



Regulating trained immunity with nanomedicine

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Abstract | Trained immunity refers to a hyperresponsive functional state of the innate immune system, which is induced by certain stimuli, such as infections or vaccination. Trained immunity plays a key part in a variety of diseases, including cancer and inflammation, and is regulated through epigenetic and metabolic reprogramming of haematopoietic stem and progenitor cells in the bone marrow, giving rise to hyperactive myeloid cells. Nanomaterials inherently interact with phagocytic myeloid cells and are thus ideal platforms with which to regulate trained immunity. In this Review, we discuss the key pathways of trained immunity and investigate nanomedicine strategies to therapeutically regulate trained immunity. Nanomedicine can be applied not only to induce trained immunity to treat cancer or to enhance resistance to infections, but also to manage hyperinflammation and maladaptive trained immunity in a variety of clinical scenarios. We conclude with an outlook to future possibilities and some remaining challenges for nanomedicine approaches in trained immunity regulation.

The clinical translation of the COVID-19 mRNA vaccines has expedited a paradigm shift in the field of nanomedicine, which has been developed from twenty-first century immuno-oncology successes. Rather than focusing on drug delivery approaches designed to evade the immune system, the field has started to embrace an engagement of the innate immune system — the body's first line of defence against invading pathogens — through nanoparticle–phagocyte interactions¹. Here, we refer to a specific application of nanomedicine involving the systemic administration of nanomaterials (size range of 10 nm to 250 nm) to achieve efficient haematopoietic system engagement and therapeutic innate immune regulation. It has long been assumed that immune memory is located only in our immune system's adaptive arm, allowing effective and antigen-specific immune responses against re-encounters. However, this paradigm has been challenged by increasing evidence for the existence of a primitive innate immune memory, referred to as trained immunity^{2,3}. Upon exposure to a pathogen-associated molecular pattern (PAMP), innate immune cells (and, in particular, myeloid cells) become more responsive to subsequent unrelated infections⁴. Trained immunity, which was first described in individuals who had been vaccinated with Bacillus Calmette–Guérin (BCG)⁵ (BOX 1), is one of the fastest growing fields in immunology.

Nanomedicine holds great promise for innate immune regulation, and may work well in conjunction

with adaptive immune system-directed immunotherapeutic approaches — such as checkpoint blockade⁶ and chimeric antigen receptor T cells, for example — through T cell co-stimulation⁷ or by modifying the tumour microenvironment⁶. In particular, trained immunity induction has the potential to mature into a compelling therapeutic approach. The use of microbial compounds to boost an immune response was explored in the late 1800s by William Coley⁸, who observed spontaneous tumour remission in a cancer patient with a concurrent *Streptococcus pyogenes* infection, which led him to hypothesize that deliberately injecting patients with this microbe could induce tumour remission⁹. However, owing to variable results and the obvious risk of life-threatening infections, the method quickly fell victim to the successes of radiation and chemotherapy¹⁰. Nevertheless, Coley's work inspired the search for alternative applications of boosting innate immunity using microbe-derived therapeutics. In the 1970s, the BCG vaccine, which was originally developed as a vaccine against tuberculosis, was approved for treating high-risk non-muscle-invasive bladder cancer¹¹ (BOX 1). This specific application is safe because the urothelium does not absorb BCG and harmful systemic uptake or bacteraemia are prevented. However, to take full advantage of the therapeutic potential of trained immunity, innovative means of safely inducing it must be developed. Trained immunity owes its longevity to progenitors in

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Box 1 | BCG vaccination

The field of trained immunity originates from research with the Bacillus Calmette–Guérin (BCG) vaccine^{178,179}, which was developed in the 1920s to enhance protection against tuberculosis. The heterologous features of this vaccine were recognized by Holmgren, who first adopted BCG as a stomach cancer treatment in 1935 (REF.¹⁸⁰). BCG has been trialled as a treatment against a range of cancers, including tumours of the kidney, colon, prostate, bladder, lung and skin, as well as haematological malignancies^{181–186}. Owing to the risks associated with systemic administration, BCG is approved by the US Food and Drug Administration (FDA) only for treating non-invasive bladder cancer, for which it is intravesically administered and kept isolated from the rest of the body. BCG is also applied to enhance protection against infections other than tuberculosis. Its nonspecific protective effects are attributed to metabolic and epigenetic reprogramming of myeloid cells and their progenitors, that is, trained immunity^{3,5}. The nonspecific benefits of regulating trained immunity have recently been demonstrated¹²⁰ in a double-blind, randomized trial. In elderly individuals, BCG vaccination reduced viral respiratory tract infections by 80% over a period of 12 months, as compared to placebo-vaccinated individuals.

During the COVID-19 pandemic, numerous BCG trials have been initiated (NCT04384549, NCT04659941, NCT04461379), with the goal of providing increased protection against SARS-CoV-2 infection to the vulnerable and frontline workers¹¹⁵. There is evidence that the age-dependency in COVID-19 severity may be related to innate immune responses¹⁸⁷. In children and young adults, the innate immune responses to a SARS-CoV-2 infection are faster and much stronger, whereas in the elderly, these responses are muted by decades of pathogen exposure. Therefore, future pandemics could be mitigated by bridge-vaccination with trained immunity-inducing vaccines, such as BCG, MTBVAC (*Mycobacterium tuberculosis* vaccine), the measles vaccine, the smallpox vaccine or the oral polio vaccine^{188–192}. These vaccines may induce protective innate immune responses for rapid viral clearance, similar to the protection children and young adults experience, limiting the detrimental damage of long-lasting infections.

the bone marrow¹², which undergo metabolic and epigenetic rewiring to continue producing ‘trained’ myeloid cells⁶, rendering these cells hyperresponsive. Therefore, technologies must be developed that directly engage myeloid progenitors¹³.

Nanomaterials inherently interact with phagocytic myeloid cells, and are thus ideal tools to regulate trained immunity by delivering drugs to myeloid cells and their progenitors in the bone marrow, including small molecules^{6,7}, polymers¹⁴, RNA therapeutics¹⁵ and immunoregulatory proteins¹⁶. Nanomedicine-based activation of trained immunity will find use in cancer treatment and will increase resistance to infection. Conversely, inhibition of trained immunity has therapeutic benefits in conditions that are characterized by an exacerbated

immune response, such as autoimmune disorders, cardiovascular disease, sepsis, transplant rejection and acute respiratory distress syndrome.

The innate immune system

The immune system of vertebrates can be divided into an innate arm and an adaptive arm. The innate arm consists of external defences (physical barriers such as the skin and stomach acid) and internal defences¹⁷. Internal defences are activated by pattern recognition receptors of innate immune cells. These receptors can sense PAMPs in case of infection, or damage-associated molecular patterns (DAMPs), which are non-microbial triggers. Once activated, innate immune cells set in motion the complement system, which ultimately results in the killing of pathogens or of infected or malignant cells through phagocytosis. Phagocytosis is a type of endocytosis — a process by which a cell engulfs a particle. Although most cells are capable of phagocytosis, it is the main function of some cells, including myeloid innate immune cells, such as monocytes, macrophages, dendritic cells and neutrophils¹⁸. Therefore, innate immunity provides a nonspecific, efficient and rapid reaction to protect the host. Certain innate immune cells, such as dendritic cells and macrophages, can also initiate an antigen-specific adaptive immune response through a combination of antigen presentation (signal 1), co-stimulation (signal 2) and cytokine regulation (signal 3), which takes several days to develop^{19–22}.

The immune cells of our host defence system are the result of haematopoiesis, that is, the highly dynamic process of blood cell production, which involves the generation of leukocytes from haematopoietic stem and progenitor cells (HSPCs) residing in the bone marrow²³. Innate immune cells include all leukocytes from the myeloid lineage, such as monocytes, macrophages and granulocytes, as well as some leukocytes from the lymphoid lineage. Besides myeloid cells, HSPCs also give rise to T and B lymphocytes, which are part of the adaptive immune system, and non-leukocytes, including thrombocytes and erythrocytes²⁴.

A growing body of evidence indicates that the innate immune system displays memory characteristics². DAMPs and PAMPs can induce functional reprogramming of innate immune cells, resulting in an altered response towards a subsequent challenge with a related or unrelated stimulus. This concept is referred to as trained immunity, in which ‘trained’ cells display a stronger response upon secondary stimulation, whereas the terms ‘tolerance’ or ‘immune paralysis’ describe muted immune responses³. Memory function of the adaptive immune system is mediated by gene recombination; by contrast, trained innate immunity relies on epigenetic reprogramming of transcriptional pathways. Shifts in cellular metabolism underlie these epigenetic changes. Myeloid-derived monocytes and macrophages were first found to display trained and tolerant phenotypes²⁵. We now know that neutrophils, natural killer (NK) cells and non-leukocytes, such as epidermal cells, also display innate immune memory features^{26–28}. Trained immunity can last for months to a few years and is systemically regulated through myelopoiesis. Indeed,

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certain HSPC populations undergo epigenetic rewiring, giving rise to 'trained' myeloid cells²⁹.

Targeting trained immunity

Trained immunity can be targeted at multiple levels, which should all be considered in the design of nanomedicines, including whole-body level, system or organ-system level, cellular level and subcellular level. On a whole-body level, myeloid cell-rich haematopoietic organs, such as the spleen and bone marrow, are important targets (FIG. 1a). HSPCs reside in the bone marrow, where they can be transcriptionally reprogrammed, for example, upon BCG vaccination, resulting in a persistent trained phenotype²⁹. Therefore, nanomaterials that can induce trained immunity in the bone marrow are particularly interesting.

The haematopoietic system

Monocytes and macrophages have pivotal roles in innate immune memory, and the molecular mechanisms underlying trained immunity in these cells have been thoroughly investigated^{25,30} (FIG. 1b). Moreover, these cells are professional phagocytes, guaranteeing the uptake of nanomaterials³¹. Immune memory has also been described in neutrophils, which make up a large proportion of myeloid cells. BCG-induced and β -glucan-induced trained immunity further increases the generation of these cells (granulopoiesis)^{12,32}, which can result in anti-tumour and antimicrobial activity^{27,33}, highlighting the therapeutic potential of neutrophil targeting. Dendritic cells, which also exhibit immune memory³⁴, can modulate both the innate and adaptive

arms of the immune system³⁵, making them a promising trained immunity target. Furthermore, non-myeloid innate immune cells, such as NK cells and other innate lymphoid cells, can undergo antigen-independent reprogramming, displaying trained immunity features^{36–40}. However, these cells do not possess phagocytic capacities, so an active targeting approach would be required, for example, through surface functionalization of nanomaterials with ligands specific to CD56. Targeting mature innate immune cells has the advantage of delivering immediate effects; however, more substantial therapeutic benefits may be achieved by HSPC reprogramming, because the durability of trained immunity relies on these cells^{12,29}. Although HSPCs make up only a tiny proportion of immune cells, they give rise to the entire immune landscape and are much longer-lived than monocytes or neutrophils²⁴.

