

Improving cancer immunotherapy through nanotechnology

Michael S. Goldberg 

Abstract | The 2018 Nobel Prize in Physiology or Medicine was awarded to pioneers in the field of cancer immunotherapy, as the utility of leveraging a patient's coordinated and adaptive immune system to fight the patient's unique tumour has now been validated robustly in the clinic. Still, the proportion of patients who respond to immunotherapy remains modest (~15% objective response rate across indications), as tumours have multiple means of immune evasion. The immune system is spatiotemporally controlled, so therapies that influence the immune system should be spatiotemporally controlled as well, in order to maximize the therapeutic index. Nanoparticles and biomaterials enable one to program the location, pharmacokinetics and co-delivery of immunomodulatory compounds, eliciting responses that cannot be achieved upon administration of such compounds in solution. The convergence of cancer immunotherapy, nanotechnology, bioengineering and drug delivery is opportune, as each of these fields has matured independently to the point that it can now be used to complement the others substantively and rationally, rather than modestly and empirically. As a result, unmet needs increasingly can be addressed with deductive intention. This Review explores how nanotechnology and related approaches are being applied to augmenting both endogenous leukocytes and adoptively transferred ones by informing specificity, influencing localization and improving function.

Therapeutic index

The ratio of efficacy to safety, which compares the amount of a drug that produces the therapeutic effect to the amount that causes toxicity.

Immune tolerance

A state of unresponsiveness by the immune system to antigens that allows for discrimination of self from non-self and is inappropriately fostered by tumours.

Area under the curve (AUC)

A pharmacokinetic parameter that represents total drug exposure by describing a quantitative relationship between drug concentration in the blood and time.

STIMIT, Cambridge, MA, USA.

e-mail: michael@stimittx.com

<https://doi.org/10.1038/s41568-019-0186-9>

Cancer immunotherapy is transforming the field of oncology, both philosophically and practically. Clinical data — most notably involving antibodies that mediate immune checkpoint blockade (ICB) — have established definitively that cancer can be treated very effectively without drugging cancer cells¹. Indeed, immunotherapy can confer superior outcomes relative to previous standards of care, whether these consisted of molecular targeted therapy² or cytotoxic chemotherapy³. As a consequence, whereas most approaches over the past 100 years have focused on treating cancer as a cell-autonomous disease, there is strong emerging interest in understanding the tumour immune microenvironment and how it impacts the response to therapy⁴.

Although certain tumours are moderately responsive to immunotherapy — including melanoma and microsatellite instable cancers (~35% objective response rate) — response rates for the majority of indications generally remain low⁵, as tumours exhibit primary, adaptive, and acquired mechanisms of resistance⁶. The therapeutic index is amongst the most relevant considerations for improving patient outcomes. Currently, dosing can be limited by the induction of autoimmune-type pathologies, as only a small percentage of the administered dose specifically acts on the leukocytes of interest at the site of interest. The ability

to break local immune tolerance without breaking systemic immune tolerance would meaningfully change patient outcomes.

Recent developments in the fields of nanotechnology and bioengineering afford new approaches⁷ that can dramatically improve not only the safety but also the efficacy of cancer immunotherapy⁸. Engineering methods can complement the biological sciences with the tools and principles of the physical sciences, improving diagnostics to monitor biomarkers as well as the development and delivery of therapeutics⁹. Biomaterials and related drug delivery solutions enable the action of potent payloads to be focused on particular cell types and at particular anatomical locations.

The consequences of these programmed interventions are noteworthy, as context matters. It is as though we have been using the framework of Newtonian gravity for the administration of cancer immunotherapy, but now we can begin to consider a framework akin to that of general relativity, wherein space–time is not constant. That is, just as location and speed affect how matter is perceived, enhancing maximum serum concentration (Cmax) in a given compartment and extending the area under the curve (AUC) of drug exposure can alter how the immune system perceives and responds to immunomodulatory agents.

Immunoengineering

A burgeoning field that incorporates the tools and approaches of bioengineering, materials science, nanotechnology, drug delivery and immunology to influence the immune system — particularly, to elicit robust antitumour immune responses — in a manner not achieved by the administration of the same molecules in solution.

Pattern recognition receptors

Host sensors that can detect molecules associated with pathogens and/or cellular damage, thus inducing innate immunity, typically through the production of pro-inflammatory cytokines.

Liposomes

Spherical vesicles composed of at least one lipid bilayer that are often used to entrap and deliver therapeutics.

Enhanced permeability and retention

(EPR). A proposed effect that suggests that molecules and particles of a certain size concentrate in tumours more so than in other tissues, owing to the leaky vasculature and poor lymphatic drainage of solid tumours.

This Review will summarize some of the recent advances in immunoengineering, focusing on widening the therapeutic index, augmenting adoptive-cell therapy, enhancing endogenous immune responses, improving vaccination, increasing nucleic acid uptake, and leveraging perioperative immunotherapy. Finally, the article will describe the first clinical trials involving nanotechnology-enhanced immunotherapy, offer perspectives on modelling immunity, and conclude with thoughts on the exciting future of this burgeoning field.

Widening the therapeutic window

When galvanized, the immune system is an extremely potent force that can mediate not only robust killing of cancer cells but also lifelong autoimmunity. Indeed, immune-related adverse events (irAEs) — such as the induction of colitis, myocarditis, pneumonitis and other self-directed inflammatory processes — are commonly observed clinical morbidities associated with cancer immunotherapy¹⁰. Systemic administration of immuno-modulatory compounds can disrupt the homeostatic functions of immune cells at nontarget sites¹¹. Notably, drug delivery technologies can focus the action of immunostimulatory payloads on particular cells or tissues, thereby minimizing systemic dissemination and undesirable side effects¹². Releasing small quantities of therapy over an extended period of time can lead to sustained target engagement locally rather than immediate focal saturation and subsequent dispersion of a compound.

Local retention is highly desirable. Notably, even in the context of local administration, extended release of immunotherapy from particles or matrices confers vastly superior safety and efficacy relative to administration of the same compounds in solution. Intratumoural or peritumoural injection of naked cytokines, antibodies or agonists of pattern recognition receptors (for example, Toll-like receptors, TLRs) results in meaningful systemic exposure as well as toxicity, as the drugs diffuse away rapidly^{12,13}. Such findings have been observed in both preclinical and clinical studies. Recombinant cytokines injected intratumourally into patients' lesions can be detected systemically within 30 minutes^{14,15}, and this manifests as toxicities that equal those following systemic injection¹⁶.

Local retention can also be important for biologic immunotherapies that modulate adaptive immunity. Antibodies that mediate ICB can be retained at the site of injection by conjugating to them super-affinity peptides that bind to components of extracellular matrix (ECM)¹⁷. This modification reduces levels of antibody in the circulation, decreasing systemic side effects. Peritumoural administration of such modified antibodies extended survival relative to unmodified antibodies in two aggressive genetically engineered mouse models of breast cancer and melanoma.

Using a nanoparticle carrier (or nanocarrier), it was shown that the anchoring of agonist anti-CD137 (a co-stimulatory molecule also known as 4-1BB) and an IL-2-Fc domain fusion protein to appropriately sized liposomes restricted the distribution of the biologics to

the tumour parenchyma and tumour-draining lymph nodes — where T cells are primed by antigen-presenting cells (APCs) — following local administration into mice, preventing leakage into the circulation¹⁸. A majority of the established primary tumours were cured, in the absence of the lethal inflammatory toxicity that was associated with intratumoural injection of an equivalent dose of the same immunotherapy combination delivered in solution.

Safer systemic administration. Notably, intravenous injection is much more readily translated to the clinic than intratumoural injection, as the majority of tumours are not readily accessible for local injection. Recently, it was shown that, even if administered systemically, such anchored liposomes could achieve a similar widening of the therapeutic window, providing antitumour efficacy in the absence of debilitating systemic toxicity¹⁹. The particles rapidly accumulated in tumours to limit systemic exposure in multiple syngeneic mouse models of cancer. Of note, the enhanced permeability and retention (EPR) effect that has been proposed as a means by which such accumulation of particles in tumours could occur may not be as relevant in human tumours, which develop over the course of years rather than days, and thus may not exhibit as leaky a vasculature.

Of note, whereas nanoparticles containing cancer cell-intrinsic drugs must deliver their cargo to the vast majority of cancer cells within a tumour in order to confer meaningful benefit, even a modest accrual of immunotherapy-loaded particles can elicit robust antitumour responses. Immune cells can proliferate as well as propagate the response by activating complementary immune cells. This inherent signal amplification may obviate the need for a substantial EPR effect in patients to realize efficacy in the context of immunoengineering; still, it remains the case that any particles that do not accrue within the tumour can lead to systemic toxicity, and one must be mindful that humans are considerably more sensitive to such toxicity than are mice.

A recent study further highlights how immunotherapies can be engineered to be safer and more effective upon systemic administration, even without a carrier. A collagen-binding domain was conjugated or fused to antibodies mediating ICB or IL-2, respectively, to concentrate these biologics at exposed tumour stroma collagen upon intravenous injection into mice²⁰. Not only were the hepatotoxicity and pulmonary oedema associated with the antibodies and cytokines, respectively, abolished in the treated mice, but also eradication of tumours was much more pronounced than that achieved upon treatment with unmodified biologics. Because the target protein is expressed on many tissues, the targeting is thought to have been achieved by leveraging the increased accessibility afforded by the greater permeability of tumour vasculature. Evaluating this approach in autochthonous models rather than implantable tumour models, whose vasculature might not be representative of naturally developing disease, would be beneficial.

Together, these studies reveal that the physical localization of immunotherapy in solution at tumour sites is insufficient to prevent systemic adverse events; extended

local release is needed and can be achieved by formulating immunotherapies in lipid and/or polymeric carriers, as well as by conjugating ECM-binding peptides.

Augmenting adoptive-cell therapy

Nanotechnology can also be applied ex vivo, with particular utility in the activation and expansion of T cells prior to their adoptive transfer (FIG. 1). Adoptive-cell therapy is becoming an increasingly important component of the antitumour arsenal: examples include tumour-infiltrating lymphocytes (TILs)^{21,22}, transgenic T cell receptor (TCR) products²³ and chimeric antigen receptor (CAR) T cells — which have been approved by the US Food and Drug Administration (FDA) for the treatment of B cell acute lymphoblastic leukaemia (tisagenlecleucel)²⁴ and B cell non-Hodgkin lymphoma (tisagenlecleucel and axicabtagene ciloleucel)^{25,26}. Notably, the standard protocol for manufacturing autologous T cells for adoptive transfer involves the use of spherical superparamagnetic polymer particles coated with antibodies that agonize CD3 and antibodies that agonize CD28^{27,28} (DynabeadsTM), which are first-generation artificial APCs (aAPCs).

aAPCs enrich T cell quantity and quality. Despite the success of autologous T cell manufacture to date, engineers continue to develop approaches that can improve the yield and function of cultured T cells. The application of a magnetic field to iron-dextran nanoparticles functionalized with proteins that activate T cells promotes the aggregation of these aAPCs, thereby clustering the TCRs to which they have bound and increasing T cell activation²⁹. Such particles, which involve antigen-specific (major histocompatibility complex (MHC)-peptide) activation rather than polyclonal (anti-CD3) activation, can further be used to enrich and expand rare tumour-specific T cells via magnetic column-mediated separation from bulk leukocyte populations³⁰ (FIG. 1a). Because antigen-specific T cells are often present at low frequencies, the ability to enhance the quantity of these desired cells is beneficial. The aAPC size, concentration, ligand density and ligand choice strongly influence the recovery, activation and expansion of such rare T cell populations³¹.

Engineering the shapes³², sizes³³ and stimuli³⁴ of aAPCs also impacts the quality of T cells for adoptive-cell therapy. Tuning the spatial arrangement and diffusivity of surface factors can promote the formation of immunological synapses and receptor clustering³⁵. Indeed, mimicking the natural antigen presentation context of physiological APCs can greatly improve the antitumour properties of the T cell product to be infused.

In addition to particle-based aAPCs³⁶, carbon nanotubes³⁷ and polymer composites thereof³⁸ have been used to great effect, leveraging the high surface area of these material systems. Recently, mesoporous silica rods, which similarly boast high surface area and enable sustained release of paracrine cues, were coated with a fluid lipid bilayer to allow for presentation of TCR stimulatory and co-stimulatory factors³⁹. This APC-mimetic scaffold substantially increased the expansion of antigen-specific or polyclonal T cell populations relative to Dynabeads,

without compromising the function of the adoptively transferred cells (FIG. 1c).

'Backpacks' to battle solid tumours. T cells can also be directly modified through conjugation of immunotherapy-loaded nanoparticles ('backpacks') to their surfaces (FIG. 1d). This approach can be used to release payloads in an autocrine-like manner to augment the function of the adoptively transferred T cells themselves^{40,41}, or to release payloads in a paracrine-like manner to modify the tumour microenvironment (TME)⁴². Both biologics and small molecules can be incorporated into the lipid-based particles. The former can be entrapped much more efficiently in liposomes³⁹, as they reside in the aqueous core that constitutes the majority of the liposomes' volume, whereas hydrophobic small molecules are restricted to the lipid bilayer and are thus well-suited to multilayer lipid nanocapsules⁴². Exemplary payloads include cytokines such as IL-21³⁹ and cytotoxic drugs such as SN-38 (the active metabolite of irinotecan)⁴². Unlike nanoparticles, which are passive entities, T cells actively migrate down chemokine gradients and can concentrate their payloads in mouse tumours by two orders of magnitude relative to free nanoparticles⁴². Notably, backpacks can be conjugated to the surface of any leukocyte population, so this approach may have broad utility once allogeneic cell products are realized.

