

Improving cancer immunotherapy using nanomedicines: progress, opportunities and challenges

John D. Martin¹, Horacio Cabral, Triantafyllos Stylianopoulos² and Rakesh K. Jain³

Abstract | Multiple nanotherapeutics have been approved for patients with cancer, but their effects on survival have been modest and, in some examples, less than those of other approved therapies. At the same time, the clinical successes achieved with immunotherapy have revolutionized the treatment of multiple advanced-stage malignancies. However, the majority of patients do not benefit from the currently available immunotherapies and many develop immune-related adverse events. By contrast, nanomedicines can reduce — but do not eliminate — the risk of certain life-threatening toxicities. Thus, the combination of these therapeutic classes is of intense research interest. The tumour microenvironment (TME) is a major cause of the failure of both nanomedicines and immunotherapies that not only limits delivery, but also can compromise efficacy, even when agents accumulate in the TME. Coincidentally, the same TME features that impair nanomedicine delivery can also cause immunosuppression. In this Perspective, we describe TME normalization strategies that have the potential to simultaneously promote the delivery of nanomedicines and reduce immunosuppression in the TME. Then, we discuss the potential of a combined nanomedicine-based TME normalization and immunotherapeutic strategy designed to overcome each step of the cancer-immunity cycle and propose a broadly applicable ‘minimal combination’ of therapies designed to increase the number of patients with cancer who are able to benefit from immunotherapy.

The widely accepted paradigm of nanomedicine — enhanced permeability and retention (EPR) — assumes that cytotoxic drugs can be delivered selectively to tumours using nanomedicines (defined as drug-loaded nanoparticles of 1–1,000 nm in diameter) to increase efficacy and minimize the risk of systemic adverse effects. However, this approach has thus far conferred only modest improvements in the survival outcomes of patients with cancer¹ (Supplementary Table 1). By contrast, immune-checkpoint inhibition (ICI) has provided unprecedented improvements in the survival outcomes of a subset of patients. However, ICI is currently estimated to benefit <13% of patients with cancer² and a substantial fraction of patients receiving these therapies will develop immune-related

adverse events³. As a result, research interest in nanomedicine is shifting rapidly towards the adaptation of delivery platforms for improving the percentage of patients who derive clinical benefit from ICI and other immunotherapies^{4,5}. Two paradigms for the application of nanomedicines to the potentiation of immunotherapy are currently emerging: systemic administration of nanomedicines that have a tumour-priming effect; and local or extratumoural administration of nanomedicines to induce local and/or systemic antitumour immunity. The first paradigm is supported by data from a successful phase III trial, in which women with metastatic triple-negative breast cancer (TNBC) received the combination of nab-paclitaxel plus the anti-programmed cell death 1 ligand 1 (PD-L1) antibody

atezolizumab⁶. Various manifestations of the second paradigm, such as the delivery of vaccines using lipid-based nanomedicines to promote antitumour immunity, are the focus of preclinical and clinical investigations (for example, NCT02410733).

We hypothesize that the pathophysiology of the tumour microenvironment (TME; Supplementary Figure 1) limits the uniform delivery of both systemically administered and locally applied nanomedicines, thus compromising their efficacy even when they accumulate in tumours^{1,7,8}. Therefore, we propose that nanomedicines should incorporate not only anticancer drugs but also agents that ‘normalize’ the various components and physiology of the TME, resulting in improved tumour perfusion and reduced levels of hypoxia. This normalization effect has the potential to facilitate not only drug delivery¹ but also that of oxygen to slow tumour progression and convert an immunosuppressed TME into an immunostimulatory TME^{9,10}. We propose that nanotechnology will improve the implementation of immunotherapies by facilitating the delivery of specific combinations and schedules of TME-normalizing agents, cytotoxic agents and immunotherapies. In this Perspective, we first summarize the evidence indicating how the TME limits the efficacy of both nanomedicines and immunotherapies, followed by discussions of how normalizing the TME can improve drug delivery and the outcomes of patients receiving immunotherapy. We then summarize how nanomedicine-based approaches might overcome the mechanisms of resistance to immunotherapies. Finally, we propose strategies that involve re-engineering and/or developing new nanomedicines with the aim of optimizing the effectiveness of immunotherapies.

Role of the TME in treatment resistance

We hypothesize that the pathophysiology of the TME of primary tumours and their distant metastases often limits the efficacy of nanomedicines and immunotherapies by limiting the accumulation, distribution and function of drugs and immune cells^{9–11}. Angiogenic and fibrotic signalling mediates this pathophysiology and directly — and indirectly through induction of hypoxia — induces immunosuppression.

Distribution of nanomedicines

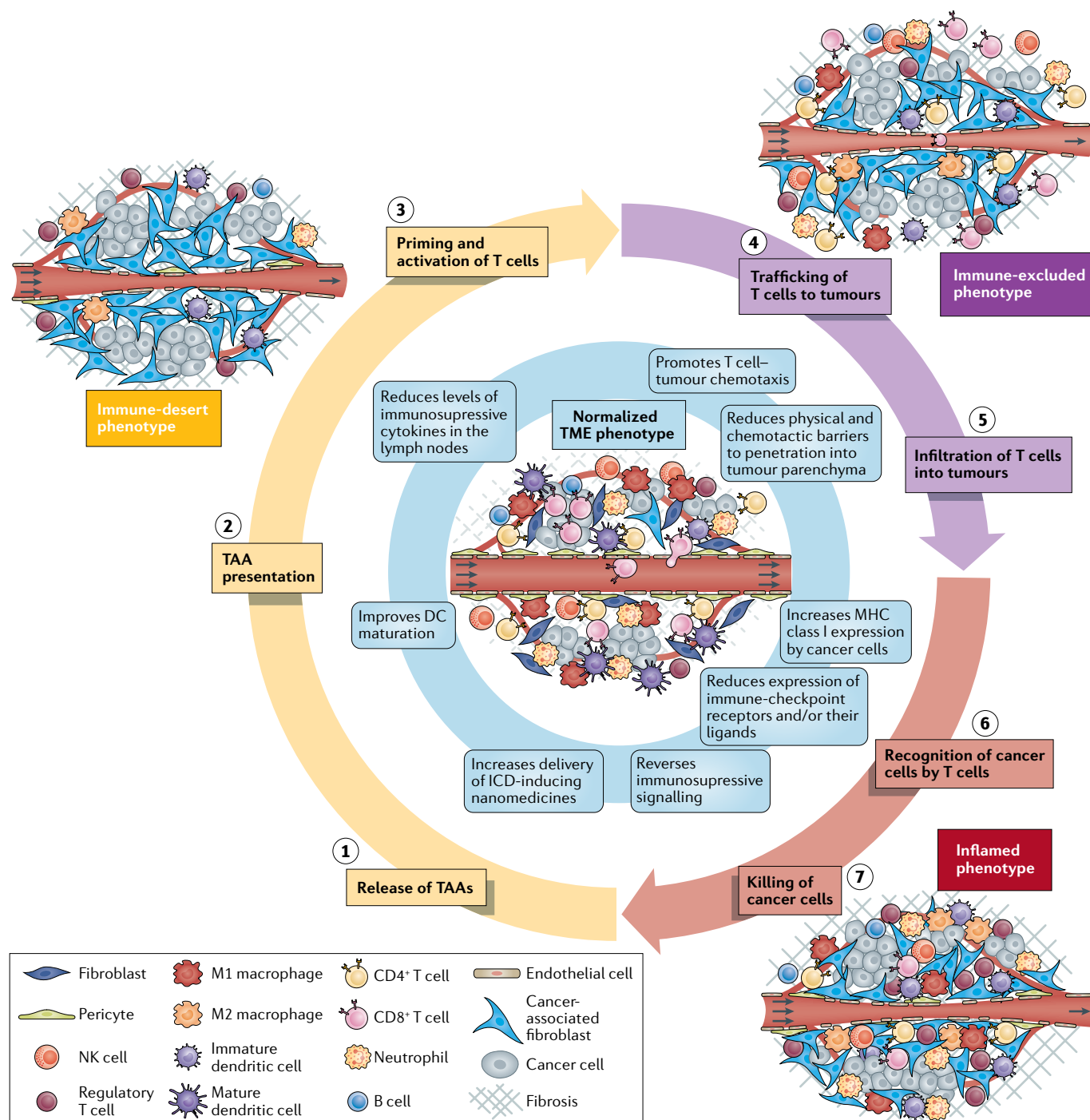
Data from clinical studies published in 2017 (REFS^{12,13}) confirm the existence of the EPR effect in patients with cancer and that this effect is correlated with the response to nanomedicines. However, the benefits of EPR are compromised by a substantial level of spatial intratumour and intertumour heterogeneity in drug distribution, both in patients with tumours of the same type and between multiple tumours in the same patient^{12,13}. This heterogeneity might explain the disparate results obtained

with nab-paclitaxel in the metastatic and adjuvant settings in patients with pancreatic ductal adenocarcinoma (PDAC; Supplementary Table 1). A dysfunctional tumour vasculature, resulting from abnormal angiogenesis and desmoplasia (leading to tumour fibrosis), limits the uniform distribution of nanomedicines independently of their physicochemical properties by reducing tumour blood flow and hindering the transport of nanomedicines from blood vessels to cancer cells (reviewed in detail elsewhere^{1,8,14,15}).

Reduced tumour blood flow also limits the availability of oxygen and induces acidity, resulting in immunosuppression^{9–11}. Similar to the distribution of nanomedicines^{12,13}, the level of immune cell infiltration can even differ between the primary tumour and metastases in the same patient^{16,17}.

Cancer-immunity phenotypes

The immune phenotype of the TME influences sensitivity to immunotherapies, including ICI and can be broadly classified into three main phenotypes¹⁸ (FIG. 1).



