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Nano dimensions/adjuvants in COVID-19 vaccines

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A favorable outcome of the COVID-19 crisis might be achieved with massive vaccination. The proposed vaccines contain several different vaccine active principles (VAP), such as inactivated virus, antigen, mRNA, and DNA, which are associated with either standard adjuvants or nanomaterials (NM) such as liposomes in Moderna's and BioNTech/Pfizer's vaccines. COVID-19 vaccine adjuvants may be chosen among liposomes or other types of NM composed for example of graphene oxide, carbon nanotubes, micelles, exosomes, membrane vesicles, polymers, or metallic NM, taking inspiration from cancer nano-vaccines, whose adjuvants may share some of their properties with those of viral vaccines. The mechanisms of action of nano-adjuvants are based on the facilitation by NM of targeting certain regions of immune interest such as the mucus, lymph nodes, and zones of infection or blood irrigation, the possible modulation of the type of attachment of the VAP to NM, in particular VAP positioning on the NM external surface to favor VAP presentation to antigen presenting cells (APC) or VAP encapsulation within NM to prevent VAP degradation, and the possibility to adjust the nature of the immune response by tuning the physico-chemical properties of NM such as their size, surface charge, or composition. The use of NM as adjuvants or the presence of nano-dimensions in COVID-19 vaccines does not only have the potential to improve the vaccine benefit/risk ratio, but also to reduce the dose of vaccine necessary to reach full efficacy. It could therefore ease the overall spread of COVID-19 vaccines within a sufficiently large portion of the world population to exit the current crisis.

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Introduction

To overcome the crisis generated by the COVID-19 pandemic, vaccination of the population seems to be one of the best options. While some vaccines are or were marketed and distributed by Moderna, Johnson and Johnson, Astra Zeneca, and Janssen, others are under development.[1] An intensification of vaccine production seems necessary to be able to vaccinate a sufficiently large portion of the world population to achieve collective immunity while limiting the number of deaths caused by the pandemic. The offered vaccines can be categorized in two different types. The first one consists of vaccines whose content should directly mimic COVID-19, comprising in this case replicating adenoviruses (AZD1222 of astrazeneca, Ad5-nCOVB from CanSino biologics, Ad26COV2.S from Janssen), inactivated COV (CoronaVac of Sinovac or BBIBP-CorV of Sinopharm), or various COV proteins (NVX-COV2373 from Novavax, COVX-19 from PTY vaccine). The second one is composed of elements containing the genetic material able to drive the cellular production of proteins or elements mimicking COVID 19 behavior, *i.e.* essentially mRNA, which is encapsulated in or associated with liposomes or lipid nanoparticles to ensure mRNA protection against degradation (mRNA-1273 of Moderna and Comirnaty of Pfizer-BioNTech), or DNA (AG0302 of AnGes).

Considerable efforts, unparalleled in the history of medicine, have been deployed to bring to market efficient vaccines against COVID-19. These efforts have been largely successful, leading to the emergence of several efficient vaccines in a record time of just a few months. However, the COVID 19 crisis has given rise to a new problem, which was largely unanticipated, *i.e.* a worldwide infection requiring massive vaccination. The vaccine does not only need to reach a high efficacy, but also to be available in large quantities within the shortest possible time period.

To improve vaccine availability, existing vaccines could be injected in smaller doses while remaining effective. Alternatively, new vaccines, which are easier to mass-produce than existing ones, could be developed. Nanomaterials (NM) can help reaching either one of these two objectives thanks to a number of their properties such as the protection of the vaccine active principle (VAP) against degradation, the improvement of VAP solubility, the successful delivery of VAP to a site of

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interest, *e.g.* mucus where COVID 19 replication is favored due to the presence of ACE2 receptors in large quantity, the combination within the same unit of several different VAP that can act synergically against COVID 19.[2,3] In fact, NM are already being introduced into Moderna's and BioNTech/Pfizer's vaccines to achieve some of these effects.[4] Other vaccines would certainly also benefit from the presence of certain safe NM in their composition.

This review complements previous reports describing the use of NM in COVID-19 vaccines,[2–7] in several respects, *i.e.* first by presenting NM under the angle of vaccine adjuvants, *i.e.* a different component from VAP essentially consisting of antigens in various forms, mRNA, or DNA, second by describing the presence of various nano-dimensions in COVID-19 vaccines, third by introducing NM as vaccine components that were originally tested against cancer before being used against COVID-19, *i.e.* that NM can in some cases undergo a shift of medical fields from being tested in cancerology to being used in virology, fourth by describing in detail the different types of NM that could be introduced in COVID-19 vaccines, fifth by pointing out NM advantages compared with standard vaccine adjuvants, and sixth by describing NM mechanisms of action, in particular immune ones.

I. Should nano components of vaccines be considered as adjuvants or active principles?

An adjuvant can be considered as a product added to a vaccine to strengthen the antigen-triggered immune response, *e.g.* by improving antigen tissue deposition. The most well-known adjuvants are Freund's adjuvant, a water-in-oil emulsion containing inactivated *Mycobacterium tuberculosis* bacteria,[8] and alum, an alumina hydroxide/phosphate with which the antigen is precipitated.[8]

While at first glance this definition may seem clear, it can in fact be prone to different interpretations, especially when it comes to applying it to nanomaterials. The role of NM in vaccines is therefore examined to determine whether these materials should be considered as adjuvants or as active principles. First, NM can have a protective role towards antigens or mRNA/DNA and avoid their degradation, *e.g.* by preventing RNA degradation by RNase.[9] Second, NM can serve as vehicle to carry VAP, either by facilitating VAP administration in a specific region of the organism, *e.g.* the mucus, or by enabling the targeting of certain cells of immune interests such as APC.[10] Concerning these first two properties, they may be associated with adjuvant functions since the dominant activity remains due to the VAP, the nano-adjuvant enhancing vaccine efficacy. Third, NM-based vaccines often contain in addition to NM other substances, which display specific immune activities, *e.g.* CPG, a TLR-9 agonist, MPL-A, a TLR-4 agonist, or anti-PD1/anti-PDL-1 (Table 1). While in some cases, only these additional substances are considered as adjuvant,[11] it seems that both such substances and NM can be classified as such.[12] Fourth, instead of being only perceived as a compound, adjuvant can in some cases be associated with one or several property(ies) resulting from a specific assembly of different VAP. In this case, an adjuvant property can be due to the presence of a complex of nanometric size in a vaccine, originating for example from the concentration/aggregation of VAP, as it is the case for ssRNA concentrates,[13] and self-aggregating peptides.[14] Such adjuvant property can ease NM and associated VAP transportation through certain physiological barriers, in particular the mucous membrane,[7] and the holes crossing angiogenic blood vessels,[15] and NM efficient targeting of specific cells/pathogens.[5] Taken as a whole, these properties can contribute to strengthen the immune response against pathogens, hence falling within the scope of the adjuvant definition.

To avoid any confusion, it may also be possible to define NM as an adjuvant by exclusion, *i.e.* consider that it represents any nanometric substance contained in a vaccine, which differs from the main/dominant active principle, *i.e.* essentially antigen or mRNA/DNA. Alternatively, a nano-adjuvant could designate a specific vaccine property, which would not be dominant, *i.e.* the vaccine might still work to the some extend in the absence of the nano-adjuvant but the latter helps the vaccine to reach its full efficacy. This presentation agrees with the separation, which is often made in the medical field between dominant and accessory effects, frequently for the sake of clarity to facilitate the understanding of the biological mechanisms involved. In any case, it is desirable to go beyond the simple definition of the adjuvant, which only conceives the adjuvant as a specific vaccine component and does not take into consideration some important properties, for example specific assemblies or interactions or transformations of the VAP, which can result from the presence of the nano-adjuvant or lead to the appearance of a property at nanometric scale and strongly enhance vaccine efficacy.

Considering the definition given above, NM contained in vaccines can generally be considered as adjuvants, except in the particular case where they act in a similar manner as the VAP, *i.e.* for example when they mimic an antigen or when an antigen or mRNA/DNA is not directly present in a nano-vaccine formulation.

II. Nano-dimensions in COVID 19 vaccines

Regarding the nanometric dimension in COVID-19 vaccines, it essentially appears in three different ways. First, the nanoscale structure can serve to protect and bring the vaccine active ingredient to the region where it can elicit an immune response against the virus, as it is the case with Pfizer/BioNTech vaccines, where mRNA is encapsulated in a liposome to allow mRNA cellular internalization followed by its processing, further yielding the production of the Spike protein. Second, it can consist of an elementary block of the vaccine, as it is the case for virus-based vaccines containing for example adenovirus or inactivated COVID-19 virus, where the nanometric elementary blocks serve to mimic the behavior of the COVID-19 virus, hence training the immune system to elicit an immune response against the real virus while avoiding its side effects. Third, it can result from the assembly at the nanometric scale of a certain quantity of active principles, *e.g.* DNA or proteins encoding or resembling parts of the COVID 19 virus,

Table 1 For different types of nano-formulated vaccines, information concerning their size, the category of vaccine to which they belong, the nano-adjuvants and active principle they contain, their mode of action, the immune response they generate with the efficacy induced against the pathogen when it is described, the treated disease, the implemented targeting when it is present, and the recommended mode of vaccine administration

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
ZnO NP + Ova	12 nm (2.5 nm pore)	Antigenic	mZnO	Ova	Antigen-specific T cell response	Multiple	↑ CD4+ and CD8+ T-cells; ↑ IgG2a, IgG2b, IFN-γ; →Th1 immune response	None	Sub-cutaneous	117
Calcium phosphate NP + DNA	10–150 nm	DNA	CaP NP	DNA encoding GRA14	Cellular and humoral immune responses against Toxoplasma infection	Toxoplasma infection	↑ IgG1 and IgG2a antibodies; ↑ lymphocyte proliferation; ↑ IFN-γ and IL-4; mice infected by parasite live 5 days longer when vaccinated	None	im	178
HMS + VLP	1 μm (silica) 20 nm (VLP)	VLP	Hollow mesoporous silica (HMS)	VLP	Cellular and humoral response against FMD	Foot and mouth disease (FMD)	↑ Antibody during >3 months; ↑ T-lymphocyte proliferation; ↑ IFN-γ	None	im	181
PLGA-chitosan + pDNA in poloxamer gel	350–450 nm	DNA	PLGA-chitosan	pDNA	Th1/Th2 immune response	Rabies	↑ GnRH antibodies induced during >9 weeks; ↑ IgG2a + IgG1 and cytokines IFN-γ and IL-4	Laser microporation (delivery through skin)	im	182
PE + PLGA NP	160 nm	NA	PLGA NP	Roasted peanut extract (PE)	NP help active principle cross mucus barrier	PE allergy	↓ Anaphylaxis symptoms ↑ Higher survival for vaccinated mice	None	Oral	183
NS + ASTING	100 nm	Cancer	Hollow polymeric nanoshell (NS)	Agonist of stimulator of intereferon gene	Adaptive immune response against tumor	Colon cancer	↑ Antigen presentation and anticancer adaptive response; ↓ tumor progression in cancer bearing mice	None	it	184
ICG + imiquimod encapsulated in PLGA + checkpoint-blockade (CTLA4)	100 nm	Cancer	PLGA	ICG (PT agent) + imiquimod (TLR-7 agonist) + checkpoint-blockade (CTLA4)	PTT combined with checkpoint-blockade immunotherapy	Breast + colorectal cancer	↑ T cells; ↓ Treg cells; ↑ INF-γ et TNF-α; ↑ Effector memory T cells (T_{EM}) PLGA-ICG-R837 + laser + anti-CTLA4: tumor growth retardation in mice	None	iv	185
Clay (heterocyte) NP + IB	75 nm	*E. coli*	Clay	*E. coli* antigen: intimin β (IB).	Humoral and cellular immune responses against EHEC antigen commercially used adjuvants – QuilA and Alum	*E. coli* infection	• Clay NP: maturation of RAW 264.7 macrophages + ↑ Cytokines; mice immunized with IB + clay NP: ↑ IgA, protects against *E. coli* infection	None	Sub-cutaneous	186
Dendritic large pore silica NP + CuS NP inside pores + resiquimod + cancer cell membrane + anti-PD1 peptide (AUNP-12)	CuS (5–10 nm) complex (150 nm)	Cancer	CuS NP in pores of silica	Resiquimod + cancer cell membrane + anti-PD1 peptide (AUNP-12)	Thermal ablation of primary tumor + vaccination effect to prevent tumor recurrence	Triple negative BC	↑ T cells to prevent TNBC recurrence and metastasis	high TNBC-targeting	iv	187
First or second LP + RA	200–7000 nm	Bacteria	First LP: 1,2-distearoyl-*sn-glycero*-3-phosphocholine stabilized with	Retinoic acid (RA)	Vaccine formulation that	Enteric infection	RA + vaccine antigens sent to local lymph nodes →	NA	SC	73

Table 1 (*continued*)

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
			PEG or Second LP cationic liposomal adjuvant CAF01		upon parenteral administration induced intestinal IgA		mucosal immune response (antigen migration through antigen presenting cells); CAF23 → antigen specific intestinal IgA response			
Polysaccharide NP + PCS5 + poly (I:C)	110–220 nm	Peptide	Poly-saccharide NP composed of chitosan or hyaluronic acid	PCS5 (protease cleavage sites peptide)	Generation of humoral and cellular responses	HIV	High anti-PCS5 antibodies; activation of antigen-presenting cells; T-cell activation	None	Intramuscular	188
Nano-11 + KAg	30 nm (Nano-11) 500 nm (Nano-11 + Kag)	Antigenic	Positively charged corned-derived NP	Kag inactivated/killed swine influenza virus antigen	Immune reaction against virus	Influenza	↑ T-helper 1 and T-helper 2 transcription factors; ↑ T cells ↑ IgA in the nasal cavity; ↑ IFN-γ; ↑ observation in pigs	None	Intramuscular/intra-nasal	189
Lipid–hyaluronic acid multi-cross-linked hybrid NP + with protein antigen	130–350 nm	Antigenic	lipids	Protein antigen	Cellular and immural response	Ebola virus (EBOV)	MVP accumulate in DC + ↑ Antigen processing; Mice immunized with MVP → CD8+ and CD4+; MVP delivering EBOV glycoprotein → 80% protection against EBOV	None	Subcutansous	190
Psi@AcDex + CCM ± check point inhibitor	NA	Anti-cancer	Porous silicon (Psi) encapsulated within a layer of acetylated dextran (AcDEX)	Cancer cell membrane (CCM) surrounding Psi@AcDex	Check point inhibitor	Melanoma	Mice bearing melanoma tumor treated twice with nanovaccine formulation → control on tumor progression (administration of nanovaccine + check point inhibitor more efficient than administration of checkpoint inhibitor alone)	None	Subcutaneous	191
YC-NPs coated with Acr, Ag85B, and HBHA (Mtb antigens)	320–350 nm	Anti-bacteria	Yellow carnauba wax NPs (YC-NPs)	3 fusion proteins	Cellular + humoral	TB	↑ Activation of CD4+/CD8+ T cell compared with BCG; generation of Ag85B-specific serum IgG and respiratory IgA;	None	Intra-nasal or intra-tracheal	192
APNP + ovalbumin (OVA) and CpG	85 nm	Anti-cancer	Nano-sized vaccine carriers AlO(OH) polymer nanoparticles (APNP)	(OVA) and CpG	Cellular + humoral	Cancer	Activation CD8+ T cell; aluminium in NP more efficient than aluminium in gel (old adjuvant); Vaccine ↑ survival of mice with melanoma	None	NA	193
Nano silicon particle + TGEV	70 nm	Virus	Nano silicon	Inactivated transmissible gastroenteritis virus (TGEV)	activate immunity through Toll-like receptor activation	Veterinary vaccine	Nano vaccine ↑ antibody titers, ↑ IL-6, ↑ TNF-α ↑ IFN-γ, ↑ CD3+ T cells, ↑ CD4+/CD8+ T lymphocyte	None	Subcutaneous	194
Phospholipid bilayer and a phosphate calcium core + cancer membrane proteins (CMP) + α-	20–30 nm	Anti-cancer	Phospholipid bilayer and a phosphate calcium core	Cancer membrane proteins (CMP) + α-helix HSP70 functional	Cellular + humoral	Cancer	Vaccination → lymph node trafficking and multi-epitope-T cells response; Vaccine + anti-PD-1 antibody → tumor	None	Subcutaneous	195

Table 1 (*continued*)

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
helix HSP70 functional peptide (αHSP70p) + CPG				peptide (αHSP70p) + CPG			regression in mice with melanoma.			
RBD-Ferritin; RBD-mi3; RBD-I53-50	30–50 nm	Virus	Ferritin NP; Mi3 NP; I53-50 NP	Spike protein receptor binding domain (RBD)	Blockage of RBD binding to ACE2	COVID 19	RBD-NP + AddaVax or Sigma adjuvant injected to mice: neutralize virus better than RBD	None	Subcutaneous	196
NE loaded with TLR7/8 agonists	150 nm	Anti-cancer	Nano-emulsion	TLR7/8 agonists	Suppress immunosuppressive TME	Cancer	Lymphocytes and innate immune cells recruited against tumor; polarization of M2 macrophages: → Tumor growth inhibition in mice	None	Subcutaneous	197
pH-Responsive polymeric NP + protein antigen + nucleic acid adjuvant	20–80 nm	Virus	polymeric NP	Protein antigen	Involvement of tissue-resident memory T cells (TRM)	vaccinia + influenza	CD8+ TRM cells in lungs; ↑ APC activation; ↑ cytokine production by TRM	None	Intra-nasal	198
IQ/PN NP + anti-PLD1	50–100 nm	Cancer	Photoresponsive polydopamine NP (PN)	Imiquimod + anti-PLD1	Targets cancer cells over-expressing PDL1; Immune check point inhibitor;	Cancer	Mice bearing CT26 injected with IQ/PN NP + anti-PLD1: tumor accumulation + tumor ablation following NIR irradiation + tumor growth prevention of secondary tumor → 100% mouse survival; ↑ T cells in tumor tissues	PDL1	Intravenous	199
PapMV	80 nm	Cancer	Papaya mosaic virus nanoparticle (PapMV)	PapMV	↑ Tumor-specific (CD8+ T) ↓ myeloid-derived suppressor cells (MDSC)	Cancer	Mice with melanoma injected it/iv with PapMV → tumor growth delay + increased survival + less lung metastases	None	Intra-tumor + intravenous	200
OMV with low endotoxins	50–150 nm	Virus	Outer membrane vesicles (OMV)	LPS	Immune stimulation by attenuated LPS	Influenza	↑ antibody + T cell, IgA, lung-resident influenza-specific T cells	None	Intra-nasal	90
MPLA + OVA encapsulated in Psomes	100 nm	Cancer	Polymersomes (Psomes)	MPLA + OVA	Polymersomes promote the cellular uptake of OVA antigens by APCs	Cancer	OVA to APC; Psomes/OVA/MPLA injected to mice→ production of IL-6 + TNF-α cytokines + OVA-specific antibody	None	Intramusculary	201
Nano-11 + antigen	70–80 nm	NA	Nano-11: cationic dendritic-like α-D-glucan NP	Antigen (OVA)	Targeting of APC (nano-11 enable progressive release of antigen at injection site for 3 weeks)	NA	↑ Monocytes; macrophages; ↑ macrophage Mc2+ phagocyte Nano-11; Nano-11 enable delivery of more antigens to APC than aluminium hydroxide adjuvant	Dendritic cells	Intramusculary	202
HAuNS + APP encapsulated in PLGA NP	400–600 nm	Cancer	Gold nanoshells (HAuNS, a photothermal agent)	An anti PD-1 peptide, APP	Heat + PD1 blockade	Cancer	Sustained release of AAP (replace frequent administration of APP); PD1 blockade immunotherapy; PTA + NP eradicate	None	Intratumoral	203

Table 1 (*continued*)

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
							primary tumors and inhibit metastatic growth in mice			
GO-Car + OVA	20–50 nm	NA	Go-Car: graphene oxide + carnosine	OVA (antigen)	Immune reaction	NA	GO-Car + OVA injected to mice: ↑ Antibody; C4+, CD8+ T cells	None	Intramuscular	204
ROP18 protein encapsulated in PLGA NP	200 nm	Pathogen	PLGA NP	ROP18 proteins	Humoral	Toxoplasmose	↑ IgA, IgG2a	None	Intra-nasal	205
TMC + α-PGA + specific antigen	200–1000 nm	Bacteria	PGA	Peptide antigen	NP improves antigen delivery	Bacteria (streptococcus)	↑ Systemic and mucosal antibody due to NP	None	Intra-nasal	206
QS-21 + subunit protein + nanopatch	Nano-patch (20 000 injection tips per cm^2)	Virus	Nano-patch (admin) Saponin adjuvant (QS-21)	Subunit protein antigen	Humoral response	Influenza	With nano-patch: • use of 1/100th of the IM antigen dose necessary with syringe; • ↑ Immune response; • less adjuvant QS-21 necessary for immune stimulation compared with IM injection	None	Nano-patch	207 and 208
Lipid NP encapsulating mRNA + LPS	50–150 nm	Cancer	Lipid NP + LPS	mRNA	mRNA in cytosol of APC: coding for tumor-associated antigen gp100 and TRP2	Cancer	CD8 T cell	APC (intracellular)	Intravenous	209
DNA + nano-aluminium	65 nm (nano-aluminium)	Toxoplasma gondii	Nano-alumium	DNA	DNA encoding ROP13 and GRA14	Toxoplasmose	In vaccinated mice: • ↑ production of IgG2a, IFN-γ, IgG antibodies/cytokines against toxoplasmosis. • ↑ survival in presence of parasite	None	Intramuscularly	210
ssRNA		NA	ssRNA (single stranded RNA)	None	Stimulation of Th1 and Th2 responses	NA	Following mouse injection: • no tissue damage; • No increase in IgE and anti-nuclear antibodies; → No sign of autoimmune response; → ssRNA safe adjuvant	None	Intramuscularly	13
PDDA/OVA NP	170 nm	NA	PDDA (poly(diallyldimethylammonium chloride))	Ovalbumin (OVA)	↑ OVA-specific antibodies compared with $Al(OH)_3$/OVA.		Mice receiving vaccine: • ↑ OVA-specific IgG1; • ↓ OVA-specific IgG2a production; • → Th-2 response; Nano-metric size favors antigen presentation by APC at the lymph nodes; PDDA advantages: simple, low concentration needed, low cytotoxicity, yields high stability	None	Intra-nasal	211
	90–130 nm	Virus						Lymph node		11

