IFNγ-inducible protein 10 (IP-10) gene delivery and adoptive T cell therapy has also shown promise in murine models of melanoma[176] and hepatocellular carcinoma[176].

In addition to DNA-based gene therapy, mRNA is being tested for vaccine applications in multiple clinical trials[177]. Compared with traditional vaccine platforms, such as protein, peptide and DNA vaccines, mRNA vaccines can be designed to contain immunostimulatory domains that stimulate pattern recognition receptors and allow for the co-delivery of danger signals and encoded antigens. Moreover, in contrast to peptides, mRNA vaccines are not restricted by the patient's human leukocyte antigen (HLA) type, and in contrast to DNA-based vaccines, mRNA does not integrate into the host genome, and thus, transient expression of mRNA-encoded proteins is possible, minimizing safety issues. Finally, large-scale good manufacturing practice production of mRNA is feasible. Messenger RNA vaccines and immune checkpoint blockers or chemotherapeutics act synergistically and exert antitumour efficacy[178] (TABLE 1). In a murine model of the EG7-OVA tumour, a protamine-mRNA vaccine elicits substantial T cell and natural killer cell responses, and in combination with anti-CTLA4, it promotes tumour regression in a subset of mice[178]. Dendritic cells overexpress mannose receptors, and thus, mannose-modified liposomes can be employed to deliver mRNA encoding the mucin 1 (MUC1) tumour antigen to dendritic cells[29]. These mRNA-loaded liposomes elicit strong antigen-specific $CD8^+$ T cell responses against a murine TNBC 4T1 tumour, and coadministration with anti-CTLA4 significantly slows tumour growth compared with the mRNA vaccine or anti-CTLA4 treatment. Alternatively, mRNA vaccination can be combined with radiation therapy, triggering strong immune responses and inhibiting tumour growth in murine models of EG7-OVA and poorly immunogenic Lewis lung cancer[179]. These studies demonstrate the versatility of gene therapies to target and modulate specific genes for combination cancer immunotherapy.

Perspectives

There has been substantial progress in the nascent field of nano-immunotherapy. However, to realize its therapeutic potential and improve patient care in the clinic, there are a number of crucial limitations and challenges that need to be addressed.

Tumour targeting. Many nanoparticle-based strategies require tumour infiltation by nanoparticles. The clinical relevance of EPR[14,21] remains controversial, and there is evidence that only a small fraction of administered

nanoparticles carrying conventional chemotherapeutic agents enter tumour tissues, thus failing to achieve a meaningful therapeutic index in clinical trials[20]. Indeed, it remains challenging to increase tumour targeting of nanoparticles despite extensive efforts to optimize nanoparticle properties and to regulate biological interactions of nanoparticles, partly owing to the complex physiology of the tumour microenvironment, which is characterized by irregular vascular distribution, high tumour interstitial fluid pressure, poor blood flow, dense extracellular matrix (ECM) and abundant stroma cells. Therefore, strategies are needed to render the tumour microenvironment more favourable to nanoparticle entry, which may be achieved by improving tumour perfusion, increasing permeability of the tumour vasculature and remodelling of ECM. For example, tumour microenvironment-targeted nanoparticles delivering agents that degrade ECM or normalize tumour vasculature[180] could prime the tumour microenvironment to generate favourable immune responses. Subsequent sequential or concomitant treatments with nano-immunotherapies or conventional immunotherapies may then provide an effective multi-modal strategy for reversing immunosuppression and eliciting strong systemic antitumour immunity[181].

If tumours are directly accessible, intratumoural injection of nanoparticles, rather than systemic injection, can address limited tumour accumulation. Retention of nanoparticles within the tumour microenvironment, followed by controlled release of immunotherapies, could bypass the initial hurdle of tumour entry, reduce treatment doses and prevent systemic off-target side effects. A number of clinical trials are currently evaluating direct intratumoural injection of immunotherapies[182,183]. Improved antitumour efficacy and safety profiles could be demonstrated in a preclinical study using immune checkpoint blockers modified with an ECM-binding peptide, which increases the retention time of immune checkpoint blockers after intratumoural administration as compared with unmodified immune checkpoint blocker administration[184]. Similarly, nanoparticles designed to bind to ECM or tumour cells can increase retention in the tumour[185] and may offer a safe and effective strategy to augment the efficacy of immunotherapies and restrict their site of action to local tumour tissues, reducing the incidence of immune-related adverse events.

Targeting immune cells. A larger fraction of systemically administered nanoparticles is taken up by non-tumour cells than by tumour cells[186], presenting an opportunity for nanoparticle-mediated immunotherapy. Phagocytic immune cells, such as dendritic cells and macrophages, can take up nanoparticles within tumours, offering the possibility to initiate antitumour immune responses and to increase penetration of T cells and antibody therapeutics into the tumour by exploiting phagocytosis-mediated signalling[187]. Similarly, immune cells residing in peripheral tissues, including the lymph nodes, spleen, skin and circulation, can be investigated as potential targets. For example, following systemic administration in tumour-bearing mice, nanoparticles encapsulating immune checkpoint blockers are mainly delivered to splenic dendritic cells and macrophages, enabling dose titration of immune checkpoint blockers and robust antitumour efficacy[188]. Therefore, nonspecific uptake of nanoparticles in the spleen, which is a major disadvantage for conventional chemotherapy-loaded nanoparticles, could be exploited to potentiate immune checkpoint blockade, alleviate immune-related adverse events and advance cancer immunotherapy. A thorough understanding of the biodistribution of nanoparticles will enable the optimal design of nano-immunotherapies targeting tumours and lymphoid tissues.

Improving efficacy and minimizing toxicity. The material composition, physicochemical parameters, dosing and injection routes of nanomedicine need to be modulated to achieve desired pharmacokinetics, tissue-specific or organ-specific delivery, optimal efficacy and minimal toxicity. In particular, administration timing and sequence of combinatorial agents need to be carefully examined, especially for cytotoxic drugs. Incorrect sequence of chemo-immunotherapy could cause elimination or inactivation of key immune cells within the tumour microenvironment or lymphoid tissues, decreasing the therapeutic efficacy of immunotherapy. For example, the timing of administration of paclitaxel or cyclophosphamide substantially impacts the induction of antitumour T cell responses by CD47 blockade[189]. In addition, safety profiles of nanoparticle platforms and nanoparticle combinations with immunotherapeutic agents should be systemically examined to identify host tissue damage or dysfunction of the immune system. Materials used to fabricate nanoparticles can further have an immunological effect, which can induce immune stimulation or immunotoxicity, possibly exacerbating immune-related adverse events associated with immunotherapy[31]. Thus, the intrinsic immune-modulatory effects of nanoparticles in relation to their physicochemical properties have to be thoroughly investigated in combination with treatment parameters, such as dose, timing, sequence and administration route, and tailored to specific clinical applications.