First, the immune-desert phenotype, which lacks antitumour immune cells, is characterized by immunological ignorance (a lack of antigens and/or their presentation), tolerance (a lack of response to antigen presentation) and a lack of T cell priming¹⁸ (FIG. 1). Tumours of this phenotype are the least responsive to ICI. Indeed, patients with tumours that lack pre-existing cytotoxic T cells and have a less clonal repertoire of T cell receptors (TCRs) have worse outcomes than those with tumours of other immune phenotypes¹⁹. The pathophysiology of the TME contributes to this phenotype. VEGF, an angiogenic growth factor induced by hypoxia, promotes immunological ignorance by inhibiting dendritic cell (DC) maturation, thereby reducing the extent of antigen presentation²⁰. Hypoxia also promotes immune tolerance by inducing the expression of chemokines that recruit pro-tumour CD4⁺CD25⁺FOXP3⁺ regulatory T (T_{reg}) cells^{21,22}. Thus, hypoxia and hypoxia-induced signalling contribute to the lack of T cell priming characteristic of the immune-desert phenotype.

Second, the immune-excluded phenotype is characterized by immune cells localized to the tumour periphery or stroma¹⁸, impeded by immature vessels and extravascular stroma and characterized by increased expression of transforming growth factor- β (TGF β), an excessive extracellular

matrix (ECM) and a high density of cancer-associated fibroblasts (CAFs)^{23–25} (FIG. 1). Tumours of this phenotype have more potential for sensitivity to ICI than those of the immune-desert phenotype because T cells present in the stroma might become active and proliferate, despite being unable to easily infiltrate the parenchyma^{19,23}. Desmoplasia is a major cause of this phenotype, as CAFs secrete chemokines such as TGF β and stromal cell-derived factor 1 α , which prevent cytotoxic T lymphocytes (CTLs) from migrating to cancer cells^{23–25}. TGF β also induces the development of a T_{reg} phenotype in naive CD4⁺ T cell precursors²². CAFs have immunosuppressive subsets and are also able to produce and maintain the dense desmoplasia that blocks CTL migration²⁶. Angiogenesis is another major cause of this phenotype, as angiogenic signalling dysregulates the expression of adhesion molecules on the vessel wall, thereby reducing the extent of leukocyte binding and limiting their flux into tumours¹¹. The demonstration that T cell motility is slower in hypoxic regions of tumours is consistent with evidence that angiogenesis and desmoplasia cause hypoxia and can directly exclude immune cells through both signalling and physical means^{27,28}.

Third, tumours of the inflamed phenotype have T cells located in the

parenchyma and express pro-inflammatory cytokines, indicating the presence of a failed antitumour immune response¹⁸. Owing to the presence of large numbers of T cells with TCRs against tumour-associated antigens, this phenotype has the most potential for sensitivity to ICI, although it often also contains many hypoxia-suppressed immune cells²³ (FIG. 1). Indeed, activation of VEGF signalling recruits immunosuppressive cells, including T_{reg} cells, myeloid-derived suppressor cells and M2-like tumour-associated macrophages (TAMs) to the tumour²⁹. Hypoxia also promotes the recruitment of T_{reg} cells²¹ and even certain CAF phenotypes can recruit immunosuppressive cells²⁶. Additionally, the expression of immune checkpoint molecules, which reduces the activity of immune cells, is a key feature of the inflamed phenotype. VEGF upregulates the expression of several immune-checkpoint proteins on T cells^{30–32}. Similarly, hypoxia — via hypoxia inducible factor 1 α signalling — upregulates the expression of immune-checkpoint receptors and/or their respective ligands on myeloid-derived suppressor cells, TAMs, DCs and cancer cells^{33,34}. Even though T cells are present in the parenchyma of tumours of the inflamed phenotype, acidity³⁵, CAFs²⁶ and collagen density³⁶ might all reduce their level of cytotoxic activity.

Abnormal angiogenesis can induce immunosuppression in tumours of all three cancer-immunity TME phenotypes and, in turn, certain immune cells can induce angiogenesis³⁷ thus creating a vicious cycle. We hypothesize that by normalizing the constituents and physiology of the TME, nanomedicine delivery will be homogenized^{1,8,14,15}, immune-cell infiltration and function will be increased^{9,10,38} and this vicious cycle will be broken^{10,38,39}, even in patients with primary tumours and/or metastases featuring heterogeneous and varied TME phenotypes^{12,13,16,17}.

Normalizing the TME

We have developed two broad strategies to ‘normalize’ the TME¹⁰: normalizing the tumour vasculature with judicious use of direct or indirect antiangiogenic therapies (AATs) so that it becomes more morphologically and functionally similar to the vasculature of nonmalignant tissues^{9,40–45}; and reprogramming CAFs to reduce ECM levels, decompress the blood vessels and improve the extent of intratumoural drug penetration^{8,14,46,47} (FIG. 1). In contrast to normalization, depletion of the TME could contribute to disease progression^{10,47}.

◀ **Fig. 1 | Cancer-immunity TME phenotypes affecting responsiveness to immunotherapy**¹⁸. Three distinct cancer-immunity phenotypes exist and can affect responsiveness to immunotherapies in different ways. These phenotypes reflect tumours at different phases of the seven-step cancer-immunity cycle that must be completed repeatedly for immunotherapies to be effective. Tumour microenvironment (TME) normalization (centre, blue shading) promotes perpetuation of the cycle. In tumours of the immune-desert phenotype (yellow shading), the TME and the often limited number of immune cells within the tumour are immunosuppressed. The host immune system permits cancer cell growth owing to a lack of antigen recognition, immune tolerance and/or a failure to prime cytotoxic T cells. Some of these processes are affected by hypoxia. In tumours of the immune-excluded phenotype (purple shading), immune cell infiltration is restricted to the periphery and/or the stroma. The stromal factors that promote this phenotype similarly inhibit the delivery of nanomedicines and/or oxygen. In tumours of the inflamed phenotype (red shading), immune cells are stimulated by pro-inflammatory cytokines and are able to move throughout the tumour parenchyma. However, various inhibitory factors, which are often induced by hypoxia, lead to a reduction in antitumour immunity. Normalization of the vasculature by targeting angiogenic factors (such as VEGF and/or angiopoietin-2) and/or immune checkpoints and normalization of the tumour extravascular compartment by reprogramming cancer-associated fibroblasts (CAFs) to produce a less dense extracellular matrix (ECM) are two strategies that can be applied alone or in combination to normalize the entire TME and improve perfusion, oxygen delivery and drug distribution. Improper use of these strategies, however, can lead to excessive vessel pruning or CAF and/or ECM depletion, which might accelerate tumour progression and metastasis. Anti-programmed cell death 1 (PD-1) or programmed cell death 1 ligand 1 (PD-L1) antibodies can also normalize blood vessels in some tumour types and could promote the growth of mature blood vessels that are protected from pruning by antiangiogenic agents, which primarily target immature vessels. Signalling pathways such as those activated by VEGF, angiopoietin-2, CXCL12/CXCR4 and transforming growth factor- β , which can all be targeted to normalize the TME, are themselves immunosuppressive. As a result of these effects, the combination of therapies targeting these pathways with immunotherapies is a promising approach. Thus, specific TME normalization strategies based on the TME immune phenotype of the target tumour could increase both the response rates and the magnitude of responses to immunotherapies. DC, dendritic cell; ICD, immunogenic cell death; NK, natural killer; TAA, tumour-associated antigen.

Normalization of tumour blood vessels

Judicious use of AATs can reduce the size of abnormally large pores in the tumour vessel wall, making the wall less permeable to fluid and repairing the chaotic structure of the vascular network without excessive vessel depletion; more than a dozen FDA-approved agents are able to mediate this effect¹⁰ (Supplementary Table 2). Decreasing the size of the pores increases tumour perfusion and reduces the interstitial fluid pressure (IFP)¹⁴. Perfusion correlates with the accumulation of nanomedicines^{48,49}, and the reduction in IFP also contributes to the increased transport of nanomedicines with a diameter of at least ~30 nm (or potentially as large as liposomes, depending on the cancer type) across the vessel wall^{50,51}. Data from more than a dozen hypothesis-generating clinical trials demonstrate that patients whose tumour blood vessels are normalized and/or those who have improved levels of tumour perfusion and/or oxygenation after AAT have better outcomes¹⁰. Nonetheless, mathematical modelling simulations suggest that AATs do not improve the vascular function of tumours with an abundance of compressed vessels, such as PDACs⁵², consistent with the clinical failure of AAT in patients with PDAC⁵³. In fact, extended use of AATs often results in excessive pruning of the tumour vasculature, which exacerbates hypoperfusion¹⁰. Unaffected by IFP and angiogenesis^{54,55}, tumour vessel compression is an effect of the accumulation of mechanical compressive forces within the tumour — termed solid stress — generated by rapid cancer cell growth and stored in intratumour and surrounding host tissue structural components⁵⁶.

Normalization of extravascular factors

Solid stress generated by proliferating cancer cells and CAFs is transmitted by the dense ECM to the tumour vasculature, resulting in compressed vessels that limit the delivery of both oxygen and therapeutics of all sizes^{14,46,56,57}. Similar to impairment of T cell migration to the tumour parenchyma^{23–25}, components of the tumour extravascular space also hinder the diffusion of therapeutics from blood vessels to cancer cells^{18,14}. Collagen is one such barrier⁵⁸, and co-injection with bacterial collagenase increases the distribution of intratumourally administered oncolytic viral vectors in a patient-derived xenograft model of melanoma⁵⁹. Similarly, the hormone relaxin modifies the structure and content of collagen to promote diffusion of macromolecular agents in mouse models of several common solid tumours⁶⁰. Sulfated

glycosaminoglycans are another barrier, as demonstrated by the observation that matrix metalloproteinase 1 (MMP1) and MMP8 overexpression reduces the density of this ECM component in a mouse model of soft-tissue sarcoma, thereby increasing the diffusion of oncolytic viral vectors⁶¹. Nonetheless, care must be exercised with such ablative strategies owing to the risk of inducing disease progression^{10,47}. Rather, TME-normalizing CAF-reprogramming agents, such as paricalcitol (which is an agonist of the vitamin D receptor on CAFs), can promote the development of a quiescent stromal phenotype⁶², can reduce the ECM density and IFP to near-normal values and can also alleviate solid stresses, thus decompressing tumour blood vessels. These agents have been referred to as ‘mechanotherapeutics’^{46,63}.

