



Macrophage-targeted nanomedicine for the diagnosis and treatment of atherosclerosis

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Abstract | Nanotechnology could improve our understanding of the pathophysiology of atherosclerosis and contribute to the development of novel diagnostic and therapeutic strategies to further reduce the risk of cardiovascular disease. Macrophages have key roles in atherosclerosis progression and, therefore, macrophage-associated pathological processes are important targets for both diagnostic imaging and novel therapies for atherosclerosis. In this Review, we highlight efforts in the past two decades to develop imaging techniques and to therapeutically manipulate macrophages in atherosclerotic plaques with the use of rationally designed nanoparticles. We review the latest progress in nanoparticle-based imaging modalities that can specifically target macrophages. Using novel molecular imaging technology, these modalities enable the identification of advanced atherosclerotic plaques and the assessment of the therapeutic efficacy of medical interventions. Additionally, we provide novel perspectives on how macrophage-targeting nanoparticles can deliver a broad range of therapeutic payloads to atherosclerotic lesions. These nanoparticles can suppress pro-atherogenic macrophage processes, leading to improved resolution of inflammation and stabilization of plaques. Finally, we propose future opportunities for novel diagnostic and therapeutic strategies and provide solutions to challenges in this area for the purpose of accelerating the clinical translation of nanomedicine for the treatment of atherosclerotic vascular disease.

Apolipoprotein B (apoB)-containing lipoproteins

Cholesterol-rich lipoproteins, such as LDL and chylomicron remnants, that have a crucial role in atherogenesis.

'Vulnerable' plaques

A type of atherosclerotic plaque composed of a lipid-rich necrotic core and thin overlying fibrous cap. These plaques are highly unstable and their rupture can trigger acute thrombosis and its consequences, namely myocardial infarction and stroke.

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Atherosclerotic cardiovascular disease is one of the most common causes of morbidity and mortality worldwide¹. Atherosclerosis is considered a chronic inflammatory disease, initiated by the retention of plasma apolipoprotein B (apoB)-containing lipoproteins in focal areas of the arterial tree^{2,3}. The cholesterol and oxidized phospholipids in these lipoproteins induce the activation of endothelial cells, which subsequently recruit monocytes into the subendothelial space^{4,5}. In the arterial intima, monocytes differentiate into pro-inflammatory macrophages that locally amplify the inflammatory response⁶. In addition, macrophages engulf lipoproteins in the intima to form lipid-laden foam cells, giving rise to early atherosclerotic lesions. If the pro-inflammatory state persists, the atherosclerotic lesions progress to an advanced stage characterized by increased macrophage apoptosis and defective clearance of apoptotic cells^{7–9}. This catastrophic combination promotes plaque necrosis, a key feature of 'vulnerable' plaques that can trigger occlusive luminal thrombosis and its consequences, namely, myocardial infarction, stroke and sudden

cardiac death^{1,10}. The risk of these cardiovascular events remains fairly high in the general population despite treatment with lipid-lowering therapies, notably statins but also PCSK9 inhibitors^{11,12}. In theory, cardiovascular disease could be eliminated if apoB-containing lipoproteins could be decreased to very low levels early in life. However, this strategy is currently not practical for widespread application mainly because of low compliance and adverse effects in certain individuals. Moreover, the safety concerns of using cholesterol-lowering drugs in childhood have not yet been fully evaluated. For this reason, novel anti-inflammatory therapies are being developed to further reduce the risk of cardiovascular disease; however, these efforts are hampered by the low bioavailability, poor target specificity and high toxicity of conventional anti-inflammatory drugs. Furthermore, identifying which individuals are at risk of developing clinically dangerous vulnerable plaques remains a challenge.