Theranostics. Precision cancer nanomedicine implies screening of tumours and stratifying of patients on the basis of their EPR status before treatment[14,21,22]. Similarly, biomarkers for immune checkpoint blockade treatments can be identified to maximize their therapeutic benefit and minimize unnecessary risks of immune-related adverse events[190]. Multifunctional theranostic nanoparticles can be explored for real-time monitoring of patient-tailored immunotherapies[187]. For example, gold nanoparticles can be loaded into T cells to track the cells in vivo by X-ray computed tomography during immunotherapy, allowing real-time monitoring of antitumour efficacy in relation to the fate of T cells[49]. Cell tracking could be combined with theranostic nanoparticles, such as gadolinium-based platforms, which can provide image-guided radiotherapy and magnetic resonance imaging[48]. The combination of theranostic nanoparticles with immunotherapy can open doors for

precision immunotherapy. Similarly, synergies between cell therapies and nanomedicine can be explored to improve intratumoural T cell infiltration and tumour cell killing[176,191,192]. For example, photothermal therapy using gold nanoshells can be applied to augment antitumour immune responses of adoptively transferred tumour-specific T cells[51].

Nanomedicines have the potential to overcome challenges associated with cancer immunotherapy. Immune checkpoint blockers generally exhibit limited efficacy in the conversion of cold tumours into hot tumours[8,9]. Nanoparticle platforms can be used to reconstitute the tumour microenvironment in favour of antitumour immune responses by reprogramming tumour-associated stromal cells and immune infiltrates or simply by debulking cold tumours. In contrast to surgical resection, which often leads to tumour recurrence from residual tumours[193], nanoparticle-based treatment modalities, such as chemotherapy, photodynamic therapy, photothermal therapy and radiotherapy, can be applied to not only ablate tumours but also trigger the release of tumour antigens and intracellular danger signals, which can initiate systemic antitumour immune responses. Therefore, in combination with immune checkpoint blockade immunotherapy, nanoparticle-based therapies could prevent tumour recurrence and eliminate metastases. Preclinical and clinical studies have already demonstrated the potential of nanomedicines for combinational cancer immunotherapy, providing a solid basis for their clinical translation.