Cellular regulation

On a cellular level, a selection of receptors can be targeted to induce or inhibit trained immunity (FIG. 1c; TABLE 1). In particular, pattern recognition receptors for PAMPs have been extensively studied, because trained immunity was first described as an altered immune response after pathogen encounters. For example, the C-type lectin receptor Dectin-1 is involved in antifungal immunity and binds various β -glucans, each resulting in different downstream effects⁴¹. β -Glucan derived from the cell wall of *Candida albicans* induces trained immunity²⁵, whereas laminarin, a mixture of short-chain β -glucans from seaweeds, competitively inhibits agonistic β -glucan binding to dectin 1 (REFS^{41,42}). These findings

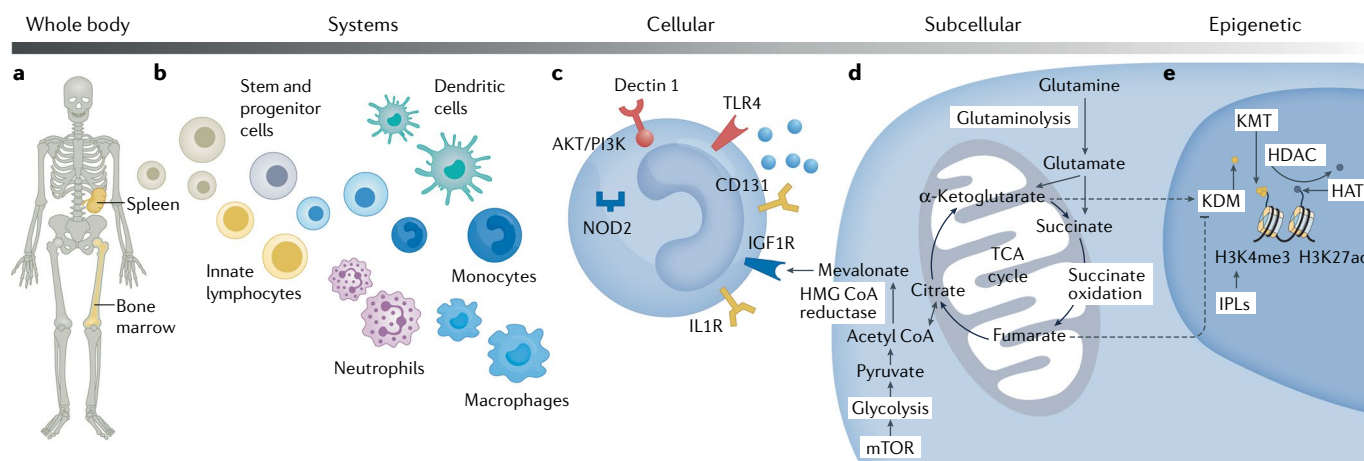


Fig. 1 | Trained immunity targeting levels. **a** | The spleen and bone marrow are important target organs, because they produce and contain large numbers of innate immune cells. **b** | Mature innate immune cells (innate lymphocytes, dendritic cells, monocytes, neutrophils and macrophages) and haematopoietic stem and progenitor cells can be targeted to prevent or enhance trained immunity. **c** | Pattern recognition receptors play an important part in trained immunity. Examples include dectin 1, Toll-like receptor 4 (TLR4) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2). These receptors recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). CD131 is the common β -subunit of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) receptors. The IL-1 receptor (IL-1R)

binds to IL-1 β . Insulin-like growth factor 1 receptor (IGF1R) recognizes extracellular mevalonate. **d** | Intracellular metabolic pathways that can be targeted include glycolysis⁴ (through interference with glycolytic enzymes or indirect through mechanistic target of rapamycin (mTOR) inhibition), cholesterol metabolism⁵⁴ (by targeting HMG CoA reductase), glutaminolysis⁶⁰ (through glutaminase inhibitors) and the tricarboxylic acid cycle (TCA) cycle (for example, by restricting succinate oxidation). **e** | H3K4me3 and H3K27ac are hallmark epigenetic signatures of trained immunity, which can be modified by targeting lysine demethylase (KDM), lysine methyltransferase (KMT), histone deacetylase (HDAC) and histone acetyltransferase (HAT) activity. Immune gene-priming long non-coding RNAs (IPLs) facilitate trimethylation of cytokine promoters⁶⁶. PI3K, phosphatidylinositol 3-kinase.

Table 1 | **Trained immunity induction**

Receptor	DAMP or PAMP	Refs
Dectin 1	β-Glucan	25
NOD2/NOD1	MTP	6,47
TLR4	Very low concentrations of LPS oxLDL Lipoprotein(a) Urate Vimentin HMGB1	7,48–50
TLR2	Low concentrations of Pam3Cys	174
TLR3	Poly I:C	175
IGF1R	Mevalonate	54

DAMP, damage-associated molecular pattern; HMGB1, high mobility group box protein 1; IGF, insulin-like growth factor; LPS, lipopolysaccharide; MTP, muramyl tripeptide; NOD, nucleotide-binding oligomerization domain-containing protein; oxLDL, oxidized low-density lipoprotein; PAMP, pathogen-associated molecular pattern; poly I:C, polyinosinic:polycytidylic acid; TLR, Toll-like receptor.

create opportunities for developing β-glucan nanoformulations. Activated Dectin-1 subsequently triggers phosphoinositide 3-kinase (PI3K) signalling, which is negatively regulated by SH2 domain-containing inositol 5'-phosphatase 1 (SHIP1)⁴³ (TABLE 2). Pharmacological inhibition of SHIP1 by 3α-amincholestane enhances *Candida albicans*-induced trained immunity⁴⁴. SHIP1 is also the primary target of microRNA-155 (miR-155)⁴⁵, and miR-155 overexpression leads to repression of SHIP1 and subsequent PI3K/Akt activation. Therefore, miR-155-containing polymeric nanoparticle formulations⁴⁶ may allow trained immunity amplification.

The nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is a pattern recognition receptor involved in trained immunity induction. This intracellular receptor recognizes bacterial peptidoglycans⁴⁷ by binding to muramyl dipeptide (MDP), which is present on Gram-positive and -negative bacteria. For example, the BCG vaccine induces trained immunity through NOD2 activation⁵. In a murine melanoma model, pharmacological stimulation of NOD2 by nanobiologics (which are apolipoprotein A1-based nanomaterials that are exclusively composed of natural molecular building blocks, decorated with muramyl tripeptide (MTP)) induces trained immunity and inhibits cancer growth⁶. Similarly, Toll-like receptor 4 (TLR4) can be targeted for trained immunity modulation. DAMPs, such as oxidized low-density lipoprotein (oxLDL), lipoprotein(a), urate, vimentin and high-mobility group box 1 (HMGB1), are endogenous stimuli that induce trained immunity^{7,48–50} and that can be recognized by TLR4 (REF.⁵¹). This receptor can be blocked by the small-molecule inhibitor TAK242 (resatorvid)⁵² and, thus, oxLDL-induced trained immunity can be suppressed by resatorvid⁵³.

In addition, cytokines and metabolites play an important part in innate immune memory regulation, making them attractive targets for immunotherapy. Upon

trained immunity induction, granulocyte–macrophage colony-stimulating factor (GM-CSF) signalling enhances progenitor proliferation in the bone marrow, and pharmacologic inhibition of GM-CSF counteracts this process¹². A similar effect can be established through pharmacologic inhibition of interleukin-1 (IL-1) by the IL-1 receptor antagonist anakinra¹². The metabolite mevalonate binds and activates the extracellular insulin-like growth factor 1 receptor (IGF1R), which leads to downstream mechanistic target of rapamycin (mTOR) signalling and upregulated glycolysis, resulting in histone modifications of pro-inflammatory enzymes and a trained immunity phenotype⁵⁴. This process can be prevented by an inhibitor or antibody targeted to IGF1R⁵⁴.

Metabolic targets

Receptor binding often results in the activation of specific metabolic pathways (FIG. 1d), which can, in turn, also be a target for trained immunity modulation. For example, induction of trained immunity strongly increases glycolysis, which can be reduced by inhibition of hexokinases with 2-deoxy-D-glucose⁵⁵. Glycolysis is not only controlled by enzyme activity, but also by mTOR signalling, and can therefore be inhibited through pharmacologic mTOR interference with rapamycin, metformin or ascorbate⁴. For example, nanoformulations containing rapamycin inhibit trained immunity and prolong graft survival in a murine heart allograft model⁷. Interference with cholesterol metabolism, for example, through 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibition with statins⁵⁴, reduces mevalonate accumulation and subsequent IGF1R and mTOR activation. The lipophilic nature of statins, such as fluvastatin and simvastatin, facilitates their incorporation into lipid-based nanoformulations⁵⁶. For example, simvastatin-loaded reconstituted high-density lipoproteins (S-HDL) are taken up by aortic plaque macrophages in atherosclerosis-prone mice^{57–59}. Interestingly, treatment with S-HDL reduces atherosclerotic plaque inflammation in mice and translational animal models⁵⁹.

In addition to glycolysis and cholesterol synthesis, glutaminolysis is upregulated in trained immunity. Glutamine is processed to glutamate, which is subsequently converted to α-ketoglutarate or succinate, which are metabolites in the tricarboxylic acid (TCA) cycle. Succinate oxidation results in the accumulation of fumarate, which inhibits histone demethylases, resulting in increased histone methylation of pro-inflammatory cytokines⁶⁰. This process can be mitigated by using bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES), a glutaminase inhibitor⁶⁰. Trained immunity can also be modulated by directly targeting the TCA cycle, for example, with dimethyl malonate, which is an inhibitor of succinate oxidation⁶¹ that can be incorporated into a nanobiologic platform⁶². By inhibiting succinate oxidation, dimethyl malonate prevents fumarate accumulation and its downstream epigenetic effects.