One promising strategy for the development of mechanotherapeutics involves repurposing commonly used drugs that inhibit TGF β signalling in CAFs for use in patients with cancer (such as tranilast^{57,64}, metformin⁶⁵, pirfenidone⁶⁶ and others; Supplementary Table 3). Of these, angiotensin system inhibitors (ASIs), which are commonly used antihypertensives, are promising TGF β -inhibiting mechanotherapeutics owing to the large numbers of retrospective studies indicating they provide benefit to patients. Indeed, data from a series of retrospective studies involving patients with non-small-cell lung cancer (NSCLC), PDAC, ovarian cancer, hepatocellular carcinoma, glioblastoma, oesophageal cancer or renal cell carcinoma (RCC) and a prospective phase II trial involving patients with locally advanced PDAC, show that repurposing angiotensin II receptor blockers (ARBs) to reprogramme the stroma has the potential to extend the survival of patients with cancer^{67–73} (Supplementary Table 4). ASIs are able to reprogramme CAFs and promote transvascular transport⁷⁴, thus improving both the intratumoural distribution and the effectiveness of nanomedicines⁷⁵. In particular, ARBs such as losartan reduce the activation of TGF β signalling and ECM production by CAFs more effectively than other ASIs^{8,74,75}. In fact, a phase II trial has shown that adding losartan to standard-of-care neoadjuvant leucovorin, 5-fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX) followed by chemoradiotherapy led to a resection rate of 61% and a median overall survival duration of 33 months in patients with locally advanced PDAC⁷⁶. These encouraging findings have led to a

multi-institutional randomized phase II trial (NCT03563248).

Normalizing the TME for immunotherapy

Improvements in tumour perfusion and oxygenation increase responsiveness to ICI⁷⁷, and multiple preclinical studies have shown that vascular normalization with AATs can increase the antitumour potency of these agents^{78–80}, whole cancer cell vaccines⁸¹ and adoptive cell therapy (ACT)⁸². Phase III trials showing the effectiveness of combining the anti-VEGFA antibody bevacizumab with atezolizumab and either paclitaxel or nab-paclitaxel in the first-line treatment of patients with metastatic nonsquamous NSCLC⁸³ and the small-molecule VEGFR1–3 inhibitor axitinib with avelumab⁸⁴ or pembrolizumab⁸⁵ in patients with advanced-stage RCC also highlight the importance of angiogenesis to the efficacy of ICI. The role of vascular normalization in increasing responsiveness to immunotherapy, in part by ameliorating hypoxia, is also supported by clinical evidence that increased tumour perfusion correlates with responsiveness to bevacizumab in patients with advanced-stage NSCLC^{10,86}. Emerging data also indicate that stimulation of immune cells via ICI can induce vascular normalization in some, but not all, preclinical models^{77,80,87}. Thus, the combination of vascular normalization and ICI could promote a positive feedback loop in which improved vascular function alleviates hypoxia and further improves the efficacy of both therapeutic modalities^{38,39,80}. This feedback loop provides an additional rationale for the combinations of AATs and ICI that are either FDA approved or currently undergoing clinical evaluation^{11,38,39} (Supplementary Table 2).

Beyond vascular normalization, mechanotherapeutics are also currently under clinical investigation in combination with nanomedicines and ICI (Supplementary Table 3), and those that interfere with TGF β signalling in CAFs (such as losartan⁷⁴, tranilast^{57,64} and pirfenidone⁶⁶) might, in particular, improve the efficacy of immunotherapies⁸⁸. TGF β signalling has multiple roles in tumour progression, although TGF β signalling through CAFs is immunosuppressive^{23,88,89}. Specifically, in mouse models of metastatic cancer, TGF β promotes immune evasion, while inhibition of TGF β signalling produces a potent T cell response⁸⁹ and potentiates the efficacy of ICI²⁵. In patients with metastatic urothelial carcinomas, TGF β expression

and desmoplasia exclude T cells from the tumour parenchyma, thereby contributing to the immune-excluded phenotype, which is associated with a poor response to ICI²³ (FIG. 1). Similarly, biopsy samples of lung metastases obtained from women with breast cancer also typically have a pattern of fibrosis that excludes T cells²⁴. In other experiments, inhibition of TGF β signalling in mouse models has been shown to promote the systemic antitumour immune response induced by radiation in mouse models — the so-called abscopal effect⁹⁰. Finally, inhibition of TGF β signalling upregulates the expression of adhesion molecules (such as intercellular adhesion molecule 1 or vascular cell adhesion molecule 1) on tumour blood vessels that are used by lymphocytes to extravasate³⁸.

In support of these mechanistic studies, a prospective analysis of samples obtained from patients with PDAC demonstrated an increase in the expression of genes associated with T cell activation, and an accompanying retrospective analysis revealed better outcomes among patients receiving long-term ARBs versus those not receiving long-term ARBs after adjustment for other covariates⁶⁸. By contrast, a retrospective analysis of samples from patients with NSCLC who received anti-programmed cell death 1 (PD-1) or anti-PD-L1 antibodies with or without angiotensin-converting enzyme (ACE) inhibitors found fewer antitumour M1 macrophages and memory-activated T cells in samples from patients who were also receiving ACE inhibitors, leading to worse patient outcomes, even after adjustment for other risk factors⁹¹. This result contradicts the findings of a preclinical study indicating that losartan reduces the incidence of lung metastases in mouse models by blocking monocyte recruitment⁹². Although ACE inhibitors, similar to ARBs, also inhibit components of the renin–angiotensin–aldosterone system, they might act in opposition to ARBs that induce stromal normalization because ACE inhibitors indirectly inhibit both angiotensin II receptor 1 and 2 signalling, unlike losartan, which inhibits receptor 1 only⁷⁴. Thus, mechanotherapeutic strategies, particularly those involving inhibition of TGF β signalling, could be used to promote the effects of both nanomedicines and immunotherapies through numerous mechanisms. This rationale supports an ongoing clinical trial in which patients with locally advanced PDAC are receiving losartan in combination with nivolumab, FOLFIRINOX and stereotactic body radiotherapy (NCT03563248).

Nanomedicines in immunotherapy

ICI has revolutionized the treatment of patients with cancer, and antibodies targeting PD-1, PD-L1 or cytotoxic T lymphocyte-associated protein 4 (CTLA-4) have been approved by various regulators as monotherapies or in combination with other treatments for more than a dozen metastatic and/or advanced-stage malignancies, including NSCLC, TNBC, melanoma, Merkel cell carcinoma, hepatocellular carcinoma, RCC, urothelial carcinoma, lymphoma, cervical cancer, gastric or gastro-oesophageal junction adenocarcinoma, colorectal cancer and head and neck squamous cell carcinoma. These agents provide durable responses in a subset of patients and some of these patients are ‘cured’; however, an estimated 87% of patients currently do not derive long-term benefit from ICI². Thus, new strategies are needed to improve the response rates to such therapies in patients with ICI-resistant disease.

Antitumour immunity must be perpetuated in order to enable durable responses to immunotherapy (FIG. 2). The cancer-immunity cycle consists of: (1) release of tumour-associated antigens; (2) antigen presentation; (3) priming and activation of T cells; (4) trafficking of T cells to tumours; (5) infiltration of T cells into tumours; (6) recognition of cancer cells by T cells; and (7) killing of cancer cells¹⁸. Tumours of the immune-desert TME phenotype cannot progress beyond steps 1–3 owing to a lack of immune cells within and surrounding the tumour, while those of the immune-excluded phenotype cannot progress beyond steps 4 and 5 owing to a lack of T cells in the tumour parenchyma. Tumours of the inflamed TME phenotype cannot overcome steps 6 and 7 owing to T cell exhaustion. Various nanomedicine-based approaches have been developed in an attempt to address steps 1–7, while TME normalization might also be beneficial in this regard.

Tumour-associated antigen release

A lack of, or limited, antigen release is a major challenge for successful immunotherapy, especially in tumours of the immune-desert phenotype^{93,94}. Underlining the importance of antigens, ICI is most effective in patients with solid tumours with a high tumour mutational burden, including melanoma, NSCLC and bladder cancer. Only isolated responses to ACT and ICI combinations have been observed among patients with tumour types with a lower tumour mutational burden⁹⁵.

Thus, varying numbers of neoantigens are produced and released across all types of solid tumours⁹⁶. Additionally, the number and type of neoantigens are unique to each lesion, which might necessitate a personalized immunotherapeutic approach⁹⁷.

Inducing immunogenic cell death (ICD) using chemotherapies with immunogenic effects or selected doses of ionizing radiation has the potential to prime tumours for a response to immunotherapy by promoting the release of signals that can be recognized by the immune system and thus enhance systemic antitumour immunity⁹⁸. Signals are released from cancer cells undergoing ICD into the microenvironment and possibly the systemic circulation, where they induce DCs to present neoantigens to T cells, a process that is required for activation of tumour-specific T cells^{99,100}. By reducing the delivery of small-molecule chemotherapies to nonmalignant tissues, and limiting the necessary dose of radiotherapy, nanomedicine-based chemotherapeutics and radiosensitizers, respectively, could improve the efficacy of ICI and minimize toxicities.