Published online: 26 April 2019

1. McCarthy, E. F. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthop. J.* **26**, 154–158 (2006).
2. Mellman, I., Coukos, G. & Dranoff, G. Cancer immunotherapy comes of age. *Nature* **480**, 480–489 (2011).
3. Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252–264 (2012).
4. Sharma, P. & Allison, J. P. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* **161**, 205–214 (2015).
5. Topalian, S. L., Drake, C. G. & Pardoll, D. M. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* **27**, 450–461 (2015).
6. Topalian, S. L., Taube, J. M., Anders, R. A. & Pardoll, D. M. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat. Rev. Cancer* **16**, 275–287 (2016).
7. Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* **168**, 707–723 (2017).
8. Herbst, R. S. et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **515**, 563–567 (2014).
9. Tumeh, P. C. et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014).
10. Wolchok, J. D. et al. Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **369**, 122–133 (2013).
11. Friedman, C. F., Proverbs-Singh, T. A. & Postow, M. A. Treatment of the immune-related adverse effects of immune checkpoint inhibitors: a review. *JAMA Oncol.* **2**, 1346–1353 (2016).
12. Weber, J. S. et al. Sequential administration of nivolumab and ipilimumab with a planned switch in patients with advanced melanoma (CheckMate 064): an open-label, randomised, phase 2 trial. *Lancet Oncol.* **17**, 943–955 (2016).
13. Postow, M. A., Sidlow, R. & Hellmann, M. D. Immune-related adverse events associated with immune checkpoint blockade. *N. Engl. J. Med.* **378**, 158–168 (2018).
14. Shi, J., Kantoff, P. W., Wooster, R. & Farokhzad, O. C. Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* **17**, 20–37 (2017).
15. Matsumura, Y. & Maeda, H. A. New concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent Smancs. *Cancer Res.* **46**, 6387–6392 (1986).
16. Peer, D. et al. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* **2**, 751–760 (2007).
17. Chou, L. Y. T., Ming, K. & Chan, W. C. W. Strategies for the intracellular delivery of nanoparticles. *Chem. Soc. Rev.* **40**, 233–245 (2011).
18. Bertrand, N., Wu, J., Xu, X., Kamaly, N. & Farokhzad, O. C. Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology. *Adv. Drug Deliv. Rev.* **66**, 2–25 (2014).
19. Venditto, V. J. & Szoka, F. C. Jr. Cancer nanomedicines: so many papers and so few drugs! *Adv. Drug Deliv. Rev.* **65**, 80–88 (2013).
20. Wilhelm, S. et al. Analysis of nanoparticle delivery to tumours. *Nat. Rev. Mater.* **1**, 16014 (2016).
21. Ojha, T. et al. Pharmacological and physical vessel modulation strategies to improve EPR-mediated drug targeting to tumors. *Adv. Drug Deliv. Rev.* **119**, 44–60 (2017).
22. Golombek, S. K. et al. Tumor targeting via EPR: strategies to enhance patient responses. *Adv. Drug Deliv. Rev.* **130**, 17–38 (2018).
23. Grippin, A. J., Sayour, E. J. & Mitchell, D. A. Translational nanoparticle engineering for cancer vaccines. *Oncoimmunology* **6**, e1290036 (2017).
24. Fan, Y. & Moon, J. J. Nanoparticle drug delivery systems designed to improve cancer vaccines and immunotherapy. *Vaccines (Basel)* **3**, 662–685 (2015).
25. Lee, I.-H. et al. Targeted chemoimmunotherapy using drug-loaded aptamer–dendrimer bioconjugates. *J. Control. Release* **155**, 435–441 (2011).
26. Conde, J. et al. Dual targeted immunotherapy via in vivo delivery of biohybrid RNAi-peptide nanoparticles to tumour-associated macrophages and cancer cells. *Adv. Funct. Mater.* **25**, 4183–4194 (2015).
27. Sato, K. et al. Spatially selective depletion of tumor-associated regulatory T cells with near-infrared photoimmunotherapy. *Sci. Transl Med.* **8**, 352ra110 (2016).
28. Zhen, Z. et al. Protein nanocage mediated fibroblast-activation protein targeted photoimmunotherapy to enhance cytotoxic T cell infiltration and tumor control. *Nano Lett.* **17**, 862–869 (2017).
29. Liu, L. et al. Combination immunotherapy of MUC1 mRNA nano-vaccine and CTLA-4 blockade effectively inhibits growth of triple negative breast cancer. *Mol. Ther.* **26**, 45–55 (2018).
30. Ochyl, L. J. et al. PEGylated tumor cell membrane vesicles as a new vaccine platform for cancer immunotherapy. *Biomaterials* **182**, 157–166 (2018).
31. Liu, Y., Hardie, J., Zhang, X. & Rotello, V. M. Effects of engineered nanoparticles on the innate immune system. *Semin. Immunol.* **34**, 25–32 (2017).
32. Wang, J. et al. Physical activation of innate immunity by spiky particles. *Nat. Nanotechnol.* **13**, 1078–1086 (2018).
33. Alshamsan, A. et al. STAT3 silencing in dendritic cells by siRNA polyplexes encapsulated in PLGA nanoparticles for the modulation of anticancer immune response. *Mol. Pharm.* **7**, 1643–1654 (2010).
34. Seth, A., Heo, M. B. & Lim, Y. T. Poly (gamma-glutamic acid) based combination of water-insoluble paclitaxel and TLR7 agonist for chemo-immunotherapy. *Biomaterials* **35**, 7992–8001 (2014).
35. Pradhan, P. et al. The effect of combined IL10 siRNA and CpG ODN as pathogen-mimicking microparticles on Th1/Th2 cytokine balance in dendritic cells and protective immunity against B cell lymphoma. *Biomaterials* **35**, 5491–5504 (2014).
36. Teo, P. Y. et al. Ovarian cancer immunotherapy using PD-L1 siRNA targeted delivery from folic acid-functionalized polyethylenimine: strategies to enhance T cell killing. *Adv. Healthc. Mater.* **4**, 1180–1189 (2015).
37. Kuai, R. et al. Dual TLR agonist nanodiscs as a strong adjuvant system for vaccines and immunotherapy. *J. Control. Release* **282**, 131–139 (2018).
38. Guo, L. et al. Combinatorial photothermal and immuno cancer therapy using chitosan-coated hollow copper sulfide nanoparticles. *ACS Nano* **8**, 5670–5681 (2014).
39. Wang, D. et al. Acid-activatable versatile micelleplexes for PD-L1 blockade-enhanced cancer photodynamic immunotherapy. *Nano Lett.* **16**, 5503–5513 (2016).
40. Kuai, R. et al. Elimination of established tumors with nanodisc-based combination chemoimmunotherapy. *Sci. Adv.* **4**, eaao1736 (2018).
41. Yang, G. et al. Smart nanoreactors for pH-responsive tumor homing, mitochondria-targeting, and enhanced photodynamic-immunotherapy of cancer. *Nano Lett.* **18**, 2475–2484 (2018).
42. Christian, D. A. & Hunter, C. A. Particle-mediated delivery of cytokines for immunotherapy. *Immunotherapy* **4**, 425–441 (2012).
43. Song, Q. et al. Tumor microenvironment responsive nanogel for the combinatorial antitumor effect of chemotherapy and immunotherapy. *Nano Lett.* **17**, 6366–6375 (2017).
44. Kong, M. et al. Biodegradable hollow mesoporous silica nanoparticles for regulating tumor microenvironment and enhancing antitumor efficiency. *Theranostics* **7**, 3276–3292 (2017).
45. Nam, J. et al. Surface engineering of inorganic nanoparticles for imaging and therapy. *Adv. Drug Deliv. Rev.* **65**, 622–648 (2013).
46. Retif, P. et al. Nanoparticles for radiation therapy enhancement: the key parameters. *Theranostics* **5**, 1030–1044 (2015).
47. Bao, Z., Liu, X., Liu, Y., Liu, H. & Zhao, K. Near-infrared light-responsive inorganic nanomaterials for photothermal therapy. *Asian J. Pharm. Sci.* **11**, 349–364 (2016).
48. Lux, F. et al. Gadolinium-based nanoparticles for theranostic MRI-radiosensitization. *Nanomedicine* **10**, 1801–1815 (2015).
49. Meir, R. et al. Nanomedicine for cancer immunotherapy: tracking cancer-specific T-cells in vivo with gold nanoparticles and CT imaging. *ACS Nano* **9**, 6363–6372 (2015).
50. Ngwa, W. et al. Targeted radiotherapy with gold nanoparticles: current status and future perspectives. *Nanomedicine* **9**, 1063–1082 (2014).
51. Bear, A. S. et al. Elimination of metastatic melanoma using gold nanoshell-enabled photothermal therapy and adoptive T Cell transfer. *PLOS ONE* **8**, e69073 (2013).
52. Toraya-Brown, S. et al. Local hyperthermia treatment of tumors induces CD8$^+$ T cell-mediated resistance against distal and secondary tumors. *Nanomedicine* **10**, 1273–1285 (2014).
53. Nam, J. et al. Chemo-photothermal therapy combination elicits anti-tumor immunity against advanced metastatic cancer. *Nat. Commun.* **9**, 1074 (2018).
54. Hwang, S. et al. Gold nanoparticle-mediated photothermal therapy: current status and future perspective. *Nanomedicine* **9**, 2003–2022 (2014).
55. Chow, J. C. L. in *Handbook of Ecomaterials* (eds Torres Martínez, L. M., Kharissova, O. V. & Kharisov, B. I.) 1–21 (Springer International Publishing, 2017).