Table 1 (*continued*)

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
VP1 protein antigen + TNF-α or CPG encapsulated in PE			Poly-electrolyte (PE) (chitosan/heparin)	VP1 protein antigen of enterovirus 71	Prolonged retention in lymph nodes;	Hand–foot–mouth disease (HFMD)	↑ Th1, Th2 immune response; ↑ IgA titers → mucosal protection		Subcutansous	
CPG (DNA) microcapsules (>4 million CPG copies)	2.5 μm (capsule diameter)	NA	$CaCO_3$ microcapsule	CPG	Microcapsules increases CPG stability	NA	*In vitro* study: • Cell internalization + endosome accumulation; • TLR-9 activated by CpG; • Microcapsule ↑ TNF-α + IL-6 by macrophages	NA	NA	212
ROM4 + GRA14 + CaP NP	90–120 nm	Parasite	CaP NP	Cocktail DNA vaccines (ROM4 + GRA14) of Toxoplasma gondii	Better immune reaction with CaP NP	Toxoplasmose	Mice immunized with cocktail DNA + CaPN NP protected against toxoplasmose	None	Intramuscular	213
Antigen + poly(I:C) adsorbed on Nano-11	214 nm	Virus	cationic alpha-D-glucan NP (Nano-11)	TLR3 agonist (poly(I:C))	Better immune response compared with commercial vaccine	Influenza	Pigs immunized with vaccine: ↑ of virus-specific SIgA antibodies in lungs compared with commercial influenza vaccine; ↑ cytokine mRNA expressions in lymph nodes compared to the commercial influenza vaccine; ↓ Lung lesions/virus load compared with commercial influenza vaccine	None	Intra-nasal	177
PLGA + H56 TB vaccine	150–500 nm	Bacteria	PLGA	H56 TB vaccine	No better immune response due to PLGA	Tuberculosis	Immunogenicity of antigen encapsulated in PLGA	None	Intra-nasal + subcutaneous	214
Hexosome containing lipid phytantriol (Phy) + immunopotentiator monomycoloyl glycerol-1 (MMG-1) encapsulating MOMP	100–150 nm	NA	Hexosomes	Antigen: Chlamydia trachomatis major outer membrane protein (MOMP)	Humoral + T-cell response;	NA	Exosomes yield stronger specific humoral responses than liposomes	None	Subcutaneous	215
TTxd-Soluplus nanoaggregates	68 nm	Bacteria	Soluplus polymeric micelles	Tetanus toxoid (TTxd)	Nano-vaccine increases antibody production	Tetanus	TTxd-Soluplus ↑ antibody response to TTxd	None	Transcutaneous	216
Outer membrane vesicles + antigen +LPS	NA	NA	Outer membrane vesicle (OMV)	Antigen	antigen program dendritic cells for cross-presentation to CD8+ T cells	NA	OMV → maturation of human monocyte-derived DC, murine bone marrow-derived DC, CD11c+ splenic DC	DC/CD8+ T cells	Intra-nasal	217
Rabies vaccine with nano-sized aluminium adjuvant	100 nm	Virus	Nano-sized aluminium	Rabbies vaccine	Enhanced antibody production with nano-sized aluminium	Rabies virus	Nano-sized aluminum → more neutralizing antibodies against rabies virus than other adjuvants	None	Intramuscular, subcutaneous, intradermal	218

Table 1 (*continued*)

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
							(BLPs, AS02, AS03, MF59 and Poly I:C)			
NanoMn	20–30 nm	Virus	Nano-sized manganese	None	Enhances interferon production	COVID 19	Nano-Mn: • M1 macrophage polarization; • Monocytes recruited into inflammatory foci; • virus-specific memory T cells; → less coronavirus-induced tissue damage. Besides	None	Intraperitoneal	219
pH-Responsive biodegradable carbonate apatite (CA) encapsulating CpG ODN + antigen	13–25 nm	Virus/cancer	Carbonate apatite NP + CPG (Cytosine-Phosphate-Guanine Oligodeoxynucleotides)	Antigen (OVA)	Enhance the production of type-I IFNs (such as IFN-α)	Influenza	Vaccination with CA-CpG + antigen: ↑ antigen-specific antibody responses; ↑ CD8+ cytotoxic T lymphocyte responses *in vivo*	None	Intravenous	45
AuSNP + FMD-VLP	110 nm	Virus	Gold-star nanoparticles (AuSNP)	Antigen: foot-and-mouth disease (FMD) virus like particle (VLP)	↑ Macrophage activation	Foot-and-mouth disease	Better immune protection than with standard adjuvant (oil, ISA205)	None	Subcutaneous; Intradermal	220
MWCNT non-covalently bound to CPG	NA	Bacteria	Multi wall carbon nanotubes (MCNT)	CPG	↑ Cytokines	Bacteria	MWCNT + CpG: more efficient/less toxic than free CpG (lower dose necessary to protect animals against bacterial infection)	None	Intraperitoneally	221
Au clusters + HEVA	5–30 nm	Virus	Au clusters	Hepatitis E vaccine (HEVA)	↑ Immune response due to gold clusters	Hepatitis E	↑ Antibody response → gold clusters aggregated vaccine; → gold clusters reduce toxicity of HEVA	None	Intraperitoneally	222
Cysteins + CPG + OVA (500 OVA per NP)	50 nm	Cancer	Cysteins attached *via* disulfide bonds	Antigen (OVA)	↑ Immune response (more antigens)	Cancer	Nano-vaccine protects 70% of mice with B16-OVA melanoma; ↑ cytotoxic T lymphocytes	None	Subcutaneous	223
GO@PEG/PEI + Ure B	50–250 nm	Cancer	Graphene oxide (GO) coated with PEG and/or PEI	Urease B (Ure B)	GO serves as antigen carrier	Cancer	Nano-vaccine → maturation of DC + cytokine production *via* TLR activation	None	Intradermal	26
DGBA + OVA + CPG	60–80 nm	Cancer	DGBA: polyamidoamine dendrimer modified with guanidinobenzoic acid	Antigen (OVA)	Nano-vaccine favors antigen cross-presentation by DC	Cancer	Cellular immunity; Nano-vaccine combined with programmed cell death protein 1 (PD-1) checkpoint-blockade efficient against B16-OVA melanoma	None	Intradermal	224
RGO@PEG +	20–30 nm	Cancer	Reduced graphene oxide (RGO) coated with PEG	NA	Intra-cellular ROS in DC		Nano-vaccine favors accumulation in LN + antigen processing/presentation to T cells	None	Subcutaneous	32
LDH-A	50–1000 nm	NA	Layered double hydroxide NP	Antigen	Activate macrophages + DC	NA	Macrophage cells exchange internalized LDH NP with external	None	Subcutaneous	225

Table 1 (*continued*)

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
							ones; Internalized LDH-antigen NP → facilitate DC maturation + antigen presentation to DC			
PLGA@CCM containing R837 + MM (mannose moiety)	140–160 nm	Cancer	PLGA NP	Antigen: cancer cell membrane + Imiquimod (R837): TLR agonist	Enhanced DC activation	Cancer	Nano-vaccine: ↑ uptake by DC; DC maturation Efficacy increased with check point blockade therapy	None	Intradermal	226
CCPS + DOX + HPPH	50–120 nm	Cancer	Chimeric cross-linked polymersome (CCPS)	DOX + HPPH (photosensitizer)	Adjuvant reduces DOX/HPPH dose necessary against tumor	Cancer	Nano-vaccine: ↑ TAA and DC recruitment; In tumor bearing mice: ↑ mature DC in tumor lymph nodes and CD8+ T cells in tumor tissues;	NA	Intravenous	227
Liposomes containing P5 + HLA-DR + MPL	120–140 nm	Cancer	Liposome	P5 (peptide Derived from T cells) + HLA-Dr (peptide antigen of CD 4+) + MPL (agonist of TLR-4)	↑ CD8+ + IFN-γ response	Cancer	Better anti-tumor activity/survival in tumor bearing mice compared with P5	NA	Subcutaneous	228
Microdisks containing antigens + TLRa	683 nm (thickness) × 5000 nm (diameter)	Cancer	Microdisks	Antigens + TLRa (Toll like receptor agonist)	Co-delivery of antigens + TLRa	Cancer	Codelivery of antigens + TLRa DC activation depends on TLRa concentration; DC antigen presentation/processing depends on antigen concentration	NA	NA	229
PSM + peptide/adjuvant	NA	Cancer	PSM: porous silicon microparticle	Peptide	Effect of shape and admin route on localization/activation	Cancer	Effect of shape: spherical PSM accumulate in circulating DC Effect of admin. route: iv admin. PSM accumulate in spleen/inguinal lymph nodes; intra-dermally admin. PSM accumulate in popliteal lymph nodes	NA	Intravenous/intradermal	230
Lipid polymer NP + IMQ + MPLA + OVA	220 nm	Cancer	Lipid polymer NP (MAN-IMNPS)	Imiquimod (IMQ) + monophoryl A (MPLA) + Ovalbumin (OVA)	Activation of DC	Cancer	Nano-vaccine in mice: • ↑ DC antigen-specific CD8+ T cells, ↑ lymphocyte activation, ↑ cross-presentation, ↑ memory T cells, ↑ antibody, ↑ IFN-γ, ↑ granzyme B; • → tumor growth delay and prolonged survival; • ↑ Anti-tumor effect with immune checkpoint blockade	DC	Subcutaneous	231
Au NP + OVA	15–100 nm	NA	Au NP	OVA	Prevents inactivation of	NA	Subcapsular sinus macrophages (SSM) capture Nano-vaccine and prevent	SSM	Intradermally	232

Table 1 (*continued*)

Nano-formulation vaccine	Size	Type of vaccine	Nano-adjuvant	Active principle	Mode of action	Treated disease	Immune response	Targeting	Admin	Ref.
					vaccine by macrophages		them from reaching lymph nodes and produce antibodies; by removing/inactivating SSM, ↑ production of antigen specific antibody by a factor of 60			
Porous silica NP + proteins (VirB9-1 and VirB9-2) in pores	50 nm	Pathogen (Anaplasma marginale)	Porous silica NP	proteins (VirB9-1 and VirB9-2)	Humor + cellular immune response	Anaplasmosis	NP enable high antigen loading capacity; Antibody titres; T-cell response	None	Subcutaneous	233
Fe_3O_4/T-MPs-CPG/Lipo (double vaccine/system)	NA	Cancer	Fe_3O_4 NP + Liposome (Lipo)	Tumor-derived antigenic microparticles (T-MPs) + CPG	strong tumor antigen-specific host immune response	Cancer	Nano-vaccine: • change macrophage polarization in TME, ↑ T-cells; • ↑ antitumor efficacy with immune checkpoint blockade PD-L1 blockade (↑ survival of melanoma bearing mice)	None	Subcutaneous	75
NanoZIF-8 containing Aluminium + antigen (OVA) + CPG	80 nm	Cancer	NanoZIF-8 + alumium	OVA + CPG (agonist of TLR-9)	pH controlled release of antigen	Cancer	Nano-vaccine injected to tumor bearing mice: • Nano-vaccine targets lymph nodes and internalize in APC; • Antigen released in APC lyposome → ↑ cross-presentation; • ↑ antigen-specific antibody + T cells; • → Tumor growth delay	None	Subcutaneous	179
Au NP (two size ranges: < 10 nm and > 10 nm)	4.5 nm <10 nm (13, 30, 70 nm) >10 nm	Cancer	Au NP	Au NP	Type of immune activation depends on NP size	Cancer	• Au NP (<10 nm) activate NLRP3 inflammasome → Caspase-1 maturation + interleukin-1β production; • Au NP (>10 nm) activate NF-κB signaling pathway	None	Subcutaneous	116
LZnP NP + $TRP2_{180-188}$ + $HPG100_{25-33}$ + MPLA	30 nm	Cancer	Lipid coated zinc phosphate NP (LZnP NP)	2 antigens ($TRP2_{180-188}$ + $HPG100_{25-33}$)	Double antigenic system	Cancer	2 epitopes improve immune response; nano-vaccine: ↑ cytokines, ↑ T cells; nano-vaccine with 2 antigens more efficient than free antigens and nano-vaccine with 1 antigen	None	Subcutaneous	12

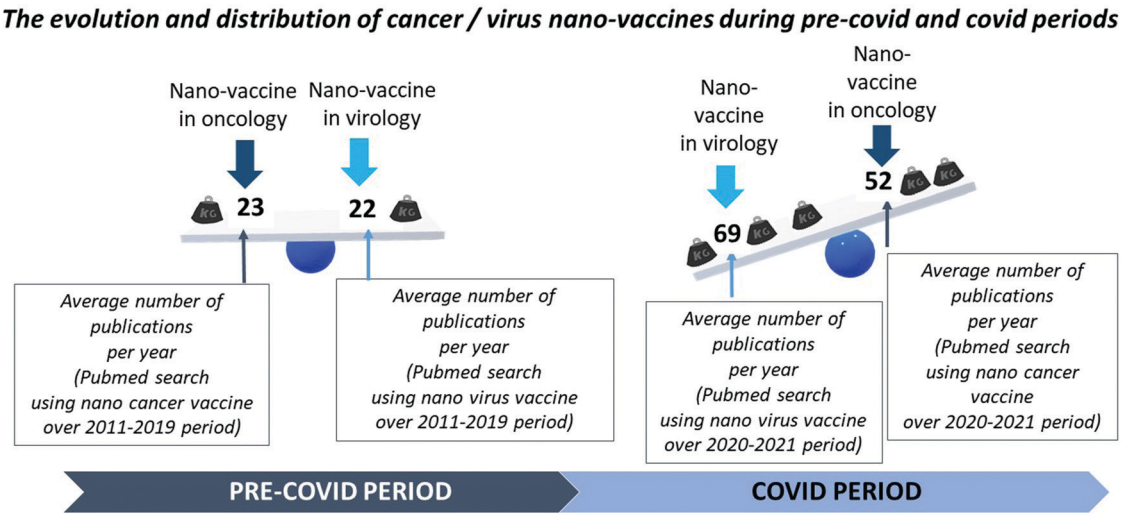

Fig. 1 A schematic representation showing the distribution between the developments of nano-vaccines in oncology and in virology during the pre-covid and covid periods, where this diagram was obtained through a pubmed search that estimated the number of publications containing the terms nano, cancer, vaccine or nano, virus, vaccine and by deducing from this search the number of publications per year containing these terms over the periods 2011–2019 (pre-covid) and 2020–2021 (covid).

where such assembly could potentially allow a better activation of the immune system than active ingredients taken individually, which are isolated from each other.[16] Following this presentation, the vaccine active principle appears to be either structurally distinct from the NM (first case), to correspond to the NM (second case), or to self-assemble in a NM (third case). In all three cases, the nanometric structure can be associated with an adjuvant function, either to allow the active principle to reach a target where it can act without being inactivated (first case), or to result in an activity, which results from its size, whether this size corresponds to that of individual blocks (second case) or assemblies (third case) of active principles, *i.e.* without such nanometric sizes, the active principles are no longer as effective.

III. Nano-adjuvants: at the crossroad between cancer and virus vaccines

As highlighted through a pubmed search, which enabled a field dependent article count, research on nano-vaccines appears to be rather limited and evenly distributed between cancer and viral applications before the COVID-19 crisis, while it has become more prominent during this crisis, resulting in a particularly large number of developments in the viral field. These trends are summarized in Fig. 1. By further comparing these two fields, NM appear at a crossroad of virology and oncology, since on the one hand NM sizes lie within the range of virus dimension and on the other hand NM can internalize in or interact with cancerous cells to destroy them. This schematic view helps to understand how similar NM could potentially be used in cancer and virus vaccines. Hence, the knowledge gained in the development of nano-vaccines against cancers could in part be used to offer a palette of efficient nano-vaccines against COVID-19. In a way, the COVID-19 crisis has shed some new light on nanotechnologies that appear as a meeting or rallying point between virologists and oncologists, who could unite their efforts together with nanotechnology specialists to fight the virus. This idea is further illustrated in the graphical abstract. In this context, I have chosen to present in this review NM, which have been tested in cancer vaccines, since they could potentially, after certain adjustments concerning the nature of the VAP, be added to COVID-19 vaccines.

IV. How to improve the benefit/risk ratio and availability of Covid-19 vaccines by using nano-adjuvant

Through their mechanisms of action described in this article, *e.g.* protection of the antigen, increased stimulation of the immune system, facilitated presentation of antigens to APC, nano-adjuvants could improve the efficacy of vaccines against COVID 19 and reduce their toxicity, thus leading to a larger benefit/risk ratio. In addition, a challenge of a relatively new nature arose during this crisis, namely the ability to have access to enough vaccines to treat a sufficiently large percentage of the population to eradicate this virus. One way to achieve this objective is to produce vaccines which operate at a lower quantity of VAP, thus enabling a reduction of the therapeutic dose necessary for obtaining efficient vaccination, and thereby, for a given vaccine production, increase the number of efficient vaccine doses. Even if this link is not always fully demonstrated experimentally, it is conceivable that an improvement in the benefit/risk ratio such as that mentioned above would be accompanied by vaccine efficacy achieved for lower therapeutic dose.

The benefits of using nano-adjuvants, which are described throughout this review, should be balanced against the risks of using them. Concerning liposomes, their toxicity is minimal.[17–19] One of the main risks associated with their use comes from their disintegration, *e.g.* if they are not stored under stabilizing conditions, hence resulting in the inactivation

of the active principle, *e.g.* by yielding mRNA degradation by RNase.[18] With regard to viruses or DNA active ingredients, the risks associated with their uses may arise from a loss of their nanometric structures through either partial/total degradation or disassembly, which can weaken their activity. Other risks may come from mistakes committed during mRNA and DNA fabrication, which prevent the production of the spike protein, or in the use of viral vaccines that don't avoid side effects, *e.g.* because the inactivation method that was used did not yield a viral structure that is sufficiently different from COVID-19. When nano-adjuvants enable to reduce the dose of VAP in vaccines, they may prevent the presence of an unwanted immune reaction, such as an auto-immune.[20] These risks can avoided through a proper design, production, and biosafety pre-clinical/clinical assessment of the nano-adjuvants, VAP and of their formulation.

V. The efficacy/toxicity of the different types of nanomaterials used as nano-adjuvants

The different types of NM used as vaccine adjuvants, whose list is provided in Fig. 2 and whose specific physico-chemical and anti-pathogenic properties are summarized in Table 1, are presented in the section below.

