Strategies for the development of metalloimmunotherapies

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Metal ions play crucial roles in the regulation of immune pathways. In fact, metallodrugs have a long record of accomplishment as effective treatments for a wide range of diseases. Here we argue that the modulation of interactions of metal ions with molecules and cells involved in the immune system forms the basis of a new class of immunotherapies. By examining how metal ions modulate the innate and adaptive immune systems, as well as host–microbiota interactions, we discuss strategies for the development of such metalloimmunotherapies for the treatment of cancer and other immune-related diseases.

Metal elements were perceived as elixirs by alchemists and have been used for hundreds of years to help treat diseases[1–3]. A notable example is platinum complexes, discovered in the 1960s[4,5]. Platinum-based drugs have become the first-line chemotherapy for many cancers[2,6]. However, as with other cytotoxic chemotherapeutics, platinum drugs have severe adverse effects on healthy tissues, and malignant cells can become resistant to them[7]. Few metal-based therapies have been successful in the clinic[2]. Strategies other than arresting the proliferation of cells are needed to spur the development of more effective and safer metal-based drugs.

Metal ions are critical in the regulation of many immune processes[8,9]. For example, K^+ contributes to the preservation of T cell stemness, and a high concentration of K^+ promotes the metabolic reprogramming of T cells and increases their in vivo persistence and multi-potency[10,11]. Ca^{2+}, whose concentration is elevated within cells after the engagement of a T cell receptor (TCR) with its antigen, facilitates the phosphorylation of TCR–CD3 complexes and acts as a second messenger for the dephosphorylation of nuclear factor of activated T cells (NFAT)[12,13]. K^+, Na^+ and Ca^{2+} play an important role in regulating activation of the nucleotide-binding oligomerization domain, leucine-rich repeats and the pyrin domain-containing protein 3 (NLRP3) inflammasome[14–16]. Mn^{2+} increases the sensitivity of cyclic GMP–AMP synthase (cGAS) and the stimulator of interferon genes (STING) against infection and cancer, whereas Zn^{2+} plays a role in the recognition of cytosol DNA by cGAS[17,18]. Platinum-based cancer drugs that induce immunogenic cell death can synergize with immune checkpoint blockade, leading to enhanced immune activation[19–21]. These immunomodulatory functions of metal ions could provide new principles and mechanisms for the design of metal-based therapies.

Metal ions and metal-ion-containing substances can modulate physiological or pathological immune responses for the treatment of disease, and thus provide opportunities for immunotherapies. Although metal ions and metal salts (such as alum adjuvant and Zn supplements) have been used to modulate immune processes for disease treatment or prevention[22,23], strategies for enhancing immunotherapies by incorporating metal ions have not been sufficiently explored. Moreover, general guidelines for the development of such metalloimmunotherapies are lacking. In this Perspective, we provide an overview of the mechanisms by which metal ions modulate the innate and adaptive immune systems and host–microbiota interactions, and leverage this knowledge to outline a set of design principles for the development of metalloimmunotherapies for use against cancer and other immune-related diseases.

Metalloimmunology

Metal ions are involved in the immune process through their structural, catalytic or regulatory interactions with immune sensors, ion transporters and enzymes and downstream effector proteins. Physiochemically, such interactions are determined by the coordination number, geometry and electrostatic charges of metal ions (which affect the stability and selectivity of metal ion–biomolecule complexing) and

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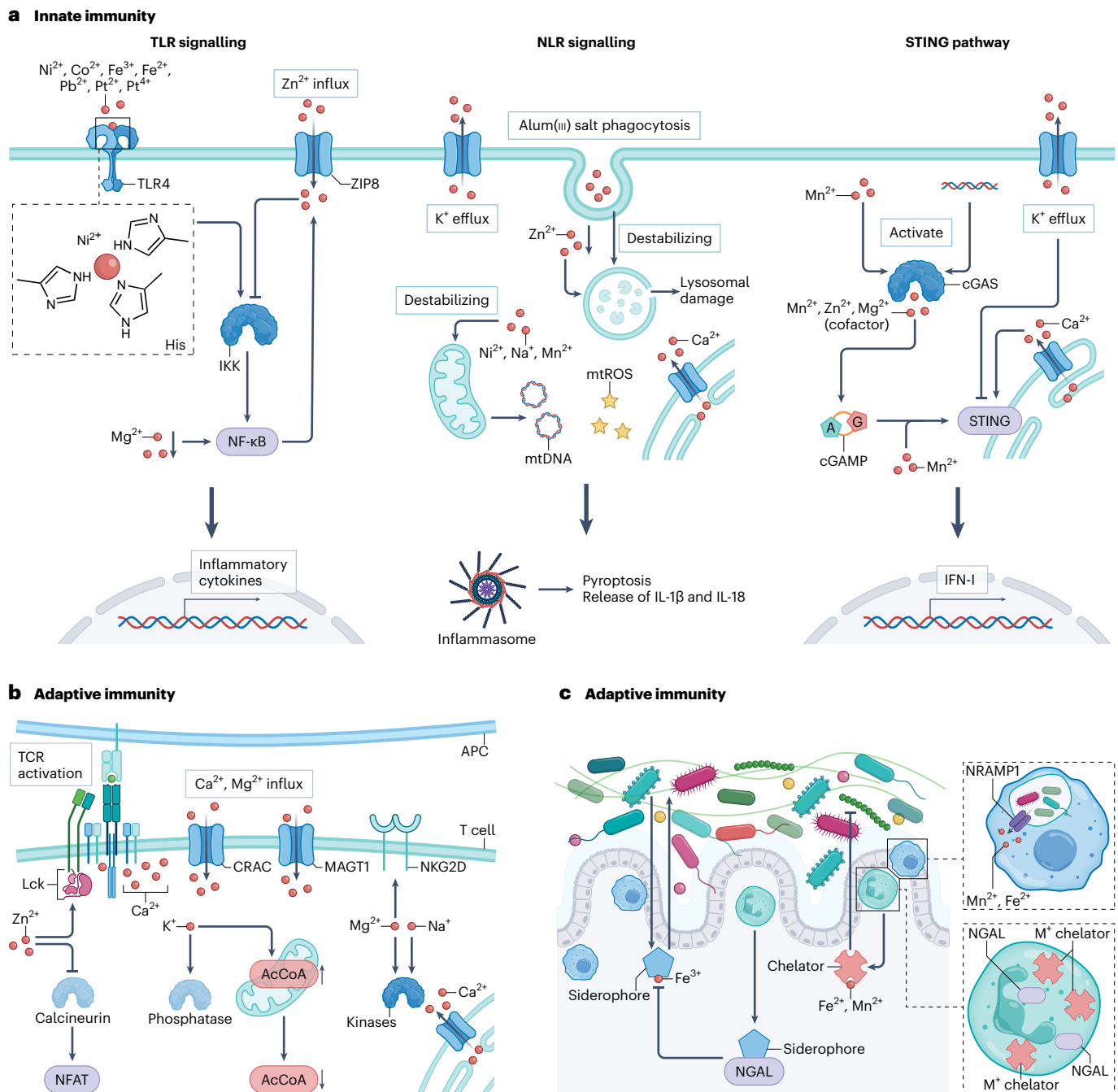
Fig. 1 | Immune processes involving metal ions. a, Metal ions and metal-ion-containing substances can modulate innate immunity. For example, Ni^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Pt^{2+}, Pt^{4+}, Zn^{2+} and Mg^{2+} can affect the TLR signalling pathway; K^+, Zn^{2+}, alum(III) salts, Ni^{2+}, Na^+, Mn^{2+} and Ca^{2+} can modulate NLR signalling; and Mn^{2+}, Zn^{2+}, Mg^{2+}, K^+ and Ca^{2+} can regulate the activation and signalling of cGAS–STING. **b**, Ca^{2+}, Mg^{2+}, Zn^{2+}, K^+ and Na^+ are involved in key signalling pathways of T cell function, and hence modulate adaptive immune responses. **c**, The immune system

of the host can control the levels of Zn^{2+}, Mn^{2+}, Fe^{2+} and Fe^{3+} at the host–microbe interface, thus regulating the composition of the microbiota and inhibiting the growth of invading pathogens. ZIP8, zinc transporter SLC39A8; CRAC, calcium release-activated channels; AcCoA, acetyl coenzyme A; mtDNA, mitochondrial DNA; mtROS, mitochondrial ROS; NGAL, neutrophil gelatinase-associated lipocalin; NRAMP1, natural resistance-associated macrophage protein 1.

by the capacity of metal ions to stabilize transition states, promote nucleophilic attacks[24] and facilitate proton transfer[25] (which function as a cofactor in catalytic processes). The concept of metalloimmunology, which was recently proposed to define the complex interactions between the immune system and metal ions[26,27], has raised awareness of metal-ion-regulated immune responses and their potential biomedical applications. In this section, we review how metal ions influence processes in innate immunity and adaptive immunity and host–microbiota interactions (Fig. 1).

Processes in innate immunity

The innate immune response is the first line of host defence against invading pathogens. The detection of microbes relies on pattern recognition receptors that sense pathogen-associated molecular patterns, such as lipopolysaccharide (LPS), flagellin, RNA and DNA[28,29]. Certain metal ions can also activate pattern recognition receptors, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs). Ni^{2+} can directly activate human TLR4 to trigger an inflammatory response that is comparable to that of LPS

(a common TLR4 ligand). Three non-conserved histidine residues within the leucine-rich repeats of TLR4 are thought to be responsible for Ni^{2+} binding. In one study, two histidine alterations substantially abolished Ni^{2+}-induced activation, whereas LPS still induced cytokine production[30], indicating the distinct TLR-binding and activation mechanisms between LPS and Ni^{2+}.