Nanotechnology is particularly advantageous for addressing these challenges. Specifically, extensive

Key points

- Because macrophages have key roles in atherosclerosis progression, macrophage-mediated pro-atherosclerotic processes are important targets for both diagnostic imaging and novel therapies for atherosclerosis.
- Nanotechnology is particularly advantageous in its capacity to substantially improve the pharmacokinetic profile and chemical stability of encapsulated diagnostic and therapeutic agents for atherosclerosis management.
- The rational design of nanoparticle-based imaging agents that can specifically target inflammatory macrophages in atherosclerotic plaques offers diagnostic potential to non-invasively quantify atherosclerosis plaque burden, evaluate the efficacy of medical interventions and serve as surrogate end points.
- Targeted nanotherapeutics that can modulate plaque macrophage functions by the activation or suppression of specific signalling pathways have shown great promise in preclinical models by improving therapeutic efficacy and reducing off-target and systemic adverse effects.
- Rapid advances in nanotechnology and bioengineering and an improved understanding of atherosclerotic pathophysiology have accelerated the development of novel nanotherapeutics for atherosclerosis diagnosis and treatment.
- The success of various nanomedicine-based approaches in preclinical studies of atherosclerosis and their use in human cancer bode well for their future application in the diagnosis and treatment of patients with cardiovascular disease.

Bioavailability

The percentage of an administered drug or substance that enters the systemic circulation and reaches its target tissue and is therefore able to have a biological effect.

Nanomedicine

Applications of nanotechnology for diagnosis, treatment and control of biological systems.

Cholesterol efflux

Process by which cells transfer excess intracellular cholesterol to other cells through either passive diffusion or dedicated mechanisms involving ABC transporters. Cholesterol efflux is important to prevent cholesterol-induced cytotoxicity, as occurs in macrophage foam cells in the arterial wall, and is an essential step in reverse cholesterol transport.

studies have shown that nanoparticles substantially improve the pharmacokinetic profile and chemical stability of the loaded therapeutics, such as small-molecule drugs, peptides, proteins, small interfering RNA (siRNA) and microRNA (miRNA)¹³. Nanotherapy could also reduce the off-target and systemic adverse effects compared with those of free drugs alone, thereby overcoming the key translational barriers for those drugs^{14–16}. Additionally, nanoparticle-mediated delivery strategies have shown great promise, in mouse models and with high specificity, in suppressing certain pathological processes, including inflammation, that are associated with atherosclerosis progression¹³. More importantly, targeted nanoparticles have been designed to improve the delivery of imaging agents and therapeutics to inflammatory macrophages in atherosclerotic plaques. In this Review, we provide a fresh perspective on atherosclerosis nanomedicine. Specifically, we discuss how the convergence of nanotechnology and a deeper understanding of the pro-atherosclerotic mechanisms mediated by macrophages in atherosclerotic plaques could drive advances in cardiovascular disease diagnosis and therapy. Moreover, we discuss the opportunities and challenges of macrophage-targeting nanomedicines that will need to be considered before clinical translation. Although nanoparticles have not yet been used for the treatment of atherosclerosis in patients, we envision that the improved understanding of atherosclerotic pathobiology

gained over the past decades, combined with the success of nanomedicines in cancer therapy, will accelerate their future clinical translation for cardiovascular disease.

Macrophage biology in atherosclerosis

In the early phase of atherogenesis, endothelial cells attract monocytes to the arterial wall through chemokine-receptor interactions and by expressing cell adhesion molecules such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1). This monocyte recruitment system has a high degree of redundancy, which explains scientific efforts to halt atherogenesis by simultaneously targeting multiple receptor-ligand interactions^{17,18}. The combined inhibition of five key adhesion molecules through nanoparticle-based RNA interference was shown in a 2016 study to be very effective in reducing monocyte recruitment to atherosclerotic lesions in a mouse model of atherosclerosis¹⁸. However, this therapy might not be of tremendous benefit in advanced atherosclerosis, when monocytes have already infiltrated the plaque. Indeed, monocyte suppression was found to reduce early but not advanced atherosclerosis in mice¹⁹, underscoring the importance of early detection of subclinical disease in humans for monocyte-directed therapies.