V.1 Nanomaterials with specific carbon atom arrangements (graphene oxide and carbon nanotube). Graphene oxide (GO) structure consists of a single two-dimensional crystalline layer containing sp^2/sp^3 hybridized carbon atoms organized within hexagonal networks, where the basal planes or edges of such layer are either largely covered by a series of oxygen functional groups such as hydroxyl, carbonyl, and epoxy groups, for non-reduced GO or not/partly covered by such groups for reduced GO (rGO).[21] Whereas GO is usually obtained by oxidizing graphite, *e.g.* using Hummers' methods,[22] rGO results from the reduction of GO, often using a toxic chemical such as hydrazine hydrate.[23] GO/rGO have further been coated or modified with various materials such as PEG to yield structures with improved hydrophilicity, solubility, stability, biocompatibility, and delivery efficacy compared with untreated structures.[24] Two very interesting aspects are derived from GO/rGO geometry. First, the large surface of GO/rGO enables the non-covalent binding of a large quantity of antigens such as human amyloid peptide (Aβ) or glioma antigen (Ag).[25] Second, such antigens could potentially be released in a controlled manner from GO/rGO surface through a transition from a folded to an un-folded state of the whole structure.[26] A series of GO/rGO properties make this material particularly well suited for being used as nano-adjuvant. Firstly, GO/rGO apparently facilitates antigen cellular internalization through endocytosis *via* specific interactions between GO/rGO and cell membrane receptors involved in endocytosis,[27] a behavior that can also be associated with the high aspect ratio of GO/rGO, which seems to facilitate GO/rGO insertion within the cell membrane. Secondly, the strength of immune stimulation can be tuned by varying the size of GO/rGO, *i.e.* it was shown that GO of 2 μm triggered a stronger immune response than GO of 350 nm.[28] Thirdly, GO/rGO can protect the antigen from enzymatic digestion, by promoting intracellular antigen trafficking *via* the cytoplasm, hence avoiding lysosome degradation.[29] Fourthly, it was demonstrated that GO/rGO could favor an immune response, by facilitating antigen cross-presentation to CD8+ T cells,[28] by increasing the quantity of cytokines and chemokines produced by DC,[26] by enhancing MHC-I expression, which is an essential step in the activation

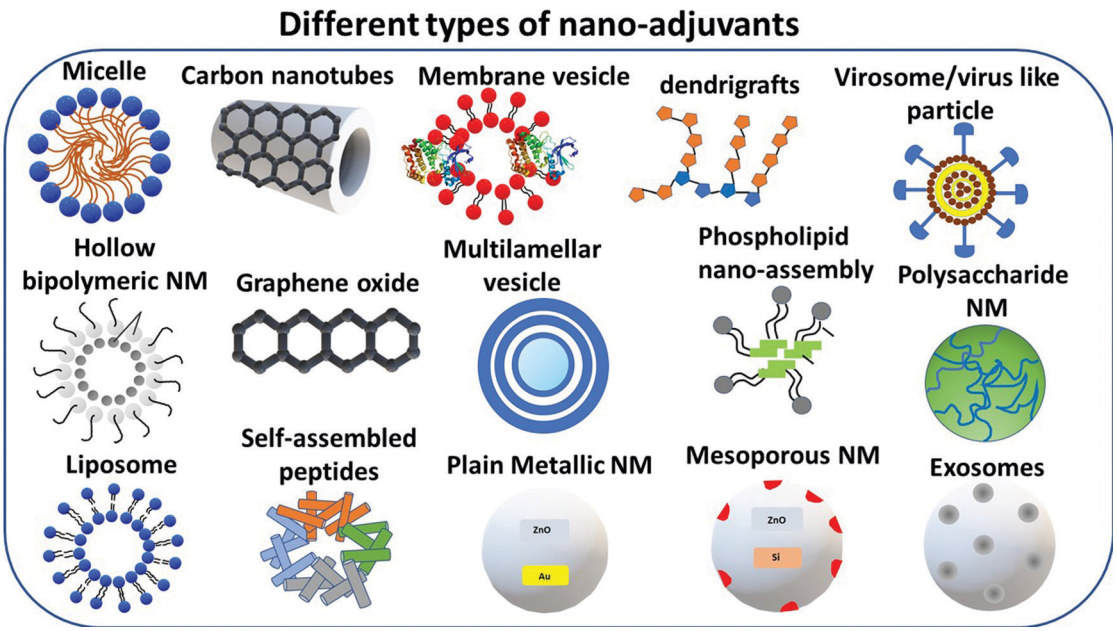

Fig. 2 Nanomaterials of different compositions and assemblies that can be used as vaccine adjuvants, including micelles, carbon nanotubes, graphene oxide, dendrigrafts, virosomes, phospholipids nano-assemblies, polysaccharides, exosomes, mesoporous or plain nanomaterial (metallic or not), self-assembled peptides, liposomes, and hollow bi-polymeric NM.

of CD8+ T cells,[21] or by stimulating the release of proinflammatory factors by macrophages.[30,31] In a very interesting study, mice suffering from B16F10 melanoma received a vaccine containing PEG/CPG functionalized rGO, where rGO apparently acted as the antigen. It led to the migration of rGO towards lymph nodes, the activation of DC, and resulted in tumor growth delay.[32]

Carbon nanotubes (CT) have also been suggested as cancer vaccine components.[33] Carbon nanotubes (CNT) are made of cylinders consisting of rolled graphene layer(s) (Fig. 1),[34] which are nanometer in width and nanometer to micrometer in length. They can be divided between single-walled CNT (SWNT) and multi-walled CNT (MWNT).[35] As for GO, CNT properties can be tuned through various functionalization. While non-covalent functionalization, *e.g.* with carboxylic groups linked to amine functions,[36] single stranded DNA (ssDNA),[37] or bovine serum albumin,[38] yielded CNT with improved hydrophilicity and aqueous dispersibility, covalent functionalization, *e.g.* with Tumor Proteins, resulted in an enhanced internalization of Tumor Proteins in DC.[39] Under general terms, CNT functionalization appears to be a means for improving their biocompatibility and for tuning their biodistribution property in the organism. For example, by functionalizing MWNT with amine functions, these structures were individualized, thus facilitating their glomerular filtration and renal clearance.[40] Cellular internalization of CNT was also reported to be more efficient than that of GO,[41] potentially highlighting the role of specific carbon atom arrangement in the mechanisms of cellular internalization. Furthermore, internalization mechanisms were in some cases associated with endocytosis, as demonstrated for SWNT–streptavidin, which were observed by microscopy in endocytic vesicles and whose internalization was reduced at 4 °C.[42] In some other cases, they were attributed to the passive diffusion of CNT through cell membrane, CNT playing the role of nano-needles inserted within the lipid bilayer, where they formed trans-membrane diffusing pores.[42] Interestingly, this type of passive diffusion of MWNT was also observed in prokaryotic cells, which lack endocytosis mechanism, thus potentially paving the way towards the specific immune activation/inactivation of these micro-organisms by CNT.[43] CNT immunogenicity can be harnessed to efficiently fight pathogenic cells, by taking advantage of the large surface area of CNT, and the various functionalization that they enable, which allow the attachment to CNT surface of many different types of immunogenic substances such as antigens, TLR agonists, CPG, αCD40, and anti-PD-1. Such multi-functional structure whose appeal is strengthened by its propensity for internalization has been shown to trigger certain specific immune mechanisms such as the cytolytic action of CD8+ T cells against pathogenic cells, which is favored by antigen cross presentation in DC.[44] As an example of preclinical study, it was shown that mice were protected against bacterial infection at a lower therapeutic dose when they received MWCNT-CpG than free CPG, revealing the interest of using this type of NM for reducing the amount of active principle that needs to be administered to reach an effective treatment.[45] As for GO, reassuring preclinical safety data of CNT are pending. They appear as a prerequisite to be able to start clinical assessment of these materials.

V.2 Vesicles (micelles, liposomes, membrane vesicles, exosomes)

Micelles. Micelles have also been suggested as vaccine adjuvant.[46] They are core/shell NP resulting from spontaneous self-assembly in water of individual amphiphilic molecules, *i.e.* molecules displaying a hydrophobic tail and a hydrophilic head. They are composed of various polymer/copolymer assemblies, *e.g.* PLA-*b*-P(NAS-*co*-NVP),[47] γ-PGA associated with hydrophobic cholesterol,[48] PEG-PLL-PLLeu,[49,50] γ-PGA polymer modified with hydrophobic L-phenylalanine ethyl-ester,[51] chitosan combined with hydrophobic phenyl alanine,[52] PEI linked to stearic acid.[53] Substances of therapeutic interest can be bound to the micelle external surface or be incorporated inside micelle core, *e.g.* HIV-1 Gag p24 antigens were attached to the micelle surface while TLR-l ligand (imiquimod) was encapsulated inside the micelle core.[47] To maintain such substances inside the micelles, the stability of the whole structures needs to be maintained. For that, it can be taken advantage of electrostatic interactions, *e.g.* between anionic DNA or mRNA and cationic PEI external micelle external surface,[51,54] Furthermore, in addition to antigens, a wide choice of other vaccine substances can be associated with micelles, such as siRNA, mRNA, or TLR agonist such as PIC,[49–51,54] allowing such structures to be multi-functional. Other appealing micelle properties should facilitate their use as vaccine adjuvants such as tunable micelle surface properties (composition and charge) to achieve biocompatibility and hydrophilicity, a nanometric size that facilitates micelle migration to lymph nodes and once there, antigen delivery to DC.[55] This results in better stimulation of the immune system when the antigens are associated with micelles than when they are free, *i.e.* in particular an enhanced stimulation/maturation of DC, followed by effective T cell activation and proliferation, a higher level of IgG/IgA antibody titers specific of the antigen associated with the micelles.[47,49,50] The efficacy of these mechanisms seems to depend on certain micellar properties such as their size, *i.e.* DC maturation was observed to be stronger for smaller micelles,[51] and their ability to release antigen in a controlled manner in lymph nodes under pH variation.[56] Among the noticeable pre-clinical data, striking results were reported on mice bearing B16F10-OVA melanoma, which received a nano-emulsion encapsulating antigens with TLR-7/TLR-8 agonists. This treatment did not only lead to common lymphocyte activation against the tumor but also to the re-programming of immunosuppressive tumor micro-environment (TME), *i.e.* a change in macrophage polarization from M2 to M1, which was concomitant with tumor growth delay.[57]

Liposomes. Liposomes are spheres comprising an aqueous core surrounded by phospholipid bilayers, made of different types of amphiphilic phospholipids, such as PC (phosphatidylcholine), PS (phosphatidylserine) and SM (sphingomyelin), which may also be combined with other lipids, such as CHO (cholesterol) or polymers (PEG), to stabilize the liposome

membrane and adjust its permeabilization.[58] A rather large range of different types of liposomes have been developed comprising deformable vesicles (Transfersome),[59] inter-bilayer cross-linked multilamellar vesicles (ICMVs),[60] solid core liposomes,[61] small unilamellar vesicles (SUV) with sizes below 200 nm, large unilamellar vesicles (LUV) with sizes comprised between 200 and 500 nm, microsized multilamellar vesicles (MLV) consisting of numerous concentric phospholipid bilayers with sizes above 1 μm, virosomes containing an envelope made of viral phospholipids and viral proteins, *e.g.* spike glycoproteins, niosomes, *e.g.*, see ref. 62 and bilosomes, consisting of bilayer vesicles incorporating bile salts.[63] Under general terms, liposomes can either be in a liquid or solid state, depending on whether their transition temperature between these two states T_c is above or below ambient temperature, *i.e.* when $T_c < 25$ °C liposomes are in a liquid state while when $T_c > 25$ °C liposomes are in a solid state. The value of T_c itself depends on certain properties of the phospholipids such as their carbon chain length, their number of unsaturation, their nature, as well as the different types of interactions taking place within the phospholipid membrane. Membrane fluidity is an important aspect determining liposome adjuvant efficacy, *i.e.* liposomes with T_c of 55 °C encapsulating antigens (*Leishmania donovani*) yielded improved adjuvant activity in mice,[64] while those with T_c of 23 °C or 41 °C loaded with antigens failed to act as adjuvants and to efficiently protect infected mice.[64] Two schematic visions of the way in which liposomes work can be presented. First, it can be considered that liposomes disintegrate or fall apart, *e.g.* when their solid–liquid transition occurs or when they encounter certain types of biological material, and that this event results in liposomes becoming therapeutically active, *e.g.* through the release of their content. Second, one can believe that liposomes protect the active principle, which remains linked in one way or another to the liposomal structure until it reaches the location or condition where it becomes fully active. It is hard to determine experimentally *in vivo* which type of mechanism is really taking place. One of the main interest of liposomes comes from the various parameters, which can be adjusted to optimize their therapeutic properties. First, they can be linked at their surface to molecules with two different specific functions, *i.e.* compounds such as PEG that enable them to avoid being captured by the immune system (macrophages),[65] and substances such as mannose, which can bind to certain cell type receptors, *e.g.* mannose receptor, hence improving liposome targeting efficacy.[66] Second, it is possible to select among the different types of liposomes, a structure that will release the active principle slowly (MLV,[67]), which seems favorable to reach a sustained immune response and achieve efficient vaccination. Third, liposomal structures are under certain conditions able to carry together molecules with various functions, *e.g.* antigens, PRR, Aluminum NP, potentially enabling to tune their immunogenic activity through the selection of the right cocktail of immunogenic entities that they incorporate.[61,68,69] Fourth, liposome surface charges can be adjusted by accurately selecting phospholipids, the charged phospholipids enabling to encapsulate in their core or to adsorb at their surface antigens of opposite charges. The resulting electrostatic interactions are believed to yield sustained/non immediate antigen release, a desired property when a long-lasting pharmaceutic effect is sought for.[70] Comparing negatively with positively charged phospholipids, it can be concluded that cationic phospholipids are more likely to interact with the negatively charged cellular membrane and to trigger an immune response that differs from that of anionic phospholipids, *i.e.* anionic liposomes where shown to more efficiently yield a Th1 responses in mice than cationic liposomes.[71] Fifth, liposome size can influence vaccine efficacy since some mechanisms of diffusion/transport and interaction with the immune system are size dependent. For example, liposomes smaller than 20 nm may enter systemic circulation, while those of sizes between 20 and 100 nm may preferentially diffuse towards draining lymph nodes, and those of sizes larger than 100 nm may remain at the injection site where they form a depot. This can further result in size dependent immune reactions, *e.g.* IL-12 cytokine production was observed for liposomes larger than 225 nm while such behavior was not reported for liposomes smaller than 155 nm.[72] Sixth, liposomes offer different modes of associations of antigens, *i.e.* antigen adsorption or covalent binding on/at liposome surface, antigen encapsulation within its inner core, or antigen intercalation between adjacent bilayers. This enables associating liposomes to antigens in a way, which depends on antigen properties, *i.e.* water-soluble antigens can be inserted in the liposome aqueous inner core, lipophilic antigens can be intercalated into the lipid bilayer, and other antigen types can be attached to the liposome surface. Although the type of association chosen shall have an impact on the immune response, this aspect, which would require a comparative experimental study between the different types of antigen association, does not seem to have been thoroughly studied. A series of striking adjuvant-like behaviors were reported pre-clinically with different types of liposomes. They include the migration to lymph nodes and the triggering of antigen specific antibody (IgA) responses in mice immunized with 200–7000 nm PEG stabilized liposomes comprising retinoic acid and CAF23 antigens,[73] multi-epitope T cells response and tumor growth delay following injection to melanoma bearing mice of 20–30 nm multi-functional liposomes consisting of a phosphate calcium core surrounded by cancer membrane proteins (CMP) and α-helix HSP70 functional peptide,[74] and a change in TME macrophage polarization after administration to melanoma bearing mice of a complex structure consisting of liposomes associated with Fe_3O_4 NP, CPG, and antigenic microparticles.[75] The properties of the various lipid nanoparticles used for delivering mRNA in mRNA vaccines are described in recent reviews.[17–19]

Exosome. On the one hand, exosomes display several properties in common with liposomes such as a relatively similar round shape, a surrounding envelope made of phospholipids and a size of 30 to 120 nm. On the other hand, they are also characterized by a series of distinctive features. First, they are released from late endosomes of eukaryotic cells. Second, their

lumen or surface content is made of various biomaterials such as specific tetraspanin proteins (CD9, CD63, CD81, CD82), nucleic acids, DNA, lipids, carbohydrate, and various types of RNA, *e.g.* mRNA, RNAs, siRNA, piRNA, microRNA.[76–78] Third, they possess the specific function of facilitating the diffusion of their biomaterial between different cells,[79,80] thereby providing a mean for cell function regulation. In this regard, exosomes appear in some cases to yield an immunosuppressive activity that can prevent tumors or pathogens from being destroyed by the immune system.[81] In some other cases, they may contain the antigens released by antigen-presenting cells (APCs), dendritic cells (DCs), and then be used in vaccines.[82] With regard to the vaccinal aspect, the case of exosomes is unique since without subsequent modification of their content in active principle following their expulsion from cells, their active compound (antigen, RNA) is incorporated in their envelope, possibly during the maturation phase of the exosomes in the endosomes.[83] By contrast to other adjuvants, in the case of exosomes, the appearance of the medically active substance takes place intracellularly more or less concomitantly with that of the adjuvant. In exosomes, due to a cell-dependent genesis, the adjuvant and active principle appear to be intrinsically linked with each other, a situation that is not so common in other vaccine components.

Membrane vesicle. A type of extracellular vesicle with a different origin from that of exosomes has been proposed as adjuvant.[84–86] It consists of outer membrane vesicles (OMV). The latter are derived from Gram negative bacteria and are characterized by a diameter lying between 20 to 250 nm depending on bacterial species.[87] They consist of a surrounding outer membrane composed of phospholipid such as LPS, proteins, which encapsulates periplasmic components.[87] They usually require bio-engineering steps to have the proper antigens expressed or incorporated in OMV and to prevent a too high level of immune reaction that can be due to the presence of LPS or other immune components, and can result in sceptic shock.[84,88] These steps can consist in modifying or adjusting the genome of the bacteria.[89] Antigens can either be inserted in OMV lumen or displayed on OMV surface.[84] Considering a simplified schematic view, both types of configurations present advantages and drawbacks. On the one hand surface exposed antigens may be more favorably accessible for antigen presenting cells (APC) but can also more easily be captured/destroyed by certain immune cells such as macrophages. The opposite holds for antigens in lumen, which could less freely interact with APC due to the phospholipidic layer separating them from APC, but this layer also protects them against immune degradation. For this reason, the choice between extra or intra vesicle antigen encapsulation or association must be made on a case-by-case basis, depending on certain vaccination parameters such as the vaccine mode of action or administration route. When OMV containing influenza antigen and a low endotoxin content were administered to mice *via* the intra-nasal route, it resulted in IgA antibody production, the activation of lung-resident influenza-specific T cells, and a certain level of protection against influenza among immunized mice.[90]

V.3 Polymer. A large number of vaccine nano-adjuvants have been inserted in the category of polymers due to the broad definition of this type of material, *i.e.* structures composed of repetitive molecular or atomic sub-units.[91–94] Since polymer structures are not necessarily characterized by the presence of a full symmetry or periodicity, it can in some cases be difficult to determine if a nanomaterial is polymeric or not. The polymeric nano-adjuvants described in the literature consist of DNA based structures, FDA approved poly(D,L-lactic-*co*-glycolic acid) (PLGA), poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) (pLHMGA), poly(L-lactic acid) (PLA), poly(glycolic acid) (PGA), polystyrene (PS), poly(propylene sulfide) (PPS), polyethyleneimine (PEI), a series of different types of polysaccharides, such as chitosan, dextran, hyaluronic acid, starch, alginate, pullulan, and inulin.[91–96] Polymeric structures are also characterized by a series of different types of arrangements such as: (i) co-polymers or the assembly of several different polymers within one structure, *i.e.* PEG-*b*-PPS amphiphile block copolymer,[97] (ii) nanogels engulfing a polymer within a nanometric matrix structure,[98] (iii) polymersomes that consist of self-assembled block copolymers,[99] (iv) dendrimers or dendrigraft, which are polymers arranged in branches, sometimes symmetrically around a core.[100–102] Antigens can be associated with polymers either *via* electrostatic interactions, *e.g.* positively charged polymers interact with negatively charged nucleotide, or through antigen encapsulation into polymeric nanoparticles.[103] In general, it is possible to select polymers that are bio-degradable, bio-compatible, easily manufacturable, and easily soluble,[104] and avoid some polymers such as PEI that can induce a certain level of toxicity.[105] Furthermore, the natural origin of polymer can be a pledge for their biocompatibility.[106] Polymer physico-chemical properties also impact their effect on the immune system, *i.e.* non-spherical nano-polymers display reduced uptake by APC compared with spherical ones,[107,108] while positively charged nano-polymers are usually reported to be more efficiently internalized in APC than negatively charged particles due to the negatively charged membrane.[109] In general, the association antigen/polymer is described as promoting antigen release as well as antigen association with other molecules of vaccinal interest such as CPG.[110] Finally, several studies also reported the use of nano-polymers to make vaccination at mucosal level more efficient than with traditional vaccines. This region of the body is of particular interest for vaccination since a stimulation of the immune system at this location can potentially prevent viruses from entering the body.[107,111]

V.4 Metallic NM (plain or mesoporous). NM with different metallic compositions, *e.g.* ZnO,[112] or Au,[113] have been suggested as vaccine adjuvant, and the use of Au NP in nano-vaccines has recently been reviewed.[114,115] Their main interest lies in their adjustable physico-chemical properties, such as their sizes, shapes, charges, which can be tuned by varying their synthesis conditions such as the types and concentrations of the used reagents, as well as pH, temperature, and duration of

the reactions. Concerning Au NM, it has notably been reported that a subcutaneous administration of such material with the OVA adjuvant resulted in immune mechanisms, which depended upon Au NP sizes, *i.e.* the smallest Au NP (<10 nm) activated NLRP3 inflammasome, caspase-1, and led to IL-1β production while the largest Au NP (>10 nm) triggered the activation of the NF-κB signaling pathway, demonstrating the possibility to tune the nature of the immune response by varying NM sizes, and hence possibly to choose the most efficient immune response against pathogens or pathogenic cells by selecting the optimal size of the Au NM contained in a vaccine as adjuvant.[116] By contrast to the previously described NM, which are plain, mesoporous ZnO NM could also be synthesized and used as vaccine adjuvant. This type of structure is particularly interestingly since it enables inserting antigens (OVA) inside the mesoporous pores through a mechanism, which seems to rely on electrostatic interactions between the positively charged surface of the pores and the negatively charged OVA. It not only leads a high a loading efficacy, but also enables a slow release of the antigen between ~5 h and ~72 h following antigen insertion. Considering this type of structure from a visual and schematic point of view, it seems that it gathers together the faculty to protect the antigen against degradation through the partial opening of its pores and the ability to present the antigen to APC and its receptors (*e.g.* TLR) by allowing local interactions between antigens and APC receptors at NM openings. The pores should be finely designed so as not to block interactions with APC. Thus, the mouse administration of ZnO NM mentioned above loaded with the OVA antigen led to an immune reaction characterized by the activation of CD4+ and CD8+ T cells, the production of IgG2a, IgG2b, IFN-γ, and a Th1 immune response.[117] Furthermore, the reaction of the immune system may be orientated in a specific direction by modifying the surface of this type of NM, *e.g.* by varying their charge through pH adjustment, with negative and positive charges obtained at high and low pH, respectively,[112] or the nature of the coating material used to stabilize metallic NM, for example using black pepper extract.[113]