Other metal ions or metals, including Co^{2+} (ref. [31]), Pb^{2+} (ref. [32]), Pt^{2+} and Pt^{4+} (ref. [33]), also induce TLR-dependent immune activation. Fe^{2+}- and Fe^{3+}-associated haeme molecules can directly activate TLR4 via a mechanism that is different from that of LPS. Both porphyrin ring and iron–ion coordination are indispensable for TLR activation[34]. The innate immune functions commonly lead to activation of the signalling pathways involving nuclear factor-κB (NF-κB) and mitogen-activated protein kinase, to stimulate cytokine production. A variety of metal ions have pro-inflammatory functions, including the activation of NF-κB and mitogen-activated protein kinase[35]. For instance, Fe^{2+}, but not Fe^{3+}, can function as an agonist to directly activate the NF-κB pathway and induce the production of tumour necrosis factor α[36]. Furthermore, decreased extracellular levels of Mg^{2+} can upregulate the expression of NF-κB[37]. As a regulatory pathway, NF-κB can also regulate the expression of zinc transporter protein and allow for Zn^{2+} intake[38]. The influx of Zn^{2+} in turn downregulates the activity of IKK by binding to a specific site in the kinase domain, which in turn negatively modulates the NF-κB pathway. In addition, chelating Zn^{2+} can augment the TLR-dependent generation of interferon β (IFNβ)[39].

Recognition by a subset of NLRs is required for the activation of caspase-1 via assembly of the multiprotein complex inflammasome[40]. This leads to the production of interleukin-1β (IL-1β) and IL-18 (ref. [41]). The canonical inflammasomes are composed of an NLR protein (such as NLRP1, NLRP3 or NLRC4), the adaptor molecule ASC and caspase-1. Activation of inflammasomes leads to cleavage of gasdermins (GSDMs), which form large pores on cell membranes. NLRs can recognize microbial stimuli, as well as endogenous markers of cellular damage, such as adenosine triphosphate (ATP) and uric acid crystals. Activation of the NLRP3 inflammasome by the crystalline form of uric acid prompted examination of whether other immunostimulatory crystals can be similarly recognized by the NLRP3 inflammasome[42]. It was found that the production of IL-1β and IL-18 in macrophages can be induced by alum adjuvant and that deficiency in each component of NLRP3 inflammasomes fails to trigger their production[22]. Mechanistically, alum(III) salts can undergo phagocytosis, resulting in the damage of lysosomes, which induces activation of the NLRP3 inflammasome[43]. Similarly, sustained Zn^{2+} depletion may cause the destabilization of lysosomes, stimulation of the NLRP3 inflammasome and secretion of IL-1β[43,44]. Mitochondrial damage has also been linked to inflammasome activation[45], and a variety of metal ions can be mediators in this process. Mn^{2+} can act as an amplifier of NLRP3 inflammasome signalling by causing mitochondrial defects in microglial cells, which further increases the release of ASC-containing exosomes and transfers the inflammasome activation from cell to cell via exosomes[46]. The Ni^{2+}-induced accumulation of mitochondrial reactive oxygen species (ROS) and the release of mitochondrial DNA[47], as well as hyperosmotic stress from high levels of NaCl-induced accumulation of mitochondrial ROS[48], can lead to the activation of inflammasomes. Apart from metal-ion-mediated lysosomal and mitochondrial defects in inflammasome activation, the influx of Ca^{2+} and efflux of K^+ are two other common signals of activation of the NLPR3 inflammasome[49,50]. Ca^{2+} can promote the assembly of components of the NLPR3 inflammasome, mediated by the G protein-coupled receptor calcium-sensing receptor[49]. When stimulated, the calcium-sensing receptor can elicit an increased cytoplasmic Ca^{2+} signal by activating phospholipase C (PLC) and via a decrease in cyclic AMP levels through the inhibition of adenylate cyclase. Both signals contribute to enhanced formation of the NLPR3 inflammasome. In addition, Gd^{3+} can function as an agonist of the calcium-sensing receptor to indirectly activate the NLRP3 inflammasome[49]. Low intracellular

levels of K^+ can contribute to activation of the NALP3 inflammasome, but not the NLRC4 inflammasome[50]. A higher concentration of K^+ can prevent the assembly of NLRP3 inflammasomes and hinder caspase-1 recruitment[51]. Cu^{2+} can induce caspase-1-dependent pyroptosis via the production of ROS in hepatocytes[52]. Additionally, Cd can induce pyroptosis mediated by GSDME in cancer cells by activating the NLRP3 inflammasome and ROS production[53].

The cytosolic surveillance mechanism of DNA can induce the production of type I IFN (IFN-I) via the activation of STING[54]. STING activation relies on cGAS, which can sense cytosolic DNA and trigger the synthesis of cyclic GMP–AMP (cGAMP) from ATP and GTP. cGAMP also induces an IFN-I response via STING, which can recruit the cytosolic kinases IKK and TBK1 to activate the downstream transcriptional factors NF-κB and IRF3 (refs. [55,56]). cGAS belongs to the nucleotidyl transferase superfamily, which is commonly dependent on divalent cations for activation[57]. This indicates that certain divalent metal ions can play important roles in cGAS-mediated innate immune pathways. As an abundant ion source in the cytosol, Mg^{2+} can serve as the catalytic cofactor of cGAS to catalyse the formation of cGAMP[58]. Mg^{2+} can be replaced by Mn^{2+}, which elicits even higher catalytic activity[17]. Mn^{2+} directly activates cGAS to generate secondary messenger cGAMP either at low concentrations of double-stranded DNA[17] or independent of it[59]. Mn^{2+} can also augment STING activity by enhancing the downstream signalling of STING[17]. Differently from Mg^{2+} in the cytosol, Mn^{2+} is released from mitochondria and the Golgi apparatus on infection by DNA viruses. These findings indicate that Mn^{2+} is a more efficient cGAS activator than Mg^{2+} (refs. [17,59]). Zn^{2+} and other metal ions stabilize the cGAS–DNA complex by binding to cGAS and increasing the generation of cGAMP[60]. In addition to mediating activation of the STING pathway, some metal ions are indirectly involved in enhancing or restraining STING activation. For example, intracellular endoplasmic reticulum stress incorporates Ca^{2+} mobilization, which can support STING activation[61]. Additionally, to decrease sustained STING activation, K^+ efflux can restrain cGAS-induced IFN-I responses by producing the pore-forming protein GSDMD[62].

Processes in adaptive immunity

A key aspect of adaptive immunity for the generation of long-lived and antigen-specific immune responses is the activation of the TCR–CD3 complex through the recognition of antigens presented by major histocompatibility complexes[63]. Metal ions serve either as structural support for the formation of the complex induced by TCR signalling or as regulatory factors for kinases or phosphatases or for transcriptional proteins that affect the associated effector programs of T cell activation. TCR stimulation is followed by the recruitment of various kinases, in particular the leukocyte-specific protein tyrosine kinase (Lck), for phosphorylation[64]. The phosphorylated proteins further activate inducible T cell kinase (ITK) and PLCγ to trigger the release of Ca^{2+} from intracellular stores, which induces activation of Ca^{2+} channels[64,65]. In fact, Ca^{2+} influx affects the signalling network in the TCR activation pathway. On the one hand, Ca^{2+} influxes lead to a local Ca^{2+} concentration in proximity to TCR that is higher than that in the cytosolic compartment, which neutralizes the negative charge of the phospholipids, promotes dissociation of the cytoplasmic domain of CD3 and exposes tyrosine groups for phosphorylation[12]. Therefore, Ca^{2+} mediation can sustain and amplify T cell activation. On the other hand, Ca^{2+} can bind to calmodulin and subsequently activate phosphatase calcineurin to dephosphorylate NFAT. Dephosphorylated NFAT can then translocate into the nucleus and induce NFAT-dependent gene expression[13]. Defects in store-operated calcium entry also cause an inability to activate NFAT and thus are associated with impaired immune responses[66]. Apart from indispensable Ca^{2+} functions, other metal ions also have roles in this signalling network. For instance, TCR stimulation also induces a robust Mg^{2+} influx through magnesium transporter 1 (MAGT1), which is critical for subsequent PLCγ activation and Ca^{2+} signalling[67]. Mg^{2+} deficiency

owing to defects in MAGT1 can lead to abnormal expression of natural killer group 2 member D (NKG2D) in cytotoxic CD8^{+} cells and natural killer cells, which undermines cytolytic responses[68]. Decreased Mg^{2+} levels also negatively affects TCR signalling by inhibiting ITK because of the unique binding ability of Mg^{2+} to the catalytic pocket of ITK. Other divalent ions, such as Ca^{2+} and Mn^{2+}, do not exhibit similar effects on ITK[69]. Because of these immune functions, Mg^{2+} supplements can enhance the expression of NKG2D[68] in T cells and the activation of lymphocyte function-associated antigen 1 (LFA-1)[70], and magnesium phosphate can increase antigen-specific T cell responses and the production of IFNγ[71]. Zn^{2+} also displays dual roles in mediating adaptive immunity. On the one hand, it has inhibitory effects on the activity of phosphatase calcineurin, which hinders the nuclear translocation of NFAT[72]. On the other hand, it displays a stimulatory effect in promoting the formation of a complex between the cytoplasmic tails of CD4/CD8a and Lck so as to induce efficient antigen-specific T cell activation via TCR complexes[73].

There are other mechanisms of metal ion mediation in adaptive immunity. In particular, increases in K^{+} levels serve as an ionic checkpoint to suppress the function of effector T cells by impairing the TCR-dependent phosphorylation of Akt–mTOR (where Akt refers to protein kinase B and mTOR is for mammalian target of rapamycin). Mechanistically, K^{+}-mediated Akt–mTOR phosphorylation impairment relies on enhanced activity of the serine/threonine phosphatase PP2A, and the inhibition of PP2A activity can rescue the hypophosphorylation of Akt and counteract the functional suppression of effector T cells caused by elevated K^{+} levels[74]. Furthermore, K^{+} elevation can also hinder the uptake of nutrients by T cells, leading to metabolic reprogramming towards autophagy and to mitochondria-dominant cellular metabolism[10]. This decreases the availability of nucleocytosolic acetyl coenzyme A and limits histone acetylation on genes that are responsible for effector functions and exhaustion, leading to dysfunction of the effector program of T cells (but preservation of their stemness)[10,75].

Serum/glucocorticoid-regulated kinase 1 is another salt-sensible regulator for Na^{+} channels and other ion channels[76]. Increasing Na^{+} concentrations can induce overexpression of this kinase, which leads to the deactivation of FOXO1. This mediates the induction of pathogenic T helper 17 (T_H17) cells by enhancing IL-23R expression[76], as well as impairment of the suppressive function of Foxp3^{+} regulatory T cells via alteration of the stability of Foxp3 and enhancement of IFNγ secretion[77].