After migrating into the arterial vessel wall, monocytes differentiate into macrophages and acquire functionally distinct phenotypes depending on signals from the local microenvironment²⁰. Macrophages can either adopt a more pro-inflammatory ('M1-like') phenotype, characterized by a high production of pro-inflammatory cytokines, reactive oxygen species (ROS) and reactive nitrogen species, or a more pro-resolving ('M2-like') phenotype, distinguished by the capacity to dampen inflammation and promote tissue repair. Atherosclerotic lesions in humans contain various macrophage populations²¹ although their functional profile is poorly understood²². Further insight into the mechanisms that drive macrophage phenotypes in atherosclerosis could provide new therapeutic opportunities. Studies indicate that pro-inflammatory lesional macrophages ingest large quantities of LDL-derived cholesterol mediated by the increased activity of cholesterol-trafficking pathways in these cells²³. Macrophage scavenger receptors facilitate the uptake of oxidized cholesterol, which can be stored in the cell to prevent cholesterol-induced cytotoxicity, resulting in the classical 'foamy' appearance of macrophages in atherosclerotic lesions. Alternatively, excess cholesterol can be transported to extracellular apolipoprotein A-I (apoA-I) and HDL through the macrophage ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, respectively^{24–27}. The expression of these membrane lipid translocases is induced by peroxisome proliferator-activated receptor- γ (PPAR γ) and the sterol-regulated transcription factor liver X receptor- α (LXR α)²⁸. By promoting macrophage cholesterol efflux, LXR α agonists ameliorate atherosclerosis in preclinical animal models^{29–31}. However, systemic LXR α activation also promotes hepatic steatosis and hypertriglyceridaemia by acting on the liver, thereby complicating LXR α -targeted therapy³². Interestingly, macrophages

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Efferocytosis

Process through which apoptotic cells are cleared by phagocytes, including pro-resolving macrophages. Impairments in efferocytosis drive atherosclerosis progression owing to secondary necrosis of uncleared apoptotic cells in the arterial wall.

Inflammation resolution

A highly coordinated process to restore tissue homeostasis following infection or tissue damage. Inflammation resolution is regulated by macrophages with a resolving–repair phenotype that produce a variety of pro-resolving mediators to dampen inflammation, repair damaged tissues and improve efferocytosis.

have been found to transfer cholesterol directly to adjacent smooth muscle cells in vitro, providing an alternative for ABC transporter-mediated cholesterol efflux³³. Whether this transcellular cholesterol movement is relevant to macrophages in atherosclerotic lesions remains to be investigated.

Cholesterol accumulation in macrophages promotes inflammatory responses, including activation of Toll-like receptor (TLR) signalling, NF- κ B-mediated activation of the NLRP3 inflammasome and pro-inflammatory cytokine production, which exacerbate the chronic inflammatory state in atherosclerosis³⁴. Accordingly, suppression of cholesterol efflux pathways through macrophage-specific deletion of *Abca1* and *Abcg1* promotes an inflammatory macrophage signature and accelerates atherosclerosis in mice³⁵. TLR signalling and NF- κ B are popular targets for anti-inflammatory therapy of atherosclerosis, although general inhibition of these mediators is risky considering their broad function in inflammation and immunity. The same is the case with inhibition of IL-1 β , a pro-inflammatory cytokine product of the NLRP3 inflammasome. The CANTOS trial³⁶ showed that canakinumab, a monoclonal antibody against IL-1 β , reduces the risk of cardiovascular events in patients with previous myocardial infarction but was also associated with a higher incidence of fatal infection compared with placebo. Additionally, results from the COLCOT trial³⁷ published in 2019 showed that systemic treatment of patients within 30 days after myocardial infarction with the anti-inflammatory drug colchicine significantly lowered the risk of ischaemic cardiovascular events by the attenuation of NLRP3 inflammasome activation and inhibition of the release of IL-1 β and IL-18 from inflammatory macrophages. However, this systemic treatment strategy also produced an immunosuppressive effect and increased the incidence of pneumonia compared with the placebo group³⁷. These findings indicate that the localized and targeted delivery of anti-inflammatory therapy might be preferred over systemic approaches to reduce adverse effects related to impaired host defence. This goal might be even more achievable with the use of nanoparticle-directed pro-resolving therapy instead of anti-inflammatory therapy (see discussion below).