Despite the tremendous potential of adoptive-cell therapy, success to date has been relatively limited in the absence of immunoengineering approaches such as backpacking. TIL-based therapy — in which TILs are recovered from patient tumours, expanded ex vivo and then reinfused — has generally been limited to patients with melanoma⁴³. Although there are creative ways to improve and extend TIL therapy⁴³, the approach is inherently autologous and therefore challenging to scale. In addition, CAR-based therapy has proven considerably less effective against solid tumours than against haematological malignancies⁴⁴. The former generally do not express targets specific only to cancer cells and nonessential tissues, such as CD19, so that targeting of antigens shared by tumours and healthy tissues poses substantial risks related to toxicity⁴⁵.

Although CAR- and TCR-based therapies have the potential to become allogeneic^{46,47}, the concentrated immunosuppressive microenvironment of solid tumours presents another formidable obstacle for any adoptive-cell therapy, and the immunoengineering approaches outlined in this section could help transferred cells overcome this harsh milieu. Backpacks can boost T cell functionality transiently, while genetic approaches can be used not only to alter specificity but also to define the cellular outputs. Synthetic biology — particularly through the use of logic gates — can improve the discrimination of cancer cells, promote persistence and immunological memory, and enable the inducible expression of pro-inflammatory cytokines, antibodies that mediate ICB and/or CAR constructs specifically within the TME⁴⁸. Notably, 'genetic' approaches need not be at the DNA level, as mRNA can be transfected into T cells to enable transient expression, as exemplified by a mesothelin-targeting CAR in a phase I trial⁴⁹. Notwithstanding, it

Mesoporous silica rods

A biomaterial that exhibits a high aspect ratio, enabling spontaneous assembly to form a three-dimensional microenvironment for host immune cells.

Synthetic biology

An intersection of biotechnology and molecular biology in which biological modules and systems are devised and created, with a particular emphasis on the incorporation of logic gates and other computer-like operations involving inputs, signal integration and outputs.

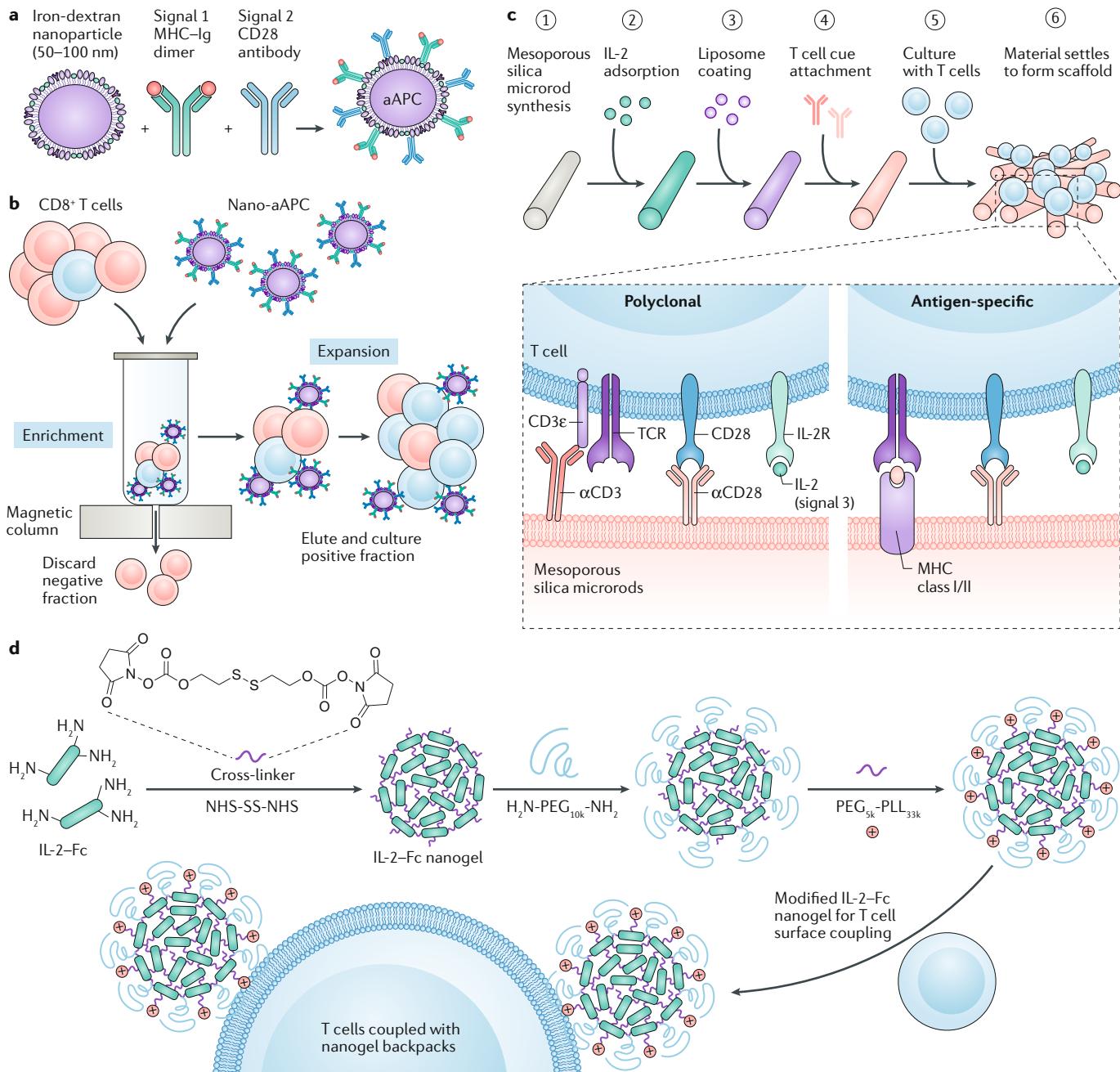


Fig. 1 | Nanotechnology can substantially improve the utility of adoptive-cell therapy. **a** Artificial antigen-presenting cells (aAPCs) can enrich and prime antigen-specific T cells. Briefly, aAPCs can be generated by coupling a major histocompatibility complex (MHC)-immunoglobulin (Ig) dimer (signal 1) and a co-stimulatory CD28 antibody (signal 2) to an iron-dextran nanoparticle. **b** Using magnetic columns, antigen-specific CD8⁺ T cells bound to aAPCs are enriched and separated from bulk leukocyte populations. **c** In addition to nanoparticles, other T cell expansion materials include nanotubes and mesoporous silica microrods (MSRs), which can be coated with fluid lipid bilayers to mimic the native dynamics of the immune synapse. For example, scaffolds that mimic APCs are synthesized from MSRs as shown. To enable polyclonal T cell expansion, activating antibodies against CD3 (α CD3) and CD28 (α CD28) are attached to the liposome-coated MSRs to act as T cell cues. For antigen-specific T cell expansion, peptide-loaded MHC and α CD28 are attached. In both contexts, interleukin 2 (IL-2) (a key regulator of T cell differentiation and proliferation) adsorbed to the MSRs is released over time, leading to paracrine-like delivery of such cytokines (signal 3) to the co-cultured T cells. **d** ‘Backpacks’ provide autocrine-like delivery of stimulatory cytokines, augmenting the function of T cells upon their entry into the immunosuppressive tumour microenvironment. Briefly, redox-responsive IL-2-Fc nanogels (NGs) prepared via chemical crosslinking are backpacked to the plasma membrane of adoptively transferred T cells. Upon tumour recognition, the T cells are activated, leading to a change in cell surface reduction potential that triggers release of their cytokine cargo. This controlled delivery method reduces the adverse events associated with using systemic IL-2 in conjunction with adoptively transferred T cells. PEG, polyethylene glycol; PLL, poly-L-lysine; TCR, T cell receptor. Parts **a** and **b** are adapted with permission from REF.³⁰, American Chemical Society. Part **c** is adapted with permission from REF.³⁹, Springer Nature Limited. Part **d** is adapted with permission from REF.¹⁰⁸, Springer Nature Limited, and from REF.¹⁴², Royal Society of Chemistry.

Lyophilization

Also known as freeze drying, a process in which water is removed from a sample under vacuum via sublimation following freezing.

Adjuvant

A molecule that augments immune responses to antigens (for example, Toll-like receptor agonists).

is important to consider that relatively simple considerations can greatly supplement sophisticated solutions. For example, *ex vivo* culturing conditions strongly impact the homing ability⁵⁰ and antitumour function⁵¹ of adoptively transferred T cells, so the components of culturing media should similarly be engineered so as to maximize efficacy.

Enhancing endogenous immune responses

T cells as targets and vectors. Off-the-shelf, acellular approaches can complement cell therapies, and may even be preferable, as the latter can be costly and cumbersome to manufacture. Technologies that enable the manipulation of endogenous leukocytes *in situ* are thus desirable and emerging (FIG. 2). In one example, nanoparticles carrying DNA were targeted to T cells in the blood of mice, leading to the expression of a defined leukaemia-specific CAR by the polyclonal population⁵² (FIG. 2b). The nanoparticle-programmed CAR T cells produced tumour regressions similar to those achieved using traditional adoptive CAR T cell therapy. Notably, the formulation was demonstrated to be functional following lyophilization and subsequent reconstitution, which is highly relevant to clinical translation but not often shown in preclinical studies.

The ability to transiently manipulate the function of endogenous T cells pharmacologically is similarly attractive and potentially safer. Such an approach could involve ‘backpacks’ that bind *in vivo* rather than *ex vivo*. Nanoparticles targeted to receptors expressed on the surfaces of particular subsets of leukocytes (for example, CD8) have been shown to bind to immune cells in the blood, lymphoid tissues and tumours of mice⁵³. Unlike epithelial cancer cells, immune cells are present in very high numbers in the circulating blood, so the effective concentration of target is very high for receptor engagement following intravenous administration. This difference likely accounts for why immune cells are efficiently targeted with such particles, whereas the many previous attempts to target cancer cells directly with nanoparticles have proven rather ineffective⁵⁴. Functionalizing nanoparticles with anti-programmed cell death protein 1 (PD1) on their surface enabled the targeting of particle-loaded transforming growth factor β receptor 1 (TGFβR1) inhibitor to PD1⁺ T cells in an autocrine-like manner. This intervention extended the survival of tumour-bearing mice, whereas free anti-PD1 in combination with free TGFβR1 inhibitor or TGFβR1 inhibitor loaded into untargeted particles had no effect at the doses administered⁵³ (FIG. 2c).

Many current immunotherapies act on TILs, and the dearth of such TILs in many solid tumours represents a major challenge⁵⁵. Converting ‘cold’ (non-T-cell-inflamed) TMEs into ‘hot’ (T cell-inflamed) ones can sensitize them to subsequent ICB⁵⁶. The aforementioned particles targeted to specific subsets of endogenous leukocytes were thus used to deliver an agonist of innate immunity to the TME in a paracrine-like manner via targeted PD1⁺ T cells⁵³. Notably, PD1 is a marker that identifies the tumour-reactive repertoire of CD8⁺ T cells in both the peripheral blood⁵⁷ and tumours⁵⁸ of human patients. Delivery of resiquimod — a TLR7 and TLR8

agonist that acts on myeloid cells but not on the targeted T cells — promoted the infiltration of CD8⁺ T cells into a cold TME, prolonged the survival of mice bearing syngeneic colorectal tumours as a monotherapy and sensitized such tumours to subsequent ICB⁵³.

Reprogramming immunosuppressive macrophages.

Repolarizing or eliminating regulatory immune cells represents a complementary approach to the direct stimulation of effector antitumour immune cells. Tumour-associated macrophages (TAMs) are an appealing target cell population for such endeavours, as these cells possess the plasticity to foster transition from a pro-tumour M2-like phenotype to an antitumour M1-like phenotype; moreover, macrophages readily internalize particles, as they are natural phagocytes, greatly improving uptake efficiency⁵⁹.

Whereas active targeting can be achieved by conjugating ligands that bind to the mannose receptor (CD206), which is highly expressed on M2-like macrophages, passive targeting can readily be achieved by tuning the charge, size, shape and stiffness of the drug-loaded particles⁶⁰. In one example, nanoparticles prepared from β-cyclodextrin were used to deliver the innate immune agonist resiquimod to TAMs in mice, resulting in polarization to IL-12-expressing M1-like macrophages within the TME⁶¹ (FIG. 2d). β-Cyclodextrin was used to solubilize the payload, to achieve high drug loading and to promote macrophage avidity. The monotherapy prevented tumour outgrowth for at least one week following even a single intravenous injection, and the nanoparticles sensitized the tumours to anti-PD1, including in the B16F10 model of melanoma, which is otherwise unresponsive to anti-PD1 therapy. Combinations that invoke both the innate and adaptive arms of the immune system are likely to enhance efficacy⁶².