Epigenetic targets

The epigenome forms the molecular basis of trained immunity and can be modified by modulating the activity of epigenetic enzymes. Various types of cancer

also display maladaptive epigenetic rewiring, and several epigenetic inhibitors have already been approved or tested in clinical trials for the treatment of haematologic malignancies and solid tumours⁶³. Nanomedicine can be applied to redirect epigenetic drugs to innate immune cells for trained immunity modulation, allowing fine-tuning of the biodistribution and cellular uptake of drugs.

A specific epigenetic hallmark of trained immunity is acetylation of histone 3 lysine 27 (H3K27ac) at distal enhancers⁶⁴ (FIG. 1e). Histone acetylation renders chromatin more accessible, facilitating gene transcription. During induction of trained immunity, these histone modifications are typically found on genes encoding pro-inflammatory cytokines and metabolic enzymes. Acetylation levels are dependent on the presence of histone acetyltransferases and deacetylases, which can be used as therapeutic targets. For example, (–)-epigallocatechin-3-gallate (EGCG), a histone acetyltransferase inhibitor, inhibits histone modifications associated with β -glucan-induced trained immunity⁴⁴. In contrast to histone acetylation, histone methylation can be either repressive or activating. Histone methyltransferases can be inhibited with 50-deoxy-50-(methylthio)adenosine (MTA)²⁵. Methylation of DNA, which is associated with repressed gene transcription, is regulated by DNA methyltransferases, which can be inhibited by azacitidine encapsulated in a polylactide-co-glycolide (PLGA)–polyethylene glycol (PEG) nanoscale delivery system. Here, incorporation into the nanomaterial improves the therapeutic effect by enhancing drug

stability and increasing cellular uptake⁶⁵, demonstrating the potential of nanoformulations to increase epigenetic drug efficacy.

Histone modifications can also be altered by long non-coding RNA (lncRNA). Upon β -glucan stimulation, a specific class of lncRNAs, called ‘immune gene-priming lncRNAs’ (IPLs), are upregulated, facilitating H3K4 trimethylation of cytokine promoters⁶⁶. Here, topologically associated domains and the specific 3D structure of lncRNAs allow interaction with multiple innate immune genes⁶⁷. The activity of UMLILO, a prototype IPL, can be inhibited by siRNA⁶⁶.

The in vivo behaviour of nanomaterials

Nanomaterials can interact with the haematopoietic system, which is responsible for leukocyte production, and with the mononuclear phagocyte system, which can be exploited in the regulation of trained immunity. In particular, the bone marrow, which has rarely been the target tissue in nanomedicine studies, is important in trained immunity. Although uptake of nanoliposomes in the bone marrow has previously been reported⁶⁸, targeting the bone marrow for immune regulation by nanomedicine has only recently been explored^{6,69}. The quest to improve drug delivery by increasing the percentage of injected drug dose at the site of action upon intravenous administration has long been central to the nanomedicine community, particularly to increase drug deposition in cancerous and inflammatory lesions. This approach requires the nanodrugs to have a long circulation half-life, which can be challenging to achieve with nanomaterials, because they are usually rapidly eliminated through complement activation and phagocytes. The blood circulation time of nanomaterials can be increased through surface coating, for example, the coating of liposomes with hydrophilic polymers, such as PEG⁷⁰, with an optimal PEG density of approximately 10 mol% (we note that very high PEG densities decrease circulation half-lives)⁷⁰. Typically, PEGylation is achieved by formulating self-assembled nanoparticles that include PEG-functionalized phospholipids⁷¹ or polymers⁷². Alternatively, nanocrystalline materials, such as gold⁷³, iron oxide⁷⁴ or semiconductor nanocrystals⁷⁵, can be applied to the material post-synthesis.

The mononuclear phagocyte system and nanoparticle clearance

Drug delivery to lesions relies on high vascular permeability and long blood circulation half-lives, which can be achieved by avoiding premature elimination by the mononuclear phagocyte system. By contrast, nanomedicines intended to regulate trained immunity require efficient interaction with myeloid cells. Therefore, the affinity of nanomaterials for phagocytes should not be prevented, but rather exploited, which necessitates in-depth knowledge of the in vivo behaviour of nanomaterials. Most nanomedicine platforms are self-assembled systems based on lipid nanoparticles comprised of phospholipids and/or mixtures of other fatty molecules, or polymeric nanoparticles, which are typically built from block copolymers. The therapeutic payload of small molecules, RNA or proteins, for example, is usually integrated by

Table 2 | Trained immunity regulation

Pathway	Target	Drug	Result	Ref.
PI3K/AKT/mTOR/HIF1 α	SHIP1	3 α -Aminocholestane	Enhancement	44
		miR-155	Enhancement	45
		Anakinra	Inhibition	12
Cholesterol synthesis	HMG-CoA reductase	Statins	Inhibition	54
Glycolysis	Hexokinases	2-Deoxy-D-glucose	Inhibition	55
		Rapamycin	Inhibition	4
	mTOR	Metformin	Inhibition	4
		Ascorbate	Inhibition	4
Glutaminolysis	Glutaminase	BPTES	Inhibition	60
	Succinate oxidation	Dimethyl malonate	Inhibition	61
Histone acetylation	Histone acetyltransferase	EGCG	Inhibition	44
Histone methylation	Histone methyltransferases (H3K4)	MTA	Inhibition	25
		Histone methyltransferases (H3K9)	BIX-01294	Enhancement
DNA methylation	DNA methyltransferases	Azacitidine	Inhibition	65

BPTES, bis-2-(5-phenylacetamido-1,2,4-thiazol-2-yl)ethyl sulfide; EGCG, epigallocatechin gallate; HIF1 α , hypoxia inducible factor 1 α ; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MTA, methylthioadenosine; mTOR, mechanistic target of rapamycin; PI3K, phosphoinositide 3-kinase; SHIP1, SH2 domain-containing inositol 5'-phosphatase 1.

a self-assembly process. The *in vivo* behaviour of such platforms is typically assessed by investigating a single nanoparticle component, that is, either the nanomaterial or the therapeutic payload. Although different components can be simultaneously labelled, for example, by using different radioisotopes or fluorophores⁵⁶ for nuclear or optical detection, respectively, nanoparticle integrity or the exchange of components with blood constituents cannot be studied using standard techniques, such as gamma counting or confocal laser scanning microscopy. However, imaging methods, in combination with *ex vivo* approaches, have been developed to holistically study nanoparticle behaviour in preclinical models (BOX 2).

The nanoparticle *in vivo* journey

The *in vivo* fate of nanomaterials depends on material properties, such as size and surface coating, and features of the circulatory system, including endothelial permeability and neovascularization. These parameters dictate nanoparticle aggregation, organ and tissue uptake, as well

as clearance rates and elimination pathways^{76,77}. Upon intravenous administration, nanoparticles are transported in the blood to the right ventricle of the heart, into the lung capillaries and back to the left ventricle, before they are distributed to vital organs in the body, such as the brain, liver and kidneys, via the aorta. In general, intravenously infused nanoparticles do not extravasate from regular blood vessels lined with an impermeable endothelium or the blood–brain barrier. Therefore, in healthy individuals, nanoparticles accumulate in organs with high microvascular permeability and/or blood filtration systems, such as in the kidneys, liver and spleen. Nanoparticles can also accumulate in tumours and inflammatory lesions, including atherosclerotic plaques, which are characterized by ongoing neovascularization and increased (micro)vascular permeability⁷⁸.

The key nanoparticle parameters that dictate *in vivo* behaviour are size, aggregation, surface charge and shape (FIG. 2). Differently sized quantum dots (semiconductor nanocrystals with size-dependent fluorescent properties) have been used to determine the size dependency of clearance, showing that nanomaterials with a hydrodynamic radius smaller than 6–8 nm are cleared within minutes by the kidneys and are excreted via the bladder^{79,80}. Kidney clearance is facilitated by the glomerular filtration membrane, which is a semipermeable membrane that filters blood. Therefore, small nanoparticles have little potential for myeloid cell targeting and trained immunity regulation. Conversely, medium-sized nanoparticles (10–100 nm) typically display longer blood circulation half-lives because they do not pass through the glomerular filtration barrier. Biodistribution studies show that medium-sized nanoparticles accumulate in (and are cleared by) the liver and spleen. These organs are part of the mononuclear phagocyte system, which filters toxins from the blood. Kupffer cells, which are the local macrophages of the liver, form a protective barrier through their scavenger and phagocytic functions, removing foreign debris and particles from portal blood flow through pattern recognition receptor interactions^{76,81,82}. In addition to Kupffer cells, B cells are involved in phagocytosis-mediated removal of certain nanomaterials, for example PEGylated quantum dots⁸¹. The spleen performs a role similar to that of the liver. Here, macrophages in the red pulp region are the primary phagocytic force driving nanoparticle removal. To achieve long blood circulation times of nanomedicines for lesion targeting, elimination by the mononuclear phagocyte system has to be prevented. Nanoparticle sizes of around 75–100 nm are optimal for long circulation times⁶⁸; by contrast, larger nanoparticles (100–250 nm) have much shorter circulation times owing to complement activation and more efficient clearance by the liver and spleen. Rapid removal can also be a result of nanoparticle aggregation upon intravenous administration, which causes the nanoparticles to become trapped in pulmonary capillaries⁸³.

Nanodelivery platforms

Nanodelivery platforms can be applied to route different classes of therapeutic payloads to myeloid cells and their progenitors in the bone marrow and spleen.