Increased inflammation not only attracts more circulating monocytes to the atherosclerotic vessel wall but also promotes a vulnerable plaque phenotype³⁸. Vulnerable plaques are characterized by a thin fibrous cap and a large necrotic core and are therefore more prone to rupture³⁹. Plaque rupture exposes pro-thrombotic substances in the plaque, which trigger the activation of platelets, leading to thrombus formation and arterial occlusion¹⁰. How to identify those individuals who are susceptible to having vulnerable and rupture-prone plaques is an important area of future research. In addition, further research is warranted into the mechanisms through which macrophages influence the stability of atherosclerotic lesions. Pro-inflammatory macrophages are known to diminish lesion stability by inhibiting collagen production by smooth muscle cells and by producing matrix metalloproteinases (MMPs) that degrade the protective fibrous cap^{40–43}. By contrast,

pro-resolving macrophages promote lesion stability by clearing apoptotic cells through the process of efferocytosis, thereby diminishing plaque necrosis^{7,44}. Efferocytosis also initiates an inflammation resolution response by macrophages, resulting in the production of resolving mediators, including IL-10, transforming growth factor- β (TGF β) and metabolites of long-chain unsaturated fatty acids called specialized pro-resolving mediators, all of which restrain inflammation and promote tissue repair⁴⁵. Importantly, macrophage-mediated resolution of inflammation is impaired in advanced atherosclerotic lesions^{8,9,46}. Boosting efferocytosis and inflammation resolution is therefore a promising approach to halt atherosclerosis progression and induce atherosclerosis regression⁴⁷ and was shown to be effective in mouse models of atherosclerosis^{48,49}.

Considering the key role of macrophages in the progression and regression of atherosclerotic lesions (FIG. 1), these cells are an important therapeutic target in atherosclerotic cardiovascular disease. However, a high degree of overlap exists between macrophage-related mechanisms involved in atherosclerosis and host defence, which makes designing therapies that do not interfere with immunity difficult. Another complicating factor is the inability of current imaging modalities to non-invasively assess atherosclerotic plaque composition and stability or to identify individuals at high risk of developing acute coronary syndrome. In the past two decades, atherosclerotic macrophage-targeted nanoparticles have emerged as a potential solution to these problems, as further explained below.

Journey of nanomedicines to macrophages

The aforementioned macrophage-mediated atherogenesis provides a plethora of compelling diagnostic and therapeutic targets for nanoparticle-assisted management of atherosclerosis. Consequently, a comprehensive investigation of the journey of nanoparticles, including their circulation in the bloodstream, extravasation into the atherosclerotic plaques, and macrophage targeting and intracellular trafficking, will aid in designing nanoparticles that specifically accumulate in lesional macrophages and thereby have more robust diagnostic and therapeutic efficacy.

Intravenous administration of nanotherapeutics is the most straightforward and effective delivery route for their systemic infiltration into atherosclerotic plaques. The diversity of nanoparticles is described in BOX 1. Following intravenous administration, the adsorption of biomolecules (such as proteins) on the surface of nanoparticles gives rise to the formation of a ‘corona’, which has a crucial role in determining the pharmacokinetics, biodistribution and cellular uptake of nanoparticles⁵⁰. For example, the adsorption of opsonin proteins, such as IgG, activates the clearance of administered nanoparticles by the mononuclear phagocyte system (MPS) in the liver and spleen^{50,51}. To increase their accumulation in atherosclerotic plaques, nanoparticles are designed to evade MPS recognition by surface coating with polymers (such as polyethylene glycol (PEG))⁵² or biomimetic materials (such as erythrocyte membranes⁵³, platelet membranes⁵⁴, platelet-derived extracellular vesicles⁵⁵,