Chemotherapy-induced ICD. The rationale for using nanomedicines rather than small molecules as ICD-inducing chemotherapy is not only the expected reduction in toxicities owing to the EPR effect, but also the potential of the former to normalize the TME. However, metronomic chemotherapy (delivered using frequent low-dose administration) can also normalize the TME, increase tumour perfusion and alleviate hypoxia, thus reprogramming an immunosuppressive TME to an antitumour immunity phenotype^{44,101} similar to the effects of certain cytotoxic nanomedicines⁶⁴. Data from mathematical modelling suggest that these two approaches induce TME normalization through similar mechanisms¹⁰¹. The key feature of both strategies is the delivery of sustained drug concentrations. Nanomedicines achieve this effect by prolonging the length of time spent in the circulation and gradually releasing the cytotoxic payload, whereas metronomic strategies achieve the same effect by maintaining consistent drug concentrations through frequent administration. The schedule, dose and type of chemotherapy administered using nanomedicine-based ICD is also important, yet still undefined. Some chemotherapeutic agents seem to promote more ICD and/or other steps of the cancer-immunity cycle than others¹⁰². Nonetheless, while the mechanisms and optimal scheduling remain unclear, the use

of nab-paclitaxel as a nanomedicine-based chemotherapy to avoid the requirement of immunosuppressive glucocorticoids and potentiate the efficacy of ICI in patients with advanced-stage TNBC fulfils the initial potential of this approach⁶.

We hypothesize that normalizing the TME using AAT and/or mechano-therapeutics in order to increase penetration of cytotoxic nanomedicines capable of both ICD induction and TME normalization could increase

the number of cancer cells undergoing ICD and, in turn, promote further TME normalization, thereby increasing responses to ICI⁶⁴ (FIG. 2). Data from a clinical trial combining metronomic chemotherapy, vascular normalization and ICI provide

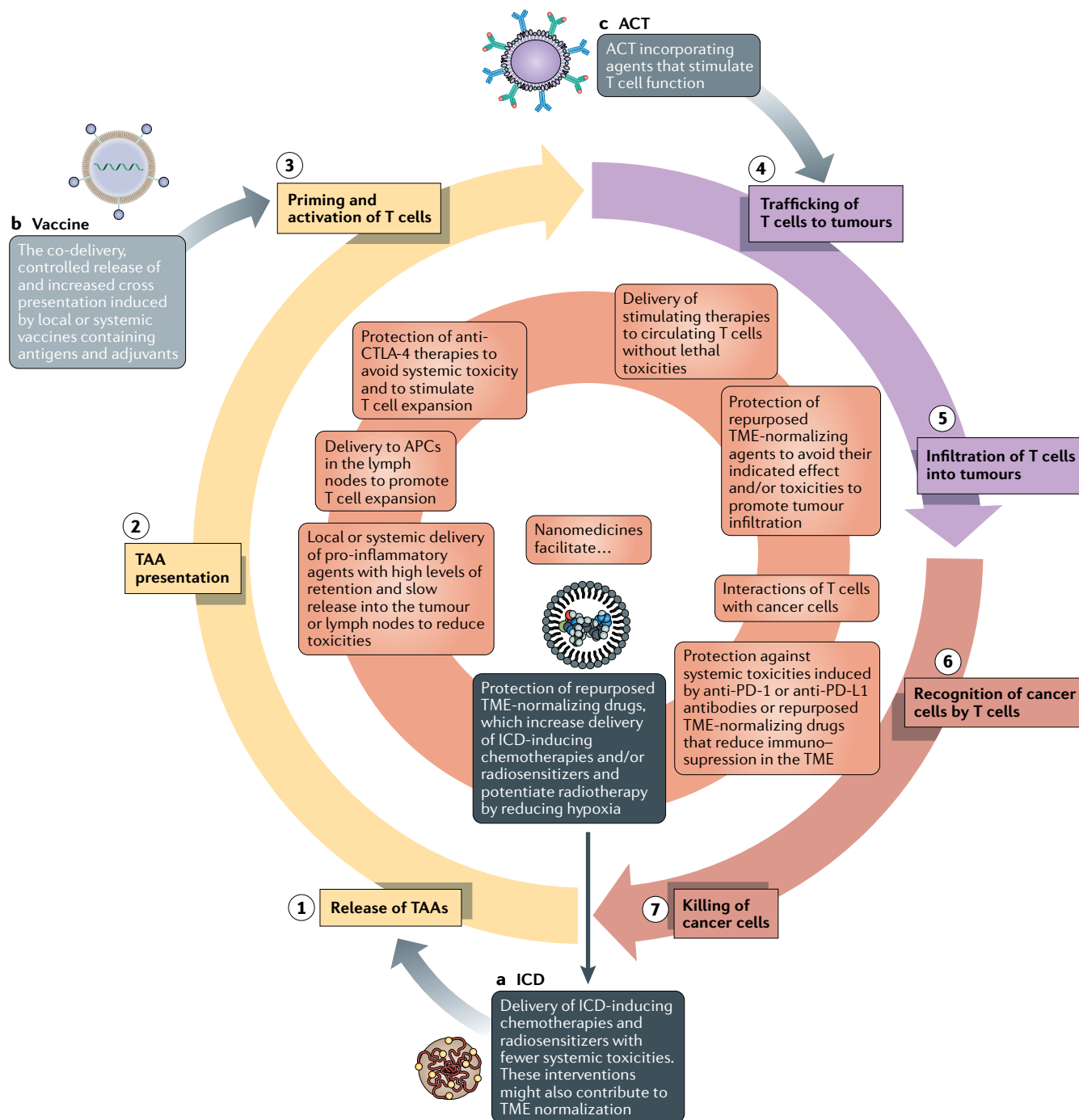


Fig. 2 | How nanomedicines can be used to perpetuate the cancer-immunity cycle. The goal of nanomedicine-based immunotherapy is to ensure that the cancer-immunity cycle (the seven numbered steps) perpetuates. Initially, in order to ensure that T cells are capable of attacking cancer cells, we highlight three nanomedicine-based starting points: **(a)** immunogenic cell death (ICD)-inducing therapy, **(b)** vaccines and **(c)** nanoparticle-loaded T cells. Therapies specifically relevant to each of these starting points are in dark grey, light grey and grey boxes, respectively. After one of these initiations, various types of nanomedicines described in this Perspective can turn the cycle forward (the inner orange circle). ACT, adoptive cellular therapy; APC, antigen-presenting cell; CTLA-4, cytotoxic T lymphocyte-associated protein 4; DC, dendritic cell; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; TAA, tumour-associated antigen; TME, tumour microenvironment.

support for further investigations of this approach¹⁰³. The preliminary results of this phase II in a small cohort of women with recurrent ovarian, fallopian tube or primary peritoneal cancers revealed that metronomic cyclophosphamide combined with bevacizumab and pembrolizumab resulted in a 37.5% overall response rate¹⁰⁵, whereas the highest response rate reported with pembrolizumab alone among women with gynaecological cancers is 8% in the KEYNOTE-100 trial¹⁰⁴.

Radiation-induced abscopal effects. The abscopal effect involves inducing ICD in one or more superficial lesions, which can then induce a systemic immune response against distant metastases^{105–109}. Besides chemotherapy, locally administered ionizing radiation can also induce ICD and stimulate the immune system to attack distant metastases. However, radiation oncologists must optimize the radiation dose and level of fractionation when scheduling radiotherapy specifically for the induction of abscopal effects, as preclinical data indicate that fractionated, rather than single-dose, radiotherapy can induce ICD^{110,111} while high-dose radiotherapy limits immunogenicity owing to activation of enzymatic digestion of DNA¹¹². When effective, radiation increases the diversity of the TCR repertoire found in tumours¹¹³. Indeed, the European Medicines Agency approval of intratumoural injections of NBTXR3 hafnium oxide nanoparticles designed to enhance the radiation-induced abscopal effect for patients with locally advanced soft-tissue sarcomas demonstrates the validity of this approach¹¹⁴ (Supplementary Table 1).

Similar to intratumoural injections of hafnium oxide nanoparticles, some elements of cancer treatment regimens could include locally administered nanomedicines. Nonetheless, these agents still must overcome the physical barriers posed by the TME of the target tumour, while the local TME and that of distant metastases will likely continue to be immunosuppressive. In this setting, nanomedicine encapsulation increases the retention of chemotherapies within the tumour, enables delayed release of the active payload and reduces the amount of drugs released into the systemic circulation, thus avoiding or reducing the risk of toxicities. Mechanotherapeutics that normalize the extravascular component of the TME increase the distribution of locally administered nanomedicines^{75,115}, although the optimal approach to normalizing the TME of distant

metastases within an appropriate period of time after local delivery of ICD-inducing therapy is unclear. TME normalization before radiotherapy improves outcomes by alleviating hypoxia^{9,116}, although the effects of TME normalization on ICD require further investigation⁶⁴. Separately, administration of nanomedicines after radiotherapy might be an effective method of perpetuating the cancer-immunity cycle. Indeed, radiotherapy improves the distribution of nanomedicines in mouse models, in part by normalizing the TME^{117,118}. An alternative approach involves the delivery of nanomedicines targeting P-selectin, which consist of fucoidan-based nanoparticles with a high level of affinity for this protein. Radiotherapy can be used to increase the cell surface expression of P-selectin, thus improving the delivery and efficacy of this formulation in preclinical models¹¹⁹.

Cancer antigen presentation

Immature DCs must capture released tumour-associated antigens, followed by maturation, lymph node migration and education of naive T cells, despite the presence of an immunosuppressive TME. Immunological adjuvants, which stimulate DCs during antigen detection and processing, can increase the strength of antigen presentation. Otherwise, immune tolerance might occur, during which an immature DC captures an antigen but is not stimulated and therefore fails to process the antigen and activate T cells¹²⁰.