V.5 Mesoporous silica NM. Mesoporous silica NM may possibly be used as nano-adjuvants, due to the various well-established methods that can be used to synthesize them, their relative biocompatibility, and their faculty to release an active principle from their pores in a controlled fashion under the application of a stimulus, *e.g.* a variation in pH or temperature, as reviewed elsewhere in more details.[118]

V.6 Cell mediated vaccines. Although the cell size is micrometric, some cell-derived compounds (proteins, *e.g.* cytokines, exosomes...) or certain cell-triggered mechanisms that occur at nanometric scale, may either directly be inserted in COVID-19 vaccines or used to design a therapeutic substance such as an antibody acting against COVID-19.[119] Under this hypothesis, cell-mediated vaccines may fall within the classification of nano-vaccines. First, exosomes, in particular those derived from mesenchymal stem cells, can act as nanosized lipid bilayer nano-systems, enabling the communication between infected and non-infected cells, and then possibly reduce the cytokine storm and/or increase/restore mitochondrial function in injured lungs.[120] Second, certain proteins fabricated by dendritic cells such as DC-SIGN and DC-SIGNR, which are believed to promote COVID-19 infection, could be neutralized by nano-vaccines triggering an antibody activation against these proteins.[121] Third, certain stem cells, in particular mesenchymal ones, could act against the cytokine storm resulting from COVID 19 infection, *e.g.* by yielding cytokines displaying regulatory instead of inflammatory immune properties.[122]

VI. Mechanisms of action of nano adjuvants

NM can be perceived as platforms serving as support to anchor not only VAP, but also other immune entities such as CPG, TLR agonist, enabling the concentration of these compounds, their protection against degradation, the controlled activation or release of the VAP from the platform, notably by breaking the bonds between the platform and the VAP, the mimicking of viruses through the platform appearance and composition, as illustrated in Fig. 3. These properties result in an efficient response of the immune system against pathogens, through the stimulation of APC, essentially dendritic and B cells, and involve different types of cells depending on the activated pathway, *i.e.* CD4+, CD8+, Th1, Th2, Th17, plasma B cells, and either the production of antibodies (IgA, IgE, IgG, IgM, IgD) or interleukines, *e.g.* IL-2 or IL-4, or interferons, *e.g.* IFN-γ, as summarized in Fig. 4.[123–125] In some cases, these immune entities directly participate in the destruction of viruses as it is the case with cytotoxic T lymphocyte through the production of perforin or granzyme, or with antibodies. In some other cases, they indirectly trigger anti-pathogenic activity as it is the case for Th cells, certain cytokines, which would activate other immune cells to capture pathogens.[125] It is also well-known that some of the activated immune cells such as Treg cells can weaken the response of the immune system against pathogens, necessitating that the immune system is activated selectively to stimulate immune cells or immune entities fulfilling the desired function.[126] Fig. 5 further illustrates how this idea can be implemented by protecting NM to avoid their capture by macrophages that would destroy them and their associated VAP (Fig. 5a) while enabling activation of CD4+ and CD8+ when the VAP is either mRNA or an antigen and the pathways involve either MHC-I or MHC-II (Fig. 5(b)).

Nano-vaccines are designed to target essentially three regions. The first one consists of mucus, located in aerodigestive and urogenital tracts, eye conjunctiva, the inner ear and the ducts of allexocrine, where an immune response takes place in so called mucosa-associated lymphoid tissue (MALT), essentially through lymphocyte or IgA antibody production.[100,111] Certain types of nanoparticles, such as positively charged ones, have been reported to enhance the immune response at mucosal site.[127] Vaccination in mucous membranes using nanoparticles is essentially described to fight viral infection.[128] In this case, it is designed to prevent pathogens from entering the organism through mucosal surfaces. Although more rarely employed, this route of vaccine administration has also been suggested against cancer, often using intranasal

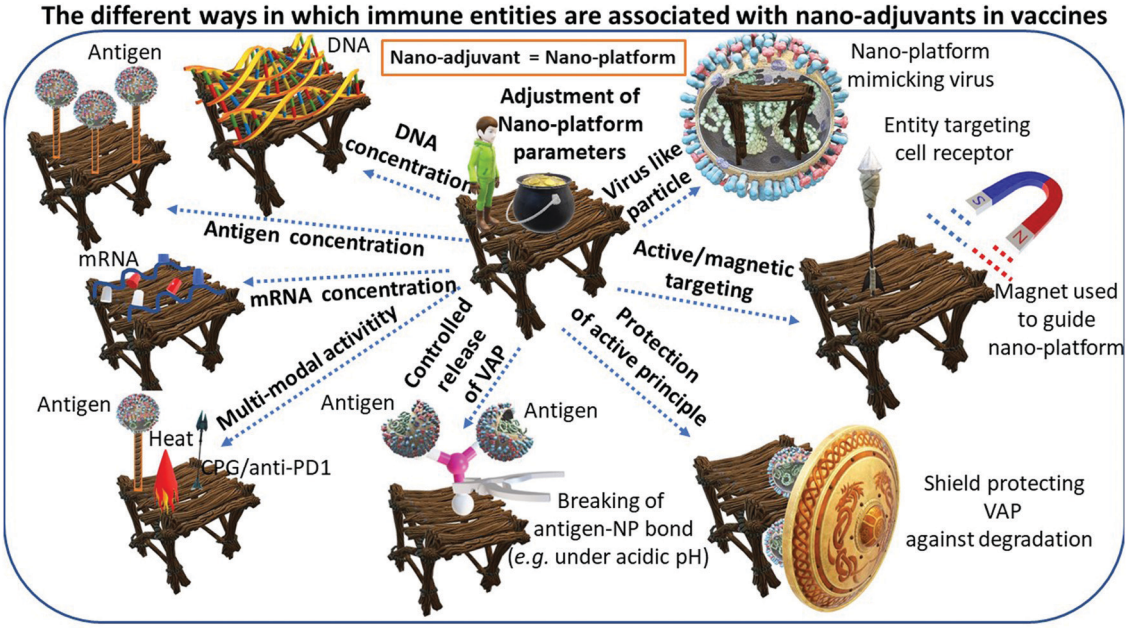

Fig. 3 The different ways in which immune entities/VAP are associated with the nano-adjuvant. Nano-adjuvants can be represented as platforms, which can concentrate VAP, gather several different types of immune entities, control VAP activation in a controlled manner for example by breaking the bonds between the antigen and NM under certain specific conditions, protect the VAP, enable targeting of specific regions/cells by using an entity attached to the NM that specifically target a cell receptor or through magnetic targeting, or reproduce through NM shapes or compositions the behaviors of a virus.

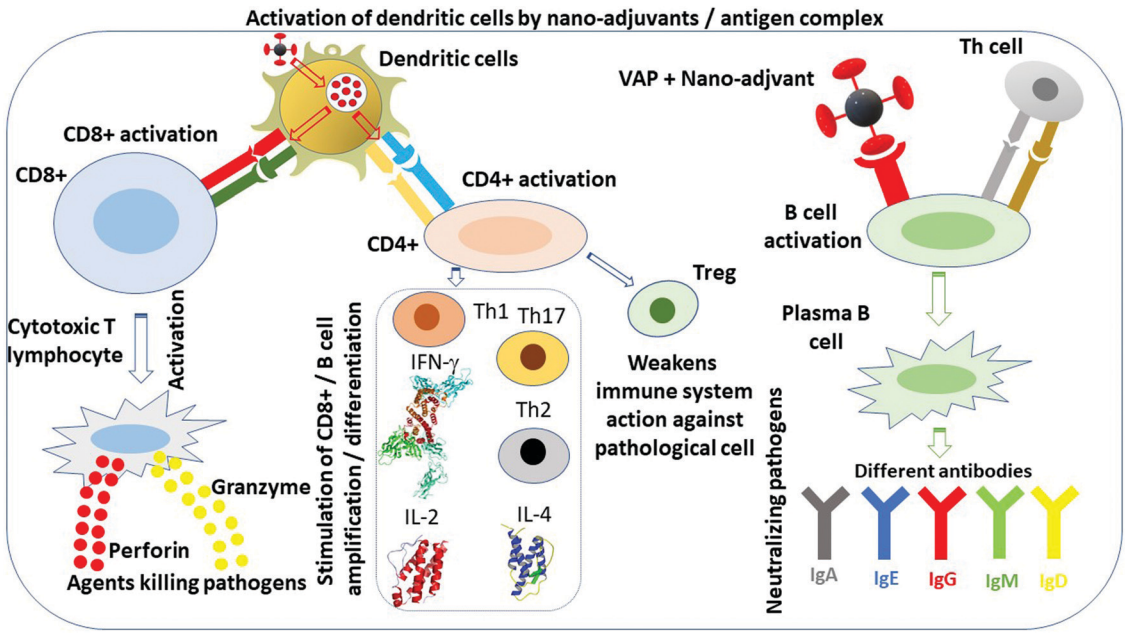

Fig. 4 Different possible activations of antigen-presenting cells, *i.e.* essentially dendritic and B cells, using nano-adjuvants, involving various cells, *e.g.* CD4+, CD8+, Th1, Th17, Th2, plasma B cells, as well as other immune entities such as antibodies (IgA, IgE, IgG, IgM, IgD), interleukins (IL-2, IL-4) and interferons, *e.g.* INF-γ.

administration.[129] For example, when emulsions of squalene-based NP of ~200 nm containing OVA were introduced in mouse mucus, it led to the recruitment of antigen-specific T-cells, not only in the injection region but also at some distance of this location such as in the spleen, further resulting in an antitumor activity characterized by the survival of 50% of mice bearing lymphoma tumors 40 days after tumor inoculation.[130] The second region of immune interest comprises lymph nodes, where APC migrate and lymphocyte priming occurs.[131] Nano-materials can favor the diffusion of antigens associated with

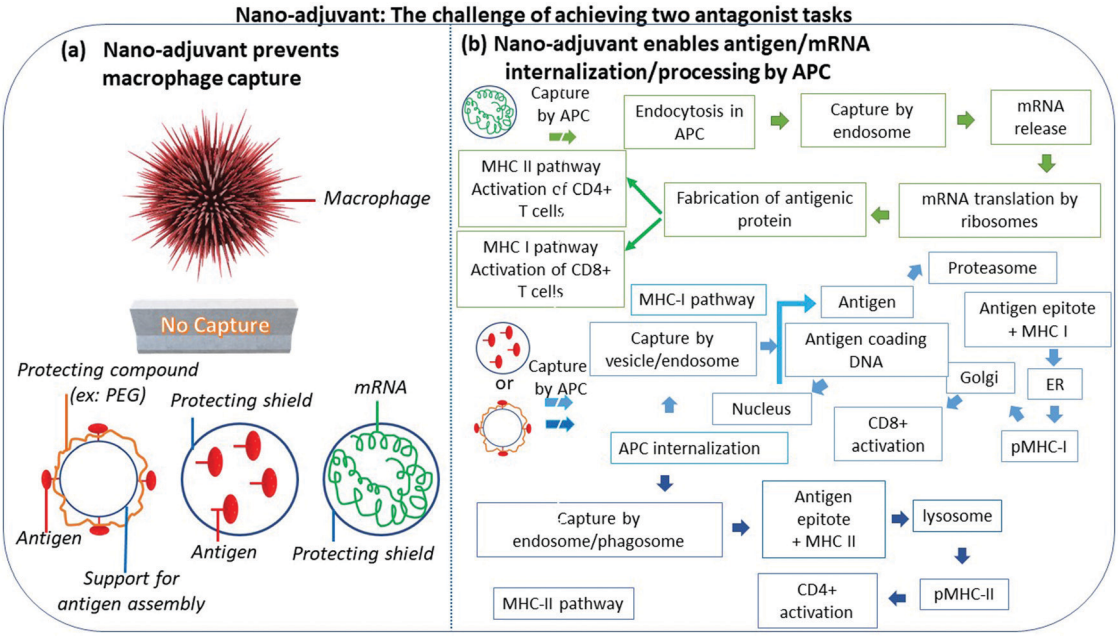

Fig. 5 A figure highlighting the challenge of achieving two antagonist tasks with nano-adjuvants. On the one hand, (a) shows that NM should prevent the capture/degradation by some immune cells such as macrophages of the VAP using a protecting shield surrounding the NM or the association with the NM of a compound such as PEG that prevents such event from occurring. On the other hand, (b) presents the ways in which nano-adjuvants and associated VAP should be presented or internalized in/to APC and further processed through the MHC-I or MHC-II pathways, to yield CD4+ and CD8+ activations.

them towards lymph nodes. Indeed, the size of the NM/antigen complex can enable the transport of this complex through the lymphatic capillary network,[132] and then antigen processing by APC in lymph nodes. To achieve such mechanism, it seems that the immune response can be triggered against tumors or viruses, using various types of nanomaterials with the antigen depending by definition on the nature of the pathological agent. The third targeted region consists of a site, which is a well irrigated site such as the muscle,[133] and/or a site of infection such as a tumor.[134] In case where this type of targeting is planned, NM can allow an efficient diffusion of the antigens in the infected zone, *i.e.* by using a targeting molecule that drives the NM/antigen complex towards this region, or through NM passive diffusion, which enables NM, due to their sizes, to cross the holes of the angiogenetic blood vessels supplying the tumor with oxygen.[135]

The use of NM in vaccines can help protecting antigens against degradation or destruction.[136] Whether the targeted disease is cancer or viral infection, the undertaking of such precaution is essential to achieve successful vaccination. It can be accomplished by encapsulating the antigen in a closed structure such as the inner aqueous core or the outer membrane of a liposome or a vesicle, or by attaching the antigen on the outer surface of a NM. The notion of non-degradation of the antigen shall be understood without forgetting that the antigen must also be internalized in APC to be presented to the MHC. In other words, NM are expected to allow selective antigen cellular interaction by allowing an absence of capture by certain cells such as macrophages, which could destroy antigens, while enabling internalization in other cells such as APC, which would allow antigen processing and further activate the immune system against pathogens. This selectivity, which could be achieved by a proper design of NM, *e.g.* using a coating material such as PEG that would prevent NM engulfment by macrophages,[137,138] by attaching molecules such as glycospingolipid, which specially recognize APC cellular receptors,[139] seems to be at the heart of a successful vaccination.

Under the general umbrella of vaccination principle, *i.e.* inducing the presence of a compound in the body which mimics certain pathological behaviors, hence training the immune system to fight against pathological cells and making it ready to prevent infection when it occurs, a large number of declinations can be considered, taking shape with nano-vaccines. The latter contain different types of antigenic proteins or other structures (mRNA and DNA) having the genetic code enabling antigen fabrication. Proteins, mRNA and DNA are either self-assembled at nanometer scale, are contained in particles mimicking a virus through their shape, *i.e.* VLP,[140] or are associated with various types of NM (vesicles, polymers, polysaccharide, liposomes). mRNA and DNA present the advantage of potentially enabling antigen fabrication for all types of pathogens once their genetic codes have been identified. mRNA can be preferred to DNA since it may be more immunogenic and in the process of antigen fabrication, mRNA seems to act at less upstream steps than DNA, *i.e.* mRNA is issued through transcription from DNA. This comparison does not however take into consideration the fragility of mRNA and the whole set

of cellular mechanisms involved in antigen fabrication, which are still largely unelucidated.[141] Whether nano-vaccines contain the antigens or their genetic code, APC involvement appears necessary to yield antigen processing, which then triggers the reaction of the immune system against pathogens. Therefore, NM properties should be tuned or chosen to reach an optimal interaction with the APC.

Another interest of NM resides in their faculty to be associated with other kinds of immunogenic substances than antigens. A first type of such substances is designed to recognize pathogen recognition receptors (PRR) such as toll like receptors (TLR) located at the surface of APC. In this case, they either consist of pathogen associated molecular patterns (PAMP) originating from viruses or danger associated molecular patterns (DAMP) coming from tumors depending on the type of targeted disease. Examples of such substances include imiquimod, detoxified LPS such as MPLA, or CPG, which are agonists of TLR7, TLR4, and TLR9, respectively.[142] It has been reported that the association of such substance with NM can favor the interaction between PRR and PRR agonist, hence preventing an uncontrolled immune reaction. This is achieved first by enabling such interaction to take place locally at PRR site, second by controlling the type of association between the antigen and the NM, *i.e.* the antigen is either adsorbed at or covalently linked to NM surface or the antigen is encapsulated within the NM, or third by triggering antigen release or dissociation from NM at a specific location of interest under physico-chemical disturbance, *e.g.* inside a cellular compartment characterized by an acidic environment. Furthermore, by using NM it may also be possible to associate a specific PRR agonists with an antigen within the same unit, hence potentially orientating the response of the immune system in a desired direction.[143] For example, by choosing TLR4 as PRR, a T cell response may be triggered, while by selecting TLR2 as PRR a Th2 response may be expected.[144] A second type of such substances is used to prevent the weakening of the immune response, which can occur at the site of infection, for example when T-cell activation by APC is blocked by so-called PD-1 receptors. To prevent the latter situation from occurring, anti-PD1 molecules have been associated with NM, leading to the successful neutralization of PD-1.[145]

Furthermore, by introducing NM in vaccines, it is feasible to tune vaccine immunogenicity by skillfully choosing the physicochemical properties of NM such as their sizes, shapes, charges, and hydrophobicity. It should be borne in mind that the immune response usually depends on the effects on several of these parameters as well as on other factors such as NM composition, concentration, route of administration, and the nature of the antigen associated with them. Thus, while the analysis of each of these parameters taken individually helps to better understand how nano-vaccines operate, the observed behaviors remain linked to specific experimental situations, and their generalization to other types of circumstances is hypothetical. Regarding sizes, it has been suggested that the type of region containing dendritic cells, which is targeted depends on NM size, with NM of 10–100 nm targeting Lymph node-resident dendritic cells, and NM larger than 500 nm targeting peripheral tissue-resident dendritic cells,[146] where they can induce adaptive T and B (humoral) cell immunity.[146] The type of cells by which NM are captured also seems to depend on NM size, *i.e.* for example smaller NM were reported to internalize in DC while larger NM were engulfed in macrophages.[147] Efficacy of cross-presentation can also be influenced by NM size and in some cases increase with decreasing NM sizes,[148,149] due for example to the smaller NM more easily escaping from endosomes to cytosol than larger NM.[148] Concerning NM shape, it can also determine the efficacy of NM cellular internalization, *i.e.* anisotropic NM such as rods were more efficiently captured by macrophages and DC than spherical or cubic NM.[150] With regard to NM charge, it is well-established that the positively charged NM interact electrostatically with the negatively charged cell membrane, a behavior that can potentially enhance the immune response, *e.g.* by resulting in a more efficient activation of B/T cells using positively than negatively charged NM.[151,152] Interestingly, NM hydrophobicity has been reported to trigger a certain type of danger signal that activates the innate immune system,[153,154] and therefore improves the strength of the immune response.[155,156] Such observation has led to the development and use of polyanydride NM, which are very hydrophobic, and therefore prone to this type of behaviors.[157]

Different types of vaccines containing essentially non-immunogenic nano-adjuvant such as SPIO, polysaccharide, polymer, CaP, liposome, associated with various types of agents activating/targeting immune cells such as folate, IgG, anti-inflammatory drugs, TLR ligands or agonists, antibodies, antigens, were developed. The immune responses that they triggered depended on the types of nano-adjuvant and targeting/active principles, as well as on the way in which these two entities were associated with each other and interacting with the external environment, *e.g.* by being sensitive or responsive to a specific stimulus such as pH or temperature variation. It was characterized by the production of cytokines, a T-cell or humoral driven immune response, the activation of APC (dendritic cells and/or macrophages), and the involvement of certain co-stimulatory molecules, as summarized in Table 2 and associated references.

VII. Tuning nm properties to adjust the anti-viral immune response

One of the main interests of introducing nano-adjuvants in vaccines comes from the fact that their properties can be adjusted to modulate their interactions with the immune system. Assuming that the vaccine active principle VAP and NM behave similarly, which implies among other things that the VAP remains attached to the NM during the various steps of NM interaction with the immune system, this aspect could be used to optimize the properties of COVID-19 vaccines. Below, the impact of NM properties on immune reactions is presented through behaviors deduced from specific examples taken outside of the COVID-19 literature that lacks to the author knowledge an experimentally-based description of these aspects.