Processes in host–microbe interactions

The roles of the gut microbiome—the largest among the microbial communities colonizing the human body[78]—in human health and disease are gradually being uncovered[79,80]. The gut microbiota mediates and trains host immunity, and microbiota and the host have a symbiotic relationship to maintain homeostasis[81,82]. Collective communities of these bacteria provide unique and powerful enzymatic capabilities for the regulation of host physiology. The mechanisms behind the crosstalk between the immune system and microbiota commonly involve various metabolites[83–85]. Metal ions, including Fe^{2+}, Zn^{2+} and Mn^{2+}, are frequently required by bacteria to produce metalloenzymes, which provide structural support or promote catalytic processes[86]. It has therefore been speculated that metal ions serve as secondary messengers at the interface of host gut–microbiota interactions, and that the ions regulate the composition of microbiota or control the growth of invading pathogens.

Levels of metal ions are carefully regulated intracellularly so that essential functions can be fulfilled while limiting toxicity. The host develops mechanisms in either restricting the availability of metal ions against unwanted pathogenic bacteria or by directing the toxicity of these metal ions to defend itself from microbial invaders[86]. For instance, to limit the access of metal ions to intracellular bacteria, the natural resistance-associated macrophage protein 1 migrates to the phagosomal membrane and pumps Fe^{2+} and Mn^{2+} out of the phagosomal compartments[87]. However, bacteria can circumvent the host immunity-mediated restriction to metal ions. The bacteria can either evolve to find alternative metal ions to support their survival (as reported for *Borrelia burgdorferi*, which can substitute Fe^{2+} for Mn^{2+}; ref. 88) or they can compete for metal ions through other means. One of the strategies is to secrete small-molecule siderophores as Fe chelators because siderophore–Fe^{3+} complexes can subsequently be transported into the bacteria[89]. As a countermeasure, activation of TLRs induces the generation of neutrophil gelatinase-associated lipocalin to bind and sequester certain siderophores[90]. In addition, bacteria have surface receptors for haeme or haemoproteins that can transport Fe^{2+} into the cytoplasm[91]. Other metal ions, including Mn^{2+} and Zn^{2+}, also play indispensable functions at host–microbe interfaces and are regulated via similar mechanisms[92]. For instance, calprotectin, which takes up nearly half of the protein compositions of the neutrophil cytoplasm, is a strong chelator of Mn^{2+} and Zn^{2+}, and restricts their access to bacteria[93]. Abnormal levels of metal ions can also cause toxicity and kill bacteria. In fact, macrophages leverage this mechanism: they release Zn^{2+} into the phagosome to eliminate bacteria[94]. Established crosstalk among metal ions, microbiota and the host immune system is one of the major host defence mechanisms against invading pathogens. As more roles of the gut microbiota in disease contexts are discovered, we expect that it will be increasingly possible to leverage metal ions to modulate the composition of the gut microbiota to treat disease[95].
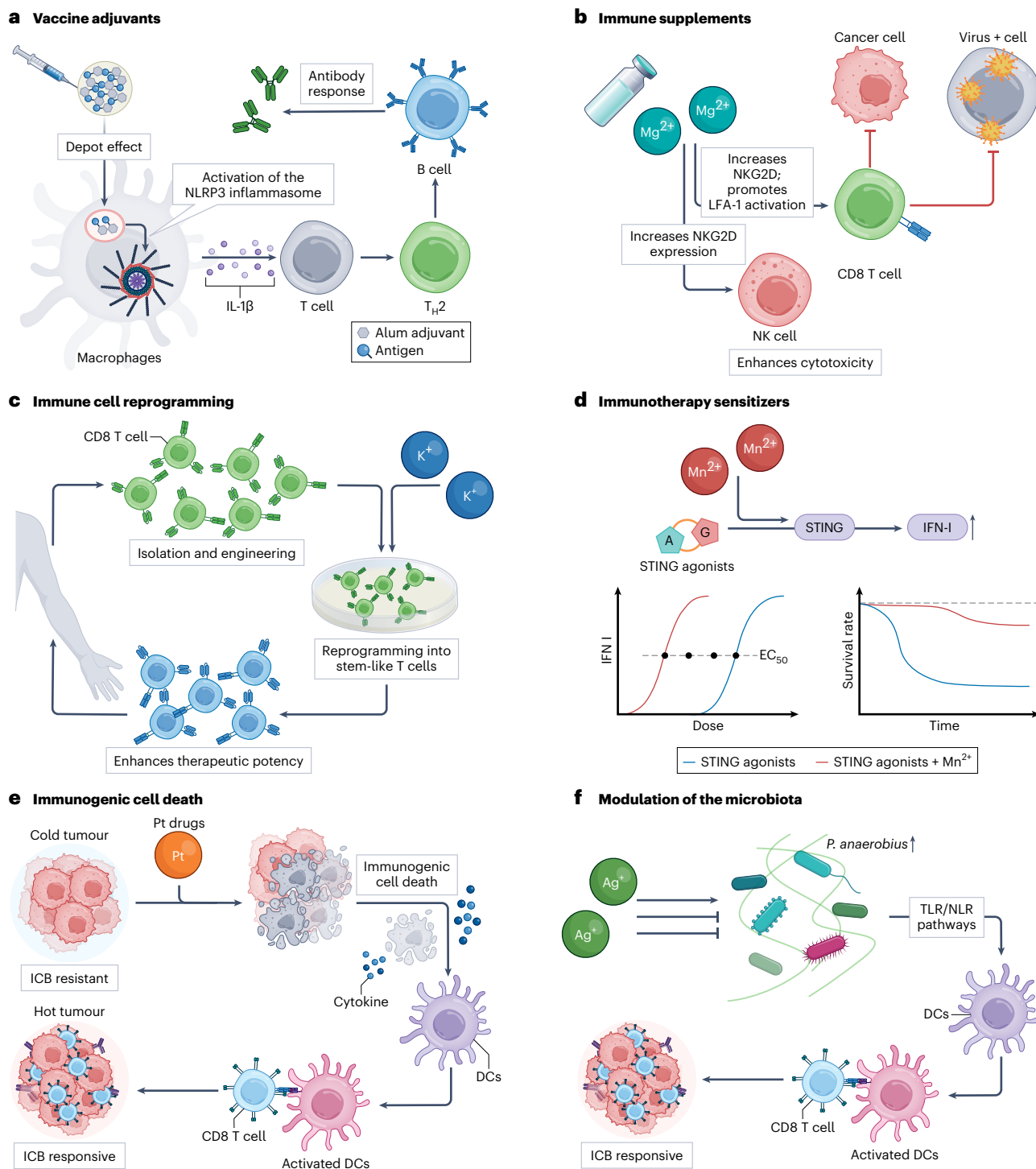
Metalloimmunotherapies

The targeted modulation of immune processes by metal ions may provide opportunities for therapy. Here we categorize such metalloimmunotherapies into six major classes: metal-based vaccine adjuvants; metal-ion-based immune supplements; metal-ion-based immune-cell reprogrammers; metal-ion-based immunotherapy sensitizers; metal-complex-induced or metal-ion-induced immunogenic-cell-death therapies; and metal ions for modulation of the microbiota. For each type of metalloimmunotherapy, we will discuss the fundamental principles, latest developments and future research directions. We seek to provide a roadmap for developing metalloimmunotherapies (Fig. 2 and Table 1).

Vaccine adjuvants

Insoluble metal salts are effective vaccine adjuvants for enhancing immune responses to vaccines. These responses are mediated via the provision of effective innate immune stimulation and the prolongation of antigen availability to B cells and antigen-presenting cells (APCs). The mechanism of action of alum hydroxide and alum phosphate, which have been widely used as vaccine adjuvants since the 1920s, had long been attributed to the depot effect and cell death-associated stimulation of the innate immune system[96]. However, alum adjuvant-induced immune stimulations were later found to be mediated in part by the NLRP3 inflammasome and production of pro-inflammatory cytokines such as IL-1β and IL-18 (ref. 22). Deficiency of NLRP3, ASC or caspase-1 compromises the effect of alum adjuvant. Moreover, other metal salts can also be used as vaccine adjuvants; for example, calcium phosphate is a vaccine adjuvant that has been used in the diphtheria–pertussis–tetanus vaccine and smallpox–yellow fever–measles–BCG–tetanus pentavalent vaccine in France[97]. Iron oxide, zinc oxide and magnesium phosphate nanoparticles have also been studied as vaccine adjuvants[71,98]. These inorganic metal salt vaccine adjuvants are often used in the form of an insoluble particle, which may have several advantages with respect to organic vaccine adjuvants, such as the sustained release of antigens and enhanced uptake by immune cells.

Based on the cGAS–STING activation property of Mn^{2+}, a colloid manganese salt called Mn jelly (MnJ) has been developed as an adjuvant[99]. MnJ has been shown to be a highly effective adjuvant that induces strong humoral immune responses, as well as cellular immune responses. Mechanistically, the adjuvant properties of MnJ

Fig. 2 | Metalloimmunotherapies. a, Metal-ion-based vaccine adjuvants[22,99]. For example, alum(III) salt can serve as an effective adjuvant and increase the efficacy of vaccines via inflammasome activation. **b**, Metal-ion-based immune supplements[68,70]. For example, Mg^{2+} supplements enhance viral control and anticancer immune responses by augmenting the cytotoxicity of CD8 T cells and NK cells. **c**, Metal-ion-mediated immune-cell reprogramming[10]. For example, K^+ can reprogramme CD8 T cells ex vivo by increasing their stemness to enhance the potency of adoptive cell transfer. **d**, Metal-ion-based immunotherapy sensitizers[18]. For example, Mn^{2+} amplifies the activation of STING and enhances the therapeutic efficacy of immunotherapies. **e**, Immunogenic cell death induced by metal complexes or metal ions[21]. For example, Pt(II) or Pt(IV) drugs, such as oxaliplatin, induce immunogenic cell death in cancer cells. **f**, Metal ions for the modulation of microbiota[147]. For example, Ag^+ can selectively allow for the growth of immune-activating microbes to augment antitumour immunity. DC, dendritic cell; EC_{50}, half-maximal effective concentration; ICB, immune checkpoint blockade.