56. Restifo, N. P., Dudley, M. E. & Rosenberg, S. A. Adoptive immunotherapy for cancer: harnessing the T cell response. *Nat. Rev. Immunol.* **12**, 269–281 (2012).
57. Kroemer, G., Galluzzi, L., Kepp, O. & Zitvogel, L. Immunogenic cell death in cancer therapy. *Annu. Rev. Immunol.* **31**, 51–72 (2013).
58. Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L. & Kroemer, G. Immunogenic cell death in cancer and infectious disease. *Nat. Rev. Immunol.* **17**, 97–111 (2017).
59. Duan, X. et al. Photodynamic therapy mediated by nontoxic core–shell nanoparticles synergizes with immune checkpoint blockade to elicit antitumor immunity and antimetastatic effect on breast cancer. *J. Am. Chem. Soc.* **138**, 16686–16695 (2016).
60. Fan, Y. et al. Immunogenic cell death amplified by co-localized adjuvant delivery for cancer immunotherapy. *Nano Lett.* **17**, 7387–7393 (2017).
61. Lu, J. et al. Nano-enabled pancreas cancer immunotherapy using immunogenic cell death and reversing immunosuppression. *Nat. Commun.* **8**, 1811 (2017).
62. Lee, E. J. et al. Nanocage-therapeutics prevailing phagocytosis and immunogenic cell death awakens immunity against cancer. *Adv. Mater.* **30**, 1705581 (2018).
63. Sahin, U. et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* **547**, 222–226 (2017).
64. Gubin, M. M. et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* **515**, 577–581 (2014).
65. Ott, P. A. et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* **547**, 217–221 (2017).
66. Rooney, M. S., Shukla, S. A., Wu, C. J., Getz, G. & Hacohen, N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* **160**, 48–61 (2015).
67. Rizvi, N. A. et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. *Science* **348**, 124–128 (2015).
68. Snyder, A. et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199 (2014).
69. Kuai, R., Ochyl, L. J., Bahjat, K. S., Schwendeman, A. & Moon, J. J. Designer vaccine nanodiscs for personalized cancer immunotherapy. *Nat. Mater.* **16**, 489–496 (2017).
70. Zhu, G. et al. Albumin/vaccine nanocomplexes that assemble in vivo for combination cancer immunotherapy. *Nat. Commun.* **8**, 1954 (2017).
71. Kuai, R. et al. Subcutaneous nanodisc vaccination with neoantigens for combination cancer immunotherapy. *Bioconjug. Chem.* **29**, 771–775 (2018).
72. Borghaei, H. et al. Nivolumab (Nivo) + platinum-doublet chemotherapy (Chemo) versus chemo as first-line (1L) treatment (Tx) for advanced non-small cell lung cancer (NSCLC) with <1% tumor PD-L1 expression: results from CheckMate 227. *J. Clin. Oncol.* **36**, 9001–9001 (2018).
73. Gandhi, L. et al. Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. *N. Engl. J. Med.* **378**, 2078–2092 (2018).
74. Paz-Ares, L. et al. Pembrolizumab plus chemotherapy for squamous non–small-cell lung cancer. *N. Engl. J. Med.* **379**, 2040–2051 (2018).
75. Schmid, P. et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N. Engl. J. Med.* **379**, 2108–2121 (2018).
76. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03036098 (2019).
77. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02807636 (2019).
78. Herbst, R. S. et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* **387**, 1540–1550 (2016).
79. Lopes, G. et al. Pembrolizumab (pembro) versus platinum-based chemotherapy (chemo) as first-line therapy for advanced/metastatic NSCLC with a PD-L1 tumor proportion score (TPS) ≥1%: open-label, phase 3 KEYNOTE-042 study [abstract]. *J. Clin. Oncol.* **36** (Suppl. 18), LBA4 (2018).
80. Pfannenstiel, L. W., Lam, S. S., Emens, L. A., Jaffee, E. M. & Armstrong, T. D. Paclitaxel enhances early dendritic cell maturation and function through TLR4 signaling in mice. *Cell. Immunol.* **263**, 79–87 (2010).
81. Machiels, J. P. et al. Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. *Cancer Res.* **61**, 3689–3697 (2001).
82. Roy, A., Singh, M. S., Upadhyay, P. & Bhaskar, S. Nanoparticle mediated co-delivery of paclitaxel and a TLR-4 agonist results in tumor regression and enhanced immune response in the tumor microenvironment of a mouse model. *Int. J. Pharm.* **445**, 171–180 (2013).
83. Heo, M. B., Kim, S. Y., Yun, W. S. & Lim, Y. T. Sequential delivery of an anticancer drug and combined immunomodulatory nanoparticles for efficient chemoimmunotherapy. *Int. J. Nanomed.* **10**, 5981–5992 (2015).
84. Lu, Y. et al. Exploiting in situ antigen generation and immune modulation to enhance chemotherapy response in advanced melanoma: a combination nanomedicine approach. *Cancer Lett.* **379**, 32–38 (2016).
85. Makkouk, A. et al. Biodegradable microparticles loaded with doxorubicin and CpG ODN for in situ immunization against cancer. *AAPS J.* **17**, 184–193 (2015).
86. Yin, Y. et al. Co-delivery of doxorubicin and interferon-gamma by thermosensitive nanoparticles for cancer immunochemotherapy. *Mol. Pharm.* **15**, 4161–4172 (2018).
87. Haque, A., Banik, N. L. & Ray, S. K. Emerging role of combination of all-*trans* retinoic acid and interferon-gamma as chemoimmunotherapy in the management of human glioblastoma. *Neurochem. Res.* **32**, 2203–2209 (2007).
88. van der Zee, J. Heating the patient: a promising approach? *Ann. Oncol.* **13**, 1173–1184 (2002).
89. Hildebrandt, B. et al. The cellular and molecular basis of hyperthermia. *Crit. Rev. Oncol. Hematol.* **43**, 33–56 (2002).
90. Evans, S. S., Repasky, E. A. & Fisher, D. T. Fever and the thermal regulation of immunity: the immune system feels the heat. *Nat. Rev. Immunol.* **15**, 335–349 (2015).
91. Dickerson, E. B. et al. Gold nanorod assisted near-infrared plasmonic photothermal therapy (PPTT) of squamous cell carcinoma in mice. *Cancer Lett.* **269**, 57–66 (2008).
92. Weissleder, R. A clearer vision for in vivo imaging. *Nat. Biotechnol.* **19**, 316–317 (2001).
93. Jung, H. S. et al. Organic molecule-based photothermal agents: an expanding photothermal therapy universe. *Chem. Soc. Rev.* **47**, 2280–2297 (2018).