Box 2 | Imaging framework

In vivo imaging methods can support multiple aspects of nanotherapy development. *In vivo* imaging allows the non-invasive study of nanoparticle biodistribution and cellular interactions *in vivo*. For example, labelling of nanoparticles with radioisotopes, such as copper-64 (⁶⁴Cu) or zirconium-89 (⁸⁹Zr), enables dynamic and quantitative positron emission tomography (PET) imaging of their biodistribution, which can also be achieved by single-photon emission computerized tomography (SPECT) imaging in combination with radioisotopes, such as technetium-99m (^{99m}Tc) and indium-111 (¹¹¹In). These tomographic nuclear imaging techniques can dynamically and longitudinally visualize nanoparticle distribution at the whole-body level, in a non-invasive and quantitative fashion. PET and SPECT imaging systems are typically integrated with computed tomography for anatomical reference and to allow attenuation corrections for tomographic purposes. Although technically more challenging, the combination of PET and SPECT with magnetic resonance imaging (MRI) has substantial advantages, in particular, for soft tissues with complex anatomical structures, such as the vessel wall⁵⁹. A variety of methods are available to radiolabel nanomaterials¹⁶⁴. In addition to radioisotopes, nanomaterials can be labelled with fluorophores, allowing *in vivo* near-infrared fluorescence (NIRF) imaging of whole-body biodistribution, and the study of nanoparticle disassembly dynamics using Förster resonance energy transfer (FRET) imaging or intravital microscopy^{58,193,194}. The latter technique can also be applied to study nanoparticle–(immune) cell interactions in live mice¹⁶⁶. In addition to *in vivo* imaging, the cellular behaviour of fluorescently labelled nanoparticles can be studied *ex vivo* in tissue sections by confocal microscopy and/or on (immune) cells using flow cytometry assays.

In addition to studying biodistribution and cellular specificity, imaging is also a valuable way of studying the induction or inhibition of trained immunity and inflammatory responses, as a result of pathological processes or (nano)therapeutic interventions. As trained immunity involves (rewiring of) programs at multiple levels, several imaging methods can be employed. Metabolic alterations of myeloid cells in the bone marrow are a hallmark of trained immunity. For example, glucose consumption and glutaminolysis are upregulated. These processes can be visualized by PET imaging using the radiotracers ¹⁸F-FDG and ¹⁸F-(2S,4R)4-fluoroglutamine PET¹⁹⁵. Histone deacetylase PET can detect epigenetic alterations in the brain and could also be adopted for the bone marrow¹⁹⁶. Immune cell proliferation in this organ can be studied using ¹⁸F-fluorothymidine PET¹⁹⁷. Changes in myeloid cell dynamics can be probed by ⁸⁹Zr-labelled nanobodies targeting CD11b^{198,199}. Many PET imaging methods and tracers are clinically approved and can therefore be readily implemented in clinical studies and may serve as companion diagnostics for trained-immunity-regulating nanotherapeutics. A range of modalities are also pre-clinically available to study inflammation. For example, fluorescence molecular tomography with computed tomography (FMT–CT) enables NIRF imaging of nanoparticle biodistribution²⁰⁰ in mice. The same method allows quantitative investigation of (macrophage) inflammation using an activatable fluorescent dye.

For example, liposomes⁸⁴ have been optimized by the drug delivery community to efficiently deliver chemotherapeutics to tumours, thereby increasing the therapeutic index of drugs and reducing side effects. The blood circulation times of liposomes⁶⁸ can be increased by coating with PEG and other surface-functionalization strategies⁶⁵. Similarly, long-circulating polymeric nanoparticles have been designed, made of diblock copolymers from PEG and biodegradable polymers, such as PLGA. By integrating PEG, recognition by the mononuclear phagocyte system and premature removal by K upffer cells in the liver can be avoided⁸⁵. The targeting of myeloid cells, particularly macrophages, has been originally explored in the context of cardiovascular disease^{86,87} and rheumatoid arthritis⁸⁸. We note that studies focusing on the specific targeting of haematopoietic organs are scarce. Following the discovery that the spleen acts as a reservoir for monocytes⁸⁹, a lipid nanoparticle has been designed to deliver siRNA (targeting CCR2) to the spleen and bone marrow, with the aim of preventing monocytes from migrating to sites of inflammation⁹⁰. In a heart transplant mouse model, mTOR-inhibiting nanobiologic therapeutics could efficiently induce immunological tolerance by targeting myeloid cells and their progenitors in the bone marrow, resulting in prolonged allograft survival⁷. This approach enabled precision inhibition of inflammation. A similar nanobiologic technology could also be applied to induce trained immunity to combat cancer. Both studies^{6,7} demonstrated that nanobiologic-facilitated regulation of trained immunity is caused by metabolic, epigenetic and transcriptomic changes of HSPCs in the bone marrow⁶.

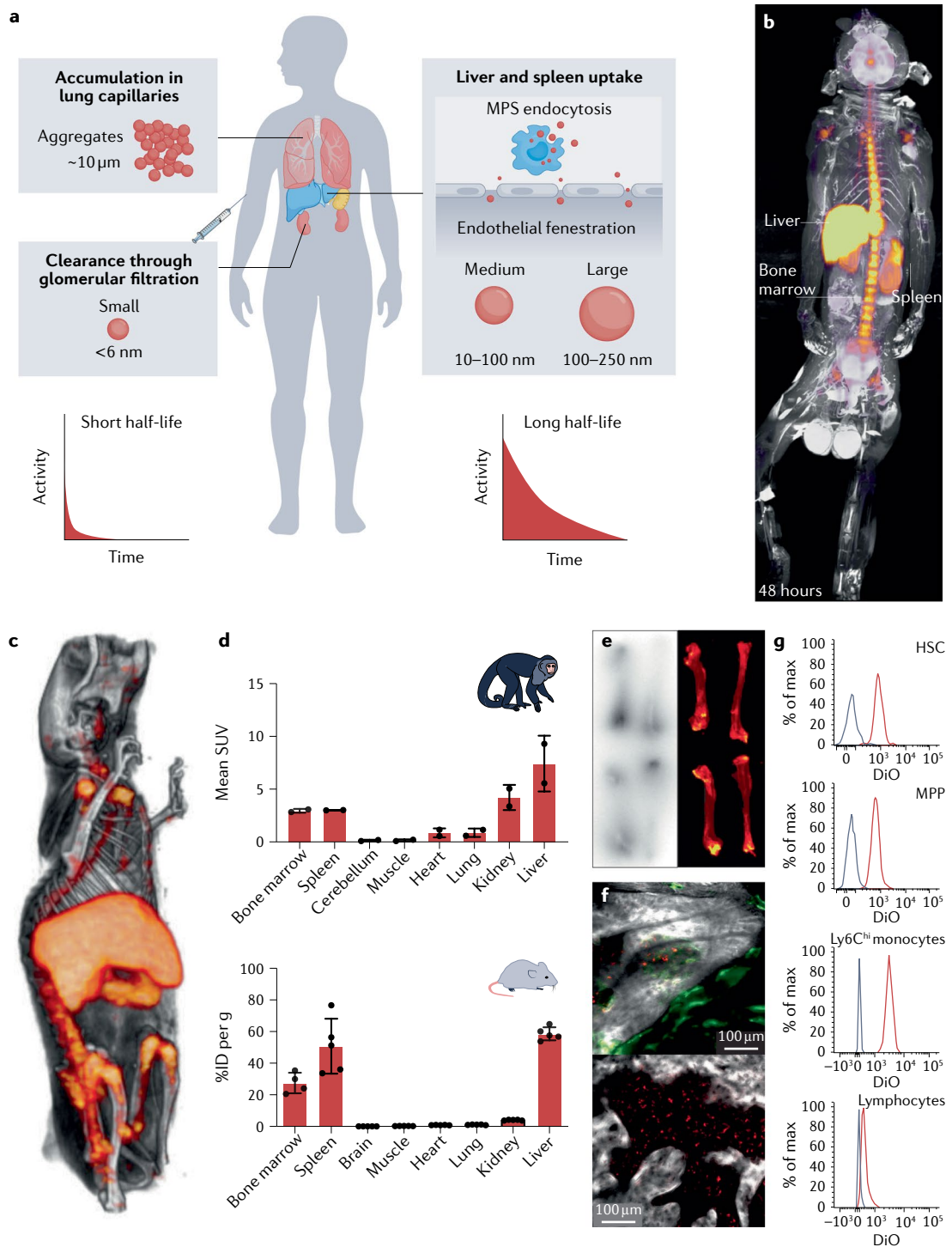
A variety of nanomedicine platforms have been developed for drug delivery. Here, we focus on those with the highest translational potential, that is, lipid-based, polymeric, lipoprotein, viral and protein-polymer nanoparticles (FIG. 3). Lipid-based nanoparticles consist of amphiphilic (phospho)lipids, which self-aggregate or self-organize owing to the dual features of these molecules, which consist of a polar, water-soluble group attached to a water-insoluble hydrocarbon chain. The most widely studied lipid-based nanomaterials are vesicles called liposomes, which are bilayered structures encapsulating an aqueous lumen. Liposomes are highly suitable to deliver hydrophilic payloads, although their true potential has been reached with the development of different loading methods. For example, liposomal doxorubicin integrates crystals of the drug, achieving very high encapsulation efficiencies of 98%⁹¹. Micelles are also lipid-based nanomaterials composed of spherical lipid aggregates, in which the amphiphilic lipids are organized with their apolar tails positioned in the core and the hydrophilic headgroups facing the aqueous exterior. Nanoemulsions are essentially 'swollen micelles', which additionally integrate apolar fatty molecules into their core, which is covered by a monolayer of amphiphilic lipids. Nanoemulsions and micelles are therefore suitable for the delivery of lipophilic payloads. A lipid-based platform also enabled the first approved RNA interference (RNAi) therapeutic⁹², and lipid nanoparticles are also integrated⁹³ in COVID-19 mRNA vaccines^{94,95}.

Polymeric nanoparticles composed of amphiphilic block copolymers include polymeric vesicles called polymersomes⁹⁶, nanostructures with a micellar morphology⁹⁷, nanoemulsions consisting of a lipophilic matrix covered by a monolayer of block copolymers⁹⁸, as well as polymeric nanoparticles for RNA delivery⁹⁹. Lipoprotein-derived and -inspired nanomaterials include high-density lipoprotein⁸⁷, low-density lipoprotein and microemulsion-like structures, as well as a apolipoprotein A1-based nanobiologic platform⁵⁶. Viral and virus-based delivery technologies have shown great potential for gene therapy and vaccination, but can also be applied for the delivery of small-molecule drugs and proteins^{100,101}. Finally, nanostructures assembled from immunomodulatory proteins or polymers^{102,103} are made of building blocks that exhibit specific immunomodulatory features.