Improving vaccination

Co-delivery of multiple components. Nanotechnology formulations can be used to load multiple modalities into the same formulation, whether these be small molecules, nucleic acids, polypeptides or cells. This ability to co-entrap has particular utility in the context of vaccines, as the co-encapsulation of both antigen and adjuvant in such nanovaccines ensures that both vaccine components are delivered concurrently to the same APCs (FIG. 3). This is important because the traditional approach, in which the epitope and danger signal are delivered in solution, can mount suboptimal immune responses. Specifically, those APCs that take up the antigen in the absence of the adjuvant become tolerogenic, counteracting the benefits of the intended vaccinal effects. Indeed, lipid⁶³ or polymeric⁶⁴ nanoparticles loaded or conjugated, respectively, with TLR agonists and peptide increased the number of primed T cells in mice by more than an order of magnitude, relative to administration of the same components in solution.

In addition to enabling co-delivery, the formulation of innate immune agonists can also be used to alter the biodistribution of these small molecules. Encapsulation of a stimulator of interferon genes (STING) agonist into polyethylene glycol (PEG)-ylated lipid nanoparticles

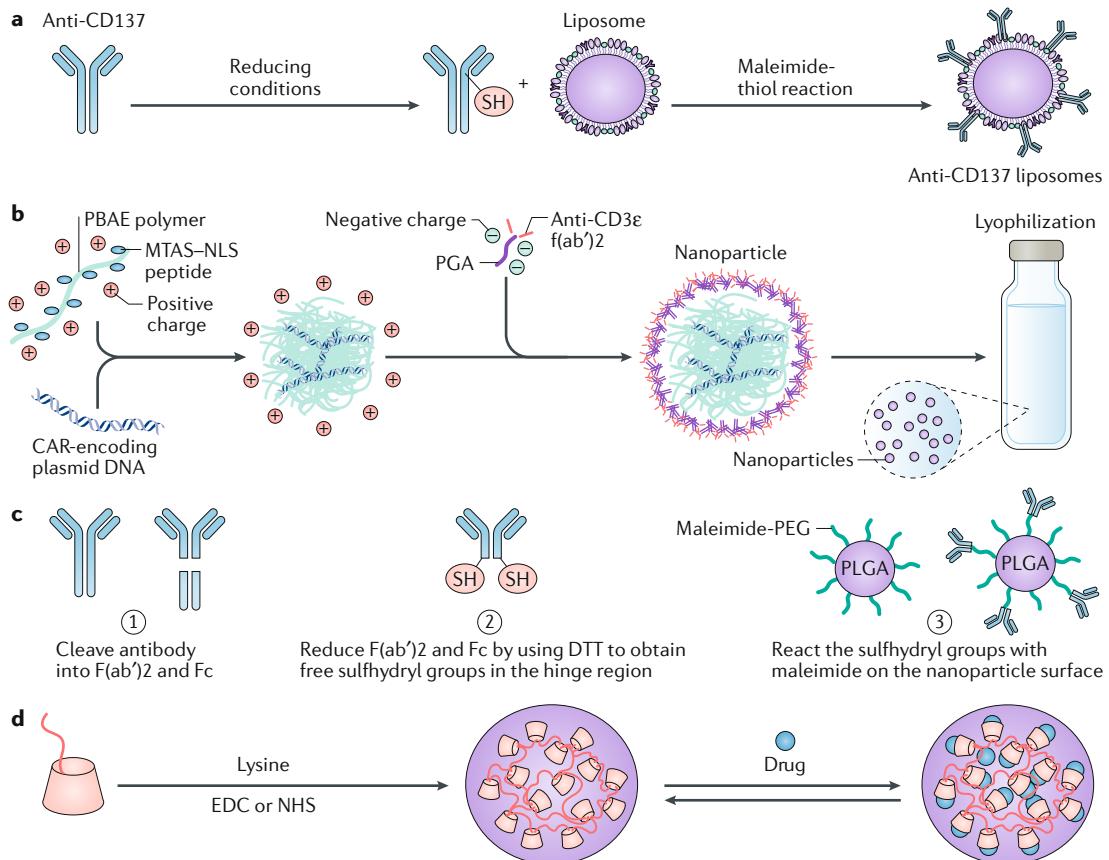
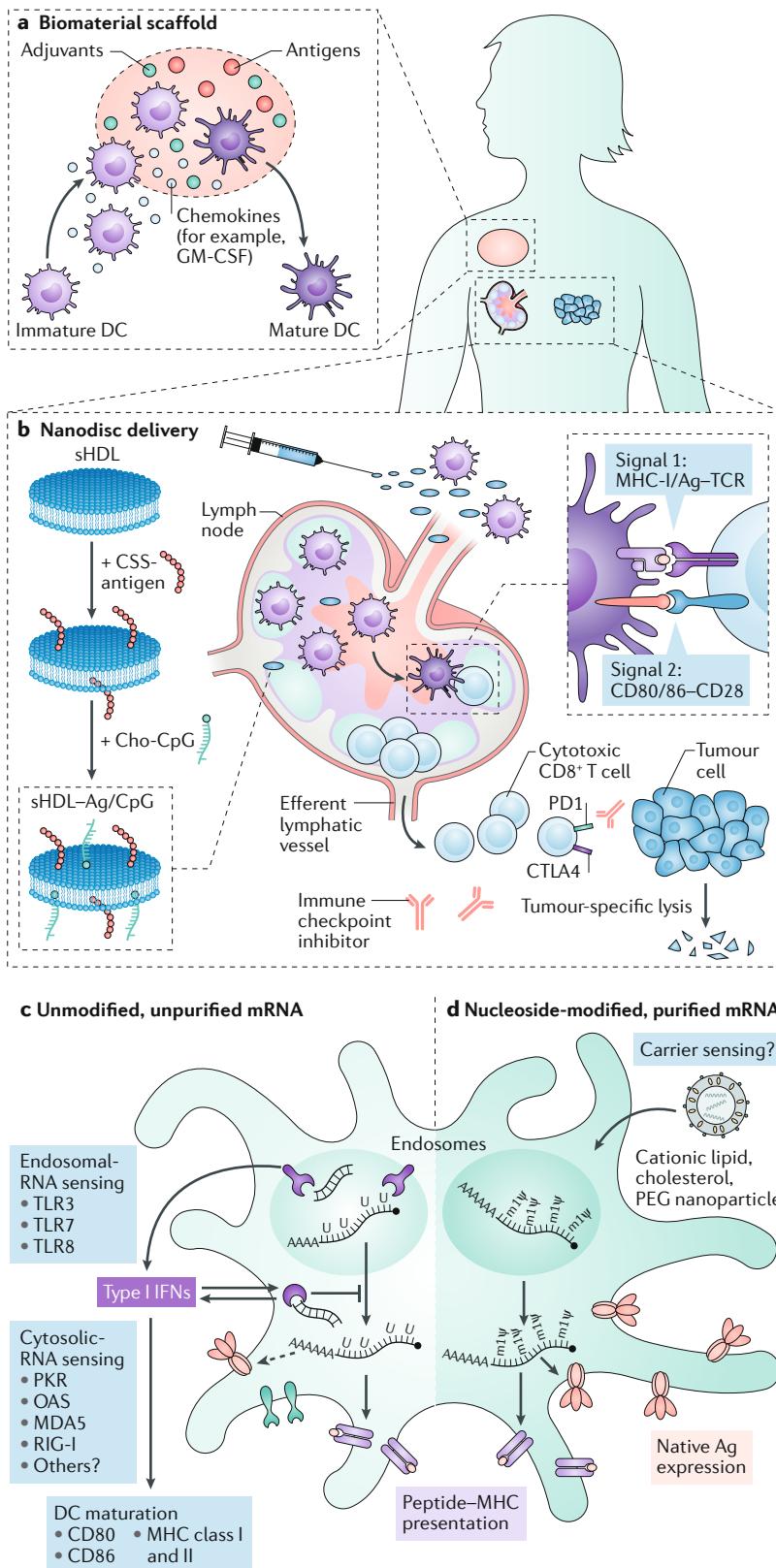


Fig. 2 | Nanoparticles can be used to modulate endogenous immune cells in situ. **a** Anchored immunoliposomes are retained locally within the tumour microenvironment to improve the therapeutic index. An example of one such immunoliposome is shown, in which a potent agonistic antibody against the co-stimulatory receptor CD137 is concentrated, retained and presented multivalently by the liposome core. **b** T cell specificity can be programmed in situ, potentially obviating the need for ex vivo manipulation of cells, which can be costly and cumbersome. An example of a DNA nanocarrier generated by encapsulating plasmid DNA in poly(β-amino ester) (PBAE)-based nanoparticles is shown in this schematic. First, to achieve effective T cell targeting, the PBAE nanoparticles are coated with antibody fragments that bind to the CD3ε chain via electrostatic interactions with polyglutamic acid (PGA). Following receptor-mediated endocytosis, the DNA cargo is transported to the T cell nucleus via the microtubule transport machinery. Such trafficking is achieved by functionalizing the PBAE polymer with a peptide containing microtubule-associated sequences (MTAS) and a nuclear localization signal (NLS). The DNA plasmid can encode various proteins of potential interest, including chimeric antigen receptors (CARs), which serve to redirect the specificity of the T cell to a tumour antigen of choice. Notably, the resulting DNA nanocarriers can be lyophilized (freeze dried) to enable stable storage that allows for future use with no change in properties or efficacy. **c** T cell functionality can be programmed in situ, by focusing the action of potent compounds on the cells of interest and/or leveraging the targeted immune cells as active delivery vehicles to concentrate the payloads within the tumour microenvironment. An example of the generation of a nanoparticle that can achieve such functionality is shown. To obtain selective T cell targeting, an antibody fragment, such as that against CD8 or programmed cell death protein 1 (PD1), is conjugated to the surface of maleimide-functionalized polyethylene glycol (PEG)-poly(lactide-co-glycolide)PLGA polymeric nanoparticles. A therapeutic payload, such as the immunomodulatory small molecule SD-208, an inhibitor of transforming growth factor β receptor I (TGFβRI), can readily be entrapped in the hydrophobic core of the PLGA nanoparticles, owing to its water insolubility. **d** Tumour-associated macrophages (TAMs) can readily be repolarized from a pro-tumour (M2-like) to an antitumour (M1-like) phenotype, thereby creating a more favourable local environment for establishing productive antitumour immunity. More specifically, certain dextran nanoparticles have been demonstrated to have avidity for macrophages, which leads to their enhanced uptake by TAMs relative to other cells present in the tumour microenvironment. Cyclodextrin nanoparticles are generated via amide-bond formation through the crosslinking of succinyl-β-cyclodextrin to lysine, which is achieved using (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) or N-hydroxysuccinimide (NHS)). Drugs such as R848, an agonist of Toll-like receptor 7 (TLR7) and TLR8 that was identified as a potent inducer of the M1 phenotype by an *in vitro* morphometric-based screen, can subsequently be loaded into the β-cyclodextrin nanoparticles for delivery to TAMs *in vivo*. DTT, dithiothreitol; SH, sulphydryls, also known as thiols. Part **a**: Copyright notice indicates the content was adapted by permission from the American Association for Cancer Research: Kwong, B. et al. Localized immunotherapy via liposome-anchored Anti-CD137 + IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity. *Cancer Res.* **73**(5), 1547–1558, <https://doi.org/10.1158/0008-5472.CAN-12-3343> (2013)¹⁸. Part **b** is adapted with permission from REF.⁵², Springer Nature Limited. Part **c** is adapted with permission from REF.⁵³, CC-BY-4.0 (<https://creativecommons.org/licenses/by/4.0/>). Part **d** is adapted with permission from REF.⁶¹, Springer Nature Limited.

prevented rapid dissemination into the circulation of mice and promoted accumulation in draining lymph nodes following subcutaneous injection, thereby reducing systemic inflammation and improving antitumour immunity as well as antibody titers⁶⁵.



Owing to their dimensions, nanoparticles naturally mimic viruses and are therefore readily recognized by various types of immune cells, including APCs. Notably, the biomaterial carrier can itself serve as an adjuvant, obviating the need to incorporate a separate agonist of innate immunity, and consequently simplifying production. An *in vivo* screen was performed to identify

Fig. 3 | Co-formulation of vaccine components dramatically improves effector responses. **a** Three-dimensional, porous biomaterial scaffolds permit one to define microenvironments, whether as implants or injectables. In the example shown, the scaffold is designed to release chemokines, such as granulocyte–macrophage colony-stimulating factor (GM-CSF), which act to recruit immature dendritic cells (DCs) to the site. The recruited DCs then undergo maturation as a result of the co-release of adjuvants and antigen (Ag) peptides from the scaffold. Once activated, the Ag-presenting DCs can migrate to local lymph nodes to prime T cells for cancer cell killing. Such scaffold-mediated vaccines can be made of sundry materials and in a variety of formats, including as cryogel-based whole-cell cancer vaccines and as spontaneously assembling inorganic scaffolds. **b** | Nanoparticles or nanodiscs can be loaded with or conjugated to both Ag and adjuvant, enabling co-delivery of both payloads to the same Ag-presenting cell. One such strategy is based on synthetic high-density lipoprotein (sHDL) nanodiscs, composed of phospholipids and apolipoprotein A1-mimetic peptides. These vaccine nanodiscs were engineered to co-deliver Ag peptides (including tumour-specific neoantigens) modified with a cysteine–serine (CSS) linker and a cholesterol-modified CpG adjuvant (Cho-CpG). Once injected, the nanodiscs reach the draining lymph nodes, where, similar to the scaffolds, they prime Ag-specific CD8⁺ T cells, which subsequently traffic to tumours and kill cancer cells. Additionally, nanodisc vaccination can be combined with immune checkpoint blockade to amplify these antitumour T cell responses. **c** | Schematic of an unmodified, unpurified mRNA-based vaccine, which both encodes Ag and serves as an inherent danger signal to drive strong vaccine responses. Such RNA is inherently immunostimulatory, stimulating the production of type I interferons (IFNs) through the engagement of endogenous RNA-sensing pattern recognition receptors (PRRs). In response to sensing foreign molecules, these PRRs also decrease protein translation, resulting in reduced Ag expression (as depicted by the dashed arrow). **d** | Schematic of a nucleoside-modified mRNA-based vaccine. The incorporation of modified nucleosides, which decreases recognition by the PRRs, allows for robust Ag presentation. The mechanisms of innate immune sensing by a DC for both types of mRNA vaccine are shown. RNA sensors are in dark purple. CTLA4, cytotoxic T lymphocyte-associated antigen 4; m1Ψ, 1-methylpseudouridine; MDA5, IFN-induced helicase C domain-containing protein 1 (also known as IFIH1); MHC, major histocompatibility complex; OAS, 2'-5'-oligoadenylate synthetase; PD1, programmed cell death protein 1; PEG, polyethylene glycol; PKR, IFN-induced, double-stranded RNA-activated protein kinase; RIG-I, retinoic-acid-inducible gene-I receptor; TCR, T cell receptor; TLR, Toll-like receptor. Part **a** is adapted with permission from REF.¹⁴³, Springer Nature Limited. Part **b** is adapted with permission from REF.⁶⁸, Springer Nature Limited. Parts **c** and **d** are adapted with permission from REF.⁷³, Springer Nature Limited.