94. Chen, W. R., Singhal, A. K., Liu, H. & Nordquist, R. E. Antitumor immunity induced by laser immunotherapy and its adoptive transfer. *Cancer Res.* **61**, 459–461 (2001).
95. Zhou, F. et al. InCVAX – a novel strategy for treatment of late-stage, metastatic cancers through photoimmunotherapy induced tumor-specific immunity. *Cancer Lett.* **359**, 169–177 (2015).
96. Wang, C. et al. Immunological responses triggered by photothermal therapy with carbon nanotubes in combination with anti-CTLA-4 therapy to inhibit cancer metastasis. *Adv. Mater.* **26**, 8154–8162 (2014).
97. Liu, Y. et al. Synergistic immuno photothermal nanotherapy (SYMPHONY) for the treatment of unresectable and metastatic cancers. *Sci. Rep.* **7**, 8606 (2017).
98. Abadeer, N. S. & Murphy, C. J. Recent progress in cancer thermal therapy using gold nanoparticles. *J. Phys. Chem. C* **120**, 4691–4716 (2016).
99. Chatterjee, D. K., Fong, L. S. & Zhang, Y. Nanoparticles in photodynamic therapy: an emerging paradigm. *Adv. Drug Deliv. Rev.* **60**, 1627–1637 (2008).
100. Korbelik, M. Induction of tumor immunity by photodynamic therapy. *J. Clin. Laser Med. Surg.* **14**, 329–334 (1996).
101. Castano, A. P., Mroz, P. & Hamblin, M. R. Photodynamic therapy and anti-tumour immunity. *Nat. Rev. Cancer* **6**, 535–545 (2006).
102. Buytaert, E., Dewaele, M. & Agostinis, P. Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. *Biochim. Biophys. Acta* **1776**, 86–107 (2007).
103. Skovsen, E., Snyder, J. W., Lambert, J. D. C. & Ogilby, P. R. Lifetime and diffusion of singlet oxygen in a cell. *J. Phys. Chem. B* **109**, 8570–8573 (2005).
104. Macdonald, I. J. & Dougherty, T. J. Basic principles of photodynamic therapy. *J. Porphyr. Phthalocyanines* **5**, 105–129 (2001).
105. Abrahamse, H. & Hamblin, M. R. New photosensitizers for photodynamic therapy. *Biochem. J.* **473**, 347–364 (2016).
106. Marrache, S., Tundup, S., Harn, D. A. & Dhar, S. Ex vivo programming of dendritic cells by mitochondria-targeted nanoparticles to produce interferon-gamma for cancer immunotherapy. *ACS Nano* **7**, 7392–7402 (2013).
107. Yu, X. et al. Inhibiting metastasis and preventing tumor relapse by triggering host immunity with tumor-targeted photodynamic therapy using photosensitizer-loaded functional nanographenes. *ACS Nano* **11**, 10147–10158 (2017).
108. Gao, L. et al. Enhanced anti-tumor efficacy through a combination of integrin αvβ6-targeted photodynamic therapy and immune checkpoint inhibition. *Theranostics* **6**, 627–637 (2016).
109. Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. *Nat. Rev. Cancer* **6**, 392–401 (2006).
110. Joyce, J. A. & Fearon, D. T. T cell exclusion, immune privilege, and the tumor microenvironment. *Science* **348**, 74–80 (2015).
111. Castano, A. P., Mroz, P., Wu, M. X. & Hamblin, M. R. Photodynamic therapy plus low-dose cyclophosphamide generates antitumor immunity in a mouse model. *Proc. Natl Acad. Sci. USA* **105**, 5495–5500 (2008).
112. Mroz, P. & Hamblin, M. R. The immunosuppressive side of PDT. *Photochem. Photobiol. Sci.* **10**, 751–758 (2011).
113. He, C. et al. Core-shell nanoscale coordination polymers combine chemotherapy and photodynamic therapy to potentiate checkpoint blockade cancer immunotherapy. *Nat. Commun.* **7**, 12499 (2016).
114. Hamblin, M. R. Upconversion in photodynamic therapy: plumbing the depths. *Dalton Trans.* **47**, 8571–8580 (2018).
115. Xu, J. et al. Near-infrared-triggered photodynamic therapy with multitasking upconversion nanoparticles in combination with checkpoint blockade for immunotherapy of colorectal cancer. *ACS Nano* **11**, 4463–4474 (2017).
116. Lu, K. et al. Chlorin-based nanoscale metal–organic framework systemically rejects colorectal cancers via synergistic photodynamic therapy and checkpoint blockade immunotherapy. *J. Am. Chem. Soc.* **138**, 12502–12510 (2016).
117. Zou, W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat. Rev. Cancer* **5**, 263–274 (2005).
118. Brown, J. M. Tumor hypoxia in cancer therapy. *Methods Enzymol.* **435**, 295–321 (2007).
119. Turan, I. S., Yildiz, D., Turksoy, A., Gunaydin, G. & Akkaya, E. U. A. Bifunctional photosensitizer for enhanced fractional photodynamic therapy: singlet oxygen generation in the presence and absence of light. *Angew. Chem. Int. Ed. Engl.* **55**, 2875–2878 (2016).
120. Chen, Z. et al. Bioinspired hybrid protein oxygen nanocarrier amplified photodynamic therapy for eliciting anti-tumor immunity and abscopal effect. *ACS Nano* **12**, 8633–8645 (2018).
121. Jin, C. S., Lovell, J. F., Chen, J. & Zheng, G. Ablation of hypoxic tumors with dose-equivalent photothermal, but not photodynamic, therapy using a nanostructured porphyrin assembly. *ACS Nano* **7**, 2541–2550 (2013).
122. Begg, A. C., Stewart, F. A. & Vens, C. Strategies to improve radiotherapy with targeted drugs. *Nat. Rev. Cancer* **11**, 239–253 (2011).
123. Sanche, L. Beyond radical thinking. *Nature* **461**, 358–359 (2009).
124. Gupta, A. et al. Radiotherapy promotes tumor-specific effector $CD8^+$ T cells via dendritic cell activation. *J. Immunol.* **189**, 558–566 (2012).
125. Reits, E. A. et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J. Exp. Med.* **203**, 1259–1271 (2006).
126. Deng, L. et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* **41**, 843–852 (2014).
127. Burnette, B. C. et al. The efficacy of radiotherapy relies upon induction of type I interferon–dependent innate and adaptive immunity. *Cancer Res.* **71**, 2488–2496 (2011).
128. Demaria, S. & Formenti, S. Radiation as an immunological adjuvant: current evidence on dose and fractionation. *Front. Oncol.* **2**, 153 (2012).
129. Demaria, S. et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int. J. Radiat. Oncol. Biol. Phys.* **58**, 862–870 (2004).
130. Formenti, S. C. & Demaria, S. Radiation therapy to convert the tumor into an in situ vaccine. *Int. J. Radiat. Oncol. Biol. Phys.* **84**, 879–880 (2012).
131. Schaue, D. & McBride, W. H. Opportunities and challenges of radiotherapy for treating cancer. *Nat. Rev. Clin. Oncol.* **12**, 527–540 (2015).