All of these platforms can be adjusted for the delivery of immunoregulatory payloads, albeit at different efficiencies (FIG. 3). For example, viral nanoparticles are inherently suitable for nucleic acid drug delivery, but can also be adopted for the delivery of small-molecule drugs. Liposomes are most suited for incorporating payloads in the aqueous lumen, but they can also carry lipophilic drugs in the phospholipid bilayer. It is important to harmonize the therapeutic goal, immunoregulatory payload and nanoparticle type when designing trained-immunity-regulating nanomedicines, and to take into account the drug's mode of action. For example, mRNA requires efficient cytosolic delivery to enable ribosomes to efficiently read the code and translate it into a protein. However, nanomaterials are usually endocytosed by cells and therefore, end up in cellular vesicles, called endosomes. Thus, endosomal escape is a prerequisite for and integral part of mRNA–lipid nanoparticle technologies¹⁰⁴. There are different types of endocytosis, of which receptor-mediated (or clathrin-mediated) endocytosis is the most relevant mechanism for nanoparticle uptake. Small nanoparticles (that is, <60 nm) can also be taken up through caveolin-mediated endocytosis⁷⁷. In addition, nanoparticle merging with the cell membrane, pinocytosis and contact-facilitated delivery have been proposed as mechanisms by which nanomaterials interact with cells¹⁰⁵.

Immunostimulatory polymers

A range of immunomodulatory polymers can serve as foundational building blocks of trained immunity-inducing nanomaterials. For example, nanoparticles exclusively composed of natural polymers, such as chitosan or hyaluronan, may innately promote trained immunity¹⁰⁶. The bacterial wall consists of peptidoglycans, which are recognized by phagocytes, inducing an immune response through NOD2 activation¹⁰⁷. Although systemic administration of such immune-activating polymers is unattractive, nanomaterial-based formulations allow controlled exposure and reduce systemic toxicity. These immunostimulatory molecules could also be templated onto nanoparticle scaffolds or function as building blocks, either by generating copolymers or through crosslinking methods¹⁰⁸.



Immunoregulatory proteins

Cytokines are key regulators of trained immunity; for example, GM-CSF and IL-1 β promote trained immunity, and many other cytokines are currently under investigation. Cytokine-based proteins (or peptides) can be incorporated into nanomedicines; for example, polymeric nanoparticles can deliver the inflammation-resolving peptide Ac2-26 (REFS^{16,109}). Cytokine receptors are typically present on the cell surface, and therefore, phagocytosis (intracellular delivery) may compromise cytokine function. To prevent phagocytosis, polymeric

nanoparticles can be decorated with collagen-targeting moieties to promote extravascular entrapment and avoid uptake by macrophages¹⁰⁹. Alternatively, nanoparticles can be surface-decorated to allow cytokines to interact freely with cell surface receptors.

Nucleic acid drugs

The development of mRNA vaccines against COVID-19 has firmly established nanotechnology’s utility in medicine. Lipid nanoparticle technology allowed the development of highly effective mRNA vaccines in record

◀ **Fig. 2 | The journey of nanomaterials upon intravenous administration.** **a** | Upon intravenous administration, injected nanoparticles travel to the right atrium and ventricle of the heart before entering the lung vasculature. Large aggregates are trapped in the pulmonary capillaries. Individual and unaggregated nanoparticles travel to the left side of the heart and enter the aorta. Small particles (<6–8 nm) are rapidly excreted through glomerular filtration in the kidneys, resulting in a short half-life in the blood. Larger formulations circulate for longer and are taken up by the liver and spleen through endocytosis by the mononuclear phagocyte system (MPS) or by endothelial fenestration. **b–g** | In vivo behaviour of high-density lipoprotein (HDL)-mimicking nanobiologics loaded with 10 mol% muramyl tripeptide (MTP₁₀-HDL). **b** | Positron emission tomography–magnetic resonance imaging (PET–MRI) scan of a non-human primate (*Macaca fascicularis*), 48 hours after intravenous ⁸⁹Zr-labelled MTP₁₀-HDL injection. There is high accumulation in the liver, spleen and bone marrow. **c** | Representative whole-body 3D-rendered PET–computer tomography image of a C57Bl/6 mouse, 24 hours after intravenous ⁸⁹Zr-MTP₁₀-HDL injection. Similar to the behaviour in non-human primates, ⁸⁹Zr-MTP₁₀-HDL accumulation in the liver, spleen and bone marrow are detected. **d** | Quantification of ⁸⁹Zr-MTP₁₀-HDL biodistribution in non-human primates (top, assessed by in vivo PET imaging, *n* = 2) and mice (bottom, assessed by ex vivo gamma counting, *n* = 5), shown as percentage injected dose (%ID). **e** | Ex vivo near-infrared fluorescence (NIRF) images and autoradiography of murine femurs and tibias, 24 hours after injection of dual-labelled Dil-⁸⁹Zr-MTP₁₀-HDL. Bone marrow uptake is concentrated at the proximal and distal ends of the bone, where the red marrow is located (*n* = 5). **f** | Intravital microscopy image of a live mouse calvarium, 8 hours post Dil-MTP₁₀-HDL administration. Fluorescein isothiocyanate (FITC)-dextran was injected intravenously to display the vasculature. **g** | Flow cytometry of murine bone marrow cells 24 hours after DiO-MTP₁₀-HDL administration (red lines) or phosphate buffered saline (PBS) control (grey lines). Representative histograms of haematopoietic stem cells (HSCs), multipotent progenitors (MPP), Ly6C^{hi} monocytes and lymphocytes indicate nanobiologic affinity for myeloid (progenitor) cells (*n* = 5). SUV, standardized uptake value. Panels **b–g** adapted with permission from REF.⁶, Elsevier.

time^{94,95}. Advances in lipid nanoparticle technology (in particular, the development of ionizable lipids) and in mRNA technology have forever changed the way vaccines will be developed^{110,111}. As an alternative to lipid nanoparticles, viral vectors can be used to deliver nucleic acids, a strategy applied for the delivery of DNA in AstraZeneca's SARS-CoV-2 vaccine¹¹². mRNA can also be delivered to myeloid cells to induce protein expression^{56,113,114}. Similarly, other RNA-based drugs have potential for trained immunity regulation, including small interfering RNA (siRNA), microRNA (miRNA), and long non-coding RNAs (lncRNA). At varying efficiencies, all of these RNAs could be integrated into lipid or polymeric nanoparticles, for example, to silence a (hub) gene involved in crucial metabolic or epigenetic pathways involved in trained immunity using siRNA or miRNA, or to induce immunoregulatory proteins using mRNA¹⁵.

Small molecules

A range of metabolic and epigenetic pathways have been identified to regulate trained immunity (FIG. 1), and a wealth of small-molecule inhibitors have been shown to inhibit trained immunity in vitro. However, delivery of these molecules to the myeloid cell compartment will require the development of targeting strategies. Water-soluble compounds can be incorporated into the aqueous interior of liposomes or polymersomes, whereas micelles and nanoemulsions can carry lipophilic molecules. For example, prodrugs can be modularly integrated into apolipoprotein A1-based nanobiologics⁶², allowing greater than 80% to 90% integration of prodrug derivatives of diethylmalonate and rapamycin. Small-molecule drugs can also be integrated into viral nanoparticles. Similarly to small-molecule inhibitors,

immunostimulatory molecules can be delivered by nanoparticles. Decorating the surface of nanoparticles with immunostimulatory molecules may be an efficient way to modulate trained immunity, as for nanobiologics decorated with muramyl dipeptide⁶.

Therapeutic applications

Nanomedicine approaches can be applied to reroute trained-immunity-regulating drugs to myeloid (progenitor) cells to either promote trained immunity or to prevent its induction (FIG. 4). Additionally, trained-immunity-regulating nanotherapies may display direct, pleiotropic pro- or anti-inflammatory effects.

Trained immunity-promoting nanotherapeutics

Trained immunity is characterized by quantitatively and qualitatively enhanced innate immune responses owing to metabolic and epigenetic rewiring of the myeloid cell compartment. Trained immunity can be induced to increase immunological resistance against a range of infections, including possibly against SARS-CoV-2, or to combat cancerous malignancies.

Infection and COVID-19. Several pathogens and vaccines can induce trained immunity and enhance the immune response to a subsequent encounter with an unrelated pathogen, and therefore, inducing trained immunity is a promising approach to combat infections. For example, BCG vaccination has been proposed as a strategy to reduce COVID-19-related morbidity and mortality^{115–117}, and retrospective observational studies showed that fewer health-care workers with a history of BCG vaccination¹¹⁸ showed positive serology to SARS-CoV-2 (IgG), as compared to individuals without BCG vaccination. In another retrospective study, it has been shown that a recent (that is, less than 5 years old) vaccination with BCG does not correlate with hyperinflammation and is not associated with increased incidence of symptoms during the COVID-19 outbreak in the Netherlands¹¹⁹. Furthermore, in a double-blind randomized controlled trial, BCG vaccination led to an 80% reduction of viral respiratory infections in 200 elderly individuals¹²⁰. Although this trial did not include SARS-CoV-2 infection, together these studies support the hypothesis that BCG vaccination may provide non-specific protection against infections. Ongoing randomized controlled trials (NCT04659941, NCT04384549, NCT04537663) will shed a light on the potential of BCG vaccination in protection against infections, and vaccines eliciting trained immunity may further mitigate the impact of emerging pathogens beyond SARS-CoV-2.

Overcoming immune paralysis. In addition to infection prevention, trained-immunity-inducing agents might also be used to overcome immune paralysis, a condition that often occurs in individuals with sepsis. Immune paralysis is characterized by a functional state in which immune responses are muted as an evolutionary mechanism to prevent damage caused by long-lasting inflammatory responses¹²¹. Through similar mechanisms, immune paralysis also occurs after severe hyperinflammatory episodes as a result of cardiovascular events, such

as stroke or myocardial infarction. Therefore, individuals suffering from immune paralysis become susceptible to (secondary) infections, such as pneumonia, which is a major cause of death in individuals hospitalized because of sepsis or a cardiovascular event^{122–124}. β -Glucan has the potential to revert immune paralysis, as assessed by ex vivo stimulation of monocytes from intensive-care sepsis patients¹²⁵. To overcome immune paralysis in vivo, sophisticated therapies must be developed to restore the balance between pro- and anti-inflammatory pathways. These therapies could, for example, be based on

β -glucan-derivatives or immunoregulatory proteins such as GM-CSF and IL-7 (REF.¹²²). We envision therapeutic opportunities for trained-immunity-inducing nanotherapies in individuals suffering from immune paralysis owing to sepsis, stroke, myocardial infarction or other acute inflammatory conditions that lead to immune paralysis.