Neoantigens

An antigen that is created by a gene fusion or somatic mutation.

Nanodiscs

Structures comprising a lipid bilayer and amphipathic membrane-stabilizing proteins.

Immunogens

Antigens that are immunogenic, provoking a cellular and/or humoral immune response.

Aptamer

An oligonucleotide, typically identified through *in vitro* selection, that binds to a target of interest.

polymers that could induce antigen-specific target-cell killing, leading to the discovery of PC7A, which activates the STING pathway⁶⁶. This polymer was used to augment antigen delivery to mouse lymph nodes and to promote cross-presentation by APCs, stimulating robust CD8⁺ T cell responses. Mixing of peptides encoding tumour-associated antigens or neoantigens with PC7A formed nanoparticles that inhibited tumour growth and extended survival in multiple mouse models of cancer.

Fostering accumulation in lymph nodes. Targeting of lymph nodes substantially enhances the efficacy and safety of systemic vaccination, and such targeting can be achieved by appending lipophilic albumin-binding tails to the antigen and adjuvant, permitting delivery to the lymphatic system by circulating albumin⁶⁷. This approach markedly increased both T cell priming and antitumour efficacy in mice, while substantially decreasing systemic toxicity. Nanodiscs⁴⁹ prepared by mixing synthetic high-density lipoprotein, which has been shown to be safe in humans at very high doses, with a cysteine-modified antigen and a cholesterol-modified CpG adjuvant prolonged antigen presentation for improved cross-priming of T cells (FIG. 3b). The nanodiscs enhanced the co-delivery of antigen and adjuvant to lymph nodes and promoted robust and sustained antigen presentation by APCs. When combined with ICB, the nanodisc vaccines eliminated established mouse colorectal and melanoma tumours⁶⁸.

Immunoengineering enables the incorporation of features that mimic natural responses to infection, and thus tune vaccination. Although nanoparticles with diameters ranging from ~10 to 100 nm naturally drain from interstitium to lymphatics, leading to their accumulation in lymph nodes⁶⁹, they are not always efficiently trafficked to sites within lymph nodes where B cells are situated. Glycoengineering the surface of nanoparticles can regulate localization not only to but also within lymph nodes⁷⁰. Recently, it was shown that inclusion of dense arrays of glycans on the surface of nanoparticles facilitated recognition by mannose-binding lectin in lymphatics, promoting transport of the particles to the B cell zone of the lymph node in mice, and thereby enhancing the quality and quantity of resultant humoral immunity^{70,71}. Multivalent nanoparticle immunogens initiated responses superior to those achieved with monomeric forms, and size, complement, and glycan density were shown to be critical to the observed effect⁷¹.

Nanovaccines can improve the effectiveness of mRNA-based vaccines considerably⁷². Nucleic acids are susceptible to nuclease-mediated hydrolysis, and carriers greatly enhance their stability⁷³. mRNA is an interesting cargo, because a single molecule contains both specificity (encoded antigen) and function (adjuvanticity), as single-stranded mRNA is a natural ligand for TLR7 and TLR8⁷⁴. Thus, every cell that takes up the mRNA is poised to induce productive immunity rather than tolerance. Lipids have been used extensively to package nucleic acids and increase the efficiency of their delivery⁷. In one example, systemically administered RNA lipoplexes were shown to concentrate in lymphoid

organs, leading to the expression of encoded neoantigens or tumour-associated antigens by local APCs, generating robust effector and memory T cell responses and inducing the rejection of established tumours in multiple mouse models of cancer⁷⁵. Interestingly, the uptake of RNA lipoplexes by dendritic cells following intravenous injection of these particles was dependent on particle charge rather than lipid composition.

The study was extended to a small number of patients with advanced malignant melanoma, in whom type I interferon (IFN) production and antigen-specific T cell responses were again observed. While these pharmacodynamic data are encouraging, the study was limited to three patients, in whom efficacy was not demonstrated. The data confirm that low doses of mRNA can be safely administered systemically in humans, but these doses may be sub-therapeutic. Moreover, mRNA vaccines are not strictly innate immune agonists, as they also encode antigens. Accordingly, it may be premature to conclude that agonists of innate immunity that do not also engage the adaptive arm of the immune system in an antigen-specific manner (for example, STING agonists) can be safely administered systemically or that therapeutic doses of agonists of innate immunity in general can be safely administered systemically. It is challenging to comment on therapeutic window in the absence of efficacy data, so additional studies will yet be required in these regards.

Increasing nucleic acid uptake

While small molecules, cytokines and antibodies can all benefit from focused delivery to relevant tissues and cell types, nucleic acids often definitively require drug delivery solutions in order to transit across hydrophobic, tightly packed cell membranes (FIG. 4). Following endocytosis or phagocytosis, nanoparticles can also facilitate payload release from membrane-bound compartments (for example, endosomes) into the cytosol. Lymphocytes are exceedingly resistant to transfection, although some initial modest successes have been reported⁷⁶. Lipid nanoparticles, polymeric particles and aptamer conjugates have been used to achieve small-interfering RNA (siRNA)-mediated silencing of transcripts in mice⁷⁶, although efficacy data remain lacking, as the efficiency of delivery is rather low. Fortunately, there are several applications of nucleic acid delivery that extend beyond sequence-specific gene silencing.

Among the most exciting of such applications is the use of targeted polymeric nanoparticles to transfet primary human T cells or haematopoietic stem cells following *in vitro* incubation⁷⁷. Transient expression of therapeutically relevant proteins — such as genome-editing agents or transcription factors — can generate permanent alterations in the absence of transgene integration. The particles developed were able to modify T cells present in a mixed population of immune cells isolated from peripheral blood by knocking out the native TCR among T cells to which a CAR was subsequently introduced. In a second application, T cells were transfected with mRNA encoding a transcription factor that promotes central memory T cell formation. The resultant improved antitumour functionality of CAR

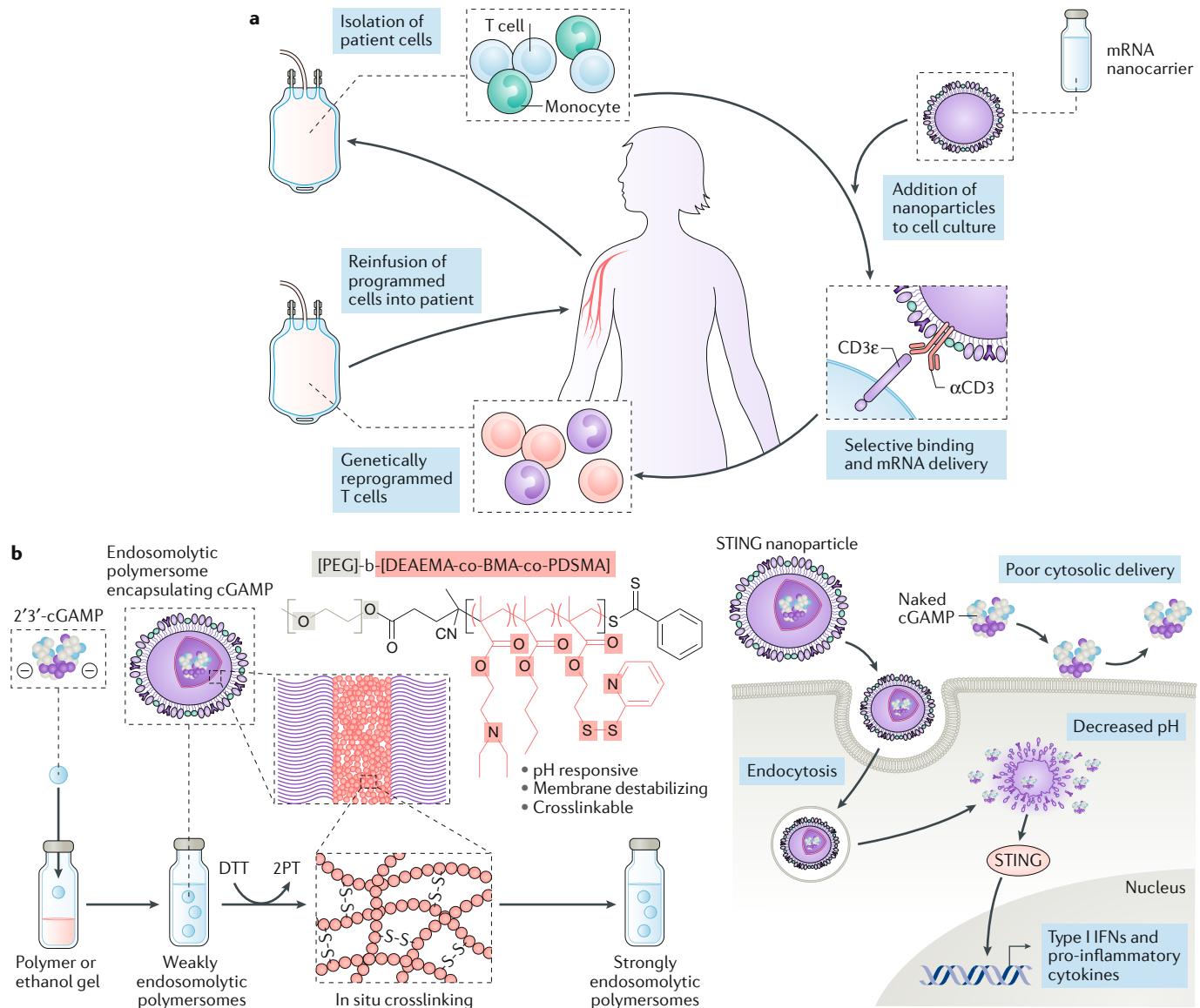


Fig. 4 | Nanoparticle carriers are needed to deliver immunomodulatory payloads efficiently into the cytosol of immune cells. **a** Human peripheral blood can be collected and incubated with mRNA-loaded polymeric nanoparticles. The primary immune cells of interest are targeted by virtue of the specific ligand coating of the nanoparticle, leading to its uptake. Following intracellular release of its mRNA cargo, transient expression of therapeutically relevant proteins by the targeted cells can promote permanent phenotypic alterations. In one example, the T cells were reprogrammed ex vivo to express a transcription factor that induced differentiation towards a central memory phenotype with enhanced antitumour activity. **b** Nanoparticles derived from block copolymers that contain a pH-responsive block promote endosomolysis, fostering the transport of innate immune agonists that bind to and activate pattern recognition receptors situated within the cytoplasm, such as stimulator of interferon genes (STING) and RIG-I. An example of the strategy for enhancing cytosolic delivery of 2'3'-cGAMP, an endogenous cyclic dinucleotide ligand for STING, is shown. cGAMP is encapsulated in endosomolytic polymersomes assembled from pH-responsive diblock copolymers. After polymersome self-assembly and 2'3'-cGAMP loading, polymer chains are crosslinked in situ via partial reduction of pyridyl disulfide groups with dithiothreitol (DTT), resulting in the formation of disulfide crosslinks. Crosslinking improves the endosomolysis capacity of the particles. The resultant nanoparticles increase the intracellular uptake and cytosolic delivery of 2'3'-cGAMP, enhancing STING activation and thus the production of type I interferon (IFN) and other pro-inflammatory cytokines. 2PT, 2-pyridinethione. Part **a** is adapted with permission from REF.⁷⁷, CC-BY-4.0 (<https://creativecommons.org/licenses/by/4.0/>). Part **b** is adapted with permission from REF.⁸⁶, Springer Nature Limited.

T cells in a xenograft mouse model of lymphoma led to enhanced survival⁷⁷. Notably, the nanoreagent has advantages relative to the current standards for nucleic acid delivery to lymphocytes and can readily be incorporated into manufacturing processes; specifically,

the particles do not involve the costly and elaborate protocols required by viral vectors, and the particles do not involve physical disruption of the cell membrane that results in the cytotoxicity observed upon electroporation.