132. Qu, Y. et al. Gamma-ray resistance of regulatory CD4+CD25+Foxp3+T cells in mice. *Radiat. Res.* **173**, 148–157 (2010).
133. Kachikwu, E. L. et al. Radiation enhances regulatory T cell representation. *Int. J. Radiat. Oncol. Biol. Phys.* **81**, 1128–1135 (2011).
134. Ngiow, S. F., McArthur, G. A. & Smyth, M. J. Radiotherapy complements immune checkpoint blockade. *Cancer Cell* **27**, 437–438 (2015).
135. Ngwa, W. et al. Using immunotherapy to boost the abscopal effect. *Nat. Rev. Cancer* **18**, 313–322 (2018).
136. Weichselbaum, R. R., Liang, H., Deng, L. & Fu, Y. X. Radiotherapy and immunotherapy: a beneficial liaison? *Nat. Rev. Clin. Oncol.* **14**, 365–379 (2017).
137. Patel, R., Czapar, A. E., Fiering, S., Oleinick, N. L. & Steinmetz, N. F. Radiation therapy combined with cowpea mosaic virus nanoparticle in situ vaccination initiates immune-mediated tumor regression. *ACS Omega* **3**, 3702–3707 (2018).
138. Demaria, S. et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. *Clin. Cancer Res.* **11**, 728–734 (2005).
139. Deng, L. et al. Irradiation and anti–PD-L1 treatment synergistically promote antitumor immunity in mice. *J. Clin. Invest.* **124**, 687–695 (2014).
140. Twyman-Saint Victor, C. et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* **520**, 373–377 (2015).
141. Kim, Y. H. et al. In situ vaccination against mycosis fungoides by intratumoral injection of a TLR9 agonist combined with radiation: a phase 1/2 study. *Blood* **119**, 355–363 (2012).
142. Brody, J. D. et al. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. *J. Clin. Oncol.* **28**, 4324–4332 (2010).
143. Lee, Y. et al. Therapeutic effects of ablative radiation on local tumor require $CD8^+$ T cells: changing strategies for cancer treatment. *Blood* **114**, 589–595 (2009).
144. Klug, F. et al. Low-dose irradiation programs macrophage differentiation to an iNOS+/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell* **24**, 589–602 (2013).
145. Tsai, C.-S. et al. Macrophages from irradiated tumors express higher levels of iNOS, arginase-I and COX-2, and promote tumor growth. *Int. J. Radiat. Oncol. Biol. Phys.* **68**, 499–507 (2007).
146. Dewan, M. Z. et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti–CTLA-4 antibody. *Clin. Cancer Res.* **15**, 5379–5388 (2009).
147. Schaue, D., Ratikan, J. A., Iwamoto, K. S. & McBride, W. H. Maximizing tumor immunity with fractionated radiation. *Int. J. Radiat. Oncol. Biol. Phys.* **83**, 1306–1310 (2012).
148. Kim, M. S. et al. Gold nanoparticles enhance anti-tumor effect of radiotherapy to hypoxic tumor. *Radiat. Oncol. J.* **34**, 230–238 (2016).
149. Liu, Y. et al. Metal-based NanoEnhancers for future radiotherapy: radiosensitizing and synergistic effects on tumor cells. *Theranostics* **8**, 1824–1849 (2018).
150. Song, G., Cheng, L., Chao, Y., Yang, K. & Liu, Z. Emerging nanotechnology and advanced materials for cancer radiation therapy. *Adv. Mater.* **29**, 1700996 (2017).
151. Xie, J. et al. Emerging strategies of nanomaterial-mediated tumor radiosensitization. *Adv. Mater.* **0**, 1802244 (2018).
152. Ngwa, W., Dougan, S. & Kumar, R. Combining nanoparticle-aided radiation therapy with immunotherapy to enhance local and metastatic tumor cell kill during pancreatic cancer treatment. *Int. J. Radiat. Oncol. Biol. Phys.* **99**, E611–E612 (2017).
153. Lu, K. et al. Low-dose X-ray radiotherapy–radiodynamic therapy via nanoscale metal–organic frameworks enhances checkpoint blockade immunotherapy. *Nat. Biomed. Eng.* **2**, 600–610 (2018).
154. Lazzari, C. et al. Combination of immunotherapy with chemotherapy and radiotherapy in lung cancer: is this the beginning of the end for cancer? *Ther. Adv. Med. Oncol.* **10**, 1758835918762094 (2018).
155. Hwang, W. L., Pike, L. R. G., Royce, T. J., Mahal, B. A. & Loeffler, J. S. Safety of combining radiotherapy with immune-checkpoint inhibition. *Nat. Rev. Clin. Oncol.* **15**, 477–494 (2018).
156. Fire, A. et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811 (1998).
157. Bumcrot, D., Manoharan, M., Koteliansky, V. & Sah, D. W. RNAi therapeutics: a potential new class of pharmaceutical drugs. *Nat. Chem. Biol.* **2**, 711–719 (2006).
158. Davidson, B. L. & McCray, P. B. Jr. Current prospects for RNA interference-based therapies. *Nat. Rev. Genet.* **12**, 329–340 (2011).
159. Huang, L. & Liu, Y. In vivo delivery of RNAi with lipid-based nanoparticles. *Annu. Rev. Biomed. Eng.* **13**, 507–530 (2011).
160. van der Waart, A. B. et al. siRNA silencing of PD-1 ligands on dendritic cell vaccines boosts the expansion of minor histocompatibility antigen-specific $CD8^+$ T cells in NOD/SCID/IL2Rg(null) mice. *Cancer Immunol. Immunother.* **64**, 645–654 (2015).
161. Van den Bergh, J. M. et al. Monocyte-derived dendritic cells with silenced PD-1 ligands and transpresenting interleukin-15 stimulate strong tumor-reactive T cell expansion. *Cancer Immunol. Res.* **5**, 710–715 (2017).
162. Iwamura, K. et al. siRNA-mediated silencing of PD-1 ligands enhances tumor-specific human T cell effector functions. *Gene Ther.* **19**, 959–966 (2012).
163. Sheng, W. Y. & Huang, L. Cancer immunotherapy and nanomedicine. *Pharm. Res.* **28**, 200–214 (2011).
164. Zheng, X. et al. Silencing IDO in dendritic cells: a novel approach to enhance cancer immunotherapy in a murine breast cancer model. *Int. J. Cancer* **132**, 967–977 (2013).
165. Brady, M. T. et al. Down-regulation of signal transducer and activator of transcription 3 improves human acute myeloid leukemia-derived dendritic cell function. *Leuk. Res.* **37**, 822–828 (2013).
166. Luo, Z. et al. Nanovaccine loaded with poly I:C and STAT3 siRNA robustly elicits anti-tumor immune responses through modulating tumor-associated dendritic cells in vivo. *Biomaterials* **38**, 50–60 (2015).
167. Hossain, D. M. et al. TLR9-targeted STAT3 silencing abrogates immunosuppressive activity of myeloid-derived suppressor cells from prostate cancer patients. *Clin. Cancer Res.* **21**, 3771–3782 (2015).
168. Marine, J. C. et al. SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. *Cell* **98**, 609–616 (1999).