have been attributed to activation of the cGAS–STING pathway and NLRP3 inflammasomes. MnJ mixed with various antigens (such as inactivated viruses, recombinant proteins or peptides) exhibited strong adjuvant activity and was superior to alum adjuvants. MnJ promoted antigen complexation, thus sharing the physical properties of alum adjuvants, and induced effective T_H1 responses (in addition to T_H2

Table 1 | Effects and applications of representative metal ions or metal-ion-containing substances

Metal ion	Pathway		Brief effects	Potential applications
Na$^+$	Innate immunity	NLRP3 and NLRC4 inflammasomes	Hyperosmotic stress induces activation of NLRP3 and NLRC4 inflammasomes	Vaccine adjuvant Immune-cell reprogramming Immunotherapy sensitizer
			Na$^+$ influxes mediated by epithelial sodium channels exacerbate NLRP3 activation in cystic fibrosis	
	Adaptive immunity	SGK1	Na$^+$ induces the overexpression of SGK1, which deactivates FOXO1 and induces activation of T_H17 cells, which impairs the suppressive function of regulatory T cells	
K$^+$	Innate immunity	NLRP3 inflammasome	Low intracellular K$^+$ induces activation of NALP3 inflammasomes	Immune-cell reprogramming Immunotherapy sensitizer
		cGAS–STING	K$^+$ effluxes inhibit responses by cGAS-dependent IFN-I via GSDMD	
	Adaptive immunity	Akt–mTOR	K$^+$ enhances Akt–mTOR phosphorylation, suppressing T cell activation	
		Mitochondrial TCA cycle	K$^+$ induces the metabolic reprogramming of T cells as a mitochondria-dominant cellular metabolic process that impairs the effector function of T cells while preserving T cell stemness	
Ca^{2+}	Innate immunity	NLRP3 inflammasome	Increased intracellular Ca^{2+} decreases cellular cyclic AMP and activates NLRP3 inflammasomes	Vaccine adjuvant Immune-cell reprogramming Immunotherapy sensitizer Immunogenic cell death
		cGAS–STING	Ca^{2+} mobilization-induced endoplasmic reticulum stress activates STING-dependent IRF3 phosphorylation	
	Adaptive immunity	TCR–CD3	Ca^{2+} neutralizes the anionic charge of phospholipids, facilitating TCR phosphorylation and potentiating the effector function of T cells	
		NFAT	Ca^{2+} activates calcineurin, inducing NFAT-dependent gene expression	
Alum(III)	Innate immunity	NLRP3 inflammasome	Alum(III) adjuvants activate NLRP3 inflammasomes	Adjuvant Immune-cell reprogramming
Zn^{2+}	Innate immunity	cGAS–STING	Zn^{2+} promotes the phase separation of DNA–cGAS complexes, which are involved in the biosynthesis of cGAMP	Immune supplements Immune-cell reprogramming Immunotherapy sensitizer Microbiota modulation
		NLRP3 inflammasome	The depletion of Zn^{2+} activates NLRP3 inflammasomes via destabilization of lysosomes	
		NF-κB	Zn^{2+} downregulates the activation of NF-κB via IKK inhibition	
	Adaptive immunity	NFAT	Zn^{2+} inhibits calcineurin and NFAT-dependent gene expression	
		TCR–Lck	Zn^{2+} promotes the formation of a complex between CD4/CD8 and Lck, which is involved in T cell activation	
	Host–microbe interface	Calprotectin	Zn^{2+} can chelate with calprotectin, restricting its access to bacteria	
Mn^{2+}	Innate immunity	cGAS–STING	Mn^{2+} increases the sensitivity of cGAS to double-stranded DNA	Vaccine adjuvants Immune supplements Immune-cell reprogramming Immunotherapy sensitizer Immunogenic cell death Microbiota modulation
			Mn^{2+} increases the affinity between STING and cGAMP	
		NLRP3 inflammasome	Mn^{2+} activates NLRP3 inflammasomes and propagates the exosomal release of ASC	
	Host–microbe interface	NRAMP1	NRAMP1 migrates to the phagosomal membrane, promoting Mn^{2+} effluxes, which influence the levels of bacteria	
		Calprotectin	Mn^{2+} can chelate with calprotectin, restricting its access to bacteria	
Fe^2 and Fe^{3+}	Innate immunity	TLR4	Haeme associated with Fe^{2+} or Fe^{3+} directly activates TLR4	Vaccine adjuvants Immune-cell reprogramming Immunotherapy sensitizer Immunogenic cell death Microbiota modulation
		NF-κB	Fe^{2+} directly activates IKK and NF-κB in Kupffer cells	
	Host–microbe interface	Haeme	Haeme can bind with Fe^{2+} to facilitate its transportation to bacteria	
		Siderophores	Bacteria secrete siderophores to chelate Fe^{3+} for their use	
		TLR–NGAL	Bacteria-activated TLR and NGAL can bind with siderophores to sequester Fe^{3+}	
		NRAMP1	NRAMP1 migrates to phagosomal membranes, promoting Fe^{2+} effluxes, which influence bacterial levels	
Co^{2+}	Innate immunity	TLR4	Co^{2+} activates TLR4	Vaccine adjuvants Immune-cell reprogramming Immunotherapy sensitizer

Table 1 (continued) | Effects and applications of representative metal ions or metal-ion-containing substances

Metal ion	Pathway		Brief effects	Potential applications
Ni^{2+}	Innate immunity	TLR4	Ni^{2+} directly activates TLR4 and triggers the production of pro-inflammatory cytokines	Vaccine adjuvants Immune-cell reprogramming Immunotherapy sensitizer Immunogenic cell death
		NLRP3 inflammasome	Ni^{2+} activates NLRP3 inflammasomes via the accumulation of mitochondrial ROS and the release of mitochondrial DNA	
Mg^{2+}	Innate immunity	NKG2D	Low intracellular levels of Mg^{2+} decrease NKG2D expression in natural killer cells and impair their cytotoxicity	Immune supplements Vaccine adjuvants Immune-cell reprogramming Immunotherapy sensitizer
		NF-κB	Decreases in the extracellular levels of Mg^{2+} upregulate the expression of NF-κB	
	Adaptive immunity	TCR–ITK	Mg^{2+} binds with ITK to enhance TCR signalling	
		NKG2D	Mg^{2+} induces NKG2D expression on CD8 T cells, enhancing their cytolytic responses	
		LFA-1	Extracellular Mg^{2+} can promote an active conformational change in the T cell co-stimulatory molecule LFA-1, augmenting T cell activation and cytotoxicity	
Pt(II) or Pt(IV)	Innate immunity	Immunogenic cell death	Pt(II) or Pt(IV) drugs induce DNA damage and the production of a wide range of DAMPs (in particular, HMGB1, heat-shock proteins, ATP, CRT and S100 proteins), activating innate immune cells	Immunogenic cell death Immunotherapy sensitizer

HMGB1, high-mobility group B1; NGAL, neutrophil gelatinase-associated lipocalin; NRAMP1, natural resistance-associated macrophage protein 1; SGK1, serum/glucocorticoid-regulated kinase 1; TCA, tricarboxylic acid.

responses). Notably, Mn salt is economical and simple to manufacture. These studies underscore that the formulation of various metal ions into appropriate delivery systems may provide new opportunities for effective adjuvants and therapeutics.

Immune supplements

Metal ion supplements could be used to treat diseases by triggering specific immunological modulation or promoting changes in the metabolism of immune cells. For example, Mg^{2+} supplements have been used to treat infection by the Epstein–Barr virus[67,68] in patients with X-linked immunodeficiency with an Mg^{2+} defect, EBV infection and neoplasia disease (XMEN). XMEN disease is associated with deficiencies in MAGT1, which impairs Mg^{2+} influxes on TCR stimulation and T cell activation. Lower intracellular concentrations of free Mg^{2+} would decrease the expression of NKG2D in natural killer cells and CD8$^+$ T cells and attenuate their cytotoxicity. These patients are thus more likely to suffer from severe chronic viral infections and lymphopoenia[68]. When these patients were treated with Mg^{2+} supplementation, the intracellular levels of free Mg^{2+} were restored and NKG2D expression was increased in a dose-dependent manner, leading to recovery of the cytotoxicity of natural killer and CD8$^+$ T cells and to a decrease in the fraction of cells infected with the Epstein–Barr virus[68]. Interestingly, Mg^{2+} can also promote a conformational change in the T cell co-stimulatory molecule LFA-1 in the context of cancer immunotherapy[70]. In an Mg^{2+}-containing environment, LFA-1 adopts an active extended conformation with an open headpiece that induces calcium fluxes, TCR signalling, metabolic reprogramming and cytotoxicity. In murine tumours, intratumoral injection of Mg^{2+} strengthened the immune responses of T cells against the cancer. Clinically, serum levels of Mg^{2+} in patients treated with chimeric antigen receptor (CAR) T cells or immune checkpoint blockers were correlated with overall survival[70]. Overall, these findings suggest that supplementation with Mg^{2+} may improve the efficacy of immunotherapies against cancer and infectious diseases.

Zn^{2+} has been widely used for cold remedies. Although the results vary across study conditions and clinical trials, Zn^{2+} seems to be effective in shortening the duration of disease when administered within 24 h of the onset of symptoms, and it may also have a prophylactic effect in children[23]. The mechanism is unclear, yet it is thought to be related to inhibition of viral replication, viral binding of ICAM-1 and improved host immunity through cytokine production and enhanced T cell function. Moreover, in patients infected with human immunodeficiency virus, Zn^{2+} supplementation resulted in increased numbers of T helper cells and decreased opportunistic infection by *Pneumocystis jiroveci* and *Candida*[100,101]. Still, the actual therapeutic effects and mechanisms of action of metal ion supplementation remain unclear.

Notably, metal ion supplements are considered as dietary supplements. These drugs are subject to strict regulations by the United States Food and Drug Administration, but the requirements for dietary supplements, including immunoregulatory supplements, are much less stringent. This underscores the need to assess the clinical utility and safety of metal-ion-based immune supplements through extensive mechanistic studies and large randomized clinical trials.