Facilitating cytosolic delivery. Nucleic acids can be used to stimulate the expression of type I IFN through the activation of pattern recognition receptors⁷⁸. Ligands of endosomal receptors such as TLR3 and TLR9, which recognize double-stranded RNA and CpG-containing oligonucleotides, respectively, do not require nanocarriers; however, ligands of cytosolic receptors, such as STING and RIG-I, demand bolstered delivery in order to access their targets. Clinical data suggest that innate immune agonism (for example, using CpG⁷⁹) is beneficial among patients with cancer, but the remarkable efficacy derived from agonism of STING in mice⁸⁰ has not been recapitulated to date in humans^{81,82}. Two recent studies revealed that uptake of these cyclic dinucleotide pharmacophores requires expression of the reduced folate carrier SLC19A1 (REFS^{83,84}), and this transporter may not be expressed at high levels within human tumours and/or may be saturated because of competing folate in the TME.

Accordingly, nanoparticles have been used to promote cytosolic delivery of cyclic dinucleotides. In one study, these immunomodulators were loaded into liposomes, which are taken up by myeloid cells⁸⁵. Relative to free drug in solution, liposome-mediated delivery of the cyclic dinucleotide cGAMP repolarized M2-like macrophages to M1-like macrophages, both *in vitro* and *in vivo*, increased the infiltration of CD8⁺ T cells and suppressed the growth of established mouse tumours following intravenous injection. In a second study, polymersomes were rationally designed to promote endosomal escape, which is a key limiting factor for the delivery of agonists of cytosolic pattern recognition receptors⁸⁶ (FIG. 4b). A block copolymer containing pH-responsive, membrane-destabilizing, crosslinkable and self-assembling blocks was used to enhance the delivery and immunostimulatory activity of cGAMP in the TME. Intratumoural injection of the nanoparticles increased the number of T cells and decreased the number of M2-like macrophages in the TME relative to free cGAMP, and the survival of tumour-bearing mice improved. Intravenous injection of these particles synergized with ICB to improve the survival of tumour-bearing mice, while free cGAMP had no effect in either context at the administered dose.

For nucleic acid oligomers and polymers that are longer than a cyclic dinucleotide, an additional block that contains a positive charge can be incorporated in order to promote ionic binding. Such a copolymer was used to form a complex with and deliver 5'-triphosphate RNA (3pRNA)⁸⁷, a ligand for RIG-I⁸⁸. It was demonstrated that inclusion of the pH-responsive block was required in order to confer endosomolytic ability, and thus receptor agonism. Cytosolic delivery of 3pRNA into dendritic cells that was mediated by the nanoparticles increased cytokine expression and CD8⁺ T cell infiltration following intratumoural injection, thereby improving the response rate upon combination with anti-PD1 relative to either monotherapy⁸⁷. Taken together, these data suggest that immunoengineering can extend the utility of nucleic acid-based immunomodulatory therapeutics at the tissue, cellular and subcellular levels; specifically, nanoparticles can be used to concentrate mRNA vaccines in lymph nodes, facilitate

cargo uptake by myeloid cells and augment delivery into the cytosol.

Leveraging perioperative immunotherapy

Addressing a favourable context. As with most approaches in oncology, cancer immunotherapy is typically deployed to confront intact primary or metastatic disease. Some groups have hypothesized that it could be easier for the immune system to clear a small number of residual cancer cells — particularly in the absence of a concentrated immunosuppressive microenvironment — than to eradicate bulky tumours, which have many more pathological cells and multiple modes of immune evasion^{6,89}. Removing cancer cells as well as immunosuppressive leukocytes and proteins is expected to favour the establishment of productive antitumour immunity. Moreover, removing intratumoural CD8⁺ T cells may not be as unfavourable as previously thought, as it was recently revealed that such cells have a rare and variable capacity to recognize tumour antigens and to be tumour reactive⁹⁰.

Importantly, breaking local immune tolerance can lead to systemic immune responses⁹¹ — which are required for effective cancer immunotherapy⁹² — and eliminate disseminated disease, including in sites considered to be cancer cell sanctuaries⁹³. Post-surgical recurrence and metastasis remain primary causes of patient mortality^{94,95}. The transient but acute immunosuppressive inflammation caused by the wound-healing response to surgery has been demonstrated to be causative of tumour progression and metastasis^{95–97}. Relatedly, the wound-healing gene signature is correlated with resistance to ICB in the clinic⁹⁸. Thus, the perioperative setting represents a high-leverage context in which to administer immunotherapy (FIG. 5).

From cell therapy to small molecules. As described above³³, cells that naturally traffic to sites of inflammation are ideal active delivery agents. Post-resection adoptive transfer of mouse platelets to which anti-programmed cell death protein 1 ligand 1 (PDL1) had been conjugated promoted accumulation of this immunotherapy at the tumour resection site, reducing post-surgical recurrence and metastasis⁹⁹ (FIG. 5b). In situ platelet activation within the wound led to release of the cargo as platelet micro-particles. Relapse of incompletely resected melanoma and breast tumours was effectively prevented by intravenous administration of the modified platelets, with mice exhibiting durable survival relative to unmodified platelets or free antibody.

Macroscale delivery devices can be used as depots for the delivery of small molecules and biologics and are particularly useful for local delivery of cells¹¹. Scaffolds provide defined cues that mimic natural immune niches, enabling *in situ* modulation of cell fate and behaviour⁸⁹. Hydrogel-based scaffolds loaded with tumour-reactive T cells, aAPCs and other relevant molecules can be placed near or at tumour resection sites, promoting the delivery, expansion and dispersal of the T cells^{100,101} (FIG. 5d). This approach was shown to be remarkably effective in the context of incompletely resected and even inoperable orthotopic disease in mice, whereas

Cyclic dinucleotide pharmacophores

Molecular structures defined by a closed ring comprising two nucleotides that are recognized by the innate immune pattern recognition receptor stimulator of interferon genes (STING).

Polymersomes

Polymeric analogues of liposomes that can serve as an artificial vesicle to entrap drug-containing solutions, often affording greater control over particle stability and drug release rate.

Block copolymer

A polymer comprising two or more homopolymer blocks, connected via covalent bonds, that can confer blended or combined properties of the individual blocks.

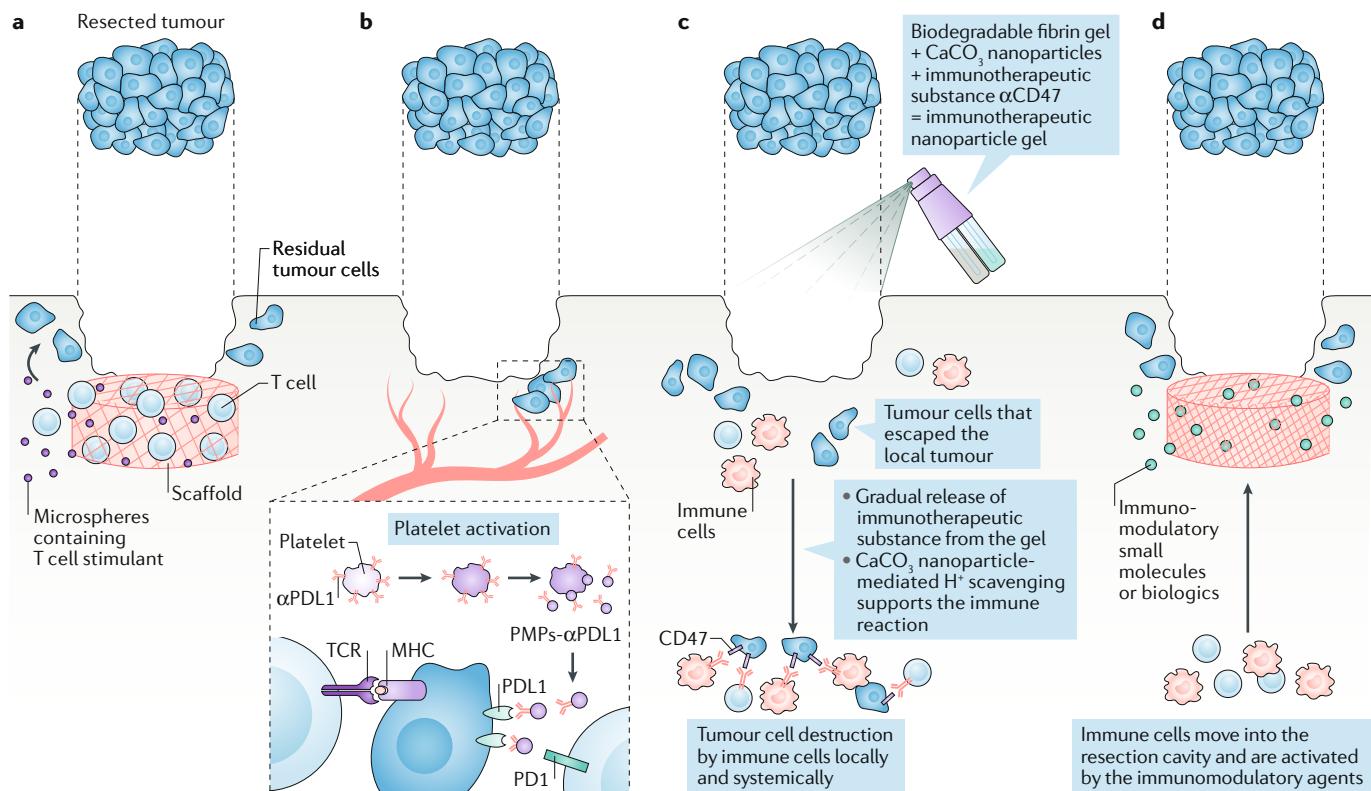


Fig. 5 | Perioperative immunotherapy represents a high-leverage context for improving the frequency and magnitude of antitumour immune responses. **a** Scaffolds can be used to deliver adoptively transferred cells locally in order to address inoperable or incompletely resected tumours. The biomaterial is first loaded with T cells and immunostimulatory molecules ex vivo. Then it is surgically implanted, either into the bed of an incompletely resected tumour or close to an inoperable tumour, where it promotes the sustained proliferation and release of tumour-reactive T cells. **b** Antibodies that induce immune checkpoint blockade, such as anti-programmed cell death protein 1 (PD1) ligand 1 (αPDL1), can be conjugated to the surface of platelets. Following intravenous injection, local activation of the platelets at the wound site generates platelet-derived microparticles (PMPs) from the plasma membrane, which can release the payload into the resection cavity. **c** Biodegradable fibrin gels containing immunotherapy-loaded calcium carbonate (CaCO₃) nanoparticles can be formed in situ after spraying the components into a resection cavity. CaCO₃ nanoparticles also scavenge protons (H⁺), thereby favouring macrophage activation and amplifying immune responses against the residual cancer cells or those that escaped the tumour prior to resection. **d** Immunotherapy-loaded hydrogel scaffolds can be used to reprogram the post-resection milieu from immunosuppressive to immunostimulatory. These scaffolds can confer dramatic survival benefits by preventing local recurrence and inducing systemic antitumour immunity that leads to the eradication of disseminated disease, even without the inclusion of cells or particles in the hydrogel. MHC, major histocompatibility complex; TCR, T cell receptor. Part **a** is adapted with permission from REF.¹⁰⁰, Springer Nature Limited. Part **b** is adapted with permission from REF.⁹⁹, Springer Nature Limited. Part **c** is adapted with permission from REF.¹⁴⁴, Springer Nature Limited.

adoptive-cell therapy lacking the scaffolds failed to control disease^{100,101}.

Still, obviating the need for cell therapy would be desirable from a manufacturing perspective. Acellular protein-based gels loaded with calcium carbonate nanoparticles containing anti-CD47 were formed in situ by spraying the components at the tumour resection site¹⁰² (FIG. 5c). CD47, which is often overexpressed on cancer cells, acts as a ‘do not eat me’ signal by engaging an inhibitory receptor signal regulatory protein-α (SIRPa) on the surface of macrophages, but the administration of anti-CD47 can restore phagocytosis and can prime adaptive antitumour responses¹⁰³. These gels effectively polarized macrophages in the post-surgical environment to an M1-like phenotype¹⁰². The nanoparticles reduced immunosuppression by buffering the acidic milieu, while the released anti-CD47 augmented phagocytosis

of cancer cells by macrophages, which can serve as APCs to initiate T cell-mediated responses. All of the components were required in order to extend mouse survival following incomplete resection, and it was further shown that the local intervention produced antitumour activity against untreated distal tumours in the mice.

Because surgeons in the clinic attempt to remove the entire tumour, confirming the benefit of intraoperative immunotherapy in the context of complete resection was of paramount importance. To this end, a hydrogel was placed in the post-resection cavity to extend locally the release of various classes of cancer immunotherapy — including antibodies that induce ICB, a cytokine that promotes lymphocyte effector function, and small molecules that stimulate the production of type I IFN — and it was shown that agonists of innate immunity were particularly useful in this context¹⁰⁴.

Notably, a durable survival benefit was conferred only if these compounds were released locally in a sustained manner from the hydrogel, as delivery of the same compounds in solution — whether systemically, intratumourally or even directly into the resection cavity — failed to extend the survival of mice.