169. Kubo, M., Hanada, T. & Yoshimura, A. Suppressors of cytokine signaling and immunity. *Nat. Immunol.* **4**, 1169–1176 (2003).
170. Shen, L., Evel-Kabler, K., Strube, R. & Chen, S. Y. Silencing of SOCS1 enhances antigen presentation by dendritic cells and antigen-specific anti-tumor immunity. *Nat. Biotechnol.* **22**, 1546–1553 (2004).
171. Heo, M. B. & Lim, Y. T. Programmed nanoparticles for combined immunomodulation, antigen presentation and tracking of immunotherapeutic cells. *Biomaterials* **35**, 590–600 (2014).
172. Xu, Z., Wang, Y., Zhang, L. & Huang, L. Nanoparticle-delivered transforming growth factor-beta siRNA enhances vaccination against advanced melanoma by modifying tumor microenvironment. *ACS Nano* **8**, 3636–3645 (2014).
173. Ahmed, M. et al. Systemic siRNA nanoparticle-based drugs combined with radiofrequency ablation for cancer therapy. *PLOS ONE* **10**, e0128910 (2015).
174. Shen, L. et al. Local blockade of interleukin 10 and C-X-C motif chemokine ligand 12 with nano-delivery promotes antitumor response in murine cancers. *ACS Nano* **12**, 9830–9841 (2018).
175. Xian, J., Yang, H., Lin, Y. & Liu, S. Combination nonviral murine interleukin 2 and interleukin 12 gene therapy and radiotherapy for head and neck squamous cell carcinoma. *Arch. Otolaryngol. Head Neck Surg.* **131**, 1079–1085 (2005).
176. Duan, S. et al. Folate-modified chitosan nanoparticles coated interferon-inducible protein-10 gene enhance cytotoxic T lymphocytes' responses to hepatocellular carcinoma. *J. Biomed. Nanotechnol.* **12**, 700–709 (2016).
177. Ulmer, J. B. & Geall, A. J. Recent innovations in mRNA vaccines. *Curr. Opin. Immunol.* **41**, 18–22 (2016).
178. Fotin-Mleczek, M. et al. Highly potent mRNA based cancer vaccines represent an attractive platform for combination therapies supporting an improved therapeutic effect. *J. Gene Med.* **14**, 428–439 (2012).
179. Fotin-Mleczek, M. et al. mRNA-based vaccines synergize with radiation therapy to eradicate established tumors. *Radiat. Oncol.* **9**, 180 (2014).
180. Siegler, E. L., Kim, Y. J. & Wang, P. Nanomedicine targeting the tumor microenvironment: therapeutic strategies to inhibit angiogenesis, remodel matrix, and modulate immune responses. *J. Cell. Immunother.* **2**, 69–78 (2016).
181. Shi, K., Haynes, M. & Huang, L. Nanovaccines for remodeling the suppressive tumor microenvironment: New horizons in cancer immunotherapy. *Front. Chem. Sci. Eng.* **11**, 676–684 (2017).
182. Marabelle, A., Tselikas, L., de Baere, T. & Houot, R. Intratumoral immunotherapy: using the tumor as the remedy. *Ann. Oncol.* **28**, xii33–xii43 (2017).
183. Aznar, M. A. et al. Intratumoral delivery of immunotherapy—act locally, think globally. *J. Immunol.* **198**, 31–39 (2017).
184. Ishihara, J. et al. Matrix-binding checkpoint immunotherapies enhance antitumor efficacy and reduce adverse events. *Sci. Transl Med.* **9**, eaan0401 (2017).
185. Raavé, R., van Kuppevelt, T. H. & Daamen, W. F. Chemotherapeutic drug delivery by tumoral extracellular matrix targeting. *J. Control. Release* **274**, 1–8 (2018).
186. Dai, Q. et al. Quantifying the ligand-coated nanoparticle delivery to cancer cells in solid tumors. *ACS Nano* **12**, 8423–8435 (2018).
187. Jiang, W. et al. Designing nanomedicine for immuno-oncology. *Nat. Biomed. Eng.* **1**, 0029 (2017).
188. Ordikhani, F. et al. Targeting antigen-presenting cells by anti–PD-1 nanoparticles augments antitumor immunity. *JCI Insight* **3**, 122700 (2018).
189. Liu, X. et al. CD47 blockade triggers T cell–mediated destruction of immunogenic tumors. *Nat. Med.* **21**, 1209–1215 (2015).
190. Gibney, G. T., Weiner, L. M. & Atkins, M. B. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol.* **17**, e542–e551 (2016).
191. Zheng, Y., Tang, L., Mabardi, L., Kumari, S. & Irvine, D. Enhancing adoptive cell therapy of cancer through targeted delivery of small-molecule immunomodulators to internalizing or noninternalizing receptors. *Acs Nano* **11**, 3089–3100 (2017).
192. Tang, L. et al. Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery. *Nat. Biotechnol.* **36**, 707–716 (2018).
193. Predina, J. et al. Changes in the local tumor microenvironment in recurrent cancers may explain the failure of vaccines after surgery. *Proc. Natl Acad. Sci. USA* **110**, 1582–1583 (2013).
194. Sharma, P. & Allison, J. P. The future of immune checkpoint therapy. *Science* **348**, 56–61 (2015).
195. Wing, K. et al. CTLA-4 control over $Foxp3^+$ regulatory T cell function. *Science* **322**, 271–275 (2008).
196. Peggs, K. S., Quezada, S. A., Chambers, C. A., Korman, A. J. & Allison, J. P. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti–CTLA-4 antibodies. *J. Exp. Med.* **206**, 1717–1725 (2009).
197. Curiel, T. J. et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat. Med.* **9**, 562–567 (2003).
198. Zou, W., Wolchok, J. D. & Chen, L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. *Sci. Transl Med.* **8**, 328rv324 (2016).
199. Dong, H. et al. Tumor-associated B7-H1 promotes T cell apoptosis: a potential mechanism of immune evasion. *Nat. Med.* **8**, 793 (2002).
200. Hodi, F. S. et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
201. Schadendorf, D. et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J. Clin. Oncol.* **33**, 1889–1894 (2015).
202. Topalian, S. L. et al. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. *N. Engl. J. Med.* **366**, 2443–2454 (2012).
203. Garon, E. B. et al. Pembrolizumab for the treatment of non–small-cell lung cancer. *N. Engl. J. Med.* **372**, 2018–2028 (2015).
204. Webster, R. M. The immune checkpoint inhibitors: where are we now? *Nat. Rev. Drug Discov.* **13**, 883 (2014).
205. Marin-Acevedo, J. A. et al. Next generation of immune checkpoint therapy in cancer: new developments and challenges. *J. Hematol. Oncol.* **11**, 39 (2018).
206. Chen, Daniel, S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1–10 (2013).
207. Zanetti, M. Tapping CD4 T cells for cancer immunotherapy: the choice of personalized genomics. *J. Immunol.* **194**, 2049–2056 (2015).