Table 2 Illustrative examples of immune responses triggered by various vaccines containing nano-adjuvants, where Table 2 indicates for each chosen nano-vaccine the treatment purpose, the active/targeting compounds and non-immunogenic nano-adjuvant contained in the vaccine, the nano-adjuvant size, various immune mechanisms triggered by the vaccine involving cytokines, lymphocytes, macrophages, dendritic cells, and co-stimulatory molecules. Here, a non-immunogenic adjuvant is an adjuvant that does not contain a compound that is usually considered as belonging to the immune system

				Immune mechanism involved					
Treatment (purpose)	Active/targeting compound	Non-immunogenic nanoadjuvant	Nano-adjuvant size	Cytokine (TNF-α, IFN-γ, IL-12…)	Lymphocyte (CD4+; CD8+) antibodies	Dendritic cells	Macrophages/ monocytes	Costimulatory molecules (CD80+; CD86+…)	Ref.
iv admin. to mice	IgG coating	SPIO	175–200 nm	NA	NA	NA	NP accumulation in monocyte/macrophage derived monocyte → enhanced by IgG	NA	234
Anti-inflammatory activity in rats with arthritis;	Folate	Dendrimer	NA	NA	NA	NA	Receptor-specific internalization in macrophages; such as ankle swelling, paw volume, cartilage damage, bone resorption, and body weight decrease.	NA	235
Treatment of obese mice	Anti-inflammatory drugs	Nano-scale polysaccharide	4–30 nm	Reduction in pro-inflammatory cytokines	NA	NA	Association with M1 macrophages: ↓ pro-inflammatory cytokine	NA	236
Delivery of NP-vaccines to DC in mice	mAb targeting CD40, CD11c, DEC-205; + TLR3/TLR7 ligands	PEG-PLGA NP	190–250 nm	↑ IL-12 production; ↑ IFN-y	CD8+ activation	activation of DC	NA	CD40, CD11c, DEC-205	237
Melanoma treatment in mice	TLR ligands (Poly:IC + CPG) + Mannose + melanoma Ag	PLGA NP	150 nm	High IgG2c/IgG1 ratios and high levels of IFN-γ and IL-2.	Th1 response; ↑ IgG2c/IgG1	Targeted	Targeted	Mannone: target MR/CD206 Expressed by DCs and macrophages	238
Immunization of mice with nano-vaccine: ↑ CD8+	Ovalbumin	pH-Sensitive polyacrylic micelles	25–30 nm	NA	CD8+ T cell activation	Membrane disruption of dendritic cells; conjugation of ovalbumin to the micelles significantly enhanced antigen cross-presentation	Macrophage uptake of nano-system	NA	239
Reduction of E.G7-OVA tumor burden in mice	Ovalbumin	EPC/DOPE/ polymer liposomes	100 nm	NA	Nano-vaccine: cytotoxic T cells	Fusion with the dendritic cell membrane; destabilization endosomes (pH variation)	NA	NA	240
Immunization of mice bearing melanoma tumors: tumor growth delay	Ag + TLR agonist	Liposomes	NA	Cytokine produced by CD8+	CD4+ and CD8+ T cell responses	Activation of TLR3 or TLR9 in dendritic cells	Macrophages/ monocytes: ⇒ transport of nano-system to lymph nodes	NA	241
Applications in preventive and therapeutic vaccines	TLR7/8 a (resiquimod) + TLR9a (CpG)	Polymer	800 nm	Nano-system favors lymph node production of cytokines (IL-12, IP-10 and IFNs)	TH1 CD4+ and CD8+ T cell immunity	Activation of endosomal TLR7/8 in dendritic cells	Nano-system sent to lymph nodes by migratory/monocyte derived dendritic cells	↑ Agonist density: higher expression of co-stimulatory molecules by macrophages/ monocytes	242
Nano-system for humoral immunity	Ag: Hen Egg Lysozyme	CaP NP	250 nm	NA	NS interact with HEL-specific B-cell receptor	NA	NA	NA	243
				NA		NA	NA	NA	244

Table 2 (*continued*)

Treatment (purpose)	Active/targeting compound	Non-immunogenic nanoadjuvant	Nano-adjuvant size	Immune mechanism involved: Cytokine (TNF-α, IFN-γ, IL-12...)	Lymphocyte (CD4+; CD8+) antibodies	Dendritic cells	Macrophages/ monocytes	Costimulatory molecules (CD80+; CD86+...)	Ref.
Mice administered with nano-system	Antigen bovine serum albumin	DMPC/Chol/ DPPE liposomes	100–600 nm		Covalent linkage of Ag: ↑ IgG and IgM; Encapsulation of Ag: ↑ IgG;				
Mice injected with nanosystems	MUC-1 (inside or at the surface of liposome)	DSPC/Chol/ DMPG/MPLA liposomes	NA	Cytokine produced by T cells	Strong antigen-specific T cell responses; Only surface conjugation: strong MUC-1 specific antibody;	IL-12 secretion by macrophages	Antigen associated with liposomes processed by APC (dendritic cells and macrophages)	NA	245

Individual NM properties have been isolated from each other to be able to draw some specific trends. Experimental proofs would be needed to confirm that these behaviors apply to COVID 19 vaccines.

First, cellular internalization of the nano-adjuvant and associated active ingredient (mRNA, DNA, spike protein, *etc.*), designated as NM-VAP, could potentially be improved by reducing the NM-VAP size, as has been shown for PLGA NM whose internalization was larger at 0.3 μm than at 1–17 μm,[158] or by choosing a NM-VAP with a negative charge that could promote electrostatic interactions between the cationic NM-VAP and anionic cell membranes,[159] where the enhanced internalization was associated with a more efficient immune response.[158]

Second, the passage of NM-VAP through lysosomes, which is essential for antigen processing, could be achieved by varying the shape ratio of NM, *e.g.* nanosheet were retained in cytoplasm and lysosomes,[160] while nano rods ended up to the nucleus.[161]

Third, the use of NM-VAP could enable targeting a large portion of the whole DC population by using NM of various sizes.[162,163]

Fourth, to enhance cytokine/chemokine/co-stimulatory molecule production as well as DC activation, hydrophobic or cationic NM-VAP could be chosen since they were shown to generate such immune reaction in a more pronounced manner than hydrophilic or anionic system nano-systems.[164–166]

Fifth, the immune response can potentially be tuned by attaching to the surface of NM molecules that yields a desired immune effect such as: (i) CD47 molecules to reduce NP internalization by phagocytic cells,[167] TLR-7, TLR-8, and TLR-9 agonists o modulate TLR-mediated immune responses,[168] TLR agonist to activate the complement pathway.[169]

VIII. Added values of nano-components in vaccines

In most cases, NM are introduced in vaccines as adjuvant. It therefore appears necessary to wonder about the added value provided by nano-adjuvants compared to standard adjuvants.[170] Adjuvants are introduced into vaccines to improve the immune response against a pathogen,[171] to yield a more sustained immune response, *e.g.* by increasing the duration of the antibody response,[170] to potentially reduce the dose of antigens needed to stimulate the immune system,[172] and to ease antigen penetration/diffusion in specific regions of immune interest such as the mucus.[173] The adjuvants which are currently used to achieve these effects suffer from certain weaknesses. First, they partially stimulate the immune system, *e.g.* saponin was reported not to trigger T Cell response whereas Alum and MF59 were described as yielding a Th2 response leading to antibody production without leading to a strong Th1 response. Second, they can display a certain level of toxicity, *e.g.* saponin can activates inflammasomes leading to APC apoptosis while Complete Freud's Adjuvant (CFA) can create a local inflammation at the site of injection.[174] Third, the administration at the site of infection, which is in some cases requested to prevent systemic toxicity as it is the case with ADU-S100 and MK-1454, can be complex to achieve. Fourth, antigen release mechanisms, which determine in large part vaccine efficacy, are not always optimal, *e.g.* adjuvants in the form of emulsions such as Montanide operate by allowing a depot of the antigen within the injection area followed by a slow diffusion of the antigen from this region without however always a full control over such behavior.[175] Nano-adjuvants allow to overcome these weaknesses in large part. Indeed, they can enable to tune the strength of the immune response by adjusting NM physicochemical properties or by associating NM with several types of different immune entities, *e.g.* anionic liposomes were associated with TLR4 and TLR7 agonists.[176] NM can also yield a reduction in vaccine toxicity. This can be due to the nature of NM, which are often less toxic than standard adjuvants. This can also come from the lower doses of VAP necessary when VAP are combined with NM than when they are associated with standard adjuvants.[177] NM also enable various routes of vaccine administration, better targeting properties than standard adjuvant, and a controlled

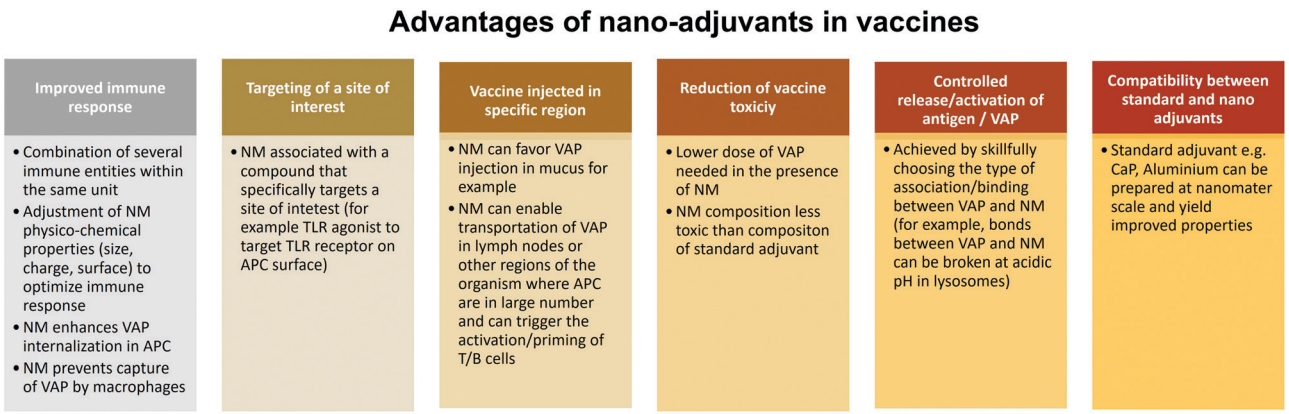

Fig. 6 The different advantages provided using NM as vaccine adjuvants, including an improved immune response, the targeting of certain sites of immune interest, the facilitated injection of the vaccine in a desired region of the organism, the reduction of vaccine toxicity, the controlled release/activation of the antigen, the compatibility or complementarity between standard and nano adjuvants.

release of the VAP by adjusting the type of association/binding between the VAP and NM, which allows breaking this binding under well-defined conditions, *i.e.* acidic pH for example. In several cases, standard adjuvants are also not incompatible with nano-adjuvants. Indeed, standard adjuvants could be prepared at the nanometric scale, either with the same composition as for CaP NM,[178] or with a slightly different one as for NM containing Zinc and Aluminum (ZA).[179] In both cases, vaccines led to the triggering of the immune system, which increased survival in mice infected with pathogens,[178] or produced tumor growth retardation in mice bearing lymphoma tumors.[179] Considering the latter approach, it presents the advantage of allowing a comparison between the efficacy of standard adjuvants and equivalent ones fabricated at nanometer scale, thus facilitating clinical trial design by offering control groups of patients with pre-established behaviors. Fig. 6 summarizes the advantages provided by nano-adjuvants.

Conclusion

In our battle against COVID 19, NM have been shown to be useful for different applications, *e.g.* in vaccines, in masks or diagnostic tools such as PCR to improve their efficacy.[180] NM incorporated in vaccines could most often be classified as adjuvants. Nano-adjuvants can often be used in vaccines against both cancer and viruses. When one examines which nano-adjuvant is best suited for use in a COVID-19 vaccine, one might therefore consider those tested against cancer to broaden its choice. Nano-adjuvants consist of NM with various compositions and structures such as graphene oxide, carbon nanotubes, various vesicles, such as micelles, liposomes, exosomes, membrane vesicles, polymers including polysaccharides, and metallic NM. The diversity of these NM makes it possible to choose the type of NM depending on the desired effect, *e.g.* vesicles to protect VAP against degradation. The different mechanisms of action of NM used as vaccine adjuvants include: (i) targeting by NM and associated VAP of a region of immune interest such as the mucus or certain specific cells such as APC, (ii) protection by NM of the antigen or mRNA/DNA against degradation in the organism, (iii) enhanced cellular internalization of VAP, (iv) improved presentation to MHC or processing by APC of antigen or mRNA/DNA, (v) synergistic effects between different immunogenic substances gathered within the same unit resulting in an improved immune response, (vi) optimization of vaccine properties through an adjustment of NM physicochemical properties such as NM sizes, surface, composition. As a whole, these behaviors result in nano-adjuvants displaying improved properties compared with standard adjuvants.

Abbreviations

ACE2	Cellular receptor necessary for entry of COVID 19 in cells
APC	Antigen presenting cell
BC	Breast cancer
CD	Cluster of differentiation
CHO	Cholesterol
CNT	Carbon nanotube
COV	Coronavirus
COVID-19	Coronavirus disease 19
DAMP	Danger associated molecular patterns
DC	Dendritic cell
DNA	Deoxyribonucleic Acid
GO	Graphene Oxide
rGO	Reduced graphene oxide
Ig	Immunoglobulin
IL	Interleukin
Im	Intramuscular
It	Intratumoral
Iv	Intravenous
IFN	Interferon
LP	Liposome
LPS	Lipopolysaccharide
NM	Nanomaterial

NP	Nanoparticle
MHC	Major histocompatibility complex
MWCNT	Multi wall carbon nanotube
OMV	Outer membrane vesicle
OVA	Ovalbumin
PAMP	Pathogen associated molecular patterns
PC	Phosphatidylcholine
PEG	Polyethylene glycol
PEI	Polyethylenimine
PGA	Polyglycolic acid
PLA	Polylactic acid
PLL	Poly-L-lysine
PRR	Pathogen recognition receptors
PS	Phosphatidylserine
mRNA	Messenger ribonucleic acid
ssRNA	Single stranded RNA
RNase	Ribonuclease
siRNA	Small interfering RNA
piRNA	PIWI-interacting RNA
SWCNT	Single wall carbon nanotube
SM	Sphingomyelin
Th	T helper cell
TLR	Toll like receptor
TME	Tumor microenvironment
VAP	Vaccine active principle
SUV	Small unilamellar vesicles
LUV	Large unilamellar vesicles
MLV	Multilamellar vesicles
VLP	Virus like particle

Conflicts of interest

Edouard Alphandéry has been working in the company Nanobacterie.

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References

1 J. Y. Chung, M. N. Thone and Y. J. Kwon, COVID-19 vaccines: The status and perspectives in delivery points of view, *Adv. Drug Delivery Rev.*, 2021, **170**, 1–25.
2 G. Chauhan, M. J. Madou, S. Kalra, V. Chopra, D. Ghosh and S. O. Martinez-Chapa, Nanotechnology for COVID-19: Therapeutics and vaccine research, *ACS Nano*, 2020, **14**, 7760–7782.
3 M. D. Shin, S. Shukla, Y. H. Chung, V. Beiss, S. K. Chan, O. A. Ortega-Rivera, D. M. Wirth, A. Chen, M. Sack, J. K. Pokorski and N. F. Steinmetz, COVID-19 vaccine development and a potential nanomaterial path forward, *Nat. Nanotechnol.*, 2020, **15**, 646–655.
4 Y. H. Chung, V. Beiss, S. N. Fiering and N. F. Steinmetz, COVID-19 vaccine frontrunners and their nanotechnology design, *ACS Nano*, 2020, **14**(10), 12522–12537.
5 M. A. Heinrich, B. Martina and J. Prakash, Nanomedicine strategies to target coronavirus, *Nano Today*, 2020, **35**, 100961.
6 C. Weiss, M. Carriere, L. Fusco, I. Capua, J. A. Regla-Nava, M. Pasquali, J. A. Scott, F. Vitale, M. A. Unal, C. Mattevi, D. Bedognetti, A. Merkoç, E. Tasciotti, A. Yilmazer, Y. Gogotsi, F. Stellacci and L. G. Delogu, Toward nanotechnology-enabled approaches against the COVID-19 pandemic, *ACS Nano*, 2020, **14**, 6383–6406.
7 S. R. Bonam, N. G. Kotla, R. A. Bohara, Y. Rochev, T. J. Webster and J. Bayry, Potential immuno-nanomedicine strategies to fight COVID-19 like pulmonary infections, *Nano Today*, 2021, **36**, 101051.
8 B. Temizoz, E. Kuroda and K. J. Ishii, Vaccine adjuvants as potential cancer immunotherapeutics, *Int. Immunol.*, 2016, **28**, 329–338.
9 H. Qian, C. Y. Tay, M. I. Setyawati, S. L. Chia, D. S. Lee and D. T. Leong, Protecting microRNAs from RNase degradation with steric DNA nanostructures, *Chem. Sci.*, 2017, **8**, 1062.
10 S. Hamdy, A. Haddadi, R. W. Hung and A. Lavasanifar, Targeting dendritic cells with nano-particulate PLGA cancer vaccine formulations, *Adv. Drug Delivery Rev.*, 2011, **63**, 943–955.
11 D. Qiao, L. Liu, Y. Chen, C. Xue, Q. Gao, H.-Q. Mao, K. M. Leong and Y. Chen, Potency of a scalable nanoparticulate subunit vaccine, *Nano Lett.*, 2018, **18**, 3007–3016.
12 X. Zhuang, T. Wu, Y. Zhao, X. Hu, Y. Bao, Y. Guo, Q. Song, G. Li, S. Tan and Z. Zhang, Lipid-enveloped zinc phosphate hybrid nanoparticles for codelivery of H-2Kb and H-2Db-restricted antigenic peptides and monophosphoryl lipid A to induce antitumor immunity against melanoma, *J. Controlled Release*, 2016, **228**, 26–37.
13 H. J. Park, H. L. Ko, D. H. Won, D. B. Hwang, Y. S. Shin, H. W. Kwak, H. J. Kim, J. W. Yun and J. H. Nam, Comprehensive analysis of the safety profile of a single-stranded RNA nano-structure adjuvant, *Pharmaceutics*, 2019, **11**, 464.
14 A. Cruz-Reséndiz, J. Zepeda-Cervantes, A. Sampieri, C. Bastián-Eugenio, G. Acero, J. I. Sánchez-Betancourt, G. Gevorkian and L. Vaca, A self-aggregating peptide: Implications for the development of thermostable vaccine candidates, *BMC Biotechnol.*, 2020, **20**, 1.
15 G. Mattheolabakis and C. M. Mikelis, Nanoparticle delivery and tumor vascular normalization: The chicken or the egg?, *Front. Oncol.*, 2019, **9**, 1227.