Furthermore, in this example, reprogramming the post-resection microenvironment from immunosuppressive to immunostimulatory induced systemic antitumour immunity that not only prevented local recurrence but also led to the eradication of existing spontaneous metastases¹⁰⁴. Administration of a single dose of a single payload in this context yielded the desired antitumour activity¹⁰⁴, even in extremely aggressive mouse models that otherwise have required combinations of up to four immunotherapies administered repeatedly^{105,106}. The approach improves efficacy by concentrating the entire effective dose at the site of interest and improves safety by markedly limiting systemic exposure that can break peripheral tolerance. Furthermore, this strategy vastly increases the number of addressable patients for highly potent immunotherapies, such as agonists of innate immunity, by obviating the need for intratumoural injection of accessible lesions. Surgery is the standard of care for most patients with solid tumours^{94,97}, so successful translation of this approach would greatly impact how, when and where cancer immunotherapy is delivered.

Initial clinical endeavours

A phase I trial (NCT03815682¹⁰⁷) of a ‘backpacking’ strategy — which involves ex vivo modification of T cells prior to adoptive-cell transfer¹⁰⁸ — was recently initiated among patients with relapsed or refractory solid tumours or lymphomas. The autologous cell therapy is primed to target several tumour-associated antigens and is loaded with a multimer of reversibly crosslinked IL-15 that is conjugated to the surface of the T cells. This version of the backpacking technology represents a major advance over the original liposome-based iterations described earlier in this Review. First, the backpacks themselves are nanogels that comprise the immunotherapy, rather than being primarily carriers that are loaded with a relatively small amount of immunotherapy. Second, the release of the cargo is actuated site-specifically in response to TCR activation, rather than by passive mechanisms such as diffusion. Specifically, antigen recognition leads to increased reduction potential at the T cell surface, leading to cleavage of a disulfide bond and self-immolation of the crosslinker, thereby resulting in release of the cytokine. The resultant system was shown to increase both efficacy and safety in mice relative to administration of free cytokine¹⁰⁸. Moreover, in contrast to CAR and transgenic-TCR T cell therapies, the process does not require genetic engineering of the T cells. Notably, the modularity of the approach enables the rapid development of iterations of the ‘backpacked’ T cells incorporating other crosslinked biologics, and even small molecules with appropriate chemical handles; indeed, IL-12 and a TLR agonist are currently under lead optimization for paracrine-like delivery to reprogram the TME.

The ability to inflame tumours with endogenous effector T cells, obviating the need for adoptive-cell transfer,

is also being investigated clinically. Intratumoural injection of a TLR9 agonist in solution restored sensitivity to anti-PD1 among patients with melanoma with anti-PD1-resistant tumours¹⁰⁹. An ongoing phase I trial (NCT03086278¹¹⁰) is assessing the safety of immuno-modulatory spherical nucleic acids (SNAs), which array a high concentration of CpG oligonucleotides around a gold or lipid core. Such SNAs induced vastly superior cellular and humoral responses, relative to free CpG, although minimal survival benefit was observed in a lymphoma mouse model relative to lipid-modified CpG, which can similarly form micellar structures¹¹¹. If subcutaneous injections of this SNA are tolerated well in healthy volunteers, then efficacy trials in patients with cancer can be undertaken.

Vaccines have entered such efficacy studies in patients with cancer. In addition to the aforementioned RNA lipoplex vaccine⁷⁵ (NCT02410733¹¹²), a vaccine scaffold is being evaluated. The first-generation ‘WDVAX’ involves loading of autologous melanoma cell lysate along with granulocyte–macrophage colony-stimulating factor (GM-CSF) and CpG into a scaffold prepared from porous poly(lactide-co-glycolide), an FDA-approved bioresorbable polymer (NCT01753089¹¹³). In a mouse model of melanoma, such scaffolds promoted the recruitment and activation of dendritic cells, production of antitumour cytokines, attenuation of immunosuppressive cells and cytokines, stimulation of potent local and systemic cytotoxic T lymphocyte responses, and regression of established tumours¹¹⁴. The platform has evolved, and the investigators have now established injectable versions, both as cryogel-based whole-cell cancer vaccines¹¹⁵ and as spontaneously assembling inorganic scaffolds¹¹⁶. Novartis has licensed aspects of this technology, signalling that there is excitement within the pharmaceutical industry to adopt and advance immuno-engineering approaches towards achieving improved safety and efficacy for cancer immunotherapies. Given the recent disappointments observed among several immunotherapy trials (for example, ECHO-301 in melanoma, EAGLE in head and neck cancer and JAVELIN Lung 200 in lung cancer), the utility of immunoengineering may receive further attention as a means of augmenting antitumour immunity.

Modelling and engineering immunity

Collaboration between basic scientists and engineers is crucial to achieving further advances. Engineers are excellent at devising interventionist strategies from first principles, developing tools to probe for information that is not otherwise readily accessible and processing data in creative ways. As useful as these contributions are, in order to make substantial rather than simply iterative progress, we must improve our understanding of both general and tumour-specific immunology¹¹⁷, as well as of how drug delivery systems interact with their target cells at the molecular level. For example, the accumulation and activity of antitumour T cells is influenced by the class of receptor that is targeted by administered particles. Specifically, immunotherapy delivered via liposomes that bind to an internalizing receptor (CD90) yielded greater efficacy than immunotherapy delivered

Nanogels

Nanoparticulate forms of a hydrogel (a crosslinked hydrophilic polymer network).

Mass cytometry

Also known as cytometry by time of flight (CyTOF), a mass spectrometry-based variation of flow cytometry in which antibodies are labelled with heavy metal ions that have discrete masses, rather than with fluorophores that can have spectral overlap.

via liposomes that bind to a non-internalizing receptor (CD45)¹¹⁸. Relatedly, a thoughtful editorial previously suggested that drug delivery research in general would benefit greatly from increased interactions with cell biologists, who could provide insights into membrane trafficking¹¹⁹.

From understanding comes intervention. Elucidating how concentration gradients of immunomodulatory compounds alter the responses of nearby leukocytes will be helpful in defining optimal release profiles, and new tools are emerging that enable such studies to be performed. Three-dimensional modelling systems are being used to recapitulate immune tissues both structurally and functionally¹²⁰. Such multiscale engineering approaches afford quantitative insights into human immunology that are expected to inform the design of next-generation immunotherapies. Immune organs ‘on a chip’ allow one to manipulate fluid flow, cytokine gradients, adhesion molecule concentration and stromal components to mimic immune organ function and immune cell interactions fairly accurately¹²¹. In one example, a microfluidic device was used to achieve high-throughput pairing of APCs and T cells with a defined contact time, allowing the characterization of early activation dynamics¹²².

Despite some extremely encouraging and exciting developments, we must acknowledge a general struggle to extrapolate findings with exactitude from *in vitro* model systems to *in vivo* biology. The immune system is highly coordinated, and *in vitro* systems are inherently reductionist. The absence or even insufficiency of a particular cytokine or leukocyte subset can dramatically impact the cells that were arbitrarily chosen for a given co-culture experiment. Furthermore, the role of mechanical forces — which contribute to immune evasion¹²³ as well as to leukocyte migration, intracellular signalling and intercellular communication¹²⁴ — is lost in suspension cultures. For example, a three-dimensional microfluidic culture of organotypic tumour spheroids has been used to profile secreted cytokines and thereby establish features associated with response and resistance to PD1 blockade¹²⁵. Although the system retained autologous lymphoid and myeloid cell populations, the ratios of various leukocytes differed from spheroid to spheroid, and the physiological architecture was lost.

Spatial relationships are vital. Circumventing some of the limitations associated with systems assembled *in vitro*, intact short-term *ex vivo* cultures represent a useful tool for maintaining not only appropriate composition but also faithful spatial relationships among cell types. A perfusion-based bioreactor generated for tissue-engineering applications was used to preserve the TME of freshly excised breast cancer tissue for more than two weeks, and thereby enabled assessment of the efficacy imparted by ICB¹²⁶. Such *ex vivo* response may one day be used to stratify patients for enrolment into appropriate clinical trials or may serve as a companion functional biomarker for prescription of an approved immunotherapy.

Spatial relationships among cells in the TME affect antitumour immunity and patient outcomes. A type of

mass cytometry termed multiplexed ion beam imaging (MIBI), which facilitates the inspection of up to 100 targets on formalin-fixed paraffin-embedded (FFPE) tissue samples¹²⁷, recently revealed that tumours exist as either immune-cell-mixed or compartmentalized environments¹²⁸. The data, which indicated that ordered immune structures along the tumour-immune boundary were correlated with compartmentalization and linked to survival, underscore that the tumour-immune microenvironment is typically organized in a manner that is structured in its cellular composition, spatial arrangement and regulatory-protein expression¹²⁸.

Mass cytometry can be complemented by imaging-based techniques, such as cyclic immunofluorescence (CyCIF), which enables standard reagents and instrumentation to be used. This multiplexing platform is compatible with live-cell imaging and additionally yields information related to cell morphology and subcellular protein localization¹²⁹. CyCIF was applied to FFPE tissues in order to generate up to 60-plex images, illuminating the spatial distribution of leukocytes within healthy or tumour samples¹³⁰. Inspection of immune checkpoint proteins permitted quantification of the extent of colocalization of the cells expressing PD1 or its ligand PDL1. Such immune profiling may yield biomarkers that help stratify patients to appropriate immunotherapies. Furthermore, the incorporation of findings from studies on spatial relationships between relevant cell populations among tissues of interest will be essential to designing tissue-engineered interventions.

Notably, synthetic immune tissues may one day be used as therapeutics themselves, serving as implants that can program immune responses by mimicking lymphoid organs^{121,131}. Examples include artificial lymph nodes to recruit leukocytes and restore adaptive immune function¹³², engineered thymic tissue to promote tolerance to donor tissue for enhanced transplantation¹³³, and engineered humanized bone marrow niches to support haematopoiesis¹³⁴. Recently, an injectable biomaterial-based scaffold was shown to mimic aspects of T cell lymphopoiesis in the bone marrow of mice¹³⁵. The cryogel may offer an off-the-shelf solution to promote T cell regeneration and reconstitution following allogeneic haematopoietic stem cell transplantation, mitigating against graft-versus-host disease and improving survival.

Together, these data underscore the importance of comprehending the dynamics between cells in native tissue. Tumour immunology is highly contextual, and tools that reveal insights into and allow the programming of molecular and cellular gradients should prove highly valuable. Temporal dynamics are similarly important, and it has been shown that altering the order and timing of combination immunotherapy can decouple efficacy from toxicity in multiple mouse cancer models¹³⁶. In addition, temporally programmed treatment schedules have revealed that delaying administration of a second immunotherapy can improve survival outcomes in mice relative to concurrent administration¹³⁷. Optimizing the duration and sequence of immunotherapy on the basis of preclinical and clinical data should dramatically improve therapeutic index¹³⁸.

Concluding remarks

In summary, nanotechnology can improve the safety and efficacy of immunomodulatory compounds by modifying their spatiotemporal release profiles¹³⁹. Concentrating the dose on relevant cells at appropriate sites enhances therapeutic index. Extending drug release locally in a controlled manner enables sustained reprogramming of the TME or post-resection milieu. Such tactics can be applied in order to provide capabilities to adoptive transferred cells or endogenous immune cells that are otherwise not accessible upon the administration of bolus immunotherapy in solution. Drug delivery solutions are disproportionately needed for nucleic acid-based therapeutics, which do not enter the cytosol efficiently in the absence of such carriers. Responses to mRNA-based and peptide-based vaccines are vastly improved by nanoparticles and scaffolds that can, respectively, localize their co-packaged subunits in lymph nodes and serve as artificial lymph nodes. Macroscale scaffolds can be deployed in the context of intraoperative immunotherapy to prevent recurrence and metastasis, and as synthetic immune tissues with defined niches that program desired immune responses.

Some immunoengineering approaches are now entering the clinic, and the preclinical data provide reason for optimism — much more so than previous nanomedicine approaches that had attempted to concentrate the action of cytotoxic therapies to cancer cells via ‘targeted’ nanoparticles⁵⁴ that do not impact biodistribution or tumour localization¹⁴⁰. Leukocytes traffic to sites of inflammation, such as tumours¹⁴¹, and thus may serve as the ultimate drug delivery systems, whether they are concentrating the dose of an exogenously administered small molecule or releasing endogenous IFNγ, perforin and granzyme. Still, the manufacturing of clinical-grade materials represents a key consideration that cannot be overlooked. Formulations should be devised with a view towards translation to good manufacturing practice (GMP)-grade production. Particles, devices and cells require reproducible and scalable chemistry, manufacturing and controls (CMC), for which simplicity is likely to be highly valuable. The convergence of cancer immunotherapy, nanotechnology and bioengineering is maturing to the point that it may impact patient lives in the not-too-distant future.