Overcoming immunosuppression in cancer. Tumours escape destruction by the immune system through a process called immunoediting, which interferes with

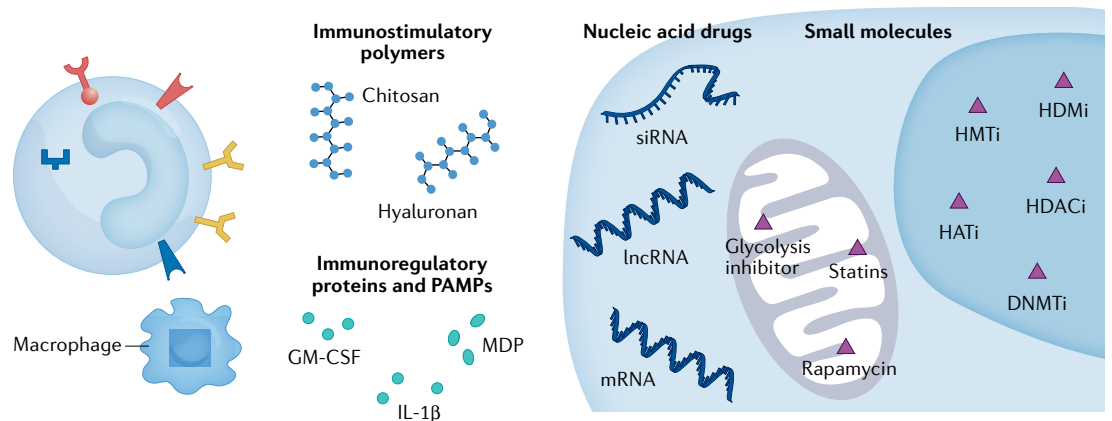
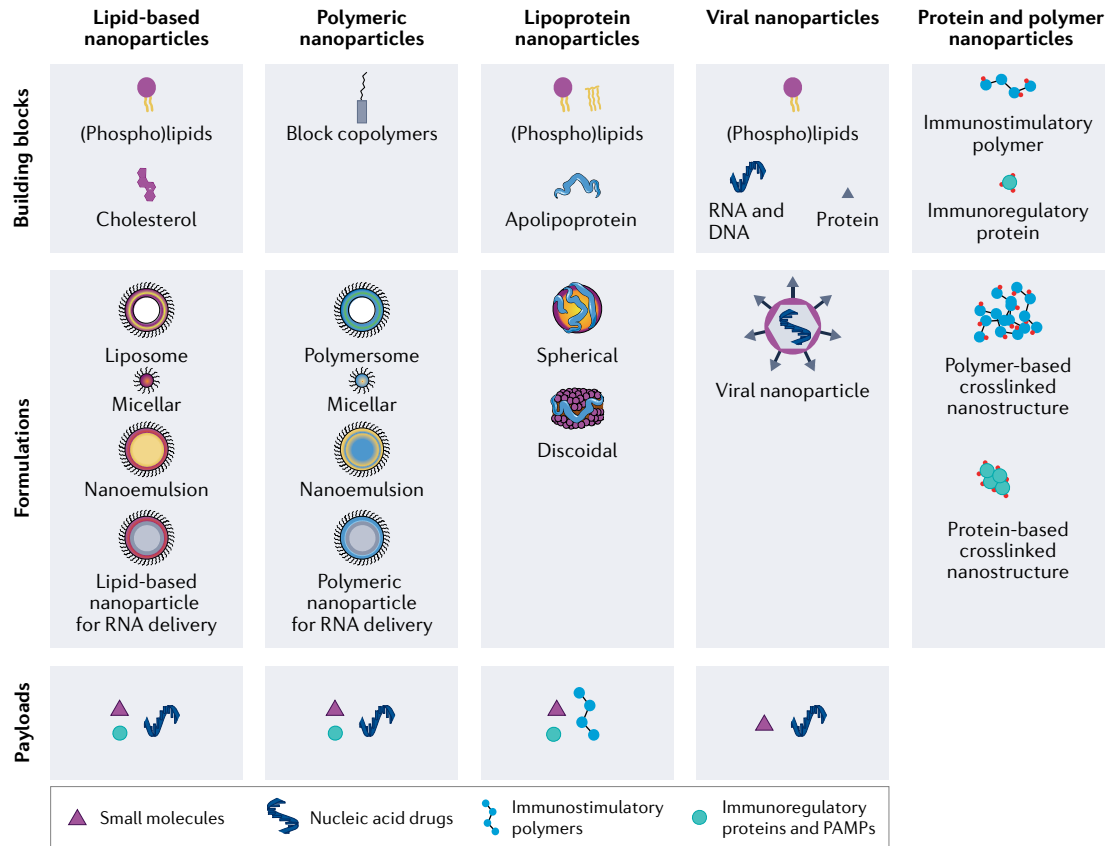


Fig. 3 | Nanoparticle platforms. An overview of nanoparticle platforms that can deliver trained-immunity-regulating payloads, including small molecules, RNA therapeutics, immunoregulatory proteins and immunostimulatory polymers. DNMTi, DNA methyltransferase inhibitor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HATi, histone acetyltransferase inhibitor; HDACi, histone deacetylase inhibitor; HDMi, histone demethylase inhibitor; HMTi, histone methyltransferase inhibitor; IL-1 β , interleukin-1 β ; lncRNA, long non-coding RNA; MDP, muramyl dipeptide; PAMP, pathogen-associated molecular pattern; siRNA, small interfering RNA.

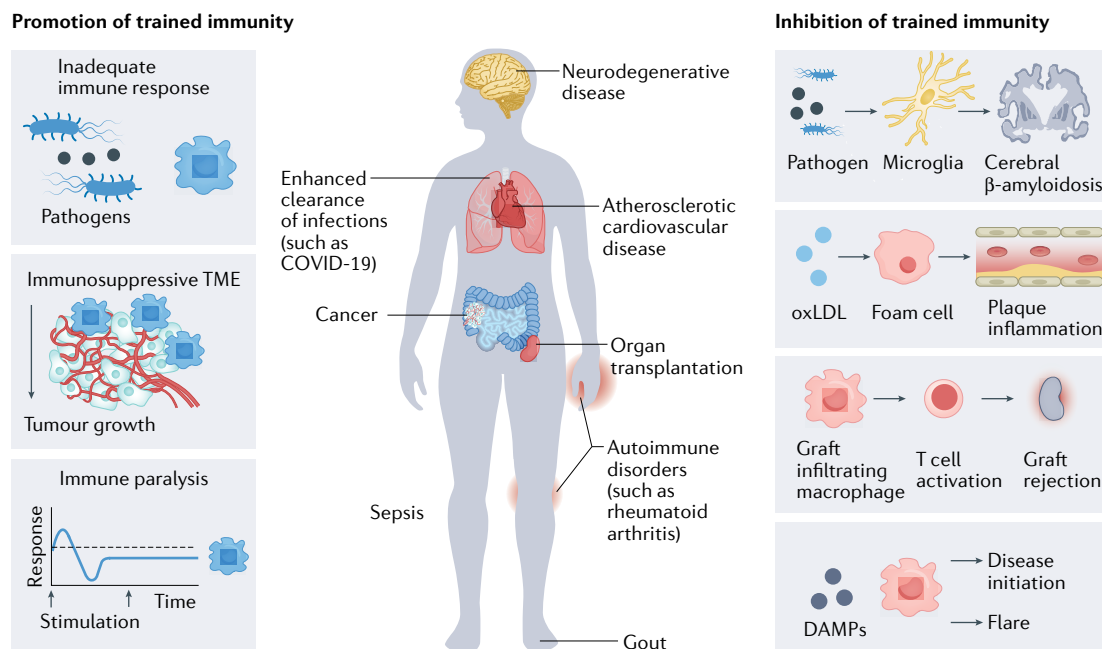


Fig. 4 | **Trained-immunity-regulating nanotherapies in clinical scenarios.** Trained immunity can be induced to combat cancer and increase resistance to infection, for example, against COVID-19, or trained immunity can be inhibited in conditions characterized by an exacerbated immune response. DAMP, damage-associated molecular pattern; oxLDL, oxidized low-density lipoprotein; TME, tumour microenvironment.

several aspects of normal immune activation¹²⁶. First, tumour cells lose their ability to effectively present antigen (signal 1) owing to downregulation of major histocompatibility complex (MHC) molecules or presentation of mutated and thus unrecognizable antigens. Functional antigen presentation would be required to trigger anti-tumour T cell responses. Second, tumours disrupt the delicate balance of co-stimulatory and co-inhibitory signals (signal 2), further impairing T cell activation^{127,128}. This loss of immunogenicity is exemplified by the upregulation of programmed cell death 1 ligand 1 (PDL1) by tumour cells. PDL1 is a co-inhibitory signal that prevents T cell-mediated tumour cell killing¹²⁹. Immunotherapies, such as chimeric antigen receptor T cell therapy and checkpoint inhibition, focus on the adaptive arm of the immune system, targeting signals 1 and 2. However, components of the innate immune system are also involved, generating an immunosuppressive tumour microenvironment consisting of tumour-associated myeloid cells and myeloid-derived suppressor cells, reducing the effectiveness of the abovementioned immunotherapies¹³⁰. The imbalance of anti-tumour and pro-tumour myeloid cells in the tumour microenvironment stems, in part, from their aberrant production in the bone marrow^{131,132}. In response to cytokines and growth factors produced by tumour cells¹³³, the bone marrow produces immature cells, which are 'polarized' following their arrival in the tumour microenvironment to assist in sustaining the immunosuppressive milieu, in essence creating an inescapable positive feedback loop. This cancer-mediated immune cell modulation by HSPCs in the bone marrow may be counteracted and/or reversed by trained immunity

induction. Rebalancing innate immune cell production bias from aberrant haematopoiesis to 'trained' myelopoiesis may overcome the immunosuppressive tumour microenvironment¹³⁴.