16 T. Kisby, A. Yilmazer and K. Kostarelos, Reasons for success and lessons learnt from nanoscale vaccines against COVID-19, *Nat. Nanotechnol.*, 2021, **16**, 843–852.
17 B. Z. Igyarto, S. Jacobsen and S. Ndeupen, Future considerations for the mRNA-lipid nanoparticle vaccine platform, *Curr. Opin. Virol.*, 2021, **48**, 65–72.
18 J. Kim, Y. Eygeris, M. Gupta and G. Sahay, Self-assembled mRNA vaccines, *Adv. Drug Delivery Rev.*, 2021, **170**, 83–112.
19 E. Kon, U. Elia and D. Peer, Principles for designing an optimal mRNA lipid nanoparticle vaccine, *Curr. Opin. Biotechnol*, 2022, **73**, 1–8.
20 Y. Ishay, A. Kenig, T. Tsemach-Toren, R. Amer, L. Rubin, Y. Hershkovitz and F. Kharouf, Autoimmune phenomena following SARS-CoV-2 vaccination, *Int. Immunopharmacol.*, 2021, **99**, 107970.
21 W. Cao, L. He, W. Cao, X. Huang, K. Jia and J. Dai, Recent progress of graphene oxide as a potential vaccine carrier and adjuvant, *Acta Biomater.*, 2020, **112**, 14–28.
22 H. Yu, B. Zhang, C. Bulin, R. Li and R. Xing, High-efficient synthesis of graphene oxide based on improved hummers method, *Sci. Rep.*, 2016, **6**, 1–7.
23 M. Mahmudzadeh, H. Yari, B. Ramezanzadeh and M. Mahdavian, Highly potent radical scavenging-antioxidant activity of biologically reduced graphene oxide using Nettle extract as a green bio-genic amines-based reductants source instead of hazardous hydrazine hydrate, *J. Hazard. Mater.*, 2019, **371**, 609–624.
24 L. Wang, D. Yu, R. Dai, D. Fu, W. Li, Z. Guo, C. Cui, J. Xu, S. Shen and K. Ma, PEGylated doxorubicin cloaked nano-graphene oxide for dual-responsive photo-chemical therapy, *Int. J. Pharm.*, 2019, **557**, 66–73.
25 D. Stauffer, N. Dragneva, W. B. Floriano, R. C. Mawhinney, G. Fanchini, S. French, O. Rubel, D. Stauffer, N. Dragneva, W. B. Floriano, R. C. Mawhinney and G. Fanchini, An atomic charge model for graphene oxide for exploring its bioadhesive properties in explicit water: An atomic charge model for graphene oxide for exploring its bioadhesive, *J. Chem. Phys.*, 2014, **141**, 044705.
26 L. Xu, J. Xiang, Y. Liu, J. Xu, Y. Luo, L. Feng, Z. Liu and R. Peng, Functionalized graphene oxide serves as a novel vaccine nano-adjuvant for robust stimulation of cellular immunity, *Nanoscale*, 2016, **8**, 3785–3795.
27 J. Russier, E. Treossi, A. Scarsi, F. Perrozzi, H. Dumortier, L. Ottaviano, M. Meneghetti, V. Palermo and A. Bianco, Evidencing the mask effect of graphene oxide: A comparative study on primary human and murine phagocytic cells, *Nanoscale*, 2013, **5**, 11234–11247.
28 H. Yue, W. Wei, Z. G. Yue, B. Wang, N. N. Luo, Y. J. Gao, D. Ma, G. H. Ma and Z. G. Su, The role of the lateral dimension of graphene oxide in the regulation of cellular responses, *Biomaterials*, 2012, **33**, 4013–4021.
29 F. Cao, X. Zhu, X. Wang, Y. Liu, G. Ma, M. Yan and H. Sun, Alum-functionalized graphene oxide nanocomplexes for effective anticancer vaccination, *Acta Biomater.*, 2018, **83**, 390–399.
30 J. Ma, R. Liu, X. Wang, Q. Liu, Y. N. Chen, R. P. Valle, Y. Y. Zuo, T. Xia and S. J. Liu, A crucial role of lateral size for graphene oxide in activating macrophages and stimulating pro-inflammatory responses in cells and animals, *ACS Nano*, 2015, **9**, 10498–10515.
31 G. Chen, H. Yang, C. Lu, Y. Chao, S. Hwang, C. Chen, K. Lo, L. Sung, W. Luo, H. Tuan and Y. Hu, Simultaneous induction of autophagy and toll-like receptor signaling pathways by graphene oxide, *Biomaterials*, 2012, **33**, 6559–6569.
32 C. Xu, H. Hong, Y. Lee, K. S. Park, M. Sun, T. Wang, M. E. Aikins, Y. Xu and J. J. Moon, Efficient lymph node-targeted delivery of personalized cancer vaccines with reactive oxygen species-inducing reduced graphene oxide nanosheets, *ACS Nano*, 2020, **14**, 13268–13278.
33 H. A. F. M. Hassana, S. S. Diebold, L. A. Smyth, A. A. Walters, G. Lombardi and K. T. Al-Jamal, Application of carbon nanotubes in cancer vaccines: Achievements, challenges and chances, *J. Controlled Release*, 2019, **297**, 79–90.
34 S. Iijima, Helical microtubules of graphitic carbon, *Nature*, 1991, **354**, 56–58.
35 E. T. Thostenson, Z. Ren and T. W. Chou, Advances in the science and technology of carbon nanotubes and their composites: A review, *Compos. Sci. Technol.*, 2001, **61**, 1899–1912.
36 D. Tasis, N. Tagmatarchis, A. Bianco and M. Prato, Chemistry of carbon nanotubes, *Chem. Rev.*, 2006, **106**, 1105–1136.
37 M. Zheng, A. Jagota, E. D. Semke, B. A. Diner, R. S. McLean, S. R. Lustig, R. E. Richardson and N. G. Tassi, DNA-assisted dispersion and separation of carbon nanotubes, *Nat. Mater.*, 2003, **2**, 338–342.
38 X. Wang, T. Xia, S. A. Ntim, Z. Ji, S. George, H. Meng, H. Zhang, V. Castranova, S. Mitra and A. E. Nel, Quantitative techniques for assessing and controlling the dispersion and biological effects of multiwalled carbon nanotubes in mammalian tissue culture cells, *ACS Nano*, 2010, **4**, 7241–7252.
39 C. H. Villa, T. Dao, I. Ahearn, N. Fehrenbacher, E. Casey, D. A. Rey, T. Korontsvit, V. Zakhaleva, C. A. Batt, M. R. Philips and D. A. Scheinberg, Single-walled carbon nanotubes deliver peptide antigen into dendritic cells and enhance igg responses to tumor-associated antigens, *ACS Nano*, 2011, **5**, 5300–5311.
40 K. T. Al-Jamal, A. Nunes, L. Methven, H. Ali-Boucetta, S. Li, F. M. Toma, M. A. Herrero, W. T. Al-Jamal, H. M. ten Eikelder, J. Foster, S. Mather, M. Prato, A. Bianco and K. Kostarelos, Degree of chemical functionalization of carbon nanotubes determines tissue distribution and excretion profile, *Angew. Chem., Int. Ed.*, 2012, **51**, 6389–6393.
41 X. Zhang, W. Hu, J. Li, L. Tao and Y. Wei, A comparative study of cellular uptake and cytotoxicity of multi-walled carbon nanotubes, graphene oxide, and nanodiamond, *Toxicol. Res.*, 2012, **1**, 62–68.
42 C. F. Lopez, S. O. Nielsen, P. B. Moore and M. L. Klein, Understanding nature's design for a nanosyringe, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 4431–4434.

43 K. Kostarelos, L. Lacerda, G. Pastorin, W. Wu, S. Wieckowski, J. Luangsivilay, S. Godefroy, D. Pantarotto, J. P. Briand, S. Muller, M. Prato and A. Bianco, Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type, *Nat. Nanotechnol.*, 2007, **2**, 108–113.

44 H. A. F. M. Hassan, L. Smyth, J. T. W. Wang, P. M. Costa, K. Ratnasothy, S. S. Diebold, G. Lombardi and K. T. Al-Jamal, Dual stimulation of antigen presenting cells using carbon nanotube-based vaccine delivery system for cancer immunotherapy, *Biomaterials*, 2016, **104**, 310–322.

45 H. Takahashi, K. Misato, T. Aoshi, Y. Yamamoto, Y. Kubota, X. Wu, E. Kuroda, K. J. Ishii, H. Yamamoto and Y. Yoshioka, Carbonate apatite nanoparticles act as potent vaccine adjuvant delivery vehicles by enhancing cytokine production induced by encapsulated cytosine-phosphate-guanine oligodeoxynucleotides, *Front. Immunol.*, 2018, **9**, 783.

46 T. Trimaille and B. Verrier, Micelle-based adjuvants for subunit vaccine delivery, *Vaccines*, 2015, **3**, 803–813.

47 G. Jiménez-Sánchez, V. Pavot, C. Chane-Haong, N. Handké, C. Terrat, D. Gigmes, T. Trimaille and B. Verrier, Preparation and *in vitro* evaluation of Imiquimod loaded polylactide-based micelles as potential vaccine adjuvants, *Pharm. Res.*, 2015, **32**, 311–320.

48 Y. W. Noh, J. H. Hong, S. M. Shim, H. S. Park, H. H. Bae, E. K. Ryu, J. H. Hwang, C. H. Lee, S. H. Cho and M. H. Sung, Polymer nanomicelles for efficient mucus delivery and antigen-specific high mucosal immunity, *Angew. Chem., Int. Ed.*, 2013, **52**, 7684–7689.

49 Z. Luo, P. Li, J. Deng, N. Gao, Y. Zhang, H. Pan, L. Liu, C. Wang, L. Cai and Y. Ma, Cationic polypeptide micelle-based antigen delivery system: A simple and robust adjuvant to improve vaccine efficacy, *J. Controlled Release*, 2013, **170**, 259–267.

50 Z. Luo, C. Wang, H. Yi, P. Li, H. Pan, L. Liu, L. Cai and Y. Ma, Nanovaccine loaded with poly I:C and STAT3 siRNA robustly elicits anti-tumor immune responses through modulating tumor-associated dendritic cells *in vivo*, *Biomaterials*, 2015, **38**, 50–60.

51 K. Hyungjin, U. Tomofumi, A. Takami, B. Masanori and A. Mitsuru, Amphiphilic poly(amino acid) nanoparticles induce size-dependent dendritic cell maturation, *Adv. Funct. Mater.*, 2010, **20**, 3925–3931.

52 B. Layek, L. Lipp and J. Singh, APC targeted micelle for enhanced intradermal delivery of hepatitis B DNA vaccine, *J. Controlled Release*, 2015, **207**, 143–153.

53 Q. Zeng, H. Jiang, T. Wang, Z. Zhang, T. Gong and X. Sun, Cationic micelle delivery of Trp2 peptide for efficient lymphatic draining and enhanced cytotoxic T-lymphocyte responses, *J. Controlled Release*, 2015, **200**, 1–12.

54 M. Zhao, M. Li, Z. Zhang, T. Gong and X. Sun, Induction of HIV-1 gag specific immune responses by cationic micelles mediated delivery of gag mRNA, *Drug Delivery*, 2015, **23**(7), 2596–2607.

55 Z. Luo, S. Shi, L. Jin, L. Xu, J. Yu, H. Chen and X. Li, Cationic micelle based vaccine induced potent humoral immuneresponse through enhancing antigen uptake and formation of germinal center, *Colloids Surf., B*, 2015, **135**, 556–564.

56 S. Keller, J. T. Wilson, G. I. Patilea, H. B. Kern, A. J. Convertine and P. S. Stayton, Neutral polymer micelle carriers with pH-responsive, endosome-releasing activity modulate antigen trafficking to enhance CD8+ T cell responses, *J. Controlled Release*, 2014, **191**, 24–33.

57 S. Y. Kim, S. Kim, J. E. Kim, S. N. Lee, I. W. Shin, H. S. Shin, S. M. Jin, Y. W. Noh, Y. J. Kang, Y. S. Kim, T. H. Kang, Y. M. Park and Y. T. Lim, Lyophilizable and multifaceted toll-like receptor 7/8 agonist-loaded nanoemulsion for the reprogramming of tumor microenvironments and enhanced cancer immunotherapy, *ACS Nano*, 2019, **13**, 12671–12686.

58 N. Wang, M. Chen and T. Wang, Liposomes used as a vaccine adjuvant-delivery system: From basics to clinical immunization, *J. Controlled Release*, 2019, **303**, 130–150.

59 A. Chopra and G. Cevc, Non-invasive, epicutaneous immunisation with toxoid in deformable vesicles protects mice against tetanus, chiefly owing to a Th_2 response, Eur, *J. Pharm. Sci.*, 2014, **56**, 55–64.

60 J. J. Moon, H. Suh, A. Bershteyn, M. T. Stephan, H. Liu, B. Huang, M. Sohail, S. Luo, S. Ho Um, H. Khant, J. T. Goodwin, J. Ramos, W. Chiu and D. J. Irvine, Inter bilayer crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses, *Nat. Mater.*, 2011, **10**, 243–251.

61 T. Wang, Y. Y. Zhen, X. Y. Ma, B. Wei and N. Wang, Phospholipid bilayer-coated aluminum nanoparticles as an effective vaccine adjuvant-delivery system, *ACS Appl. Mater. Interfaces*, 2015, **7**, 6391–6396.

62 R. N. Germain, Vaccines and the future of human immunology, *Immunity*, 2010, **33**, 441–450.

63 A. E. Gregory, R. Titball and D. Williamson, Vaccine delivery using nanoparticles, *Front. Cell. Infect. Microbiol.*, 2013, **3**(13), DOI: 10.3389/fcimb.2013.00013.

64 T. Mazumdar, K. Anam and N. Ali, Influence of phospholipid composition on the adjuvanticity and protective efficacy of liposome-encapsulated *Leishmania donovani* antigens, *J. Parasitol.*, 2005, **91**, 269–274.

65 R. Kaur, V. W. Bramwell, D. J. Kirby and Y. Perrie, Pegylation of DDA:TDB liposomal adjuvants reduces the vaccine depot effect and alters the Th1/Th2 immune responses, *J. Controlled Release*, 2012, **158**, 72–77.

66 T. Wang and N. Wang, Preparation of the multifunctional liposome-containing microneedle arrays as an Oral cavity mucosal vaccine adjuvant-delivery system, *Methods Mol. Biol.*, 2016, **1404**, 651–667.

67 B. S. Pattni, V. V. Chupin and V. P. Torchilin, New developments in liposomal drug delivery, *Chem. Rev.*, 2015, **115**, 10938–10966.

68 N. Wang, Y. Zhen, Y. Jin, X. Wang, N. Li, S. Jiang and T. Wang, Combining different types of multifunctional liposomes loaded with ammonium bicarbonate to fabricate microneedle arrays as a vaginal mucosal vaccine

adjuvant-dual delivery system (VADDS), *J. Controlled Release*, 2017, **246**, 12–29.

69 F. S. Hassane, A. Phalipon, M. Tanguy, C. Guerreiro, F. Belot, B. Frisch, L. A. Mulard and F. Schuber, Rational design and immunogenicity of liposome-based diepitope constructs: application to synthetic oligosaccharides mimicking the Shigella flexneri 2a O-antigen, *Vaccine*, 2009, **27**, 5419–5426.

70 A. Vangala, V. W. Bramwell, S. McNeil, D. Christensen, E. M. Agger and Y. Perrie, Comparison of vesicle based antigen delivery systems for delivery of hepatitis B surface antigen, *J. Controlled Release*, 2007, **119**, 102–110.

71 M. T. Orr, C. B. Fox, S. L. Baldwin, S. J. Sivananthana, E. Lucas, S. Lin, T. Phan, J. J. Moon, T. S. Vedvick, S. G. Reeda and R. N. Coler, Adjuvant formulation structure and composition are critical for the development of an effective vaccine against tuberculosis, *J. Controlled Release*, 2013, **172**, 190–200.

72 C. R. Alving, M. Rao, N. J. Steers, G. R. Matyas and A. V. Mayorov, Liposomes containing lipid A: An effective, safe, generic adjuvant system for synthetic vaccines, *Expert Rev. Vaccines*, 2012, **11**, 733–744.

73 D. Christensen, L. B. Hansen, R. Leboux, W. Jiskoot, J. P. Christensen, P. Andersen, J. Dietrich and A. Liposome-Based, Adjuvant containing two delivery systems with the ability to induce mucosal immunoglobulin A following a parenteral immunization, *ACS Nano*, 2019, **13**, 1116–1126.

74 T. Kang, Y. Huang, Q. Zhu, H. Cheng, Y. Pei, J. Feng, M. Xu, G. Jiang, Q. Song, T. Jiang, H. Chen, X. Gao and J. Chen, Necroptotic cancer cells-mimicry nanovaccine boosts anti-tumor immunity with tailored immune-stimulatory modality, *Biomaterials*, 2018, **164**, 80–97.

75 H. Zhao, B. Zhao, L. Wu, H. Xiao, K. Ding, C. Zheng, Q. Song, L. Sun, L. Wang and Z. Zhang, Amplified cancer immunotherapy of a surface-engineered antigenic microparticle vaccine by synergistically modulating tumor microenvironment, *ACS Nano*, 2019, **13**, 12553–12566.

76 X. Huang, T. Yuan, M. Tschannen, Z. Sun, H. Jacob, M. Du, M. Liang, R. L. Dittmar, Y. Liu and M. Kohli, *et al.*, Characterization of human plasma-derived exosomal RNAs by deep sequencing, *BMC Genom.*, 2013, **14**, 319.

77 T. Kogure, I. K. Yan, W. L. Lin and T. Patel, Extracellular vesicle-mediated transfer of a novel long noncoding RNA TUC339: A mechanism of intercellular signaling in human hepatocellular cancer, *Genes Cancer*, 2013, **4**, 261–272.

78 Y. Sato-Kuwara, S. A. Melo, F. A. Soares and G. A. Calin, The fusion of two worlds: Non-coding RNAs and extracellular vesicles—Diagnostic and therapeutic implications, *Int. J. Oncol.*, 2014, **46**, 17–27.

79 A. K. Ludwig and B. Giebel, Exosomes: Small vesicles participating in intercellular communication, *Int. J. Biochem. Cell Biol.*, 2012, **44**, 11–15.

80 M. Simons and G. Raposo, Exosomes – vesicular carriers for intercellular communication, *Curr. Opin. Cell Biol.*, 2009, **21**, 575–581.

81 F. Chalmin, S. Ladoire, G. Mignot, J. Vincent, M. Bruchard and J.-P. Remy-Martin, *et al.*, Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells, *J. Clin. Invest.*, 2010, **120**, 457.

82 S. Montaner-Tarbes, F. E. Borras, M. Montoya, L. Fraile and H. A. Del Portillo, Serum-derived exosomes from non-viremic animals previously exposed to the porcine respiratory and reproductive virus contain antigenic viral proteins, *Vet. Res.*, 2016, **47**, 59.

83 K. Cheng, Q. Kang and X. Zhao, Biogenic nanoparticles as immunomodulator for tumor treatment, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2020, **12**, e1646.

84 M. J. H. Gerritzen, D. E. Martens, R. H. Wijffels, L. Van der Pold and M. Storka, Bioengineering bacterial outer membrane vesicles as vaccine platform, *Biotechnol. Adv.*, 2017, **35**, 565–574.

85 S. Stremersch, S. C. De Smedt and K. Raemdonck, Therapeutic and diagnostic applications of extracellular vesicles, *J. Controlled Release*, 2016, **244**, 167–183.

86 K. Tan, R. Li, X. Huang and Q. Liu, Outer membrane vesicles: Current status and future direction of these novel vaccine adjuvants, *Front. Microbiol.*, 2018, **9**, 783.

87 A. Kulp and M. J. Kuehn, Biological functions and biogenesis of secreted bacterial outer membrane vesicles, *Annu. Rev. Microbiol.*, 2010, **64**, 163–184.

88 R. S. Munford, Endotoxemia—Menace, marker, or mistake?, *J. Leukoc. Biol.*, 2016, **100**, 687–698.

89 A. Zariri, E. Pupo, E. Van Riet, J. P. Van Putten and P. Van der Ley, Modulating endotoxin activity by combinatorial bioengineering of meningococcal lipopolysaccharide, *Sci. Rep.*, 2016, **6**, 36575.

90 T. Y. Lee, C. U. Kim, E. H. Bae, S. H. Seo, D. G. Jeong, S. W. Yoon, K. T. Chang, Y. S. Kim, S. H. Kim and D. J. Kim, Outer membrane vesicles harboring modified lipid A moiety augment the efficacy of an influenza vaccine exhibiting reduced endotoxicity in a mouse model, *Vaccine*, 2017, **35**, 586–595.

91 A. Comberlato, K. Paloja and M. M. C. Bastings, Nucleic acids presenting polymer nanomaterials as vaccine adjuvants, *J. Mater. Chem. B*, 2019, **7**, 6321.

92 S. Guo, D. Fu, A. Utupova, D. Sun, M. Zhou, Z. Jin and K. Zhao, Applications of polymer-based nanoparticles in vaccine field, *Nanotechnol. Rev.*, 2019, **8**, 143–155.

93 A. Gutjahr, C. Phelip, A.-L. Coolen, C. Monge, A.-S. Boisgard, S. Paul and B. Verrier, Biodegradable polymeric nanoparticles-based vaccine adjuvants for lymph nodes targeting, *Vaccines*, 2016, **4**, 34.

94 J. Han, D. Zhao, D. Li, X. Wang, Z. Jin and K. Zhao, Polymer-based nanomaterials and applications for vaccines and drugs, *Polymers*, 2018, **10**, 31.

95 B. Sun, S. Yu, D. Zhao, S. Guo, X. Wang and K. Zhao, Polysaccharides as vaccine adjuvants, *Vaccine*, 2018, **36**, 5226–5234.

96 X. Wei, J. Liao, Z. Davoudi, H. Zheng, J. Chen, D. Li, X. Xiong, Y. Yin, X. Yu, J. Xiong and Q. Wang, Folate

receptor-targeted and GSH-responsive carboxymethyl chitosan nanoparticles containing covalently entrapped 6-mercaptopurine for enhanced intracellular drug delivery in leukemia, *Mar. Drugs*, 2018, **16**, 439, DOI: 10.3390/md16110439.

97 E. A. Scott, A. Stano, M. Gillard, A. C. Maio-Liu, M. A. Swartz and J. A. Hubbell, Dendritic cell activation and T cell priming with adjuvant- and antigen-loaded oxidation-sensitive polymersomes, *Biomaterials*, 2012, **33**, 6211–6219.

98 P. Li, Z. Luo, P. Liu, N. Gao, Y. Zhang, H. Pan, L. Liu, C. Wang, L. Cai and Y. Ma, Bioreducible alginate-poly(ethylenimine) nanogels as an antigen-delivery system robustly enhance vaccine-elicited humoral and cellular immune responses, *J. Controlled Release*, 2013, **168**, 271–279.

99 B. M. Discher, Y. Y. Won, D. S. Ege, J. C. M. Lee, F. S. Bates, D. E. Discher and D. A. Hammer, Polymersomes: tough vesicles made from diblock copolymers, *Science*, 1999, **284**, 1143–1146.

100 R. Sharma, U. Agrawal, N. Mody and S. P. Vyas, Polymer nanotechnology based approaches in mucosal vaccine delivery: Challenges and opportunities, *Biotechnol. Adv.*, 2015, **33**, 64–79.

101 K. Zhao, R. Guangyu, Q. Teng, X. Li, H. Lan, L. Yu, S. Yu, Z. Jin, G. Chen and Z. Li, Dendrigraft poly-L-lysines delivery of DNA vaccine effectively enhances the immunogenic responses against H9N2 avian influenza virus infection in chickens, *Nanomedicine*, 2020, **27**, 102209.

102 J. S. Chahal, O. F. Khan, C. L. Cooper, J. S. McPartlan, J. K. Tsosie, L. D. Tilley, S. M. Sidik, S. Lourido, R. Langer, S. Bavari, H. L. Ploegh and D. G. Anderson, Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, E4133–E4142.

103 A. M. Carmona-Ribeiro and Y. Pérez-Betancourt, Cationic nanostructures for vaccines design, *Biomimetics*, 2020, **5**, 32.

104 R. J. C. Bose, M. Kim, J. H. Chang, R. Paulmurugan, J. J. Moon, W. G. Koh, S. H. Lee and H. Park, Biodegradable polymers for modern vaccine development, *J. Ind. Eng. Chem.*, 2019, **77**, 12–24.

105 M. Khansarizadeh, A. Mokhtarzadeh, M. Rashedinia, S. M. Taghdisi, P. Lari, K. H. Abnous and M. Ramezani, Identification of possible cytotoxicity mechanism of polyethylenimine by proteomics analysis, *Hum. Exp. Toxicol.*, 2016, **35**, 377–387.