Immune-cell reprogramming

The immune activity of metal ions can be used to reprogramme immune cells and improve the efficacy of immune-cell therapies, such as CAR T cell therapy. T cells exposed to high K^+ conditions undergo autophagy and mitochondrially dominant metabolism, which leads to decreased nucleocytosolic acetyl coenzyme A, as well as histone modification and epigenetic remodelling, restricting the terminal differentiation of effector T cells and preserving T cell stemness[10]. This enhances the stemness of T cells and thereby their self-renewal, differentiation potential and persistence. Hence, treatment with K^+ ex vivo reprogrammes T cells into stem cell-like T cells and improves the efficacy of adoptive T cell immunotherapy. As a proof of concept, activated Pmel T cells ex vivo in the presence of a high concentration of K^+ were adoptively transferred into mice with established B16 melanoma tumours[10]. The K^+-treated T cells exhibited substantially enhanced persistence in the secondary lymphoid system and the tumour microenvironment, and were maintained in a more multipotent and less differentiated state. The adoptive transfer of K^+-reprogrammed T cells substantially inhibited tumour growth and prolonged animal survival compared with the conventional transfer of T cells that had not been exposed to K^+ (ref. 10). Secondary transfer of tumour-infiltrating lymphocytes isolated from the mice that received K^+-reprogrammed T cells led to enhanced immune memory responses to tumour antigens. This study therefore improved the mechanistic understanding of K^+ metalloimmunology and provided evidence of the feasibility of K^+-reprogrammed T cell therapy. Further work could focus on reprogramming other immune-cell types and exploring additional metal ions for reprogramming. For example, in view of their APC stimulation activity[18,22,36], Mn^{2+}, alum(III) and $Fe^{2+/3+}$ might be useful for reprogramming dendritic cells for therapy.

The reprogramming of immune cells with metal ions has unique advantages. First, ex vivo reprogramming can be performed with precise and adjustable doses of metal ions. It sidesteps the challenges of delivering metal ions to specific sites and to cells in vivo. Furthermore, it is technically complex and time consuming to genetically reprogramme CAR T cells with armours (that is, pro-inflammatory ligands and cytokines) in order to limit T cell exhaustion[102], and the necessary gene-editing agents come with safety risks. Instead, metal-ion-mediated cell reprogramming could serve as metal armours that can increase the persistence of CAR T cell therapy in a simple yet effective manner, as shown by the K^+-mediated reprogramming of T cells[10]. Moreover, metal armours of cell therapy can be applied to other immune-cell therapies, including those based on dendritic cells, natural killer cells and macrophages. For example, macrophages and natural killer cells have been engineered with CARs for the treatment of solid tumours[103,104]. Mn^{2+} can promote the polarization of macrophages from the M2 phenotype to the M1 phenotype, as well as natural killer cell activation via the cGAS–STING pathway[105]. This type of development may broaden the applicability of metalloimmunotherapy.

Immunotherapy sensitizers

Metal ions that can improve the sensitivity of the immune system to immune therapeutics are classified as immunotherapy sensitizers. Sensitized responses can be achieved in two ways: metal ions can directly modulate drug target interactions; or they can act on specific immune pathways that synergize with immunotherapy. Different from metal-based immune supplements, metal-ion-based immunotherapy sensitizers should be considered as drugs. Thus, metal-ion-based immunotherapy sensitizers should be regulated strictly, which would involve specific disease indications, precise doses, complete clinical safety and effectiveness testing, evidence-based approval and prescription-based use.

Mn^{2+} and Co^{2+} can increase the sensitivity of dendritic cells to STING agonists and induce the production of IFN-I[18]. Specifically, Mn potentiated cGAMP, an endogenous STING agonist, by 12- to 77-fold across different human STING haplotypes. The combination of STING agonists and Mn^{2+} increased tumour antigen-specific T cell responses and tumour inhibition effects in vivo. Mechanistic studies revealed that Mn^{2+} augmented downstream molecular events, leading to STING-independent p65 phosphorylation, STING-independent TBK1 phosphorylation and STING-dependent IRF3 phosphorylation. Highly activated p65 and IRF3 in turn formed a transcriptional IFNβ enhanceosome for further potentiation of the IFN-I response[18]. We have shown that the co-loading of a CDN-based STING agonist and Mn into a nanoparticle led to remarkable increases in the therapeutic efficacy of STING agonists[18]. In mice with immune checkpoint blocker-resistant tumours, this therapy (administered intratumourally or intravenously) led to tumour regression and the establishment of anticancer immune memory. Moreover, this therapy outperformed other clinical-stage STING agonists and led to superior antitumour therapeutic effects. This work illustrates the feasibility of discovering bioactive metal ions and their utilization for the formulation of metalloimmunotherapies.

In addition to directly potentiating the activity of STING agonists, Mn^{2+} can synergize with immune checkpoint blockers. For example, intranasal administration of Mn^{2+} promoted dendritic cell maturation, antigen presentation and the activation of T cells and natural killer cells, and synergized with therapy targeting programmed cell death protein 1 (PD-1) in a murine tumour model[105]. In a phase I clinical trial, the combination of anti-PD-1 antibody with intranasal administration or inhalation of Mn^{2+} led to IFN-I induction[105]. Notably, partial responses and stable disease were observed in patients with advanced solid tumours who had failed to respond to an anti-PD-1 antibody combined with either chemotherapy or radiotherapy. Encouragingly, the researchers did not report any major toxicity. This clinical example of metalloimmunotherapy underscores that such a simple yet efficient strategy can in principle be used to modulate the therapeutic effects of immune checkpoint inhibitors. Also, many Mn-based nanomaterials, such as MnO_2 nanoparticles[106–108], hybrid MnO_2 nanoparticles[109–111], Mn-doped inorganic nanoparticles[112,113] and Mn-doped organic nanoparticles[114,115], could potentially synergize with immune checkpoint blockade. Moreover, Mn-based nanoparticles can also alleviate tumour hypoxia[106,115], induce immunogenic cell death[116] and potentiate chemotherapies and phototherapies[106,117,118].

Immunogenic cell death

Immunogenic cell death is a type of cell death-inducing immune activation involving the release of damage-associated molecular pattern (DAMP) molecules, such as high-mobility group B1, heat-shock proteins, calreticulin and ATP[19]. These DAMP molecules can further activate APCs (such as dendritic cells) in the tumour microenvironment and prime T cell immune responses against cancer[20]. Certain metal drugs, notably oxaliplatin, have been found to be inducers of immunogenic cell death[20,119]. Oxaliplatin, in addition to causing DNA damage and inducing collateral endoplasmic reticulum stress, triggers the expression of a wide range of immunogenic cell death markers and promotes the release of various DAMP molecules from tumour tissues. Notably, new Pt(IV)–indoleamine-2,3-dioxygenase (IDO) inhibitor conjugates have been developed for immuno-chemotherapy. Pt(IV)–(D)-1-methyltryptophan conjugates induced effective DNA crosslink-triggered apoptosis and led to IDO-mediated T cell inhibition[120]. In addition to Pt(II) or Pt (IV) metallodrugs[119], several other metal complexes targeting the endoplasmic reticulum (such as the iridium(III) complex[121], ruthenium(II) complex[122] and copper(II) complex[123]) induce immunogenic cell death. Moreover, radiotherapy based on metal radioisotopes[124] or radiotherapy sensitized via metal nanomaterials[125] can also induce immunogenic cell death, activate the cGAS–STING pathway and turn cold tumours into hot tumours.

Because tumours are generally resistant to apoptosis, cancer treatments may also benefit from new forms of immunogenic cell death, such as ferroptosis and pyroptosis[126,127]. Ferroptosis is an iron-dependent cell death process associated with iron accumulation, lipid peroxidation and membrane damage. For example, a p53 plasmid-encapsulated metal–organic network induced ferroptosis via the Fenton reaction of Fe^{3+}-based MON, which led to the production of ROS, as well as p53 expression and oxidase stress[128]. Additionally, a glucose oxidase and doxorubicin-loaded biomimetic metal–organic framework (MOF) triggered antitumour immunity based on the regulation of ROS–ferroptosis–glycolysis[129]. Also, transferrin-modified MgO_2 nanosheets delivered ROS to kill tumour cells[130]. A ruthenium(II) complex exhibited antitumour activity in vivo. Pyroptosis involves the formation of plasma membrane pores by members of the GSDM protein family, such as GSDMD and GSDME[131]. Ca^{2+} modulators could cause mitochondrial Ca^{2+} overload and subsequently trigger the generation of ROS, as well as GSDME cleavage, leading to pyrolysis[132]. Also, iron ions combined with carbonyl cyanide *m*-chlorophenyl hydrazone (a ROS-inducing drug) can activate ROS and induce pyroptosis via the Tom20–Bax–caspase–GSDME pathway in melanoma with promising in vivo antitumour effects[133]. On the basis of the photosensitivity of various metal ions, metal ion complexes have been developed as photosensitizers for inducing ferroptosis and pyroptosis. For instance, the iridium(III) complex serves as a photosensitizer and induces ferroptosis under hypoxic conditions[134]. Rhenium(I) was also anchored by carbonic anhydrase IX to elicit GSDMD-mediated pyroptotic cell death on irradiation, which led to dendritic cell maturation and T cell activation[135]. Platinum(II) was also developed as a photocontrollable cGAS–STING activator that damages mitochondrial and nuclear DNA on light irradiation and induces pyroptosis among cancer cells[136]. Cuproptosis, a newer form of programmed cell death, is copper and mitochondria dependent[137]. Although cuproptosis seems to be mechanically different from other cell death pathways, it remains unclear which role cuproptosis plays in the immune system[138].

Modulation of the microbiota

In humans, the microbiota modulates immunity and immunotherapies[79,82]. Specifically, the gut microbiota and its metabolites can influence the function of T cells and lead to improved outcomes of cancer immunotherapy[116,139,140]. Because of the capability of metal ions to interfere with the host–microbe interface[80,86], it is expected that metal ions can be leveraged to promote the outgrowth of favourable microbes or to alter the composition of the host microbiota for the treatment of disease. For example, sodium tungstate selectively inhibits molybdenum cofactor-dependent microbial respiratory pathways only during periods of inflammation[141]. Feeding mice with tungstate water resulted in inhibition of inflammation-induced blooming of the *Enterobacteriaceae* population, which ameliorated the severity of gut inflammation. Notably, tungstate treatment had minimal effects on the composition of the gut microbiota under homeostatic conditions. To modulate the gut microbiota more efficiently, colon retentive gels[142] or micelles[143] and other engineered products could be developed to enable the efficient delivery of metal ions into the gut so as to enhance their biological functions on the host gut–microbe interface.