208. Im, S. J. et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* **537**, 417–421 (2016).
209. Ribas, A. et al. PD-1 blockade expands intratumoral memory T cells. *Cancer Immunol. Res.* **4**, 194–203 (2016).
210. Takeuchi, Y. et al. Clinical response to PD-1 blockade correlates with a sub-fraction of peripheral central memory CD4+ T cells in patients with malignant melanoma. *Int. Immunol.* **30**, 13–22 (2018).
211. Bauer, C. A. et al. Dynamic Treg interactions with intratumoral APCs promote local CTL dysfunction. *J. Clin. Invest.* **124**, 2425–2440 (2014).
212. Munn, D. H., Sharma, M. D., Johnson, T. S. & Rodriguez, P. I. D. O. PTEN-expressing Tregs and control of antigen-presentation in the murine tumor microenvironment. *Cancer Immunol. Immunother.* **66**, 1049–1058 (2017).
213. Ni, X., Langridge, T. & Duvic, M. Depletion of regulatory T cells by targeting CC chemokine receptor type 4 with mogamulizumab. *Oncoimmunology* **4**, e1011524 (2015).
214. Gabrilovich, D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. *Nat. Rev. Immunol.* **4**, 941–952 (2004).
215. Perrot, I. et al. Dendritic cells infiltrating human non-small cell lung cancer are blocked at immature stage. *J. Immunol.* **178**, 2763–2769 (2007).
216. Kretz-Rommel, A. et al. In vivo targeting of antigens to human dendritic cells through DC-SIGN elicits stimulatory immune responses and inhibits tumor growth in grafted mouse models. *J. Immunother.* **30**, 715–726 (2007).
217. Macri, C., Dumont, C., Johnston, A. P. R. & Mintern, J. D. Targeting dendritic cells: a promising strategy to improve vaccine effectiveness. *Clin. Transl Immunol.* **5**, e66 (2016).
218. Dahlberg, C. I. M., Sarhan, D., Chrobok, M., Duru, A. D. & Alici, E. Natural killer cell-based therapies targeting cancer: possible strategies to gain and sustain anti-tumor activity. *Front.Immunol.* **6**, 605 (2015).
219. Zamai, L. et al. NK cells and cancer. *J. Immunol.* **178**, 4011–4016 (2007).
220. Vanherberghen, B. et al. Classification of human natural killer cells based on migration behavior and cytotoxic response. *Blood* **121**, 1326–1334 (2013).
221. Munn, D. H. & Bronte, V. Immune suppressive mechanisms in the tumor microenvironment. *Curr. Opin. Immunol.* **39**, 1–6 (2016).
222. Gonda, K. et al. Myeloid-derived suppressor cells are increased and correlated with type 2 immune responses, malnutrition, inflammation, and poor prognosis in patients with breast cancer. *Oncol. Lett.* **14**, 1766–1774 (2017).
223. Genard, G., Lucas, S. & Michiels, C. Reprogramming of tumor-associated macrophages with anticancer therapies: radiotherapy versus chemo- and immunotherapies. *Front. Immunol* **8**, 828 (2017).
224. Arlauckas, S. P. et al. In vivo imaging reveals a tumor-associated macrophage–mediated resistance pathway in anti–PD-1 therapy. *Sci. Transl Med.* **9**, eaal3604 (2017).
225. Scodeller, P. et al. Precision targeting of tumor macrophages with a CD206 binding peptide. *Sci. Rep.* **7**, 14655 (2017).
226. Fujimura, T., Kambayashi, Y., Fujisawa, Y., Hidaka, T. & Aiba, S. Tumor-associated macrophages: therapeutic targets for skin cancer. *Front. Oncol.* **8**, 3 (2018).

Acknowledgements
This work was supported in part by the US National Institutes of Health (NIH) (R01AI127070, R01EB022563, R01CA210273, R01CA223804 and U01CA210152), the Michigan Translational Research and Commercialization (MTRAC) for Life Sciences Hub, a University of Michigan (UM) Forbes Institute for Cancer Discovery Pilot Grant and the Emerald Foundation. J.J.M. is a Young Investigator supported by the Melanoma Research Alliance (348774), the US Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP) Peer Reviewed Cancer Research Program (W81XWH-16-1-0369) and a US National Science Foundation (NSF) CAREER Award (1553831). C.P. acknowledges financial support from the UM TEAM Training Program (DE007057 from the US National Institute of Dental and Craniofacial Research (NIDCR)). Opinions interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the DoD.

Author contributions
J.N., S.S. and J.J.M. discussed content, researched data and wrote the manuscript. K.S.P. aided in the figure design and prepared the table. W.Z. and L.D.S. contributed to the revision of the manuscript. All authors reviewed and edited the manuscript.

Competing interests
A patent application for nanodisc technology has been filed with J.J.M. as an inventor, and J.J.M. is a co-founder of EVOQ Therapeutics, which develops nanodisc technology for cancer immunotherapy. All other authors declare no competing interests.