Published online: 06 September 2019

1. Khalil, D. N., Smith, E. L., Brentjens, R. J. & Wolchok, J. D. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat. Rev. Clin. Oncol.* **13**, 273–290 (2016).
2. Motzer, R. J. et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N. Engl. J. Med.* **378**, 1277–1290 (2018).
3. Reck, M. et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N. Engl. J. Med.* **375**, 1823–1833 (2016).
4. Binnewies, M. et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat. Med.* **24**, 541–550 (2018).
5. Yarchoan, M., Hopkins, A. & Jaffee, E. M. Tumor mutational burden and response rate to PD-1 inhibition. *N. Engl. J. Med.* **377**, 2500–2501 (2017).
6. Sharma, P., Hu-Lieskovsky, S., Wargo, J. A. & Ribas, A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* **168**, 707–723 (2017).
7. Jeanbart, L. & Swartz, M. A. Engineering opportunities in cancer immunotherapy. *Proc. Natl Acad. Sci. USA* **112**, 14467–14472 (2015).
8. Riley, R. S., June, C. H., Langer, R. & Mitchell, M. J. Delivery technologies for cancer immunotherapy. *Nat. Rev. Drug Discov.* **18**, 175–196 (2019).
9. Mitchell, M. J., Jain, R. K. & Langer, R. Engineering and physical sciences in oncology: challenges and opportunities. *Nat. Rev. Cancer.* **17**, 659–675 (2017).
10. Winer, A., Bodor, J. N. & Borghaei, H. Identifying and managing the adverse effects of immune checkpoint blockade. *J. Thorac. Dis.* **10**, S480–S489 (2018).
11. Dellacherie, M. O., Seo, B. R. & Mooney, D. J. Macroscale biomaterials strategies for local immunomodulation. *Nat. Rev. Mater.* (in the press).
12. Milling, L., Zhang, Y. & Irvine, D. J. Delivering safer immunotherapies for cancer. *Adv. Drug Deliv. Rev.* **114**, 79–101 (2017).
13. Kwong, B., Liu, H. & Irvine, D. J. Induction of potent anti-tumor responses while eliminating systemic side effects via liposome-anchored combinatorial immunotherapy. *Biomaterials* **32**, 5134–5147 (2011).
14. Pfreundschuh, M. G. et al. Phase I study of intratumoral application of recombinant human tumor necrosis factor. *Eur. J. Cancer Clin. Oncol.* **25**, 379–388 (1989).
15. van Herpen, C. M. et al. Intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients induces B cell activation. *Int. J. Cancer* **123**, 2354–2361 (2008).
16. Bartsch, H. H., Pfizenmaier, K., Schroeder, M. & Nagel, G. A. Intralesional application of recombinant human tumor necrosis factor alpha induces local tumor regression in patients with advanced malignancies. *Eur. J. Cancer Clin. Oncol.* **25**, 287–291 (1989).
17. Ishihara, J. et al. Matrix-binding checkpoint immunotherapies enhance antitumor efficacy and reduce adverse events. *Sci. Transl. Med.* **9**, eaan0401 (2017).
18. Kwong, B., Gai, S. A., Elkhader, J., Wittrup, K. D. & Irvine, D. J. Localized immunotherapy via liposome-anchored Anti-CD137: IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity. *Cancer Res.* **73**, 1547–1558 (2013).
19. Zhang, Y., Li, N., Suh, H. & Irvine, D. J. Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity. *Nat. Commun.* **9**, 6 (2018).
20. Ishihara, J. et al. Targeted antibody and cytokine cancer immunotherapies through collagen affinity. *Sci. Transl. Med.* **11**, eaau3259 (2019). **This study illustrates that conjugation or fusion of a collagen-binding domain to biologics promotes their accumulation in tumours, improving the therapeutic index of systemically administered immunotherapy.**
21. Bobisse, S. et al. Sensitive and frequent identification of high avidity neo-epitope specific CD8+ T cells in immunotherapy-naïve ovarian cancer. *Nat. Commun.* **9**, 1092 (2018).
22. Tran, E. et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. *Science* **344**, 641–645 (2014).
23. Robbins, P. F. et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. *Clin. Cancer Res.* **21**, 1019–1027 (2015).
24. Maude, S. L. et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Engl. J. Med.* **378**, 439–448 (2018).
25. Neelapu, S. S. et al. Axicabtagene ciloleucel CAR-T-cell therapy in refractory large B-cell lymphoma. *N. Engl. J. Med.* **377**, 2531–2544 (2017).
26. Schuster, S. J. et al. Chimeric antigen receptor t cells in refractory b-cell lymphomas. *N. Engl. J. Med.* **377**, 2545–2554 (2017).
27. Hollyman, D. et al. Manufacturing validation of biologically functional T cells targeted to CD19 antigen for autologous adoptive cell therapy. *J. Immunother.* **32**, 169–180 (2009).
28. Huang, X. et al. Sleeping beauty transposon-mediated engineering of human primary T cells for therapy of CD19+ lymphoid malignancies. *Mol. Ther.* **16**, 580–589 (2008).
29. Perica, K. et al. Magnetic field-induced T cell receptor clustering by nanoparticles enhances T cell activation and stimulates antitumor activity. *ACS Nano* **8**, 2252–2260 (2014).
30. Perica, K. et al. Enrichment and expansion with nanoscale artificial antigen presenting cells for adoptive immunotherapy. *ACS Nano* **9**, 6861–6871 (2015).
31. Hickey, J. W. et al. Efficient magnetic enrichment of antigen-specific T cells by engineering particle properties. *Biomaterials* **187**, 105–116 (2018). **This study demonstrates the importance of optimizing particle size, concentration, ligand and ligand density for maximal enrichment, activation and expansion of antigen-specific T cells.**
32. Sunshine, J. C., Perica, K., Schneck, J. P. & Green, J. J. Particle shape dependence of CD8+ T cell activation by artificial antigen presenting cells. *Biomaterials* **35**, 269–277 (2014).
33. Hickey, J. W., Vicente, F. P., Howard, G. P., Mao, H. Q. & Schneck, J. P. Biologically inspired design of nanoparticle artificial antigen-presenting cells for immunomodulation. *Nano Lett.* **17**, 7045–7054 (2017).
34. Kosmidis, A. K., Necochea, K., Hickey, J. W. & Schneck, J. P. Separating T cell targeting components onto magnetically clustered nanoparticles boosts activation. *Nano Lett.* **18**, 1916–1924 (2018).
35. Ben-Akiva, E., Meyer, R. A., Wilson, D. R. & Green, J. J. Surface engineering for lymphocyte programming. *Adv. Drug Deliv. Rev.* **114**, 102–115 (2017).
36. Siebert, A. L., Fahmy, T. M. & Kim, D. Artificial antigen-presenting cells for immunotherapies. *Methods Mol. Biol.* **1530**, 343–353 (2017).
37. Fadel, T. R. et al. Adsorption of multimeric T cell antigens on carbon nanotubes: effect on protein structure and antigen-specific T cell stimulation. *Small* **9**, 666–672 (2013).
38. Fadel, T. R. et al. A carbon nanotube-polymer composite for T-cell therapy. *Nat. Nanotechnol.* **9**, 639–647 (2014).
39. Cheung, A. S., Zhang, D. K. Y., Koshy, S. T. & Mooney, D. J. Scaffolds that mimic antigen-presenting cells enable ex vivo expansion of primary T cells. *Nat. Biotechnol.* **36**, 160–169 (2018).
40. Stephan, M. T., Moon, J. J., Um, S. H., Bershteyn, A. & Irvine, D. J. Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. *Nat. Med.* **16**, 1035–1041 (2010).
41. Stephan, M. T., Stephan, S. B., Bak, P., Chen, J. & Irvine, D. J. Synapse-directed delivery of immunomodulators using T-cell-conjugated nanoparticles. *Biomaterials* **33**, 5776–5787 (2012).
42. Huang, B. et al. Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells. *Sci. Transl. Med.* **7**, 291ra294 (2015).
43. Hinrichs, C. S. & Rosenberg, S. A. Exploiting the curative potential of adoptive T-cell therapy for cancer. *Immunol. Rev.* **257**, 56–71 (2014).
44. Wing, A. et al. Improving CART-cell therapy of solid tumors with oncolytic virus-driven production of a