Local microbiomes (in tumours[144] and several organs[145,146]) have been implicated in disease prognosis. Therefore, metal-ion-based therapeutics could be developed to favourably alter the local microbiota. For example, silver nanoparticles incorporated within a mucoadhesive hydrogel have been shown to modulate the oral microbiota, which then synergized with PD-1 blockade for the treatment of oral squamous cell carcinoma[147]. Silver nanoparticles are unique in allowing for the growth of *Peptostreptococcus anaerobius* while inhibiting the growth of other bacteria. The abundance of *P. anaerobius* is positively correlated with the prognosis of patients with oral squamous cell carcinoma. Mechanistically, *P. anaerobius* exerts immune-activating effects by promoting the maturation of dendritic cells via TLR and NLR pathways and the subsequent activation of T cells. An absence of T cells weakens the efficacy of *P. anaerobius*, indicating the relevance of crosstalk between the microbes and immune system.

Precision metalloimmunotherapy

We believe that metal ions and metal-ion-containing substances could engender a range of metalloimmunotherapies (Table 1). Also, metalloimmunotherapies may provide therapeutic advantages owing to a number of factors: many metal ions and substances containing them are abundant and cost effective[96,99]; metalloimmunotherapy can act through mechanisms of action that are distinct from those of traditional immunotherapies, thus allowing for complementary and synergistic effects[18]; and metal ions have unique physicochemical properties that can be harnessed to design novel, multifunctional therapeutics[148].

Metal ions have unique pharmacokinetic and pharmacodynamic profiles. Some metal ions, such as $Fe^{2+/3+}$, Cu^{2+} and Zn^{2+}, are essential nutrients and perform essential functions[149]. Also, a metal ion may play different tissue-dependent roles, such as the diverse roles of Ca^{2+} in T cell signalling, bone health, muscle contraction and neurotransmitter release. Some metal ions, such as Pb^{2+} and Cd^{2+}, are non-physiological and have inherent toxicity. Therefore, non-targeted systemic administration of metal ions could lead to physiological imbalances and toxicity. Thus, strategies for the precise control of metal ions in vivo will be needed (Fig. 3).

Leveraging molecular engineering

Ionophores—chelating small-molecule agents—have a long history of medical use for the modulation of levels of metal ions in the body. They are widely used for the elimination of heavy metal ions from blood and tissues, as well as for the treatment of excessive heavy metal accumulation or poisoning[150]. Small-molecule ionophores can also selectively chelate metal ions and mobilize them to a disease area for targeted metal ion modulation in the context of disease treatment. For example,

elesclomol can target Cu^{2+} and transport Cu^{2+} for the treatment of Menkes disease[151] (a lethal condition, caused by a genetic deficiency in the copper-transporting adenosine triphosphatase ATP7A, that is associated with progressive neurological injury owing to impaired activity of cytochrome *c* oxidase in the brain). Interestingly, elesclomol can transport Cu^{2+} across the cell membrane and escort it into mitochondria, thereby restoring the levels of cytochrome *c* oxidase in the brain. In animal models, the elesclomol–Cu^{2+} complex prevented neurodegeneration in cortical and hippocampal regions and led to long-term control of the disease. This suggests that targeting metal ions and delivering them to a specific tissue, cell or subcellar compartment is a feasible therapeutic strategy. Small-molecule ionophores could be designed to selectively increase or decrease immune-active metal ions in a targeted disease area. Drug repurposing[151] and structure-based rational drug design[152] could be applied to accelerate the development of such ionophores.

Furthermore, the conjugation of targeting ligands with metal complexes could be used for the targeted delivery of metal ions for precision metalloimmunotherapy. Antibody–metal ion isotope conjugates have been used for targeted imaging with positron emission tomography (PET)[153] and single-cell mass cytometry[154]. For PET imaging, antibodies or antibody fragments can deliver metal ions to specific tissues, such as tumour sites, with high contrast[154]. For single-cell mass cytometry, various immune cell populations can be differentiated and tagged at high resolution via antibody–metal ion isotope conjugates[154,155]. This suggests that antibody–metal ion isotope conjugates are feasible for the selective delivery of metal ions to specific tissues and cells. Polymeric chelators may also be used to increase the payload capability of antibodies[154], and chelators could be optimized to avoid the non-specific release of metal ions before they arrive at the target site. In addition to antibodies, other targeting ligands, including peptides[156], antibody fragments[153] and aptamers[157], might also be used for the targeted delivery of metal ions.
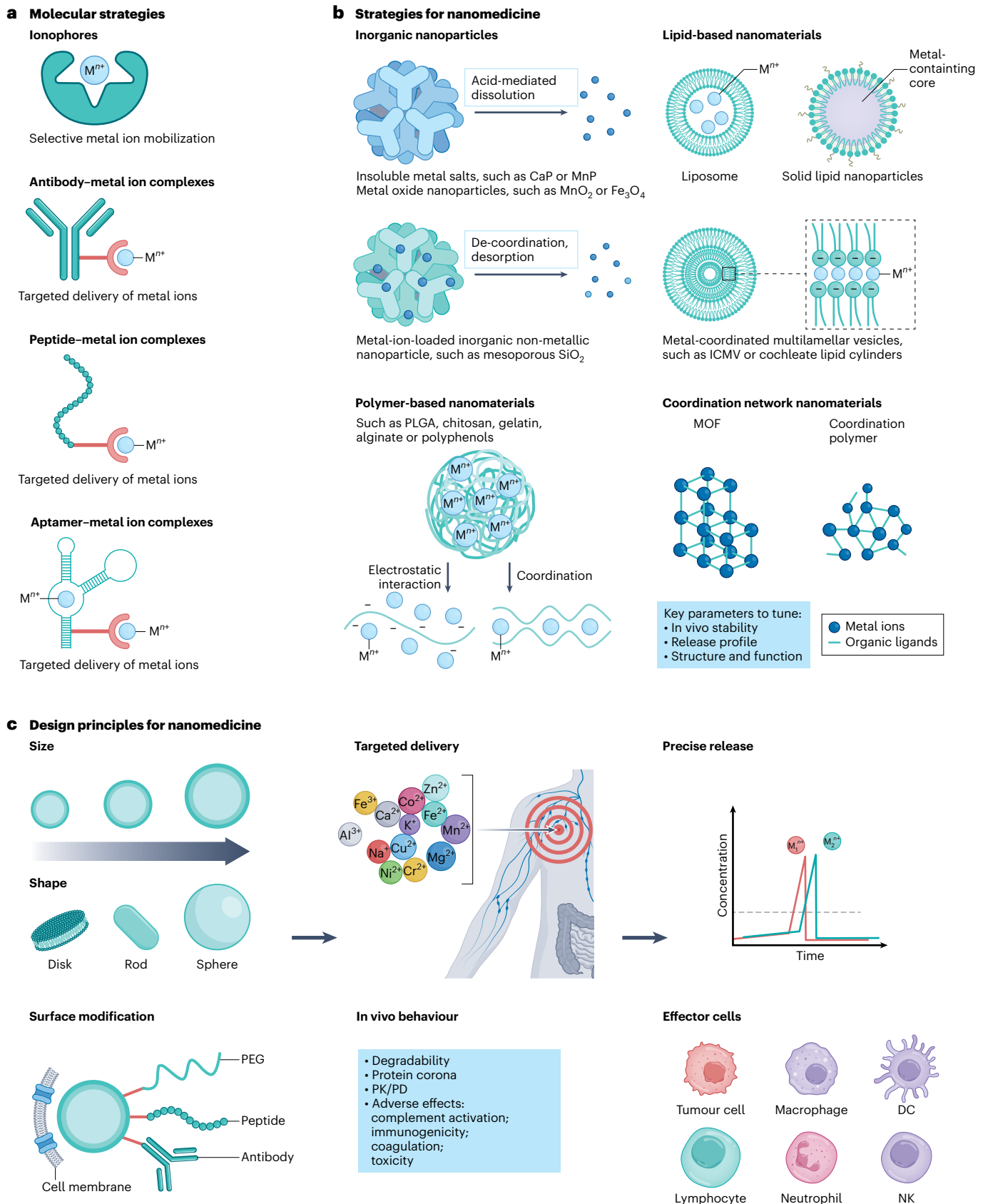
Metal-ion-containing drug conjugates could also be leveraged for combined metalloimmunotherapy. For example, Pt(IV) prodrugs have been conjugated to immunomodulator molecules (for example, an IDO inhibitor and a formyl peptide receptor peptide) to amplify antitumour immune responses[120,158]. This strategy induced immunogenic cell death and synergistically amplified the immune activation cascade.

Leveraging nanobiotechnology

Strategies developed for nanomedicines may also aid the delivery of metal ions in patient-acceptable dosage forms with appropriate stability and release profiles[148], as well as the targeted delivery of ions across physiological barriers to specific tissues and cells[159]. We believe that nanobiotechnology-based metalloimmunotherapy is a suitable strategy for the development of effective and safe metalloimmunotherapies.

Nanomaterials for metalloimmunotherapy. Advances in the development of nanomaterials and nanomedicines over the past few decades provide a solid foundation for metalloimmunotherapeutics[148,160–162]. Nanoparticles of various sizes and shapes are available for the delivery of metal ions[148]. Such metal-ion-containing nanoparticles can be inorganic nanoparticles, polymer-based nanomaterials[161,163,164], lipid-based nanomaterials[165,166], MOFs[129,167] or coordination polymers[166,168].