The trained immunity inducer BCG is used as immunotherapy in individuals with non-muscle-invasive bladder cancer¹³⁴. This intravesical treatment induces a local immune response that is based on the activation of trained immunity¹⁹. Because drugs administered to the bladder are not systemically distributed in the body, BCG, a live bacterium, can be safely applied. In addition to this local effect, a systemic effect has been reported, highlighted by the increased production of several key trained immunity cytokines by blood-derived monocytes¹³⁵. To develop bone-marrow-engaging, trained-immunity-inducing nanotherapeutics that can be safely applied intravenously, we decorated nanobiologics with the smallest molecular structure of BCG capable of inducing trained immunity, that is, the NOD2-activating molecule muramyl dipeptide (MDP)⁶. Screening of a library of nanobiologics surface-functionalized with MDP identified a formulation, named MTP₁₀-HDL (FIG. 5a), with potent trained-immunity-inducing features (FIG. 5b) and a favourable biodistribution profile. Upon intravenous administration, MTP₁₀-HDL immunotherapy significantly inhibited tumour growth and sensitized the resistant B16F10 melanoma tumour mouse model to checkpoint inhibition therapy (FIG. 5c). Furthermore, the anti-tumour effect of this nanobiologic immunotherapy is transferable through bone marrow transplantation into untreated mice. In-depth analysis of the bone marrow uncovered a shift in bone marrow immune cell production

towards ‘trained’ myelopoiesis. The resulting increase in ‘trained’ monocytes and neutrophils rebalanced the tumour microenvironment, leading to tumour growth inhibition^{6,33}. Similarly, β -glucan has been shown to prophylactically induce trained immunity against cancer, achieving anti-tumour responses perpetuated by innate immune training through granulopoiesis and neutrophil reprogramming.

Trained immunity as an adjuvant. Trained-immunity-induced activation of the innate immune system could also enhance the efficacy of cancer vaccines. Adjuvant technology for most infection-preventing vaccines consists of potent pattern recognition receptor ligands, which are locally injected with the poorly immunogenic vaccine antigens to kick-start the immune cascade¹³⁶. In contrast to classical vaccines that use foreign antigens, therapeutic cancer vaccines use self-antigen and have to

overcome the immunosuppressive tumour microenvironment. The additional immunological boost provided by trained immunity induction could be advantageous in overcoming this hurdle.

Trained immunity induction as an anti-cancer strategy has been established for BCG, β -glucan and the trained immunity-inducing nanobiologic immunotherapy. Nanomedicine strategies could also be based on a clinically used oncolytic virus coding for GM-CSF. This oncolytic virotherapy, called talimogene laherparepvec (T-VEC), inhibits histone deacetylase, and has considerable off-target effects, resulting in remission of metastases, which is often contributed to immunogenic cell death. Trained immunity induction through GM-CSF signalling could be a key mechanism in the observed remission¹³⁷. In addition, encapsulation of other trained immunity inducers, such as β -glucan and epigenetic drugs, could be effective.

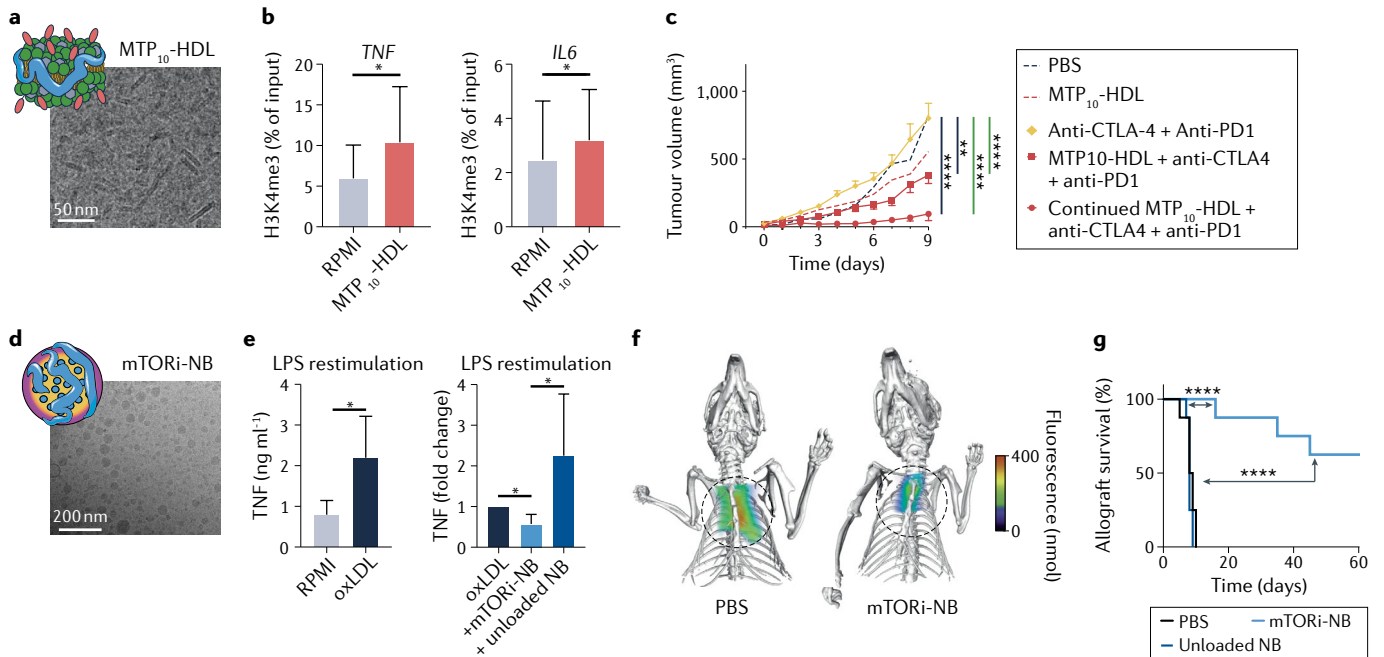


Fig. 5 | Applications of nanoimmunotherapies. **a** | Schematic representation (left) and cryo-transmission electron microscopy (cryo-TEM) image (right) of high-density lipoprotein (HDL)-mimicking nanobiologics (NB) loaded with 10 mol% muramyl tripeptide (MTP₁₀-HDL)⁶. **b** | Chromatin immunoprecipitation (ChIP)-quantitative polymerase chain reaction (qPCR) of peripheral blood mononuclear cells shows increased H3K4 methylation on the tumor necrosis factor (TNF) and interleukin-6 (IL-6) promoters after incubation with MTP₁₀-HDL as compared to control (Roswell Park Memorial Institute, RPMI; n = 3). Error bars represent the standard deviation. **c** | Tumour growth curve of B16F10 melanoma in C57Bl/6 mice. Mice were inoculated with 10⁵ B16F10 cells at day -7 and MTP₁₀-HDL (MTP at 1.5 mg kg⁻¹) was intravenously administered at days 0, 2 and 4. In one group, MTP₁₀-HDL treatment was continued; mice were also injected on days 6 and 8. Checkpoint inhibitors anti-cytotoxic T lymphocyte antigen 4 (anti-CTLA4) and anti-programmed cell death 1 (anti-PD1) (both 200 µg per mouse) were administered intraperitoneally twice per week, starting at day 2. The primary outcome was the comparison between checkpoint inhibitor monotherapy versus combination with MTP₁₀-HDL. Significance was calculated for tumour growth rate (black) and tumour size (green). Data are represented at mean ± standard error of the mean. **d** | Schematic representation and cryo-TEM image of HDL-mimicking NB loaded with the mechanistic target

of rapamycin (mTOR) inhibitor (mTORi-NB). **e** | Human primary monocytes were incubated with oxidized LDL (oxLDL) for 24 hours, and after a 5-day rest, cells were restimulated with lipopolysaccharide (LPS). oxLDL amplifies the TNF production upon LPS stimulation, as measured by enzyme-linked immunosorbent assay (ELISA). When human monocytes are incubated with oxLDL in combination with mTORi-NB, TNF production is reduced, as compared to unloaded NB or oxLDL only (n = 6). **f** | Atherosclerosis-prone *ApoE*^{-/-} mice were held on a Western diet for 12 weeks to develop advanced plaques. The mice received four intravenous injections of mTORi-NB (mTORi at 5 mg kg⁻¹) or phosphate buffered saline (PBS) for one week, before being subjected to fluorescence molecular tomography (FMT) imaging¹⁷⁷. mTORi-NB-treated mice showed less fluorescence in the aortic root area, indicative of reduced protease activity — a measure of macrophage inflammation. **g** | Assessment of heart allograft survival in C57Bl/6 mice treated with mTORi-NBs (mTORi at 5 mg kg⁻¹). The mice received unloaded 35 nm NB or PBS directly before as well as 2 days and 5 days post-transplantation, n = 8 per group. Allograft survival was monitored by micro-ultrasound imaging. P values were calculated using a log-rank test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Panels **a–c** adapted with permission from REF.⁶, Elsevier. Panels **d** and **g** adapted with permission from REF.⁶², AAAS. Panels **e** and **f** adapted with permission from REF.¹⁴⁰, AAAS.

Trained immunity-inhibiting nanotherapeutics

Many immune-mediated conditions could be combated through the prevention or reversal of 'dysfunctional' trained immunity.

Inflammatory disease. Maladaptive trained immunity mechanisms have been reported in atherosclerosis, a chronic inflammatory disease, which is the main underlying cause of cardiovascular pathologies. Non-microbial stimuli relevant to atherosclerosis, such as oxLDL, induce training in human monocytes, reflected in the increased production of pro-inflammatory cytokines and underlying epigenetic changes⁴⁸. Similar monocyte phenotypes have been detected in individuals with coronary artery disease¹³⁸ or dyslipidaemia¹³⁹. Prevention of oxLDL-induced trained immunity can be achieved *in vitro* through mTOR inhibition with rapamycin. Similarly, nanobiologics containing the mTOR inhibitor (mTORi-NB) prevent oxLDL-induced pro-inflammatory cytokine production in human monocytes *in vitro*¹⁴⁰ (FIG. 5d,e). Importantly, in atherosclerosis-prone *Apoe*^{-/-} mice that were fed a Western diet for 12 weeks, a one-week mTORi-NB treatment regimen reduced plaque inflammation (FIG. 5f). Such highly effective, anti-inflammatory nanotherapeutics could also be applied in an acute cardiovascular event, such as myocardial infarction, or to treat high-risk patients with elevated biomarkers indicative of systemic inflammation¹⁴¹.