- bispecific T-cell engager. *Cancer Immunol. Res.* **6**, 605–616 (2018).
45. Rosenberg, S. A. & Restifo, N. P. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* **348**, 62–68 (2015).
 46. Sommer, C. et al. Preclinical evaluation of allogeneic CAR T cells targeting BCMA for the treatment of multiple myeloma. *Mol. Ther.* **27**, 1126–1138 (2019).
 47. MacLeod, D. T. et al. Integration of a CD19 CAR into the TCR alpha chain locus streamlines production of allogeneic gene-edited CAR T cells. *Mol. Ther.* **25**, 949–961 (2017).
 48. Lim, W. A. & June, C. H. The principles of engineering immune cells to treat cancer. *Cell* **168**, 724–740 (2017).
 49. Beatty, G. L. et al. Activity of mesothelin-specific chimeric antigen receptor T cells against pancreatic carcinoma metastases in a phase 1 trial. *Gastroenterology* **155**, 29–32 (2018).
 50. Sackstein, R., Schatton, T. & Barthel, S. R. T-lymphocyte homing: an underappreciated yet critical hurdle for successful cancer immunotherapy. *Lab Invest.* **97**, 669–697 (2017).
 51. Vodnala, S. K. et al. T cell stemness and dysfunction in tumors are triggered by a common mechanism. *Science* **363**, eaau0135 (2019).
 52. Smith, T. T. et al. In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers. *Nat. Nanotechnol.* **12**, 813–820 (2017). **This study shows that circulating T cells can be transfected in vivo to express a CAR transgene and thereby target and kill leukaemic cells, affording an off-the-shelf alternative to adoptive cell transfer.**
 53. Schmid, D. et al. T cell-targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity. *Nat. Commun.* **8**, 1747 (2017).
 54. Wilhelm, S. et al. Analysis of nanoparticle delivery to tumours. *Nat. Rev. Mater.* **1**, 16014 (2016).
 55. Gajewski, T. F. The next hurdle in cancer immunotherapy: overcoming the non-T-cell-inflamed tumor microenvironment. *Semin. Oncol.* **42**, 663–671 (2015).
 56. Ribas, A. et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. *Cell* **170**, 1109–1119 (2018).
 57. Gros, A. et al. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. *Nat. Med.* **22**, 433–438 (2016).
 58. Gros, A. et al. PD-1 identifies the patient-specific CD8⁺ tumor-reactive repertoire infiltrating human tumors. *J. Clin. Invest.* **124**, 2246–2259 (2014).
 59. Amoozgar, Z. & Goldberg, M. S. Targeting myeloid cells using nanoparticles to improve cancer immunotherapy. *Adv. Drug Deliv. Rev.* **91**, 38–51 (2015).
 60. C., N., Gustafson, H. H. & Pun, S. H. Progress in tumor-associated macrophage (TAM)-targeted therapeutics. *Adv. Drug Deliv. Rev.* **114**, 206–221 (2017).
 61. Rodell, C. B. et al. TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy. *Nat. Biomed. Eng.* **2**, 578–588 (2018).
 62. Crusz, S. M. & Balkwill, F. R. Inflammation and cancer: advances and new agents. *Nat. Rev. Clin. Oncol.* **12**, 584–596 (2015).
 63. Li, A. V. et al. Generation of effector memory T cell-based mucosal and systemic immunity with pulmonary nanoparticle vaccination. *Sci. Transl. Med.* **5**, 204ra130 (2013).
 64. Nembrini, C. et al. Nanoparticle conjugation of antigen enhances cytotoxic T-cell responses in pulmonary vaccination. *Proc. Natl. Acad. Sci. USA* **108**, E989–E997 (2011).
 65. Hanson, M. C. et al. Nanoparticulate STING agonists are potent lymph node-targeted vaccine adjuvants. *J. Clin. Invest.* **125**, 2532–2546 (2015).
 66. Luo, M. et al. A STING-activating nanovaccine for cancer immunotherapy. *Nat. Nanotechnol.* **12**, 648–654 (2017).
 67. Liu, H. et al. Structure-based programming of lymph-node targeting in molecular vaccines. *Nature* **507**, 519–522 (2014). **This study reveals that antigens and adjuvants can be efficiently delivered to lymph nodes by conjugating them to lipophilic albumin-binding tails using a polar linker that promotes solubility, thereby dramatically improving antitumour efficacy and markedly reducing systemic toxicity.**
 68. Kuai, R., Ochyl, L. J., Bahjat, K. S., Schwendeman, A. & Moon, J. J. Designer vaccine nanodisks for personalized cancer immunotherapy. *Nat. Mater.* **16**, 489–496 (2017).
 69. Reddy, S. T. et al. Exploiting lymphatic transport and complement activation in nanoparticle vaccines. *Nat. Biotechnol.* **25**, 1159–1164 (2007).
 70. Wilson, J. T. A sweeter approach to vaccine design. *Science* **363**, 584–585 (2019).
 71. Tokatlian, T. et al. Innate immune recognition of glycans targets HIV nanoparticle immunogens to germinal centers. *Science* **363**, 649–654 (2019).
 72. Zhu, G., Zhang, F., Ni, Q., Niu, G. & Chen, X. Efficient nanovaccine delivery in cancer immunotherapy. *ACS Nano* **11**, 2387–2392 (2017).
 73. Pardi, N., Hogan, M. J., Porter, F. W. & Weissman, D. mRNA vaccines — a new era in vaccinology. *Nat. Rev. Drug Discov.* **17**, 261–279 (2018).
 74. Sahin, U., Karikó, K. & Türeci, Ö. mRNA-based therapeutics — developing a new class of drugs. *Nat. Rev. Drug Discov.* **13**, 759–780 (2014).
 75. Kranz, L. M. et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* **534**, 396–401 (2016). **This study illustrates that charge-optimized lipoparticles can efficiently deliver RNA to lymphoid organs and APCs in mice; the ability of this technique to effectively prime antigen-specific T cells was confirmed in patients with cancer.**
 76. Ramishetti, S. & Peer, D. Engineering lymphocytes with RNA. *Adv. Drug Deliv. Rev.* <https://doi.org/10.1016/j.addr.2018.12.002> (2018).
 77. Moffett, H. F. et al. Hit-and-run programming of therapeutic cytoreagents using mRNA nanocarriers. *Nat. Commun.* **8**, 389 (2017).
 78. Junt, T. & Barchet, W. Translating nucleic acid-sensing pathways into therapies. *Nat. Rev. Immunol.* **15**, 529–544 (2015).
 79. Ribas, A. et al. SD-101 in combination with pembrolizumab in advanced melanoma: results of a phase Ia, multicenter study. *Cancer Discov.* **8**, 1250–1257 (2018).
 80. Corrales, L. et al. Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. *Cell Rep.* **11**, 1018–1030 (2015).
 81. Harrington, K. J. et al. Preliminary results of the first-in-human (FIH) study of MK-1454, an agonist of stimulator of interferon genes (STING), as monotherapy or in combination with pembrolizumab (pembro) in patients with advanced solid tumors or lymphomas [abstract LBA15]. *Ann. Oncol.* **29** (Suppl. 8) (2018).
 82. Meric-Bernstam, F. et al. Phase I dose-finding study of MIW815 (ADU-S100), an intratumoral STING agonist, in patients with advanced solid tumors or lymphomas. *Soc. Immunother. Cancer Abstr.* **2018**, 462–463 (SITC, 2018).
 83. Luteijn, R. et al. SLC19A1 is a cyclic dinucleotide transporter. Preprint at *bioRxiv* <https://www.biorxiv.org/content/10.1101/539767v1> (2019).
 84. Ritchie, C., Cordova, A. F., Hess, G. T., Bassik, M. C. & Li, L. SLC19A1 is an importer of the immunotransmitter cGAMP. *Mol. Cell* <https://doi.org/10.1016/j.molcel.2019.05.006> (2019).
 85. Cheng, N. et al. A nanoparticle-incorporated STING activator enhances antitumor immunity in PD-L1–insensitive models of triple-negative breast cancer. *JCI Insight* **3**, 120638 (2018).
 86. Shae, D. et al. Endosomal polymeric nanoparticles increase the activity of cyclic dinucleotide STING agonists to enhance cancer immunotherapy. *Nat. Nanotechnol.* **14**, 269–278 (2019). **This study demonstrates that rationally designed, multiblock, pH-sensitive polymersomes increase cytosolic delivery of cyclic dinucleotides, thereby enhancing antitumour immunity.**
 87. Jacobson, M. E., Wang-Bishop, L., Becker, K. W. & Wilson, J. T. Delivery of 5'-triphosphate RNA with endosomal polymeric nanoparticles potently activates RIG-I to improve cancer immunotherapy. *Biomater. Sci.* **7**, 547–559 (2019).
 88. Hornung, V. et al. 5'-triphosphate RNA is the ligand for RIG-I. *Science* **314**, 994–997 (2006).
 89. Spranger, S. & Gajewski, T. F. Impact of oncogenic pathways on evasion of antitumour immune responses. *Nat. Rev. Cancer* **18**, 139–147 (2018).
 90. Scheper, W. et al. Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers. *Nat. Med.* **25**, 89–94 (2019).
 91. Sagiv-Barfi, I. et al. Eradication of spontaneous malignancy by local immunotherapy. *Sci. Transl. Med.* **10**, eaan4488 (2018).
 92. Spitzer, M. H. et al. Systemic immunity is required for effective cancer immunotherapy. *Cell* **168**, 487–502 (2017).
 93. Marabelle, A. et al. Depleting tumor-specific Tregs at a single site eradicates disseminated tumors. *J. Clin. Invest.* **123**, 2447–2463 (2013).
 94. 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**, E415–E424 (2013).
 95. Horowitz, M., Neeman, E., Sharon, E. & Ben-Eliyahu, S. Exploiting the critical perioperative period to improve long-term cancer outcomes. *Nat. Rev. Clin. Oncol.* **12**, 213–226 (2015).
 96. Krall, J. A. et al. The systemic response to surgery triggers the outgrowth of distant immune-controlled tumors in mouse models of dormancy. *Sci. Transl. Med.* **10**, eaan3464 (2018).
 97. Hiller, J. G., Perry, N. J., Poulogiannis, G., Riedel, B. & Sloan, E. K. Perioperative events influence cancer recurrence risk after surgery. *Nat. Rev. Clin. Oncol.* **15**, 205–218 (2018).
 98. Hugo, W. et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* **165**, 35–44 (2016).
 99. Wang, C. et al. In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy. *Nat. Biomed. Eng.* **1**, 1–10 (2017).
 100. Stephan, S. B. et al. Biopolymer implants enhance the efficacy of adoptive T-cell therapy. *Nat. Biotechnol.* **33**, 97–101 (2015).
 101. Smith, T. T. et al. Biopolymers codelivering engineered T cells and STING agonists can eliminate heterogeneous tumors. *J. Clin. Invest.* **127**, 2176–2191 (2017).
 102. Chen, Q. et al. In situ sprayed bioresponsive immunotherapeutic gel for post-surgical cancer treatment. *Nat. Nanotechnol.* **14**, 89–97 (2019).
 103. Tseng, D. et al. Anti-CD47 antibody-mediated phagocytosis of cancer by macrophages primes an effective antitumor T-cell response. *Proc. Natl. Acad. Sci. USA* **110**, 11103–11108 (2013).
 104. Park, C. G. et al. Extended release of perioperative immunotherapy prevents tumor recurrence and eliminates metastases. *Sci. Transl. Med.* **10**, eaar1916 (2018). **This study shows that reprogramming the post-resection milieu from immunosuppressive to immunostimulatory not only prevents local recurrence but also eradicates disseminated disease, and that sustaining drug exposure locally is required in order to achieve a durable survival benefit.**
 105. Kim, K. et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proc. Natl. Acad. Sci. USA* **111**, 11774–11779 (2014).
 106. Moynihan, K. D. et al. Eradication of large established tumors in mice by combination immunotherapy that engages innate and adaptive immune responses. *Nat. Med.* **22**, 1402–1410 (2016).
 107. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT03815682> (2019).
 108. Tang, L. et al. Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery. *Nat. Biotechnol.* **36**, 707–716 (2018). **This study reveals that cytokines can be conjugated to the surface of T cells using a reversible crosslinker that enables triggered release of native protein in the context of TCR activation, and that adoptive transfer of such ‘backpacked’ cells confers antitumour responses vastly superior to those from unmodified cells plus free cytokine.**
 109. Milhem, M. et al. Intratumoral toll-like receptor 9 (TLR9) agonist, CMP-001, in combination with pembrolizumab can reverse resistance to PD-1 inhibition in a phase Ib trial in subjects with advanced melanoma [abstract CT144]. *Cancer Res.* **78** (13 Suppl.) (2018).
 110. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT03086278> (2019).
 111. Radovic-Moreno, A. F. et al. Immunomodulatory spherical nucleic acids. *Proc. Natl. Acad. Sci. USA* **112**, 3892–3897 (2015).
 112. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT02410733> (2019).
 113. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT01753089> (2019).

114. Ali, O. A., Emerich, D., Dranoff, G. & Mooney, D. J. In situ regulation of DC subsets and T cells mediates tumor regression in mice. *Sci. Transl. Med.* **1**, 8ra19 (2009).
115. Bencherif, S. A. et al. Injectable cryogel-based whole-cell cancer vaccines. *Nat. Commun.* **6**, 7556 (2015).
116. Kim, J. et al. Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy. *Nat. Biotechnol.* **33**, 64–72 (2015).
117. Nature Collection: Nobel Prize in Physiology or Medicine 2018, <https://www.nature.com/collections/gqznlfngkz> (2018).
118. Zheng, Y., Tang, L., Mabardi, L., Kumari, S. & Irvine, D. J. Enhancing adoptive cell therapy of cancer through targeted delivery of small-molecule immunomodulators to internalizing or noninternalizing receptors. *ACS Nano* **11**, 3089–3100 (2017).
119. Editorial. Time to deliver. *Nat. Biotechnol.* **961**, 10 (2014).
120. Kim, S., Shah, S. B., Graney, P. L. & Singh, A. Multiscale engineering of immune cells and lymphoid organs. *Nat. Rev. Mater.* **4**, 355–378 (2019).
121. Gosselin, E. A., Eppler, H. B., Bromberg, J. S. & Jewell, C. M. Designing natural and synthetic immune tissues. *Nat. Mater.* **17**, 484–498 (2018).
122. Dura, B. et al. Profiling lymphocyte interactions at the single-cell level by microfluidic cell pairing. *Nat. Commun.* **6**, 5940 (2015).
123. Jain, R. K., Martin, J. D. & Stylianopoulos, T. The role of mechanical forces in tumor growth and therapy. *Annu. Rev. Biomed. Eng.* **16**, 321–346 (2014).
124. Huse, M. Mechanical forces in the immune system. *Nat. Rev. Immunol.* **17**, 679–690 (2017).
125. Jenkins, R. W. et al. Ex vivo profiling of PD-1 blockade using organotypic tumor spheroids. *Cancer Discov.* **8**, 196–215 (2018).
126. Muraro, M. G. et al. Ex-vivo assessment of drug response on breast cancer primary tissue with preserved microenvironments. *Oncimmunology* **6**, e1331798 (2017).
127. Angelo, M. et al. Multiplexed ion beam imaging of human breast tumors. *Nat. Med.* **20**, 436–442 (2014).
128. Keren, L. et al. A structured tumor-immune microenvironment in triple negative breast cancer revealed by multiplexed ion beam imaging. *Cell* **174**, 1373–1387 (2018). **This study demonstrates that novel technologies can be applied in order to elucidate spatial relationships and features in the context of human tumour tissue, providing insights into patient stratification.**
129. Lin, J. R., Fallahi-Sichani, M. & Sorger, P. K. Highly multiplexed imaging of single cells using a high-throughput cyclic immunofluorescence method. *Nat. Commun.* **6**, 8390 (2015).
130. Lin, J. R. et al. Highly multiplexed immunofluorescence imaging of human tissues and tumors using t-CyCIF and conventional optical microscopes. *eLife* **7**, e31657 (2018).
131. Weiden, J., Tel, J. & Figdor, C. G. Synthetic immune niches for cancer immunotherapy. *Nat. Rev. Immunol.* **18**, 212–219 (2018).
132. Kobayashi, Y. & Watanabe, T. Gel-trapped lymphorganogenic chemokines trigger artificial tertiary lymphoid organs and mount adaptive immune responses in vivo. *Front. Immunol.* **7**, 316 (2016).
133. Fan, Y. et al. Bioengineering thymus organoids to restore thymic function and induce donor-specific immune tolerance to allografts. *Mol. Ther.* **23**, 1262–1277 (2015).
134. Reinisch, A. et al. A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. *Nat. Med.* **22**, 812–821 (2016).
135. Shah, N. J. et al. An injectable bone marrow-like scaffold enhances T cell immunity after hematopoietic stem cell transplantation. *Nat. Biotechnol.* **37**, 293–302 (2019). **This study illustrates that a scaffold can be used to release factors that promote the recruitment of specific cells and induce differentiation of progenitor cells to particular lineages, underscoring the utility of macroscale devices that can establish defined niches to program immunity in situ.**
136. Rothschilds, A. et al. Order of administration of combination cytokine therapies can decouple toxicity from efficacy in syngeneic mouse tumor models. *Oncimmunology* **8**, e1558678 (2019).
137. Messenheimer, D. J. et al. Timing of PD-1 blockade is critical to effective combination immunotherapy with anti-OX40. *Clin. Cancer Res.* **23**, 6165–6177 (2017).
138. Rothschilds, A. M. & Wittrup, K. D. What, why, where, and when: bringing timing to immuno-oncology. *Trends Immunol.* **40**, 12–21 (2019).
139. Goldberg, M. S. Immunoengineering: how nanotechnology can enhance cancer immunotherapy. *Cell* **161**, 201–204 (2015).
140. Bartlett, D. W., Su, H., Hildebrandt, I. J., Weber, W. A. & Davis, M. E. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. *Proc. Natl. Acad. Sci. USA* **104**, 15549–15554 (2007).
141. Grivennikov, S. I., Greten, F. R. & Karin, M. Immunity, inflammation, cancer. *Cell* **140**, 883–899 (2010).
142. Xie, Y. Q. et al. Redox-responsive interleukin-2 nanogel specifically and safely promotes the proliferation and memory precursor differentiation of tumor-reactive T-cells. *Biomater Sci.* **7**, 1345–1357 (2019).
143. Wang, H. & Mooney, D. J. Biomaterial-assisted targeted modulation of immune cells in cancer treatment. *Nat. Mater.* **17**, 761–772 (2018).
144. Liebers, R. & Jager, D. Surgical wound immunotherapy. *Nat. Nanotechnol.* **14**, 7–8 (2019).

Acknowledgements

The author thanks W. Rindler for helpful comments.

Competing interestsM.S.G. is an inventor on patent applications related to REF⁵⁵ (Schmid et al., 2017) and REF¹⁰⁴ (Park et al., 2018). M.S.G. is also an employee of STIMIT.**Peer review information***Nature Reviews Cancer* thanks J. T. Wilson, J. Tel and the other, anonymous, reviewer for their contribution to the peer review of this work.**Publisher's note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.