Inorganic nanomaterials can be insoluble metal salt nanoparticles, metal oxide nanoparticles or inorganic non-metallic nanomaterials. For insoluble metal salt nanoparticles, immune-active metal ions could be easily loaded inside nanoparticles during synthesis. For example, Ca^{2+}, Mn^{2+} and Al^{3+} could be incorporated inside phosphate salt nanoparticles via nanoprecipitation synthesis[165,169–171]. In these nanoparticles, metal ions also serve as the structural component, which usually leads to good formulation stability and high loading capacity. Metal ions could be released under acidic conditions, such as the tumour microenvironment and endosomes[165].

a **Molecular strategies**

Ionophores

Selective metal ion mobilization

Antibody–metal ion complexes

Targeted delivery of metal ions

Peptide–metal ion complexes

Targeted delivery of metal ions

Aptamer–metal ion complexes

Targeted delivery of metal ions

b **Strategies for nanomedicine**

Inorganic nanoparticles

Acid-mediated dissolution

Insoluble metal salts, such as CaP or MnP
Metal oxide nanoparticles, such as MnO_2 or Fe_3O_4

De-coordination, desorption

Metal-ion-loaded inorganic non-metallic nanoparticle, such as mesoporous SiO_2

Polymer-based nanomaterials

Such as PLGA, chitosan, gelatin, alginate or polyphenols

Electrostatic interaction — Coordination

Lipid-based nanomaterials

Metal-containing core

Liposome — Solid lipid nanoparticles

Metal-coordinated multilamellar vesicles, such as ICMV or cochleate lipid cylinders

Coordination network nanomaterials

MOF — Coordination polymer

Key parameters to tune:
• In vivo stability
• Release profile
• Structure and function

● Metal ions
— Organic ligands

c **Design principles for nanomedicine**

Size

Shape

Disk — Rod — Sphere

Targeted delivery

Precise release

Surface modification

PEG
Peptide
Antibody
Cell membrane

In vivo behaviour

• Degradability
• Protein corona
• PK/PD
• Adverse effects:
  complement activation;
  immunogenicity;
  coagulation;
  toxicity

Effector cells

Tumour cell — Macrophage — DC

Lymphocyte — Neutrophil — NK

Fig. 3 | Precision metalloimmunotherapy. a, Molecular engineering of ionophores and metal-ion-containing drug conjugates for the precise modulation of metal ions in vivo. **b**, Different classes of nanomedicines may be used to deliver metal ions in patient-acceptable dose forms. **c**, Design principles for precision metalloimmunotherapy that may allow for targeted delivery across physiological barriers and for the controlled release of metal ions in the target tissue at the appropriate time. M^{n+}, a metal ion with a positive charge of 'n'; ICMV, interbilayer crosslinked multilamellar vesicles; MOF, metal–organic framework, PK, pharmacokinetic; PD, pharmacodynamic.

Metal oxide nanoparticles can also release metal ions under acidic conditions. For example, iron oxide nanoparticles have been used as an iron supplement in patients[172], and MnO_2 nanoparticles can alleviate the hypoxia in tumours and release Mn^{2+} for the activation of STING[107].

Regarding inorganic non-metallic nanomaterials, metal ions can be loaded inside via physical sorption or chemical bonds. Physical sorption, including absorption and adsorption, refers to the process whereby metal ions attach to nanomaterials through physical forces, such as charge differences and polarizability, whereas chemical bonds provide interactions between the metal ions and heteroatom functional groups (consisting of oxygen, sulfur and nitrogen, for example) on the surface of nanomaterials. For optimal stability during metal ion loading and in vivo delivery, it is beneficial to employ both physical sorption and chemical bonds. For example, metal ion radioisotopes loaded into amorphous silica nanoparticles via physical interactions (involving surface charge and porosity) and chemical bonds (via coordination with oxygen atoms arranged in a variety of symmetries) resulted in excellent in vivo stability of the nanoparticles for PET imaging[173]. Under certain conditions, the physical interactions and chemical bonds between the metal ions and nanoparticles can be disrupted, facilitating release of the ions. For example, the pH of the solution could change the surface electric potential of the nanoparticles and affect their electrostatic interactions with the metal ions (physical release)[174], or cations, anions or biomolecules in the microenvironment could induce de-coordination of metal ions from the nanomaterials (chemical release)[175]. These phenomena could be harnessed to devise strategies for the selective release of metal ions at target sites.

Lipid-based nanoparticles, such as unilamellar vesicles, multilamellar vesicles, liposomes and solid lipid nanoparticles, are widely used because they are biocompatible, easy to prepare and manufacture and suitable for the delivery of a wide range of payloads[176]. Metal ions can be loaded into the hydrophilic interior of unilamellar and multilamellar liposomes by hydrating a lipid film in metal-ion-containing solutions[177]. Divalent metal cations (such as Mg^{2+}, Ca^{2+} and Mn^{2+}) can bind phospholipid head groups and induce the fusion of unilamellar lipid vesicles composed of certain phospholipids[178], such as phosphatidylserine, thus producing new lipid structures, including cochleate lipid cylinders[179] and multilamellar vesicles[180]. Different from lipid vesicles, solid lipid nanoparticles have a solid core and an outer lipid shell. Metal ions can be embedded in the core matrix[165,166]. The inner core improves the stability of the metal ions, whereas the outer lipid surface provides specific biophysical properties. Metal ions can also be chelated in hydrophobic molecules and inserted into lipid layers[181].

Many natural and synthetic polymers have been developed for use as components of nanomedicines. Metal ions can be entrapped in the polymer matrix via coordination or electrostatic interactions or complexed on its surface via chelation[182]. Polylactic-co-glycolic acid (PLGA) is a commonly used polymer in drug delivery because of its biocompatibility, biodegradability, safety and approval records[183]. PLGA nanoparticles have been used to encapsulate metal ions (Ba^{3+}, Gd^{3+} and Ce^{3+}) as surrogates of radionuclides[184]. Insoluble metal salts can also be encapsulated in PLGA nanoparticles by using the water-in-oil-in-water emulsion–solvent–evaporation method. For example, calcium phosphate was added in the inner aqueous phase of a water-in-oil-in-water emulsion to make calcium phosphate-loaded PLGA nanoparticles[185]. Both strategies could be applied for loading immune-active metal ions in PLGA nanoparticles for metalloimmunotherapy. Other well-studied biocompatible polymers, such as polylactic acid[186], chitosan[187], gelatin[188], alginate[189] and hyaluronic acid[190], may also be employed. These polymers typically have free coordination groups that can complex with metal ions and entrap them in the polymer matrix. A key advantage of polymer-based nanomedicines is the flexibility that they offer to integrate into the construct multiple monomers, functional groups or stimuli-responsive linkers for the precise modulation of key formulation parameters, such as stability, responsivity, degradation profiles, release kinetics and drug combinations[148].

Nanoscale MOFs[129,167] and nanometric coordination polymers (NCPs)[166,168], based on metal–ligand coordination, are promising systems for metalloimmunotherapy. Nanoscale MOFs are organized MOFs that are crystalline and porous, whereas NCPs are amorphous coordination polymer nanoparticles that can either be porous or non-porous[162]. Nanoscale MOFs and NCPs are built from metal ions or clusters bridged by organic linkers through coordination interactions, which readily combines the beneficial features of organic and inorganic nanoparticles. Leveraging the features of nanoscale MOFs and NCPs, immune-active metal ions and various drugs could be loaded easily in nanoparticles with high encapsulation efficacy and tunable release profiles. However, because coordination bonds are mostly unstable under physiological conditions, further surface modifications need to be performed to increase nanoparticle stability. For example, nanoscale MOFs and NCPs can be coated with liposomes[166] or silica[191] for use in cancer treatment[162]. Nanoscale MOFs and NCPs may exhibit unique advantages: their compositions and structures are readily tunable; the coordination bonds within them are relatively labile, rendering them biodegradable; and various drugs and metal ions, especially those with porous structures with high surface area, can be loaded into them.

To develop a successful metalloimmunotherapy, appropriate nanoparticles as delivery vehicles should be selected on the basis of their physical properties (including size and shape) and biocompatibility, as well as according to specific needs regarding the loading efficacy or capacity, stability and release kinetics of the metal ions in the desired physiological environments. From a translatability viewpoint, each metalloimmunotherapy faces distinct challenges that must be addressed. For inorganic nanoparticles, as well as for polymer- and lipid-based nanomaterials, challenges related to large-scale manufacturing, reproducibility and stability would need to be addressed[192,193]. Although MOFs and coordination polymers are tunable and multifunctional systems, their sensitivity to environmental and physiological factors, complex synthesis and surface modification and biocompatibility are hurdles to overcome[194,195]. Antibody–metal ion conjugates would benefit from their specificity and targeted approach, but they face challenges in payload capability, potential immunogenicity and the standardization of their synthesis[154,196,197]. For all forms of metalloimmunotherapy, it will be essential to address regulatory requirements for safety and efficacy[192,193,196]. Overall, the clinical translation of metal-ion-containing nanomedicines will require a comprehensive understanding of each material's properties, the optimization of synthesis techniques and the mitigation of safety concerns.