Dendritic cell maturation. Cancer antigen presentation can be increased by agents that induce DC maturation, although agents designed to induce this effect have, thus far, shown poor pharmacokinetics when administered systemically¹²¹. One clinically investigated, non-nanomedicine-based strategy involves intratumourally injecting an agonist of Toll-like receptor 9 (TLR9) (such as SD-101) to induce DC maturation and subsequent antigen presentation. This strategy has been shown to be well-tolerated and leads to promising response rates in the injected lesion as well as in distant metastases when administered in combination with ICI in patients with anti-PD-1-antibody-naïve advanced-stage melanomas in a phase Ib/II trial¹²². Similarly, intratumourally injected activators of stimulator of interferon-genes (STING) agonists (such as SD-100) are currently in early-phase clinical trials with preliminary data indicating limited response rates¹²³. Indeed, a potential problem involves the overstimulation of T cells,

resulting in their death and a reduction in antitumour immunity¹²⁴. Nonetheless, such therapies could be useful in combination with ICI in patients with tumours with an immune-desert TME phenotype.

Compared with small molecules, locally administered nanomedicines are likely to be retained in the TME for longer periods of time, thereby increasing the ratio of intratumour to systemic drug distribution. For example, a nanomedicine-conjugated TLR7 and TLR8 agonist promotes DC maturation and potentiates the effects of ICI in mouse models¹²⁵. STING agonists are also candidates for encapsulation in locally and systemically administered nanomedicine formulations^{126,127}. Such cytokines can also be targeted to collagen, thus enabling them to be retained in tumours more effectively^{128,129}.

Nanomedicine-based vaccines induce antigen presentation and make release dispensable. Vaccines are able to initiate antigen presentation, although their effects upon cancer treatment have, thus far, been modest. Nanomedicine-based approaches confer various advantages over conventional methods of delivery of subunit vaccines, which contain only the antigenic parts. First, nanomedicine-based vaccines improve the delivery of antigens to lymphoid tissues¹³⁰. Without nanotechnology, the delivery of subunit vaccines can require repeat local injections, such as injections of mRNAs into inguinal lymph nodes¹³¹ and peptides into subcutaneous tissues¹³², which can be invasive and lead to antigen tolerability¹³³. Subcutaneously administered nanomedicines, particularly of a diameter of 10–100 nm, enable more efficient delivery and retention of vaccines in draining lymph nodes and facilitate the controlled release of antigens and adjuvants, thus increasing the level of antigen presentation¹²⁰. Second, nanomedicine-based vaccines might be targeted for uptake by DCs and other antigen-presenting cells (APCs) using targeting moieties and by tuning their physicochemical properties¹³⁴. Third, nanomedicine-based vaccines can promote antigen cross-presentation and increase the level of CTL activation that otherwise skews T cells towards a CD4⁺ phenotype when exposed to conventional vaccines. Specifically, nanomedicine-based vaccines promote endosomal release via pH-sensitive delivery systems that release antigens in the endosome, which enables antigen engulfment by APCs for presentation to CTLs by MHC class I molecules, while conventional vaccines rely on extracellular

antigens (soluble proteins or peptides) that are presented to CD4⁺ T cells via MHC class II molecules¹³⁵. Fourth, nanomedicine-based vaccines enable the co-delivery of antigens and adjuvants, which increases the extent of antigen presentation and cross-presentation and promotes the development of CD4⁺ T helper antitumour phenotypes¹³⁶.

Various sources of antigens for antitumour vaccines are available. Whole-cell vaccines increase the scope of antigens delivered, are personalized and benefit from nanomedicine-based approaches¹³⁷. For example, in a preclinical study, ICD was induced in cancer cells that were removed from mice that had been previously loaded with a nanomedicine-based adjuvant. Upon subsequent reintroduction, this vaccine elicited systemic antitumour responses that increased the efficacy of ICI¹³⁸. Furthermore, intravenously administered DC-based vaccines generated by ex vivo exposure to tumour antigens are also capable of priming T cells in patients¹³⁹. Besides cells, DNA and mRNA can also be used as subunit vaccines to encode protein-based or peptide-based antigens. mRNA can serve as both antigen and adjuvant because, as a single strand, it is a ligand for TLR7 and TLR8 (REF.¹⁴⁰). DNA and mRNA can be administered with or without the use of viral vectors. A general consensus exists that nanomedicine-based approaches protect nucleic acids from degradation and are safer than viral vectors. Nanomedicine-based strategies designed to improve the intravenous administration of RNA-based vaccines have entered clinical development. Specifically, intravenously administered RNA lipoplexes can be selectively targeted to DCs without ligands by adjusting the net charge to slightly negative, which targeted the particles to the spleen and generated de novo T cell responses in three patients with melanoma¹⁴¹. Nanomedicines are generally the same, or a similar, size as pathogens so they can be efficiently taken up by DCs^{142,143}. Furthermore, controlling the diameter of the nanoparticles promotes distribution to tissues enriched with APCs. In mouse models, 200 nm diameter RNA lipoplexes are retained in the spleen, although smaller nanoparticles with a diameter of 70 nm preferentially accumulate in the lymph nodes after systemic injection¹⁴⁴.

Nanomedicine-based delivery of antigens to DCs can be well controlled using subcutaneous or intranodal administration¹⁴⁵. Indeed, subcutaneous

injections can preferentially target the uptake of nanomedicines to lymph nodes via the lymphatic drainage by adjusting the diameter of the nanoparticles to <100 nm (REF.¹⁴⁶). Once in lymph nodes, surface characteristics of nanovaccines also affect distribution within lymph nodes. Specifically, an array of glycans on the nanomedicine surface can enable antigens to reach the follicles in which B cells reside¹⁴⁷. Besides size and surface decoration, another consideration for the optimal delivery of vaccines is that soluble tumour antigens must be released into the cytosol of DCs in order to be processed for presentation to CTLs, although such antigens are often trapped and retained in endosomes. As mentioned previously, nanomedicines sensitive to the acidic pH of endosomes can be controlled to enable antigen release into the cytosol of APCs. This construct works as a monotherapy and also synergizes with ICI in tumour-bearing mice¹⁴⁸. Additionally, the nanocarrier itself can act as an adjuvant to the vaccine. Specifically, researchers have screened polymers¹⁴⁸ and lipids¹⁴⁹ that can assemble into nanocarriers for antigens and themselves act as adjuvants stimulating the STING signalling pathway while also identifying characteristics of the chemical structure that lead to this effect. Vascular normalization can also improve the efficacy of vaccines⁸¹, although the benefits likely arise from improvements in T cell infiltration and activity, suggesting that the administration of AATs after vaccines is worth exploring.

Priming and expanding T cells

Nanomedicines that promote antigen presentation often also result in increased priming and expansion of T cells, as the process of antigen presentation is very closely related to priming, and DCs are able to directly stimulate naive T cells. Antigen presentation is completed when naive T cells in lymphoid organs are primed to become effector or memory T cells and expand into differentiated cytotoxic T cells; therefore, drugs that stimulate this process are desirable. Ipilimumab, an anti-CTLA-4 antibody, promotes the priming and expansion of T cells, although it also introduces substantial toxicities especially when simultaneously administered with anti-PD-1 antibodies¹⁵⁰. The ability of DCs to prime T cells is dependent on the TCRs, co-stimulatory molecules and cytokine stimulation. Delivering such stimuli to lymphoid organs could stimulate the priming and expansion of T cells, although such an approach could also induce

autoimmune toxicities if lacking selectivity. Thus, encapsulating the stimuli in a nanomedicine could prove beneficial.

Systemically administered IL-2 globally activates the immune system and can lead to autoimmune toxicities. Unfortunately, IL-2 and other simulators of T cell expansion and activity, such as antibodies that activate CD137, also result in a 'cytokine storm' effect when binding to circulating lymphocytes. Fortunately, anchoring IL-2 and CD137 stimulatory antibodies to the surface of liposomes enables the passive delivery of these agents to tumours and tumour-draining lymph nodes to induce a therapeutic effect without notable toxicities in a mouse model¹⁵¹. Similar results were observed with IL-2, anti-PD-1 antibodies and anti-CTLA-4 antibodies bound to a collagen-binding domain that accumulates within tumours¹²⁹. Besides inducing toxicities, systemic IL-2 mediates the expansion of immunosuppressive cells that could limit the effectiveness of ICI. Thus, bempegaldesleukin, a PEGylated CD122-preferential IL-2 pathway agonist, was developed to target antitumour T cells as opposed to pro-tumour T_{reg} cells and is currently being tested in combination with nivolumab in a phase III clinical trial involving patients with metastatic RCC (NCT03729245). Only 11% of patients had grade 3–4 adverse events in early-phase trials of this agent as a monotherapy¹⁵². The promising results achieved with such agents in clinical trials support the notion that stimulating the priming and expansion of T cells can enhance the effectiveness of ICI.

Agents that prime and expand T cells could be delivered to lymphoid organs as nanomedicines and thus potentially avoid encountering the TME, although many patients with advanced-stage cancers have undergone surgery and might not have an intact lymphatic system at one or more tumour locations. Indeed, such agents carried in nanomedicines engineered to bind with albumin and traffic to lymph nodes enable more effective targeting of APCs towards expanding T cells and boost the effectiveness of ACT¹⁵³. Additionally, circulating T cells can be programmed with a chimeric antigen receptor (CAR) in vivo using polymeric nanomedicines carrying DNA¹⁵⁴. In combination with, or separate from, these strategies, normalization of the TME could reduce the secretion of immunosuppressive cytokines from the tumour to the draining lymph nodes, thereby also promoting the expansion of T cells in lymph nodes. However, once these expanded T cells reach an

immunosuppressive TME, they could lose their activity. This concern remains relevant, even for ACT, which reduces the need for priming and expansion of T cells in patients by doing so *ex vivo*. Thus, strategies that aim to prime and expand T cells in lymph nodes could still benefit from TME normalization and/or intratumour T cell stimulation.