106 F. G. Torres, O. P. Troncoso, A. Pisani, F. Gatto and G. Bardi, Natural polysaccharide nanomaterials: An overview of their immunological properties, *Int. J. Mol. Sci.*, 2019, **20**, 5092.

107 G. Sharma, D. T. Valenta, Y. Altman, S. Harvey, H. Xie, S. Mitragotri and J. W. Smith, Polymer particle shape independently influences binding and internalization by macrophages, *J. Controlled Release*, 2010, **147**, 408–412.

108 J. A. Champion and S. Mitragotri, Role of target geometry in phagocytosis, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 4930–4934.

109 Z. G. Yue, W. Wei, P. P. Lv, H. Yue, L. Y. Wang, Z. G. Su and G. H. Ma, Surface charge affects cellular uptake and intracellular trafficking of chitosan-based nanoparticles, *Biomacromolecules*, 2011, **12**, 2440–2446.

110 A. L. Siefert, M. J. Caplan and T. M. Fahmy, Artificial bacterial biomimetic nanoparticles synergize pathogen associated molecular patterns for vaccine efficacy, *Biomaterials*, 2016, **97**, 85–96.

111 Z. Jin, S. Gao, X. Cui, D. Sun and K. Zhao, Adjuvants and delivery systems based on polymeric nanoparticles for mucosal vaccines, *Int. J. Pharm.*, 2019, **572**, 118731.

112 P. Sharma, N. Y. Jang, J. W. Lee, B. C. Park, Y. K. Kim and N. H. Cho, Application of ZnO-based nanocomposites for vaccines and cancer immunotherapy, *Pharmaceutics*, 2019, **11**, 493.

113 S. A. C. Carabineiro, Applications of gold nanoparticles in nanomedicine: Recent advances in vaccines, *Molecules*, 2017, **22**, 857.

114 L. A. Dykman, Gold nanoparticles for preparation of antibodies and vaccines against infectious diseases, *Expert Rev. Vaccines*, 2020, **19**, 465–477.

115 S. A. C. Carbineiro, Applications of gold nanoparticles in nanomedicine: Recent advances in vaccines, *Molecules*, 2017, **22**, 857, DOI: 10.3390/molecules22050857.

116 M. Zhu, L. Du, R. Zhao, H. Y. Wang, Y. Zhao, G. Nie and R. F. Wang, Cell-penetrating nanoparticles activate the inflammasome to enhance antibody production by targeting microtubule-associated protein 1-light chain 3 for degradation, *ACS Nano*, 2020, **14**, 3703–3717.

117 S. Afroz, H. Medhi, S. Maity, G. Minhas, S. Battu, J. Giddaluru, K. Kumar, P. Paik and N. Khan, Mesoporous ZnO nanocapsules for the induction of enhanced antigen-specific immunological responses, *Nanoscale*, 2017, **9**, 14641.

118 G. C. Carvalho, R. M. Sábio, T. D. C. Ribeiro, A. S. Monteiro, D. V. Pereira, S. J. L. Ribeiro and M. Chorilli, Highlights in mesoporous silica nanoparticles as a multifunctional controlled drug delivery nanoplatform for infectious diseases treatment, *Pharm. Res.*, 2020, **37**, 191, DOI: 10.1007/s11095-020-02917-6.

119 Q. Wang, H. Cheng, H. Peng, H. Zhou, P. Y. Li and R. Langer, Non-genetic engineering of cells for drug delivery and cell-based therapy, *Adv. Drug Delivery Rev.*, 2015, **91**, 125–140, DOI: 10.1016/j.addr.2014.12.003.

120 S. Gurunathan, M. H. Kang and J. H. Kim, Diverse Effects of Exosomes on COVID-19: A Perspective of Progress From Transmission to Therapeutic Developments, *Front. immunol.*, 2021, **12**, 716407.

121 M. K. Saadeldin, A. K. Abdel-Aziz and A. Abdellatif, Dendritic cell vaccine immunotherapy; the beginning of the end of cancer and COVID-19. A hypothesis, *Med. Hypotheses*, 2021, **146**, 110365.

122 M. S. Choudhery and D. T. Harris, Stem cell therapy for COVID-19: Possibilities and challenges, *Cell Biol. Int.*, 2020, **44**, 2182–2191.

123 K. T. Gause, A. K. Wheatley, J. Cui, Y. Yan, D. J. Kent and F. Caruso, Immunological principles guiding the rational

design of particles for vaccine delivery, *ACS Nano*, 2017, **11**, 54–68.
124 A. C. Gomes, M. Mohsen and M. F. Bachmann, Harnessing nanoparticles for immunomodulation and vaccines, *Vaccines*, 2017, **5**, 6.
125 S. Singha, K. Shao, K. K. Ellestad, Y. Yang and P. Santamaria, Nanoparticles for immune stimulation against infection, cancer, and autoimmunity, *ACS Nano*, 2018, **12**, 10621–10635.
126 A. Tanaka and S. Sakaguchi, Targeting Treg cells in cancer immunotherapy, *Eur. J. Immunol.*, 2019, **49**, 1140–1146.
127 C. Thomas, V. Gupta and F. Ahsan, Influence of surface charge of PLGA particles of recombinant hepatitis B surface antigen in enhancing systemic and mucosal immune responses, *Int. J. Pharm.*, 2009, **379**, 41–50.
128 H. K. Dewangan, Rational application of nanoadjuvant for mucosal vaccine delivery system, *J. Immunol. Methods*, 2020, **481–482**, 112791.
129 N. Csaba, M. Garcia-Fuentes and M. J. Alonso, Nanoparticles for nasal vaccination, *Adv. Drug Delivery Rev.*, 2009, **61**, 140–157.
130 C. H. Huang, C. Y. Huang, H. M. Ho, C. H. Lee, P. T. Lai, S. C. Wu, S. J. Liu and M. H. Huang, Nanoemulsion adjuvantation strategy of tumor-associated antigen therapy rephrases mucosal and immunotherapeutic signatures following intranasal vaccination, *J. Immunother. Cancer*, 2020, **8**, e001022.
131 S. Hirosue and J. Dubrot, Modes of antigen presentation by lymph node stromal cells and their immunological implications, *Front. Immunol.*, 2015, **6**, 446.
132 S. N. Mueller, S. Tian and J. M. DeSimone, Rapid and persistent delivery of antigen by lymph node targeting PRINT nanoparticle vaccine carrier to promote humoral immunity, *Mol. Pharmaceutics*, 2015, **12**, 1356–1365.
133 S. H. Wang, J. Chen, D. Smith, Z. Cao, H. Acosta, Y. Fan, S. Ciotti, A. Fattom and J. Baker, A novel combination of intramuscular vaccine adjuvants, nanoemulsion and CpG produces an effective immune response against influenza A virus, *Vaccine*, 2020, **38**, 3537–3544.
134 A. Marabelle, H. Kohrt, C. Caux and R. Levy, Intratumoral immunization: A new paradigm for cancer therapy, *Clin. Cancer Res.*, 2014, **20**, 1747–1756.
135 Q. Zhoua, C. Dong, W. Fan, H. Jiang, J. Xiang, N. Qiu, Y. Piao, T. Xie, Y. Luo, Z. Li, F. Liu and Y. Shen, Tumor extravasation and infiltration as barriers of nanomedicine for high efficacy: The current status and transcytosis strategy, *Biomaterials*, 2020, **240**, 119902.
136 B. Slütter, L. Plapied, V. Fievez, M. A. Sande, A. des Rieux, Y. J. Schneider, E. Van Riet, W. Jiskoot and V. Préat, Mechanistic study of the adjuvant effect of biodegradable nanoparticles in mucosal vaccination, *J. Controlled Release*, 2009, **138**, 113–121.
137 V. Shalgunov, D. Zaytseva-Zotova, A. Zintchenko, T. Levada, Y. Shilov, D. Andreyev, D. Dzhumashev, E. Metelkin, A. Urusova, O. Demin, K. McDonnell, G. Troiano, S. Zale and E. Safarova, Comprehensive study of the drug delivery properties of poly(L-lactide)-poly(ethylene glycol) nanoparticles in rats and tumor-bearing mice, *J. Controlled Release*, 2017, **261**, 31–42.
138 *Shi*2021.
139 S. Ahmad, A. A. Zamry, H. T. T. Tana, K. K. Wong, J. Lim and R. Mohamud, Targeting dendritic cells through gold nanoparticles: A review on the cellular uptake and subsequent immunological properties, *Mol. Immunol.*, 2017, **91**, 123–133.
140 T. Tong, Y. Qi, L. B. Bussiere, M. Wannemuehler, C. L. Miller, Q. Wang and C. Yu, Transport of artificial virus-like nanocarriers through intestinal monolayers *via* microfold cells, *Nanoscale*, 2020, **12**, 16339–16347.
141 M. A. Liu, A comparison of plasmid DNA and mRNA as vaccine technologies, *Vaccines*, 2019, **7**, 37.
142 R. Madan-Lala, P. Pradhan and K. Roy, Combinatorial delivery of dual and triple TLR agonists *via* polymeric pathogen-like particles synergistically enhances innate and adaptive immune responses, *Sci. Rep.*, 2017, **7**, 2530.
143 A. Vasou, N. Sultanoglu, S. Goodbourn, R. E. Randall and L. G. Kostrikis, Targeting pattern recognition receptors (PRR) for vaccine adjuvantation: From synthetic PRR agonists to the potential of defective interfering particles of viruses, *Viruses*, 2017, **9**, 186.
144 H. Fang, B. Ang, X. Xu, X. Huang, Y. Wu, Y. Sun, W. Wang, N. Li, X. Cao and T. Wan, TLR4 is essential for dendritic cell activation and anti-tumor T-cell response enhancement by DAMPs released from chemically stressed cancer cells, *Cell. Mol. Immunol.*, 2014, **11**, 150–159.
145 Y. Zhang, K. R. Hughes, R. M. Raghani, J. Ma, S. Orbach, J. S. Jeruss and L. D. Shea, Cargo-free immunomodulatory nanoparticles combined with anti-PD-1 antibody for treating metastatic breast cancer, *Biomaterials*, 2021, **269**, 120666.
146 A. Schudel, D. M. Francis and S. N. Thomas, Material design for lymph node drug delivery, *Nat. Rev. Mater.*, 2019, **4**, 415–428.
147 T. Fifis, A. Gamvrellis, B. Crimeen-Irwin, G. A. Pietersz, J. Li, P. L. Mottram, I. F. C. McKenzie and M. Plebanski, Size-dependent immunogenicity: Therapeutic and protective properties of nano-vaccines against tumors, *J. Immunol.*, 2004, **173**, 3148–3154.
148 T. Hirai, Y. Yoshioka, H. Takahashi, K. Ichihashi, T. Yoshida, S. Tochigi, K. Nagano, Y. Abe, H. Kamada, S. Tsunoda, H. Nabeshi, T. Yoshikawa and Y. Tsutsumi, Amorphous silica nanoparticles enhance cross-presentation in murine dendritic cells, *Biochem. Biophys. Res. Commun.*, 2012, **427**, 553–556.
149 A. Mant, F. Chinnery, T. Elliott and A. P. Williams, The pathway of cross-presentation is influenced by the particle size of phagocytosed antigen, *Immunology*, 2012, **136**, 163–175.
150 K. Niikura, T. Matsunaga, T. Suzuki, S. Kobayashi, H. Yamaguchi, Y. Orba, A. Kawaguchi, H. Hasegawa, K. Kajino, T. Ninomiya, K. Ijiro and H. Sawa, Gold nanoparticles as a vaccine platform: Influence of size and shape

on immunological responses in vitro and in vivo, *ACS Nano*, 2013, **7**, 3926–3938.

151 C. Foged, C. Arigita, A. Sundblad, W. Jiskoot, G. Storm and S. Frokjaer, Interaction of Dendritic Cells with Antigen-Containing Liposomes: Effect of Bilayer Composition, *Vaccine*, 2004, **22**, 1903–1913.

152 C. Foged, B. Brodin, S. Frokjaer and A. Sundblad, Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model, *Int. J. Pharm.*, 2005, **298**, 315–322.

153 S. Y. Seong and P. Matzinger, Hydrophobicity: An ancient damage-associated molecular pattern that initiates innate immune responses, *Nat. Rev. Immunol.*, 2004, **4**, 469–478.

154 P. Matzinger, The danger model: A renewed sense of self, *Science*, 2002, **296**, 301–305.

155 (*a*) D. F. Moyano, M. Goldsmith, D. J. Solfiell, D. Landesman-Milo, O. R. Miranda, D. Peer and V. M. Rotello, Nanoparticle hydrophobicity dictates immune response, *J. Am. Chem. Soc.*, 2012, **134**, 3965–3967; (*b*) B. Pulendran, P. S. Arunachalam and D. T. O. Hagan, Emerging concepts in the science of vaccine adjuvants, *Nat. Rev. Drug Discovery*, 2021, **20**, 454–475.

156 Y. Liu, Y. Yin, L. Y. Wang, W. F. Zhang, X. M. Chen, X. X. Yang, J. J. Xu and G. H. Ma, Surface hydrophobicity of microparticles modulates adjuvanticity, *J. Mater. Chem. B*, 2013, **1**, 3888–3896.

157 L. K. Petersen, A. E. Ramer-Tait, S. R. Broderick, C. S. Kong, B. D. Ulery, K. Rajan, M. J. Wannemuehler and B. Narasimhan, Activation of innate immune responses in a pathogen-mimicking manner by amphiphilic polyanhydride nanoparticle adjuvants, *Biomaterials*, 2011, **32**, 6815–6822.

158 V. B. Joshi, S. M. Geary and A. K. Salem, Biodegradable particles as vaccine delivery systems: size matters, *AAPS J.*, 2013, **15**, 85–94.

159 C. Foged, B. Brodin, S. Frokjaer and A. Sundblad, Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model, *Int. J. Pharm.*, 2005, **298**, 315–322.

160 M. Pei, J. Liang, C. Zhang, X. Wang, C. Zhang, G. Ma and H. Sun, Chitosan/calcium phosphates nanosheet as a vaccine carrier for effective cross-presentation of exogenous antigens, *Carbohydr. Polym.*, 2019, **224**, 115172.

161 J. Yue, L. Liang, Y. Shen, X. Guan, J. Zhang, Z. Li, R. Deng, S. Xu, C. Liang, W. Shi and W. Xu, Investigating dynamic molecular events in melanoma cell nucleus during photodynamic therapy by SERS, *Front. Chem.*, 2019, **6**, 665.

162 Y. Nishioka and H. Yoshino, Lymphatic targeting with nanoparticulate system, *Adv. Drug Delivery Rev.*, 2001, **47**, 55–64, DOI: 10.1016/S0169-409X(00)00121-6148.

163 V. Manolova, A. Flace, M. Bauer, K. Schwarz, P. Saudan and M. F. Bachmann, Nanoparticles target distinct dendritic cell populations according to their size, *Eur. J. Immunol.*, 2008, **38**, 1404–1413, DOI: 10.1002/eji.200737984.

164 R. S. Raghuvanshi, Y. K. Katare, K. Lalwani, M. M. Ali, O. Singh and A. K. Panda, Improved immune response from biodegradable polymer particles entrapping tetanus toxoid by use of different immunization protocol and adjuvants, *Int. J. Pharm.*, 2002, **245**, 109–121, DOI: 10.1016/S0378-5173(02)00342-3.

165 F. Shima, T. Akagi and M. Akashi, Effect of hydrophobic side chains in the induction of immune responses by nanoparticle adjuvants consisting of amphiphilic poly(γ-glutamic acid), *Bionconjugate Chem.*, 2015, **26**, 890–898.

166 H. W. Despres, A. Sabra, P. Anderson, U. D. Hemraz, Y. Boluk, R. Sunasee and K. Ckless, Mechanisms of the immune response cause by cationic and anionic surface functionalized cellulose nanocrystals using cell-based assays, *Toxicol. In Vitro*, 2019, **55**, 124–133.

167 M. P. Chao, C. H. Takimoto, D. D. Feng, K. McKenna, P. Gip, J. Liu, J. P. Volkmer, I. L. Weissman and R. Majeti, Therapeutic targeting of the macrophage immune checkpoint CD47 in myeloid malignancies, *Front. Oncol.*, 2020, **9**, 1380.

168 S. Agrawal and E. R. Kandimalla, Synthetic agonists of Toll-like receptors 7, 8 and 9, *Biochem. Soc. Trans.*, 2007, **35**, 1461–1467.

169 G. Hajishengallis and J. D. Lambris, Crosstalk pathways between Toll-like receptors and the complement system, *Trends Immunol.*, 2010, **31**, 154–163.

170 B. Pulendran, P. S. Arunachalam and D. T. O. Hagan, Emerging concepts in the science of vaccine adjuvants, *Nat. Rev. Drug Discovery*, 2021, **20**(6), 454–475.

171 Z. B. Wang and J. Xu, Better adjuvants for better vaccines: Progress in adjuvant delivery systems, modifications, and adjuvant–antigen codelivery, *Vaccines*, 2020, **8**, 128.

172 R. Billeskov, Y. Wang, S. Solaymani-Mohammadi, B. Frey, S. Kulkarni, P. Andersen, E. M. Agger, Y. Sui and J. A. Berzofsky, Low antigen dose in adjuvant-based vaccination selectively induces CD4 T cells with enhanced functional avidity and protective efficacy, *J. Immunol.*, 2017, **198**, 3494–3506.

173 Y. W. Noh, J. Hyun Hong, S. M. Shim, H. S. Park, H. H. Bae, E. Kyoung, R. Jung, H. Hwang, C. H. Lee, S. H. Cho, M. H. Sung, H. Poo and Y. T. Lim, Polymer nanomicelles for efficient mucus delivery and antigen-specific high mucosal immunity, *Angew. Chem., Int. Ed.*, 2013, **52**, 7684–7689.

174 N. Petrovsky, Comparative safety of vaccine adjuvants: A summary of current evidence and future needs, *Drug Saf.*, 2015, **38**, 1059–1074.

175 K. D. Brewer, G. M. Weir, I. Dude, C. Davis, C. Parsons, A. Penwell, R. Rajagopalan, L. Sammatur, C. V. Bowen and M. M. Stanford, Unique depot formed by an oil based vaccine facilitates active antigen uptake and provides effective tumour control, *J. Biomed. Sci.*, 2018, **25**, 7.

176 C. B. Fox, S. J. Sivananthan, M. S. Duthie, J. Vergara, J. A. Guderian, E. Moon, D. Coblentz, S. G. Reed and D. Carter, A nanoliposome delivery system to synergistically trigger TLR4 AND TLR7, *J. Nanobiotechnol.*, 2014, **12**, 17.

177 S. Renu, N. Feliciano-Ruiz, F. Lu, S. Ghimire, Y. Han, J. Schrock, S. Dhakal, V. Patil, S. Krakowka,

H. HogenEsch and G. J. Renukaradhya, A nanoparticle-poly(I:C) combination adjuvant enhances the breadth of the immune response to inactivated influenza virus vaccine in pigs, *Vaccines*, 2020, **8**, 229.

178 E. Ahmadpour, S. Sarvi, M. B. H. Soteh, M. Sharif, M. T. Rahimid, R. Valadan, M. Tehranic, A. Khalilian, M. Montazeri, M. Fasihi-Ramandi and A. Daryani, Enhancing immune responses to a DNA vaccine encoding Toxoplasma-gondii GRA14 by calcium phosphate nanoparticles as an adjuvant, *Immunol. Lett.*, 2017, **185**, 40–47.

179 X. Zhong, Y. Zhang, L. Tan, T. Zheng, Y. Hou, X. Hong, G. Du, X. Chen, Y. Zhang and X. Sun, An aluminum adjuvant-integrated nano-MOF as antigen delivery system to induce strong humoral and cellular immune responses, *J. Controlled Release*, 2019, **300**, 81–92.

180 E. Alphandéry, The potential of various nanotechnologies for coronavirus diagnosis/treatment highlighted through a literature analysis, *Bioconjugate Chem.*, 2020, **31**, 1873–1882.

181 M. Bai, H. Dong, X. Su, Y. Jin, S. Sun, Y. Zhang, Y. Yang and H. Guo, Hollow mesoporous silica nanoparticles as delivery vehicle of foot-and-mouth disease virus-like particles induce persistent immune responses in guinea pigs, *J. Med. Virol.*, 2019, **91**, 941–948.

182 A. Bansal, W. Gamal, I. J. Menon, V. Olson, X. Wu and M. J. D'Souzaa, Laser-assisted skin delivery of immunocontraceptive rabies nanoparticulate vaccine in poloxamer gel, *Eur. J. Pharm. Sci.*, 2020, **155**, 105560.

183 A. Brotons-Canto, C. Gamazo, N. Martín-Arbella, M. Abdulkarim, M. Gumbleton, G. Quincoces, I. Peñuelas and J. M. Irache, *J. Pharm. Sci.*, 2019, **108**, 2421–2429.

184 S. Chattopadhyay, Y. H. Liu, Z. S. Fang, C. L. Lin, J. C. Lin, B. Y. Yao and C. M. J. Hu, Synthetic immunogenic cell death mediated by intracellular delivery of STING agonist nanoshells enhances anticancer chemoimmunotherapy, *Nano Lett.*, 2020, **20**, 2246–2256.