Design principles of nanomedicine for metalloimmunotherapy. Nanoparticle size affects the systemic circulation of nanomedicines, their ability to cross physiological barriers and their biodistribution, tissue penetration, cellular uptake and subcellular distribution. For intravenous injection, particles smaller than the renal filtration cut-off of 5.5 nm may be excreted fast through the kidney[198], whereas large particles are taken up by the reticuloendothelial system and exhibit poor tissue penetration[148]. Therefore, for intravenous metalloimmunotherapy, nanoparticle size should be tuned to obtain appropriate circulation times and suitable accumulation levels in target tissues. Naturally, the size of a nanoparticle also affects the in vivo behaviour of the nanomedicine when administered via other routes of administration. For example, nanoparticles of 10–30 nm administered subcutaneously drain passively to lymphatics and accumulate in lymph nodes, thus allowing for targeted delivery of immune modulators[199]. At the cellular level, 50 nm was reported to be the optimal nanoparticle size for cell internalization[200] (however, this is cell type dependent; for example, phagocytic immune cells can internalize nanoparticles about 400 nm in diameter more efficiently than 130-nm nanoparticles[201]).
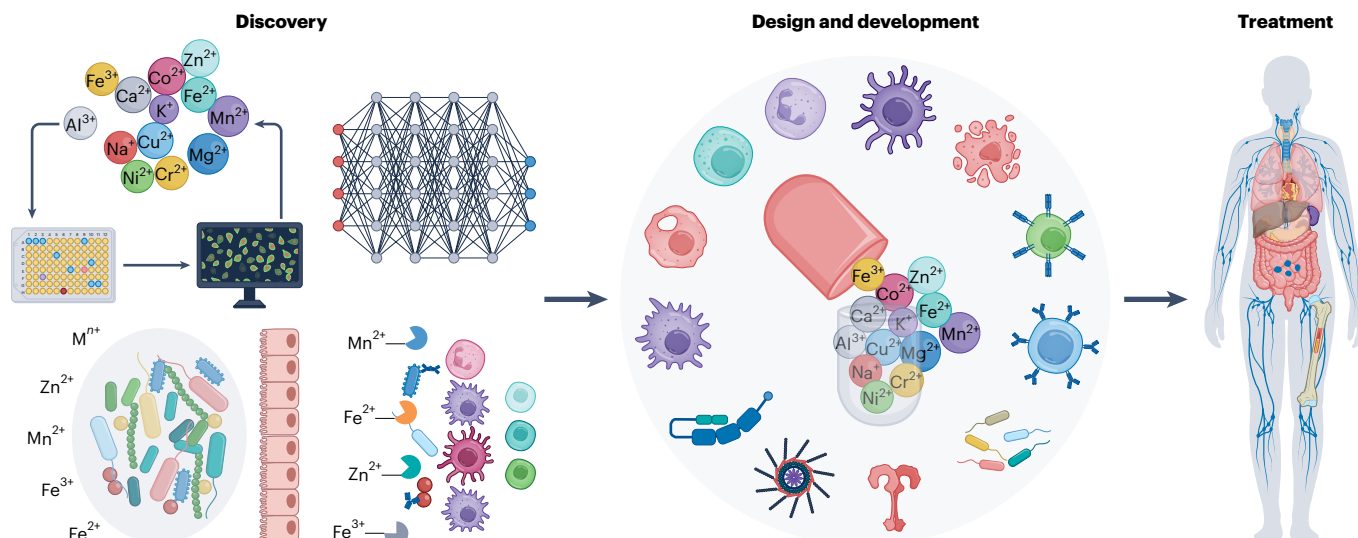
Fig. 4 | Future research directions in metalloimmunotherapy. Top left: the discovery of metalloimmunological processes and mechanisms via metallomics (that is, the use of -omics tools to understand how metal or metalloid elements interact with immune processes) and metallomics-integrated multi-omics. Bottom left: the deciphering and modulation of the interaction of metal ions with microbiota. Centre: the design of suitable strategies for the development of metalloimmunotherapies, and the leveraging of molecular engineering and nanobiotechnology to develop precision metalloimmunotherapies. Right: metalloimmunotherapies may be tested and optimized for the treatment of cancer, inflammation, infectious diseases, autoimmune diseases and other immune-related conditions.

The shape of nanoparticles also plays an important role in the pharmacokinetics and pharmacodynamics of nanomedicines. First, their shape affects their interactions with blood fluidics. For example, nanorods localize better to blood vessels than nanospheres[202,203] (this is because nanorods have higher aspect ratios, and blood flow will induce rolling in shapes with higher ratios; in this process, edge margination in nanorods will happen at a faster rate than in nanospheres[159]). Second, nanoparticle shape affects interactions with cells. For example, owing to their elongated shape, rod-shaped nanoparticles are more efficiently taken up by immune cells[148]. In mice, polystyrene spherical nanoparticles carrying ovalbumin antigen induced a T_H1-biased humoral immune response, whereas rod-shaped polystyrene particles induced a T_H2-biased response[204].

The surface modification of nanoparticles is crucial for the efficacy and safety of nanomedicines[205]. In fact, PEGylation—the modification of nanoparticles with poly(ethylene glycol)—is a widely used strategy. PEG provides a stealth coating[206] with multiple functions: it increases nanoparticle stability and compatibility; it works as a brush that prevents the shielding of nanoparticles from serum proteins, such as complement compounds and immunoglobulins, thus limiting opsonization and clearance; and it increases the circulation half-life of the nanoparticles. However, because PEGylated drugs can lead to the formation of anti-PEG antibodies[206], alternative polymers are under development[207]. Another approach is the use of cell membrane coatings, which can mimic the natural structure of cells and help nanoparticles evade detection by the immune system[208]. For example, red blood cell membrane-coated nanoparticles can circulate in blood for a long time without inducing anti-drug antibodies[209]. Additionally, the cloaking of nanoparticles with membrane material from platelets decreases complement activation and can be used to target wound sites, owing to the inherent ligands displayed on platelets[210]. Also, nanoparticles have been functionalized with the CD47 peptide to present a don't eat me signal to macrophages[211]. The surface properties of nanoparticles can also affect the formation of a protein corona in vivo, which can mask the nanoparticle with endogenous biomolecules. Protein coronas can mediate biological recognition and thus change the tissue and cellular biodistributions of nanoparticles[205].

Nanomedicines can target specific organs or tissues by either passive targeting or active targeting. In passive targeting, nanoparticles of specific biophysical properties accumulate at a target site via inherent physiological or pathological processes. For example, leaky tumour vasculature brings about the enhanced permeability and retention effect[212], which has been exploited to promote the intratumoral accumulation of nanoparticles after intravenous injection. In addition, owing to the inherent lymphatic draining process, nanoparticles 5–50 nm in diameter can effectively drain to lymph nodes after subcutaneous injection[213,214]. The surface charge of nanoparticles is also an important factor for organ selectivity[215]. In particular, the pKa of nanoparticles controlled by anionic, cationic or zwitterionic lipids dictates the composition of the protein corona, affecting the accumulation of nanoparticles in the spleen, lung or liver[216]. These inherent passive targeting mechanisms can therefore be exploited by adjusting the biophysical properties of nanoparticles. In active targeting, nanomedicines are modified with ligands[217]—typically, antibodies, peptides, carbohydrates or aptamers—for the delivery of cargo to specific immune cells. Nanoparticles with magnetic properties can also be attracted to a target tissue via an external magnetic field[218]. Because the immune system is cellularly heterogeneous, cell-specific delivery of metal ions will be crucial to eliciting specific immune functions. Hence, appropriate conjugate chemistry, type of ligand and surface ligand density should all be taken into consideration when designing nanomedicines for metalloimmunotherapy that leverage active targeting.

The delivery of metal ions could be spatiotemporally manipulated through suitably functionalized nanoparticles. Although the systemic administration of metal ions may induce therapeutic effects[68], it is desirable to regulate the release of metal ions in target tissues so as to minimize side effects and maintain the physiological homeostasis of metal ions. Many nanobiotechnology strategies can be employed for the controlled release of metal ions. For example, nanomedicines endowed with stimuli-responsive properties can release metal ions on demand in response to endogenous stimulation[106,168,219–222] (involving pH, ROS, enzymes or concentration gradients, for example) or to external stimulation[221,223–226] (with light, X-ray radiation, temperature, ultrasound or magnetic fields) at the desired location and time. For nanomedicines

responsive to endogenous stimuli, the design principles can be based on responsive linkers or functional groups on polymer-based nanoparticles[148], nanoscale MOFs[162,167] and NCPs[162,168,226,227]. For nanomedicines responsive to external stimuli, wireless techniques based on advanced electronics and actuators (such as acoustic waves[228,229], electric fields[225], magnetic fields[230] and electromagnetic radiation[231]) could be applied for the on-demand release of metal ions. Nanoparticles could also be functionalized with imaging properties for imaging-guided metalloimmunotherapy[224]. Moreover, nanomedicines with inherent therapeutic activity can synergize with immune-active metal ions for combination metalloimmunotherapy. For instance, metal-containing nanoparticles with catalytic properties and X-ray sensitizer properties could augment metalloimmunotherapy[167,232]. Overall, the unique physical, electrical, magnetic and optical properties of nanoparticles may be exploited to develop stimuli-responsive metalloimmunotherapies.

Outlook

Many immune functions of metal ions have been discovered over the past few decades. However, a more systematic approach is needed to broaden our understanding of the immunological roles of metal ions (Fig. 4), for example by understanding how metal or metalloid elements interact with biological processes[233]. Such a metallomics approach, and in particular its application in relation to the immune system[234], is in its infancy, yet may shed new light on metalloimmunology and facilitate the development of metalloimmunotherapies. High-throughput screening could also be leveraged for the systemic examination of the immune functions of metal ions. Furthermore, how metal ions affect commensal microbiota is yet to be examined. What's more, we believe that a next frontier for metalloimmunotherapy will be the modulation of interactions between metal ions and the commensal microbiome.

The development of metalloimmunotherapies has mainly focused on cancer treatments. However, all diseases involving immune-related physiology or pathology, such as infectious, inflammatory and autoimmune diseases, could be treated by metalloimmunotherapy in the future (Fig. 4). For example, ranitidine bismuth citrate, which is commonly used for the treatment of *Helicobacter pylori* infection, has been used to treat COVID-19 (ref. 235). Mg^{2+}-mediated upregulation of the NKG2a pathway[70] and Mn^{2+}-mediated activation of the cGAS–STING pathway[17,18] could also be used for treatments against infection. For the treatment of inflammatory and autoimmune conditions, metalloimmunotherapy could play an anti-inflammatory role (as exemplified by Zn^{2+}, which inhibits the NF-κB response[236], and disrupts the IL-6–JAK2–STAT3 pathway to dampen T_H17 responses[237]). Moreover, the selective depletion of key active metal ions may also serve as a treatment for inflammatory and autoimmune diseases (for example, the intracellular chelation of Ca^{2+} with the calcium-specific aminopolycarboxylic acid BAPTA can suppress Ca^{2+} and signalling processes involving calcium-modulated protein[238,239]).

Metalloimmunotherapy provides multiple pathways for the development of metal-ion-containing drugs. We expect that further discovery-led research on metalloimmunology, and the leveraging of strategies of molecular engineering and nanobiotechnology, will advance the development of metal-ion-based immunotherapies.

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Author contributions

X. Sun, X.Z. and J.J.M. discussed the content, researched the data and wrote the paper. X. Shi, O.A.A., X.A. and Y.L.L. contributed to the discussion. All authors reviewed and edited the manuscript.

Competing interests

X. Sun is an employee and shareholder of Editas Medicine. Y.L.L. is a co-founder of Saros Therapeutics and serves on its scientific advisory board. J.J.M. declares financial interests in EVOQ Therapeutics and Saros Therapeutics as a board member, paid consultant and equity holder, and as a recipient of research funding. The University of Michigan also has financial interest in EVOQ Therapeutics.

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