T cell trafficking and infiltration

Trafficking. Once primed, T cells must traffic out of lymph nodes and through the bloodstream to tumours, followed by infiltration from the vasculature or tumour periphery into the tumour parenchyma to encounter cancer cells. DCs located in tumour-draining lymph nodes present antigens to and stimulate naive T cells, which then enter the bloodstream. Unfortunately, solid tumours often produce chemokines that reduce the extent of T cell trafficking into tumours¹⁵⁵. Additionally, the chemokines that solid tumours do produce often do not match the chemokine receptors expressed by T cells, thereby resulting in limited chemotaxis¹⁵⁶. Vascular normalization, which either polarizes TAMs to an antitumour M1-like phenotype that is associated with secretion of the chemokine CXCL9 that binds to CXCR3 on T cells¹⁵⁷ or modifies the TME and peripheral T cells to express CX₃CL1 and its receptor³² to stimulate trafficking to the tumour, is one strategy that might overcome this problem (FIG. 1).

Infiltration. After trafficking to the site of the tumour, T cells must infiltrate from blood vessels into the parenchyma in order to reach cancer cells. Because tumours often have an abnormal vasculature with limited blood flow, increasing the density and distribution of perfused vessels enables T cells to flow closer to cancer cells before extravasating and migrating. Thus, perfusion-enhancing vascular and stromal normalization improves the distribution of T cells within the TME^{24,25,81}. Even after flowing deep into the tumour vasculature, T cells¹⁵⁸ and natural killer cells¹⁵⁹ both face barriers to extravasation, owing to reduced expression of adhesion molecules in the immature tumour vasculature. Thus, vascular normalization with AATs that increase the maturity of perivascular and endothelial cells can enhance T cell adhesion, extravasation and infiltration^{32,82,160}.

Once extravasated, T cell migration from blood vessels to the parenchyma is often limited by the desmoplasia. Thus, reprogramming CAFs to reduce the levels

of ECM, beyond increasing perfusion and intravascular T cell transport, increases extravascular T cell migration towards cancer cells^{23–25}. Potential stroma-normalizing drugs have been approved by the FDA for other diseases or indications. These drugs include losartan, as described previously^{74,75}, plerixafor²⁴ for stem cell mobilization prior to haematopoietic stem cell transplantation and pirfenidone⁶⁶ for the treatment of idiopathic pulmonary fibrosis. These drugs have a proven record of clinical use in other indications; therefore, data from retrospective studies could support their application to the treatment of patients with cancer⁷¹ (Supplementary Table 4). Moreover, these stroma-normalizing drugs could also reduce the toxicities of cancer therapies¹⁶¹. Incorporating these drugs into nanomedicines could reduce the systemic effects of these agents (for example, the antihypertensive effects of losartan), while enhancing their normalizing effects within tumours. As an example, we developed a nanoformulation consisting of an ARB (valsartan) bound to pH-sensitive polymers, thus creating a nano-ARB, which abrogates the blood-pressure-reducing effect of losartan while increasing the extent of TME normalization²⁵. Accordingly, this nano-ARB improved the antitumour effects of ICI in multiple mouse models of metastatic breast cancer²⁵. Other examples of ARB-containing nanoformulations include telmisartan glycolipid micelles¹⁶² and brush-arm star polymers¹⁶³. Apart from the repurposing of approved drugs, nanomedicines can deliver experimental agents that reprogramme the tumour-associated stroma to a quiescent phenotype. For example, PEGylated polyethylenimine-coated gold nanoparticles that deliver all-*trans* retinoic acid and small interfering RNAs (siRNAs) against heat-shock protein 47 are able to reprogramme CAFs in mouse xenograft models of PDAC¹⁶⁴. Furthermore, the widely used AAT sunitinib (Supplementary Table 2) has been encapsulated into a polymeric micelle delivery system that enhanced the efficacy of an antitumour vaccine in a mouse model of advanced-stage melanoma¹⁶⁵. However, a major challenge for the clinical implementation of these nanoformulations will be to demonstrate meaningful clinical benefit over relatively inexpensive, small-molecule generic drugs such as ARBs, which have already led to unprecedented levels of benefit in a phase II clinical trial in patients with locally advanced PDAC⁷⁶.

Recognition of cancer cells by T cells

In addition to the delivery of antigens and immune-activating compounds to APCs for the promotion of memory T cell responses against tumours, nanomedicines could potentially be engineered to promote the recognition of cancer cells by T cells following infiltration of the TME (FIG. 2). The potential of this strategy is supported by the clinical success of bispecific antibody technology in the clinic for the treatment of lymphoma and some types of leukaemia¹⁶⁶. These bispecific T cell engagers (BiTEs) bind to T cells using a conserved component of the TCR (mainly CD3) and to antigens expressed by cancer cells. The first BiTEs to reach the clinic were catumaxomab, which binds to EpCAM on the cell surface of carcinomas¹⁶⁷, and blinatumomab, which targets CD19 and has been approved by the FDA for patients with acute lymphoblastic leukaemias¹⁶⁶.

The ongoing experience with anti-EGFRvIII CAR T cell therapy in patients with glioblastoma illustrates the importance of recognition of cancer cells by T cells and the potential of BiTEs. Histological evaluations of tumour material from a cohort of ten patients with glioblastoma who received intravenously administered anti-EGFRvIII CAR T cells demonstrated that, although the treatment was not effective in inducing disease regression and resulted in the recruitment of T_{reg} cells, CAR T cells were successfully delivered to the tumour, and a loss of cancer cells harbouring the target antigen was observed¹⁶⁸. In order to improve this approach, researchers engineered these CAR T cells to secrete BiTEs that target CD3⁺ T cells to the wild-type antigen and administered them intraventricularly to mouse models of glioblastoma. This approach increased the range of cancer cells targeted without increasing the incidence of toxic effects on nonmalignant cells bearing the wild-type antigen¹⁶⁹. The local administration of BiTE-secreting CAR T cells provides an example of how overactivation of T cells can be avoided, as demonstrated by the lack of nonspecific effects on nonmalignant cells and the ability of the BiTE to direct T_{reg} cell cytotoxicity against *EGFR*-mutant cancer cells. A nanoparticle has been conjugated with an anti-human epidermal growth factor receptor 2 (HER2) antibody and calreticulin, which provides an ICD signal that promotes phagocytosis. This multivalent bispecific nanobioconjugate engager stimulated the phagocytosis of cancer cells. Thus, although HER2 targeting was used for recognition, HER2-negative cancer cells were also killed because the phagocytosis of

cancer cells released additional cancer cell antigens¹⁷⁰.

TME normalization could aid in the recognition of cancer cells by T cells. Both T cells and natural killer cells recognize cancer cells via MHC class I molecules, yet hypoxia causes cancer cells to shed these molecules^{171,172}. Vascular normalization, achieved by inhibiting either nitric oxide^{171,172} or VEGF signalling, increases the expression of MHC class I molecules³². Indeed, vascular normalization also promotes the antigen-specific migration of T cells³². Thus, combining TME normalization with one or more of the strategies described in this section might alleviate hypoxia and immunosuppression in the TME and enable increasing levels of cancer cell recognition by T cells.

Killing of cancer cells

If the cancer-immunity cycle proceeds until T cells recognize yet fail to kill cancer cells, inhibition of the PD-1 immune checkpoint, which suppresses T cell activity, using an anti-PD-1 or anti-PD-L1 antibody could stimulate antitumour immunity. However, these inhibited T cells then produce enzymes such as indoleamine 2,3-dioxygenase (IDO) that reduce their activity, thus making IDO a rational target. Nonetheless, attempts to inhibit this enzyme in combination with pembrolizumab in a phase III trial involving patients with unresectable or metastatic melanoma failed to provide any improvements in efficacy, which raises questions about the dosing and scheduling of these agents¹⁷³ as well as the extent of co-expression of PD-L1 and IDO¹⁷⁴. These questions must be addressed before nanomedicines that combine inhibition of PD-L1 and IDO can be considered¹⁷⁵.

Besides stimulating a desmoplastic reaction that causes solid stress in CAFs, vessel compression and hypoxia (FIG. 1), TGF β also suppresses T cell killing by promoting the development of T cells of a tumour-promoting phenotype. Thus, nanomedicines have been developed that inhibit TGF β signalling in T cells by actively targeting CD8⁺ T cells¹⁷⁶, ex vivo in preparing cells for ACT¹⁷⁷, or passively through the EPR effect¹⁷⁸. Indeed, cells loaded with delivery platforms that become activated after cellular recognition are promising approaches for supporting T cell-mediated cancer cell killing. Specifically, loading nanomedicines into T cells enables the delivery of T cell-stimulating agents without accumulation in nonmalignant tissues, with activation upon cancer cell binding observed in mouse models^{179–181}.

Along with TGF β , hypoxia might inhibit cancer cell lysis, in part by inducing epithelial-to-mesenchymal transition in NSCLC cells, and thus interfere with the receptors and signalling molecules at the T cell–cancer cell junction¹⁸². In this scenario, hypoxia delays the development of CTLs, although in general it is considered to enhance cancer cell lysis³⁴. Mechanotherapeutics that inhibit TGF β signalling could alleviate hypoxia while also interfering with signalling pathways that limit T cell signalling. Furthermore, because impaired cancer cell lysis occurs in the tumour parenchyma, we hypothesize that agents that aim to modulate exhausted T cells must penetrate deep into tumours. Thus, using TME normalization strategies to increase the penetration of nanomedicines that reverse T cell exhaustion is worth exploring.