In addition to atherosclerosis, trained immunity plays a part in neurodegenerative diseases. For example, peripheral inflammatory stimuli can induce training or tolerance in brain-resident macrophages, also known as microglia, through epigenetic reprogramming¹⁴². Here, training increases β -amyloidosis and inflammation, whereas tolerized microglia have the opposite effect. Interestingly, tolerized microglia also reduce neuronal damage after stroke. Given that HSPCs in the bone marrow have a key role in the regulation of trained immunity, the blood-brain barrier does not pose any restrictions for this therapeutic approach. Gout, a form of inflammatory arthritis, is caused by the deposition of urate crystals in joints. These urate crystals as well as soluble urate can cause epigenetic and translational reprogramming of monocytes, resulting in increased IL-1 β production^{50,143}, indicating a contribution of trained immunity to this disease. The inhibition of mTOR signalling may be a promising approach to inhibit trained immunity, because mTOR activation is involved in both pathways⁵⁰.

Autoimmune disorders. Many autoimmune and autoinflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, sarcoidosis, diabetes mellitus type 1, and familial Mediterranean fever, display features of a trained immunity phenotype¹⁴⁴. For example, changes in cellular metabolism and/or epigenetic rewiring underlie the increased cytokine production of innate immune cells in these diseases. Trained immunity could therefore have a crucial role in the initiation of these diseases, but also in the persistence or aggravation of symptoms. Nanotherapies could be developed for specific trained

immunity pathways in these autoimmune diseases. For example, a nanotherapeutic treatment has been tested for experimental autoimmune encephalitis, a mouse model of multiple sclerosis¹⁴⁵. Here, lipid nanoparticle formulations have been designed that contain modified autoantigen-encoding mRNA, which is taken up by CD11c⁺ cells in the spleen, resulting in antigen-specific tolerance. Although it is different to antigen-unspecific trained immunity regulation, nanoparticle-innate immune cell interaction holds great promise for the treatment of autoimmune diseases.

Organ transplantation. The ultimate goal in transplantation medicine is optimal graft function and the prevention of allograft rejection through the induction of immunological tolerance. The current standard of care is chronic immunosuppression, which is associated with major side effects, such as increased cancer incidence and susceptibility to infection. Allograft rejection is driven by the adaptive immune system. However, the onset of allograft rejection is triggered by innate immune cell activation¹⁴⁶. Therefore, induction of an immune memory could be prevented by targeting myeloid cell inflammation. Moreover, allograft transplantation induces trained innate immunity through vimentin and HMGB1 signalling⁷, which can be inhibited by myeloid-cell-avid mTORi-NBs. Indeed, a brief mTORi-NB treatment regimen consisting of three intravenous injections significantly increased allograft survival without the need for chronic immunosuppression in a mouse heart transplantation model^{7,62} (FIG. 5g). Mechanistically, mTORi-NB treatment rebalances the immune system and prevents the induction of trained immunity, resulting in expansion of CD4⁺ regulatory T (T_{reg}) cells at the expense of CD8⁺ cytotoxic T cells, subsequently delaying or completely preventing allograft rejection.

Hyperinflammatory episodes. The initiation and resolution of inflammation are complex mechanisms, which must be precisely calibrated to achieve the best possible clinical outcome¹⁴⁷. For example, excessive inflammation after myocardial infarction can cause local tissue damage and worsen cardiac outcome¹⁴⁸⁻¹⁵⁰. Importantly, a hyperinflammatory episode can have major systemic consequences through the induction of trained immunity-like programs. Similar harmful cascades can also occur following infections. For example, an epidemic of cardiovascular events follows the normal influenza season, most probably caused by infection-induced rewiring of immune programs, which exacerbate ongoing cardiovascular inflammation, thereby increasing the likelihood of atherothrombotic events¹⁵¹. Individuals suffering from a hyperinflammatory episode, following cardiovascular events or infection, could benefit from anti-trained-immunity (nano)therapy that prevents immunological rewiring and circumvents detrimental downstream consequences. Although more research is needed to prove this hypothesis, an anti-inflammatory therapy with canakinumab, a therapeutic monoclonal antibody targeting IL-1 β , has already been shown to reduce the recurrence rate of cardiovascular events in a clinical trial¹⁵².

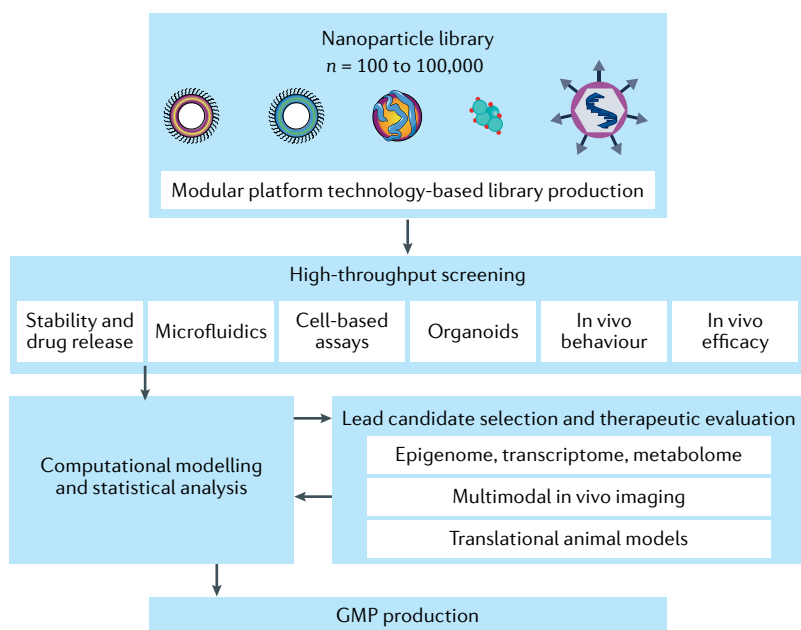


Fig. 6 | Translation from preclinical concept to clinical trials. Modular platform technology facilitates the seamless incorporation of drugs into a particular platform. High-throughput screening of nanomaterial libraries includes *in vitro* systems and *in vivo* assays. Lead candidate selection is supported by computational modelling, and therapeutic evaluation of the lead candidate includes omics approaches, multimodal *in vivo* imaging and the use of translational animal models. This approach will generate large data sets from which meaningful information can be extracted using non-biased computational modelling methods, before translational steps to good manufacturing practice (GMP) production can be made.

Outlook

In the flood of twenty-first century immuno-oncology successes using checkpoint blockade therapies that activate T lymphocytes, innate immune regulation is increasingly being studied^{130,153}. In this context, the fields of nanomedicine and immunotherapy are converging. Indeed, the full potential of nanomedicine may not be realised in drug delivery, but rather in immunotherapy^{1,154–156}. We anticipate that nanomedicine will develop into a versatile immunotherapy modality, particularly for the regulation of inflammation and trained immunity. The latter is one of the fastest-growing fields within immunology³; however, the field is still very young¹⁵⁷.

To unlock nanomedicine's full potential for immunotherapeutic purposes, in particular, for trained immunity regulation, we need to learn from the (mistakes of the) drug delivery nanomedicine field and the success of the COVID-19 mRNA nanovaccines. Importantly, the relation of nanomaterial properties and *in vivo* behaviour needs to be better understood, including biodistribution, cellular specificity and toxicity¹⁵⁸. Most studies investigating the physicochemical property–function relationships of nanomaterials are based on *in vitro* observations^{159–161}, which are often not directly transferrable to the behaviour of the material *in vivo*. For example, shape has been proposed to be essential for nanoparticle uptake by cells; however, upon intravenous administration, nanoparticle shape features are substantially affected by the adsorption of proteins, that is, the protein corona¹⁶². In light of

these discrepancies between *in vitro* and *in vivo* nanoparticle behaviour, we advocate a two-pronged approach that involves the systematic screening of large libraries of nanomaterials, concurrently *in vitro* and in mouse models *in vivo* (FIG. 6). Thus, from a single platform, libraries of differently composed, sized, shaped or charged nanoparticles could be established¹⁶³. Nanoparticles in the library could be individually labelled, for example, by radiolabelling their therapeutic payloads or molecular building blocks, allowing quantitative detection by nuclear imaging techniques¹⁶⁴. Similarly, fluorophores or stable isotopes could be integrated to study nanoparticle behaviour using optical techniques (NIRF imaging¹⁶⁵, intravital microscopy¹⁶⁶, immunofluorescence¹⁶⁷ and flow cytometry⁶) and methods based on mass spectrometry, such as liquid chromatography–mass spectrometry or mass cytometry^{168,169} (BOX 2). Additionally, non-invasive imaging could provide dynamic and longitudinal biodistribution information at the whole-organism level. Finally, multi-omics approaches, including chromatin immunoprecipitation followed by sequencing (ChIP-seq) and assay for transposase-accessible chromatin using sequencing (ATAC-seq) (epigenome), RNA-sequencing (transcriptome) and cytometry methods (immune landscape) should be applied to understand the interaction of nanomaterials with the immune system, from the molecular to the cellular level and beyond¹⁷⁰. These studies will generate large data sets from which meaningful information can be extracted using non-biased computational modelling methods, including supervised and unsupervised machine learning¹⁷¹.

An additional level of refinement can be achieved by a concept called modularity of functionalization, that is, designing nanoparticle platforms that allow straightforward integration of therapeutic functionalities, without compromising the platform's *in vivo* features. Modularity is an innate feature of RNA-based therapeutics¹⁷², but can also be implemented for other types of therapeutics, for example, smart pro-drug approaches for small-molecule drugs⁶². Regulatory bodies, such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), should develop an approval process for nanomedicine platforms that can be modularly functionalized with diverse therapeutic payloads. Indeed, one reason that the COVID-19 mRNA vaccines were rapidly developed and deemed effective and safe is because the nanoparticle carrier system had previously been extensively evaluated, albeit carrying different nucleic acids¹⁷³.

As new pathways and mechanisms are being unravelled, an increasingly defined picture of trained immunity is emerging³. The therapeutic regulation of trained immunity requires innovative therapeutics with the ability to change the functional programs of HSPCs (in the bone marrow and spleen). Nanomedicine has the potential to develop valuable trained-immunity-regulating methods, and the merging of nanomedicine, immunology, immunotherapy, *in vivo* imaging and artificial intelligence will yield nanoparticle-based immunotherapies that should revolutionize the treatment of immune-mediated diseases.

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M.M.T.v.L., B.P. and W.J.M.M. wrote the manuscript. All authors researched data for the article, provided substantial contributions to discussion of content and reviewed and edited the manuscript before submission.

Competing interests

W.J.M.M. and M.G.N. are scientific founders and shareholders of Trained Therapeutics Discovery.

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