185 Q. Chen, L. Xu, C. Liang, C. Wang, R. Peng and Z. Liu, Photothermal therapy with immune-adjuvant nanoparticles together with checkpoint blockade for effective cancer immunotherapy, *Nat. Commun.*, 2016, **7**, 13193.

186 W. Chen, H. Zuo, T. J. Mahony, B. Zhang, B. Rolfe and Z. P. Xu, Efficient induction of comprehensive immune responses to control pathogenic *E. coli* by clay nanoadjuvant with the moderate size and surface charge, *Sci. Rep.*, 2017, **7**, 13367.

187 Y. Cheng, Q. Chen, Z. Guo, M. Li, X. Yang, G. Wan, H. Chen, Q. Zhang and Y. Wang, An intelligent biomimetic nanoplatform for holistic treatment of metastatic triple-negative breast cancer via photothermal ablation and immune remodeling, *ACS Nano*, 2020, **14**, 15161–15181.

188 T. G. Dacoba, R. W. Omange, H. Li, J. Crecente-Campo, M. Luo and M. J. Alonso, Polysaccharide nanoparticles can efficiently modulate the immune response against an HIV peptide antigen, *ACS Nano*, 2019, **13**, 4947–4959.

189 S. Dhakal, F. Lu, S. Ghimire, S. Renu, Y. S. Lakshmanappa, B. T. Hogshead, D. Ragland, H. HogenEsch and G. J. Renukaradhya, Corn-derived alpha-D-glucan nanoparticles as adjuvant for intramuscular and intranasal immunization in pigs, *Nanomedicine*, 2019, **16**, 226–235.

190 Y. Fan, S. M. Stronsky, Y. Xu, J. T. Steffens, S. A. Van Tongeren, A. Erwin, C. L. Cooper and J. J. Moon, Multilamellar vaccine particle elicits potent immune activation with protein antigens and protects mice against ebola virus infection, *ACS Nano*, 2019, **13**, 11087–11096.

191 F. Fontana, M. Fusciello, C. Groeneveldt, C. Capasso, J. Chiaro, S. Feola, Z. Liu, E. E. Mäkilä, J. J. Salonen, J. T. Hirvonen, V. Cerullo and H. A. Santos, Biohybrid Vaccines for Improved Treatment of Aggressive Melanoma with Checkpoint Inhibitor, *ACS Nano*, 2019, **13**, 6477–6490.

192 P. Hart, A. Copland, G. R. Diogo, S. Harris, R. Spallek, W. Oehlmann, M. Singh, J. Basile, M. Rottenberg, M. J. Paul and R. Reljic, Nanoparticle-fusion protein complexes protect against *Mycobacterium tuberculosis* infection, *Mol. Ther.*, 2018, **26**, 822–833.

193 H. Jiang, Q. Wang, L. Li, Q. Zeng, H. Li, T. Gong, Z. Zhang and X. Sun, Turning the old adjuvant from gel to nanoparticles to amplify CD8+ T cell responses, *Adv. Sci.*, 2018, **5**, 1700426.

194 X. H. Jin, L. L. Zheng, M. R. Song, W. S. Xu, Y. N. Kou, Y. Zhou, L. W. Zhang, Y. N. Zhu, B. Wan, Z. Y. Wei and G. P. Zhang, Nano silicon adjuvant enhances inactivated transmissible gastroenteritis vaccine through activation the Toll-like receptors and promotes humoral and cellular immune responses, *Nanomedicine*, 2018, **14**, 1201–1212.

195 T. Kang, Y. Huang, Q. Zhu, H. Cheng, Y. Pei, J. Feng, M. Xu, G. Jiang, Q. Song, T. Jiang, H. Chen, X. Gao and J. Chen, Necroptotic cancer cells-mimicry nanovaccine boosts anti-tumor immunity with tailored immune-stimulatory modality, *Biomaterials*, 2018, **164**, 80e97.

196 Y. F. Kang, C. Sun, Z. Zhuang, R. Y. Yuan, Q. Zheng, J. P. Li, P. P. Zhou, X. C. Chen, Z. Liu, X. Zhang, X.-H. Yu, X. W. Kong, Q. Y. Zhu, Q. Zhong, M. Xu, N. S. Zhong, Y. X. Zeng, G. K. Feng, C. Ke, J. C. Zhao and M. S. Zeng, Rapid development of SARS-CoV-2 spike protein receptor-binding domain self-assembled nanoparticle vaccine candidates, *ACS Nano*, 2021, **15**, 2738–2752.

197 S. Y. Kim, S. Kim, J. E. Kim, S. N. Lee, I. W. Shin, H. S. Shin, S. M. Jin, Y. W. Noh, Y. J. Kang, Y. S. Kim, T. H. Kang, Y. M. Park and Y. T. Lim, Lyophilizable and multifaceted toll-like receptor 7/8 agonist-loaded nanoemulsion for the reprogramming of tumor microenvironments and enhanced cancer immunotherapy, *ACS Nano*, 2019, **13**, 12671–12686.

198 F. C. Knight, P. Gilchuk, A. Kumar, K. W. Becker, S. Sevimli, M. E. Jacobson, N. Suryadevara, L. Wang-Bishop, K. L. Boyd, J. E. Crowe, S. Joyce and J. T. Wilson, Mucosal immunization with a pH-responsive nanoparticle vaccine induces protective CD8+ lung-resident memory T cells, *ACS Nano*, 2019, **13**, 10939–10960.

199 Q. V. Le, J. Suh, J. J. Choi, G. T. Park, J. W. Lee, G. Shim and Y. K. Oh, In situ nanoadjuvant-assembled tumor vaccine for preventing long-term recurrence, *ACS Nano*, 2019, **13**, 7442–7462.

200 M. E. Lebel, K. Chartrand, E. Tarrab, P. Savard, D. Leclerc and A. Lamarre, Potentiating cancer immunotherapy using papaya mosaic virus-derived nanoparticles, *Nano Lett.*, 2016, **16**, 1826–1832.

201 J. W. Lim, W. Na, H. O. Kim, M. Yeom, A. Kang, G. Park, C. Park, J. Ki, S. Lee, B. Jung, H. H. Jeong, D. Park, D. Song and S. Haam, Co-delivery of antigens and immunostimulants via a polymersome for improvement of antigen-specific immune response, *J. Mater. Chem. B*, 2020, **8**, 5620.

202 F. Lu, Y. Y. C. Mosley, R. J. R. Rosales, B. E. Carmichael, S. Elesela, Y. Yao and H. HogenEsch, Alpha-D-glucan nanoparticulate adjuvant induces a transient inflammatory response at the injection site and targets antigen to migratory dendritic cells, *npj Vaccines*, 2017, **2**, 4.

203 L. Luo, C. Zhu, H. Yin, M. Jiang, J. Zhang, B. Qin, Z. Luo, X. Yuan, J. Yang, W. Li, Y. Du and J. You, Laser immunotherapy in combination with perdurable PD-1 blocking for the treatment of metastatic tumors, *ACS Nano*, 2018, **12**, 7647–7662.

204 C. Meng, X. Zhi, C. Li, C. Li, Z. Chen, X. Qiu, C. Ding, L. Ma, H. Lu, D. Chen, G. Liu and D. Cui, Graphene oxides decorated with carnosine as an adjuvant to modulate innate immune and improve adaptive immunity in vivo, *ACS Nano*, 2016, **10**, 2203–2213.

205 H. Nabi, I. Rashid, N. Ahmad, A. Durrani, H. Akbar, S. Islam, A. A. Bajwa, W. Shehzad, K. Ashraf and N. Imran, Induction of specific humoral immune response in mice immunized with ROP18 nanospheres from Toxoplasma gondii, *Parasitology*, 2017, **116**, 359–370.

206 R. J. Nevagi, Z. G. Khalil, W. M. Hussein, J. Powell, M. R. Batzloff, R. J. Capon, M. F. Good, M. Skwarczynski and I. Toth, Polyglutamic acid-trimethyl chitosan-based intranasal peptide nanovaccine induces potent immune responses against group A streptococcus, *Acta Biomater.*, 2018, **80**, 278–287.

207 H. I. Ng, G. J. P. Fernando, A. C. I. Depelsenaire and M. A. F. Kendall, Potent response of QS-21 as a vaccine adjuvant in the skin when delivered with the Nanopatch, resulted in adjuvant dose sparing, *Sci. Rep.*, 2016, **6**, 29368.

208 D. Jenkins, S. Corrie, C. Flaima and M. Kendallac, High density and high aspect ratio solid micro-nanoprojection arrays for targeted skin vaccine delivery and specific antibody extraction, *RSC Adv.*, 2012, **2**, 3490–3495.

209 M. A. Oberli, A. M. Reichmuth, J. R. Dorkin, M. J. Mitchell, O. S. Fenton, A. Jaklenec, D. G. Anderson, R. Langer and D. Blankschtein, Lipid nanoparticle assisted mRNA delivery for potent cancer immunotherapy, *Nano Lett.*, 2017, **17**, 1326–1335.

210 A. S. Pagheh, A. Daryani, P. Alizadeh, H. Hassannia, S. M. R. Oliveira, T. Kazemi, F. Rezaei, M. D. L. Pereira and E. Ahmadpour, Protective effect of a DNA vaccine cocktail encoding ROP13 and GRA14 with Alum nano-adjuvant against Toxoplasma gondii infection in mice, *Int. J. Biochem. Cell Biol.*, 2021, **132**, 105920.

211 Y. Pérez-Betancourt, B. C. L. F. Távora, M. Colombini, E. L. Faquim-Mauro and A. M. Carmona-Ribeiro, Simple nanoparticles from the assembly of cationic polymer and antigen as immunoadjuvants, *Vaccines*, 2020, **8**, 105.

212 Y. Qu, Y. Ju, C. Cortez-Jugo, Z. Lin, S. Li, J. Zhou, Y. Ma, A. Glab, S. J. Kent, F. Cavalieri and F. Caruso, Template-mediated assembly of DNA into microcapsules for immunological modulation, *Small*, 2020, **16**, 2002750.

213 M. T. Rahimi, S. Sarvi, M. Sharif, S. Abediankenari, E. Ahmadpour, R. Valadan, R. Fasihi- Ramandie, S. A. Hosseini and A. Daryani, Immunological evaluation of a DNA cocktail vaccine with co-delivery of calcium phosphate nanoparticles (CaPNs) against the Toxoplasma gondii RH strain in BALB/c mice, *Parasitol. Res.*, 2017, **116**, 609–616.

214 C. B. Roces, M. T. Hussain, S. T. Schmidt, D. Christensen and Y. Perrie, Investigating prime-pull vaccination through a combination of parenteral vaccination and intranasal boosting, *Vaccines*, 2020, **8**, 10.

215 L. Rodrigues, K. N. Raftopoulos, S. T. Schmidt, F. Schneidere, H. Dietze, T. Radesc, H. Franzyk, A. E. Pederseng, C. M. Papadakis, D. Christensen, G. Winter, C. Foged and M. Hubert, Immune responses induced by nano-self-assembled lipid adjuvants based on a monomycoloyl glycerol analogue after vaccination with the Chlamydia trachomatis major outer membrane protein, *J. Controlled Release*, 2018, **285**, 12–22.

216 M. Saydam, W. P. Cheng, N. Palmer, R. Tierney, R. Francis, K. MacLellan-Gibson, A. Khan and F. Mawas, Nano-sized Soluplus polymeric micelles enhance the induction of tetanus toxin neutralising antibody response following transcutaneous immunisation with tetanus toxoid, *Vaccine*, 2017, **35**, 2489–2495.

217 S. T. T. Schetters, W. S. P. Jong, S. K. Horrevorts, L. J. W. Kruijssen, S. Engels, D. Stolk, M. H. Daleke-Schermerhorn, J. Garcia-Vallejo, D. Houben, W. W. J. Unger, J. M. M. den Haan, J. Luirink and Y. Van Kooyk, Outer membrane vesicles engineered to express membrane-bound antigen program dendritic cells for cross-presentation to CD8+ T cells, *Acta Biomater.*, 2019, **91**, 248–257.

218 W. Shi, Y. Kou, J. Xiao, L. Zhang, F. Gao, W. Kong, W. Su, C. Jiang and Y. Zhang, Comparison of immunogenicity, efficacy and transcriptome changes of inactivated rabies virus vaccine with different adjuvants, *Vaccine*, 2018, **36**, 5020–5029.

219 Y. Sun, Y. Yin, L. Gong, Z. Liang, C. Zhu, C. Ren, N. Zheng, Q. Zhang, H. Liu, W. Liu, F. You, D. Lu and Z. Lin, Manganese nanodepot augments host immune response against coronavirus, *Nano Res.*, 2020, 1–13.

220 Z. Teng, S. Sun, H. Chen, J. Huang, P. Du, H. Dong, X. Xu, S. Mua, Z. Zhang and H. Guo, Golden-star nanoparticles as adjuvant effectively promotes immune response to foot-and-mouth disease virus-like particles vaccine, *Vaccine*, 2018, **36**, 6752–6760.

221 J. Tomporowski, J. M. Heer, B. Allan, S. Gomis and P. Aich, Carbon nanotubes significantly enhance the biological activity of CpG ODN in chickens, *Int. J. Pharm.*, 2019, **561**, 135–147.

222 H. Wang, Y. Ding, S. Su, D. Meng, A. Mujeeb, Y. Wu and G. Nie, Assembly of hepatitis E vaccine by 'in situ' growth of gold clusters as nano-adjuvants: An efficient way to enhance the immune responses of vaccination, *Nanoscale Horiz.*, 2016, **1**, 394.

223 K. Wang, S. Wen, L. He, A. Li, Y. Li, H. Dong, W. Li, T. Ren, D. Shi and Y. Li, ''Minimalist'' nanovaccine constituted from near whole antigen for cancer immunotherapy, *ACS Nano*, 2018, **12**, 6398–6409.

224 J. Xu, H. Wang, L. Xu, Y. Chao, C. Wang, X. Han, Z. Dong, H. Chang, R. Peng, Y. Cheng and Z. Liu, Nanovaccine based on a protein-delivering dendrimer for effective antigen cross-presentation and cancer immunotherapy, *Biomaterials*, 2019, **207**, 1–9.

225 S. Yan, K. Xu, L. Li, W. Gu, B. E. Rolfe and Z. P. Xu, The pathways for layered double hydroxide nanoparticles to enhance antigen (cross)-presentation on immune cells as adjuvants for protein vaccines, *Front. Pharmacol.*, 2018, **9**, 1060.

226 R. Yang, J. Xu, L. Xu, X. Sun, Q. Chen, Y. Zhao, R. Peng and Z. Liu, Cancer cell membrane-coated adjuvant nanoparticles with mannose modification for effective anticancer vaccination, *ACS Nano*, 2018, **12**, 5121–5129.

227 W. Yang, G. Zhu, S. Wang, G. Yu, Z. Yang, L. Lin, Z. Zhou, Y. Liu, Y. Dai, F. Zhang, Z. Shen, Y. Liu, Z. He, J. Lau, G. Niu, D. O. Kiesewetter, S. Hu and X. Chen, In situ dendritic cell vaccine for effective cancer immunotherapy, *ACS Nano*, 2019, **13**, 3083–3094.

228 P. Zamani, J. G. Navashen, A. R. Nikpoor, M. Hatamipour, R. K. Oskueee, A. Badiee and M. R. Jaafari, MPL nanoliposomal vaccine containing P5 HER2/neu-derived peptide pulsed PADRE as an effective vaccine in a mice TUBO model of breast cancer, *J. Controlled Release*, 2019, **303**, 223–236.

229 Q. Zeng, P. Zhang, X. Zeng, L. H. Tostanoski and C. M. Jewell, Advanced manufacturing of microdisk vaccines for uniform control of material properties and immune cell function, *Biomater. Sci.*, 2018, **6**, 115.

230 J. Zhang, J. Mai, F. Li, J. Shen, G. Zhang, J. Li, L. E. Hinkle, D. Lin, X. Liu, Z. Li, R. F. Wang, E. A. Mittendorf, M. Ferrari and H. Shen, Investigation of parameters that determine Nano-DC vaccine transport, *Biomed. Microdevices*, 2019, **21**, 39.

231 Y. N. Zhang, W. Poon, E. Sefton and W. C. W. Chan, Suppressing subcapsular sinus macrophages enhances transport of nanovaccines to lymph node follicles for robust humoral immunity, *ACS Nano*, 2020, **14**, 9478–9490.

232 Y. N. Zhang, W. Poon, E. Sefton and W. C. W. Chan, Suppressing subcapsular sinus macrophages enhances transport of nanovaccines to lymph node follicles for robust humoral immunity, *ACS Nano*, 2020, **14**, 9478–9490.

233 L. Zhao, D. Mahony, A. S. Cavallaro, B. Zhang, J. Zhang and J. R. Deringer, *et al.*, Immunogenicity of outer membrane proteins VirB9-1 and VirB9-2, a novel nanovaccine against anaplasma marginale, *PLoS One*, 2016, **11**, e0154295.

234 A. Beduneau, Z. Ma, C. B. Grotepas, A. Kabanov, B. E. Rabinow, N. Gong, R. L. Mosley, H. Dou, M. D. Boska and H. E. Gendelman, Facilitated monocyte-macrophage uptake and tissue distribution of superparmagnetic iron-oxide nanoparticles, *PLoS One*, 2009, **4**, e4343.

235 T. P. Thomas, S. N. Goonewarden, I. J. Majoros, A. Kotlyar, Z. Cao, P. R. Leroueil and J. R. Baker, Folate-targeted nanoparticles show efficacy in the treatment of inflammatory arthritis, *Arthritis Rheumatism*, 2011, **63**, 2671–2680.

236 L. Ma, T. W. Liu, M. A. Wallig, I. T. Dobrucki, L. W. Dobrucki, E. R. Nelson, K. S. Swanson and A. M. Smith, Efficient targeting of adipose tissue macrophages in obesity with polysaccharide nano-carriers, *ACS Nano*, 2016, **10**, 6952–6962, DOI: 10.1021/acsnano.6b02878.

237 L. J. Cruz, R. A. Rosalia, J. W. Kleinovink, F. Rueda, C. W. G. M. Löwik and F. Ossendorp, Targeting nanoparticles to CD40, DEC-205 or CD11c molecules on dendritic cells for efficient CD8+ T cell response: A comparative study, *J. Controlled Release*, 2014, **192**, 209–218.

238 M. Silva, E. Zupancic, G. Vandermeulen, V. G. Oliveira, A. Salgado, M. Videira, M. Gaspar, L. Graca, V. Préat and H. F. Florindo, In vivo delivery of peptides and Toll-like receptor ligands by mannose-functionalized polymeric nanoparticles induces prophylactic and therapeutic antitumor immune responses in a melanoma, *J. Controlled Release*, 2015, **198**, 91–103, DOI: 10.1016/j.jconrel.2014.11.033.model.

239 S. Keller, J. T. Wilson, G. I. Patilea, H. B. Kern, A. J. Convertine and P. S. Stayton, Neutral polymer micelle carriers with pH-responsive, endosome-releasing activity modulate antigen trafficking to enhance CD8+ T cell responses, *J. Control. Release*, 2014, **191**, 24–33, DOI: 10.1016/j.jconrel.2014.03.041.

240 E. Yuba, A. Harada, Y. Sakanishi, S. Watarai and K. Kono, A liposome-based antigen delivery system using pH-sensitive fusogenic polymers for cancer immunotherapy, *Biomaterials*, 2013, **34**, 3042–3052.

241 K. Zaks, M. Jordan, A. Guth, K. Sellins, R. Kedl, A. Izzo, C. Bosio and S. Dow, Efficient immunization and cross-priming by vaccine adjuvants containing TLR3 or TLR9 agonists complexed to cationic liposomes, *J. Immunol.*, 2006, **176**, 7335–7345.

242 G. Lynn, R. Laga, P. A. Darrah, A. S. Ishizuka, A. J. Balaci, A. E. Dulcey, M. Pechar, R. Pola, M. Y. Gerner, A. Yamamoto, C. R. Buechler, K. M. Quinn, M. G. Smelkinson, O. Vanek, R. Cawood, T. Hills, O. Vasalatiy, K. Kastenmüller, J. R. Francica, L. Stutts, J. K. Tom, K. A. Ryu, A. P. Esser-Kahn, T. Etrych, K. D. Fisher, L. W. Seymour and R. A. Seder, In vivo characterization of the physicochemical properties of polymer-linked TLR agonists that enhance vaccine immunogenicity, *Nat. Biotechnol.*, 2015, **33**, 1201–1210, DOI: 10.1038/nbt.3371.

243 V. V. Temchura, D. Kozlova, V. Sokolova, K. Überla and M. Epple, Targeting and activation of antigen-specific

B-cells by calcium phosphate nanoparticles loaded with protein antigen, *Biomaterials*, 2014, **35**(23), 6098–6105, DOI: 10.1016/j.biomaterials.2014.04.010.

244 E. Shahum and H. M. Thérien, Immunopotentiation of the humoral response by liposomes: Encapsulation versus covalent linkage, *Immunology*, 1988, **2**, 315–317.

245 H. H. Guan, W. Budzynski, R. R. Koganty, M. J. Krantz, M. A. Reddish, J. A. Rogers, B. M. Longenecker and J. Samuel, Liposomal formulations of synthetic MUC1 peptides: Effects of encapsulation versus surface display of peptides on immune responses, *Bioconjugate Chem.*, 1998, **9**, 451–458.