Re-engineering nanomedicines

In most patients with solid tumours, the TME of both the primary tumour and the metastases compromises responsiveness owing to limited drug delivery and increased levels of immunosuppression. Thus, in this Perspective we have described how various nanomedicines and TME normalization strategies can facilitate progression through the cancer-immunity cycle. Nonetheless, treatments that overcome only one step of this cycle are insufficient for most patients with cancer. Indeed, even in patients with primary tumours with increased levels of nanomedicine uptake and a favourable immune TME phenotype, the metastases could have different distributions of both nanomedicines^{12,13} and immune cells^{16,17}. Even in patients with a single primary lesion, treatments designed to overcome one step of the cancer-immunity cycle might simply reveal the next mechanism of treatment resistance. Thus, we propose using minimal combinations of agents that could facilitate progression through the entire cancer-immunity cycle while limiting the incidence of adverse events. In order to initiate the cycle, nanomedicine-based vaccines or ACT incorporating nanomedicines could be administered either locally or systemically. Alternatively, nanomedicine-based TME-normalization strategies that precede ICD induction, either involving nanomedicine-based chemotherapy or radiotherapy-sensitizing agents, can initiate the cycle. If an adjuvant is not administered before, or with, the vaccine, additional nanomedicines should be administered that stimulate DC maturation and thus enable antigen presentation and subsequent priming and

expansion of antitumour T cells (FIG. 2). TME normalization following T cell activation can increase T cell trafficking to, and infiltration into, tumours. Finally, TME normalization and nanomedicines targeting T cells can also promote the recognition and killing of cancer cells by T cells. Thus, combinations of drugs will likely be necessary to potentiate the effectiveness of immunotherapies in patients with solid tumours (FIGS 2, 3). Nonetheless, the extent of resistance mediated by the various steps of the cancer-immunity cycle varies between patients, within patients and likely also within a single tumour over time¹⁸³. Thus, in order to avoid the risk of toxicities in patients with tumours with a high level of nanomedicine uptake and a favourable immune TME phenotype, the number of agents should be reduced.

Combination nanomedicines

Given the various drugs necessary to facilitate progression of the cancer-immunity cycle in patients with solid tumours, extensive research has led to the development of nanomedicines that combine multiple immunotherapies, including: chemotherapy and immune-modulators; radiosensitizers, including photothermal and photodynamic therapies and immune-modulators; RNA interference-based immune-modulators; and RNA-based or other vaccines loaded with adjuvants¹⁸⁴. In fact, several features of nanomedicines, such as coordinating drug interactions within the core of nanomedicines, controlling the release chemistry of a drug–polymer linker, and/or conjugating a drug or antibody on the surface of the nanomedicine, might enable the synchronous administration of multiple agents, including nucleic acids, into single nanomedicines. These features enable the control of pharmacokinetics, drug concentrations, drug ratios and release rates of bioactive payloads, thus enabling the optimization of synergistic effects^{185,186}. For example, self-assembled micelles carrying oxaliplatin in the core and dihydroartemisinin in the shell are able to synergistically induce ICD and increase the efficacy of ICI in subcutaneously grown tumours¹⁸⁷. A similar approach, relying on the delivery of external radiation, involves the delivery of two agents that promote ICD (chemotherapy and a photodynamic therapy sensitizer), which are located in the nanoparticle core and shell, respectively¹⁰⁹. A nanomedicine consisting of a hollow MnO₂ nanoparticle containing doxorubicin plus a photodynamic agent that

decomposes to eventually supply oxygen to the TME has been engineered to deliver a photodynamic therapy sensitizer to provide an additional level of functionality¹⁸⁸.

A nanomedicine featuring four components (a tumour antigen-targeting antibody, a recombinant form of IL-29 with an extended half-life, an anti-PD-1 antibody and a T cell stimulatory vaccine) demonstrated robust antitumour effects in mouse models of melanoma with a high tumour burden¹⁸⁹. Fucoidan-based nanoparticles are antiangiogenic and have been combined with an anti-PD-L1 antibody and anti-CD3 and anti-CD28 moieties, thereby forming another quadruple combination¹⁹⁰. Another formulation involving a three-in-one liposome-based nanomedicine has been developed with the aim of normalizing the TME prior to the administration of CAR T cells to patients with solid tumours. This liposome binds to angiogenic tumour vessels via an iRGD ligand that enhances the transit of tumour-specific nanomedicines across the vessel wall followed by the delivery of a PI3K inhibitor to inhibit immunosuppressive cancer cells and an α -galactosylceramide agonist that stimulates T cells¹⁹¹. Indeed, direct modifications such as equipping nanomedicines with targeting agents^{192,193} and providing nanomedicines with triggers that are either TME-responsive (such as tumour pH, redox status or specific enzyme activity)^{25,194} or that can be activated by external stimuli (such as heat, light, magnetic fields or sound waves) to enable spatiotemporal control of drug activity^{195,196} have been pursued by several investigators. However, an extensive discussion of this research is mostly outside the scope of this Perspective because these strategies are unlikely to reach their full potential without prior systemic TME normalization (FIG. 3). Furthermore, it is unclear to what extent specifically targeted nanomedicines increase the intratumour distribution of these agents, especially for nanomedicines administered after TME normalization.

The specific clinical implementation of these various nanomedicine-based combination therapy approaches will depend on whether these therapies should be scheduled simultaneously, sequentially or are contraindicated. All the drugs described in this Perspective, even repurposed drugs like losartan that already have regulatory approval for other indications, can unfortunately have serious adverse effects in certain patients; therefore, combination nanomedicines and treatment schedules incorporating more than one agent must be carefully designed to avoid

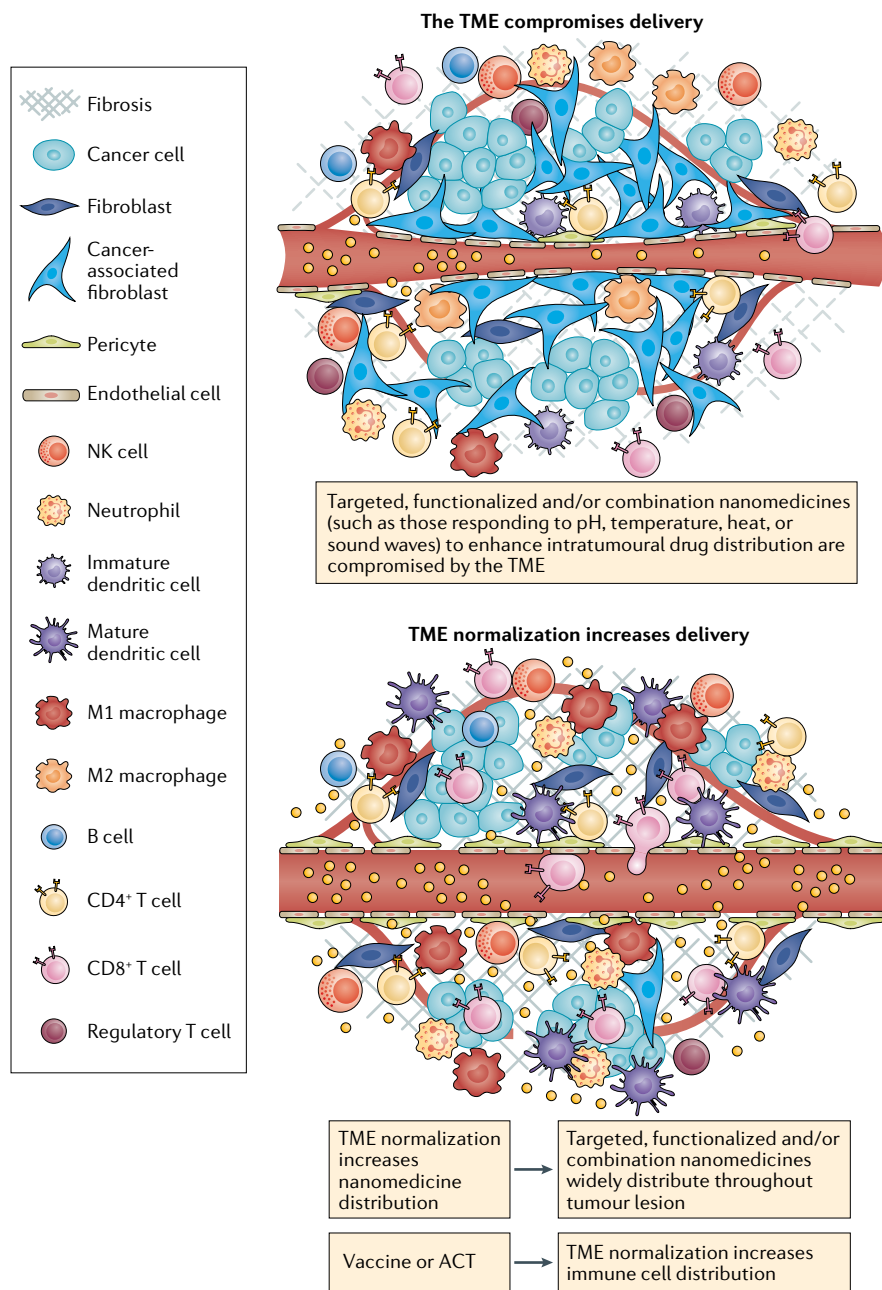


Fig. 3 | Normalizing the TME to increase the penetration of combination therapies. Targeted or stimuli-responsive nanomedicines often have a limited level of distribution within tumours because the tumour microenvironment (TME) limits blood flow and therefore the extent of tumour penetration. With insufficient penetration and a limited density of antitumour immune cells, the advantage of these nanomedicines compared with passively accumulating and releasing nanomedicines is reduced. TME-normalizing therapies increase and homogenize the intratumour distribution of immune cells and nanomedicines. Nanomedicine-based vaccines and autologous transferred T cells carrying nanomedicines increase the number of antitumour T cells in the host. If followed by TME normalization, a larger percentage of these T cells can migrate to the tumour parenchyma. Similarly, functionalized nanomedicines following TME normalization can penetrate the tumour parenchyma in higher fractions thereby reaching their target and/or stimuli and demonstrating a larger improvement over passive nanomedicine. Thus, normalizing the TME could increase the effectiveness of targeted nanomedicines that combine cytotoxic agents and immunotherapies. ACT, adoptive cellular therapy; NK, natural killer.

administering an unnecessary number of drugs to patients. For example, because TME normalization is beneficial throughout the cancer-immunity cycle whereas ICI

can be toxic and is more important at specific stages of the cycle, strategies that combine repurposed TME-normalizing agents with an ICI in a single nanomedicine

could be counterproductive. Similarly, agents designed to normalize the TME need not precede vaccines, so there is no need to integrate them into a single nanomedicine. The experience with combined administration of ipilimumab plus nivolumab to patients with metastatic melanoma, in whom the combination was more efficacious but also more toxic than sequential administration, demonstrates the risks associated with combining different immunotherapies¹⁵⁰. Nonetheless, the FDA approval of the liposomal combination of daunorubicin and cytarabine for patients with newly diagnosed therapy-related acute myeloid leukaemia or acute myeloid leukaemia with myelodysplasia-related changes confirms the validity of using nanomedicines to concurrently deliver more than one drug to patients with cancer.

Imaging nanomedicines

Clinical imaging of nanomedicines that emit PET or MRI signals has confirmed the heterogeneous distribution of nanomedicines in patients with solid tumours, as originally discovered in preclinical studies^{12,13}. Further development of these targeted imaging approaches could also provide information on TME status¹⁹⁷ and the activity of immunotherapies in patients¹⁹⁸. These probes will be useful in evaluating the benefit of TME-normalizing strategies in reducing the heterogeneity of nanomedicine distribution and testing predictive markers of delivery and responsiveness to nanomedicines. Current predictive approaches, including tumour histology, flow cytometry and whole-genome sequencing, provide a partial view of the TME conditions that affect the antitumour immune response. Direct imaging of nanomedicines has the potential to facilitate the direct visualization of dynamic events that could complement, or possibly even replace, these other methods. Thus, advances in TME normalization could help advance the development of nanomedicines and vice versa.

Gene therapies as nanomedicines

The encapsulation of oligonucleotide vaccines in nanomedicines might help avoid nucleic acid degradation, immune detection, renal clearance and the difficulties associated with cell entry. In the example of mRNA-loaded nanomedicines, encapsulation might also enable the production of therapeutic proteins in tumours, as demonstrated in mouse models of PDAC¹⁹⁹. The silencing effects of siRNAs or the protein translation

from mRNAs are transient, although more permanent alterations of the TME and long-lasting therapeutic responses can be achieved by modifying the DNA of tumour cells. This strategy can be achieved by using nanomedicines to deliver DNA to the nuclei of tumour cells, as well as by nanomedicines that incorporate CRISPR–Cas9 gene editing technology. CRISPR–Cas9 systems are an important tool that enables precision genome editing. For *in vivo* use, however, delivery systems are required that transport the necessary components to the nucleus of the target cells. PEGylated nanoparticles have been developed that can deliver CRISPR–Cas9 and have antitumour efficacy in cell lines and in mouse models²⁰⁰. However, owing to the bacterial origins of the enzymes, humans often have pre-existing adaptive immunity to Cas9 proteins²⁰¹. Hence, novel strategies must be developed to overcome this and various other challenges²⁰².

Innovative approaches involving the encapsulation of oligonucleotides have been developed and used to deliver several anticancer drugs to experimental animals and a drug to patients with Alzheimer disease¹⁸⁶. One strategy involves the combination of ICD-inducing chemotherapy with a nanomedicine enabling the delivery of plasmid DNA encoding a PD-L1 trap with expression localized to the TME, which enables reduced levels of toxicity compared with systemic ICI²⁰³. Another strategy involves the potentiation of a DC vaccine by interfering with the adenosine signalling pathway using a nanomedicine containing an siRNA against CD73 (also known as 5′-nucleotidase, an enzyme that converts AMP into adenosine). This approach enhances T cell activity and reduces that of immunosuppressive cells in the TME²⁰⁴. Other strategies include the layer-by-layer self-assembly of polymers encapsulating siRNAs and chemotherapy²⁰⁵, a polymer construct of metformin harbouring siRNA in a core-membrane structured lipid–polycation–hyaluronic acid nanomedicine²⁰⁶ and poly-ion complex (PIC) micelles. In this latter approach, the charge of nucleic acids can be neutralized by oppositely charged block ionomers, thereby facilitating the formation of stable micelles^{186,207}. Various researchers have conjugated cyclic arginine–glycine–aspartate (cRGD) ligands to PIC micelles in an attempt to knockdown VEGF and other targets in various cancer models^{208–210}. In order to improve the uptake and minimize accumulation in nontarget organs, smaller diameter PIC micelles have been developed

involving a Y-shaped block cationer with a specific chain length designed to match the negative charges of the oligonucleotide strand²¹¹. Similar to the strategy of using cRGD ligands on the surface of PIC micelles to target endothelial cells, plasmid DNA encoding tumour necrosis factor-related apoptosis-inducing ligand encapsulated in branched polyethylenimine can be targeted to pancreatic stellate cells via uptake through the fibroblast growth factor receptor²¹². The clinical success, including safety, of the lipid-based RNA interference nanomedicine patisiran in patients with amyloidosis demonstrates the feasibility of using such approaches to deliver oligonucleotides²¹³; however, the experience with phase I trials of nanomedicines designed to deliver siRNAs to solid tumours indicates the need to improve both the safety and efficiency of delivery^{214,215}.


Conclusions

The use of cytotoxic nanomedicines is based on the assumption of selective accumulation in tumours relative to most nonmalignant tissues. This targeting enables chemotherapies to accumulate more selectively in tumours relative to nonmalignant tissues, which reduces the risk of toxicities. We previously suggested, approximately 30 years ago, that the efficacy of this strategy is limited by the physical barriers posed by tumours⁷. Indeed, various clinical studies have since confirmed our preclinical observations of the heterogeneity of nanomedicine accumulation and distribution in single tumours, between multiple tumours within the same patient and between patients with the same cancer type. Nonetheless, strategies to promote the delivery of nanomedicines have not received clinical approval, and much of the research in this area is now focused on the delivery of immunotherapies. Pathological angiogenesis and desmoplasia compromise drug and oxygen delivery alike, thus resulting in hypoxia and acidity and causing immunosuppression. Here, we propose that normalizing the TME in order to improve the delivery of nanomedicines will also reverse immunosuppression, thereby potentiating the effectiveness of immunotherapies delivered using nanomedicines. Furthermore, nanomedicines designed to deliver repurposed FDA-approved drugs should be used to normalize the TME.

Despite intense clinical investigation, much more research is still needed in order to effectively combine immunotherapies, including identifying predictive biomarkers

and biomarkers of response and gaining a better understanding of the temporal effects of the various therapies, thus enabling the development of more effective nanomedicines that have fewer toxicities. A better understanding of these aspects will guide the combination and personalization of therapies that is necessary to increase the percentage of patients with cancer who derive benefit from ICI from the current level of 13%². The limited percentage of patients who currently benefit from ICI alone indicates an urgent need for novel combination strategies.

Personalization — requiring validation and measurement of biomarkers beyond expression of the immune-checkpoint receptors or their ligands — could take years to develop and be expensive to implement. Furthermore, many patients cannot afford and/or tolerate biopsy sampling and/or other invasive procedures for the procurement of samples for biomarker analysis. Finally, the TME immune phenotype can change with treatment, over time and can also vary by location; even the TME of the metastases can differ from that of other metastases and of the primary tumour in the same patient. In other words, the biomarkers used to select a treatment for a patient's primary tumour might be ineffective against their metastases. In this Perspective, we propose a minimum combination of therapies that potentially could address all seven steps of the cancer-immunity cycle in patients with systemic, heterogeneous disease. Treatment can be initiated using either ACT, a vaccine carrying an antigen and an adjuvant, or using a repurposed TME-normalizing agent followed by ICD-inducing chemotherapy and/or radiotherapy. Once T cells are primed and expanded, TME normalization combined with an anti-PD-1 or anti-PD-L1 antibody could perpetuate the cycle. Nanomedicines have the potential to enhance the efficacy and safety of each of these treatment modalities.

John D. Martin¹ , Horacio Cabral¹,
Triantafyllos Stylianopoulos^{1,2*} and
Rakesh K. Jain^{1,3*}

¹Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, Bunkyo, Tokyo, Japan.

²Cancer Biophysics Laboratory, Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus.

³Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

*e-mail: tstylian@ucy.ac.cy; jain@stele.mgh.harvard.edu

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Author contributions

J.D.M. researched data for this article. All authors made a substantial contribution to the discussion of content, writing the manuscript and reviewing and/or editing the manuscript prior to submission.

Competing interests

J.D.M. became a full-time employee of NanoCarrier during the preparation of this manuscript. R.K.J. has received honoraria from Amgen, has acted as a consultant for Chugai, Merck, Ophthotech, Pfizer, SPARC, SynDevRx and XTuit, owns equity in Enlight, Ophthotech and SynDevRx and serves on the Boards of Trustees of Tekla Healthcare Investors, Tekla Life Sciences Investors, Tekla Healthcare Opportunities Fund and Tekla World Healthcare Fund. H.C. and T.S. declare no competing interests.

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