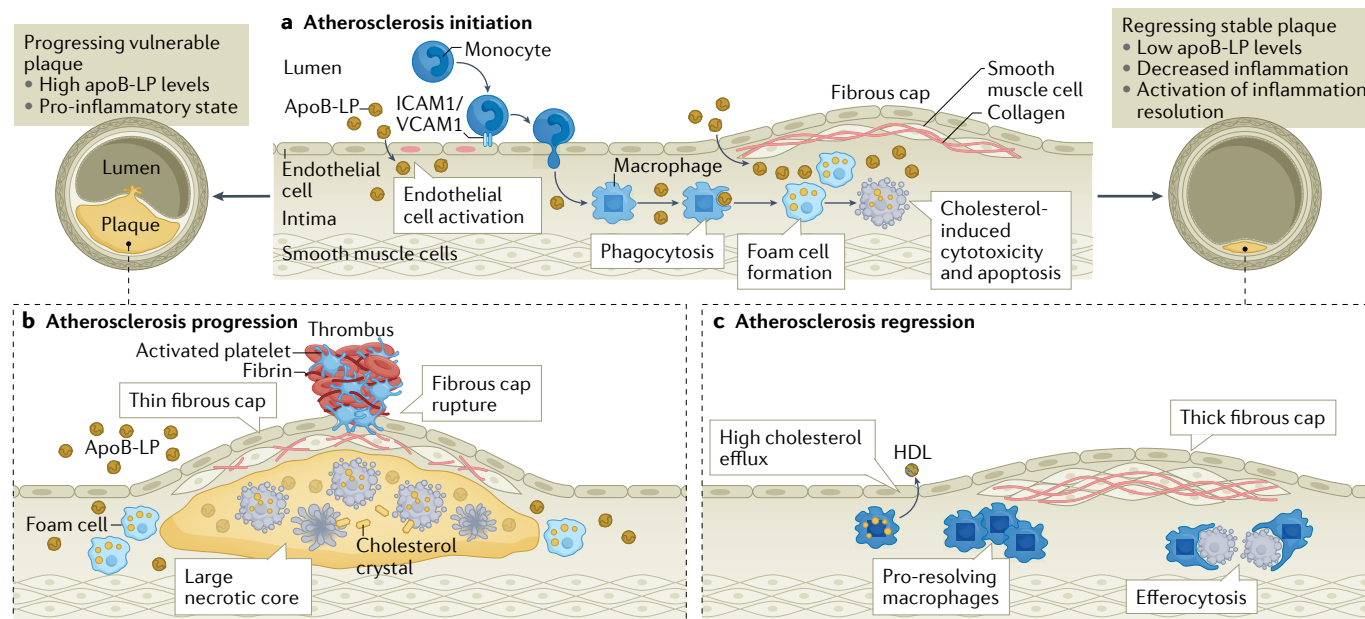


Fig. 1 | Role of macrophages in the progression and regression of atherosclerosis. **a** | Atherosclerosis development is initiated by the retention and aggregation of apolipoprotein B-containing lipoproteins (apoB-LPs) in the subendothelial space. ApoB-LPs activate endothelial cells, resulting in the upregulation of adhesion molecules (such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1)) that mediate monocyte adhesion to endothelial cells and migration into the arterial vessel wall. In the arterial intima, monocytes differentiate into macrophages, which engulf lipoprotein-derived cholesterol, leading to foam cell formation. Macrophages that cannot process the large amounts of cholesterol undergo cholesterol-induced cytotoxicity and apoptosis. **b** | If circulating apoB-LP levels remain elevated and the pro-inflammatory state persists, monocyte infiltration and

macrophage apoptosis continue, producing so-called 'vulnerable' atherosclerotic plaques with large necrotic cores and thin fibrous caps. Vulnerable plaques are prone to rupture, which can lead to thrombus formation, arterial occlusion and sudden cardiac death. **c** | However, if the plasma cholesterol level is sufficiently lowered and/or the pro-inflammatory state subsides and resolution processes are activated, atherosclerosis regression is possible. Regressing atherosclerotic lesions are characterized by a high cholesterol efflux from macrophages to HDL, large numbers of pro-resolving macrophages that clear apoptotic cells through efferocytosis, reduced plaque necrosis and a thick protective fibrous cap. Regressing atherosclerotic plaques are relatively stable and less likely to rupture than vulnerable plaques and, most importantly, are associated with a lower risk of coronary artery disease in humans.

macrophage membranes⁵⁶ and macrophage-derived exosomes⁵⁷). In addition, other natural nanomaterials, such as HDL^{58,59}, ferritin nanocages⁶⁰ and heat shock protein⁶¹, which can evade MPS recognition and thereby prolong circulation in the blood, have been extensively used to deliver imaging and therapeutic agents to macrophages in atherosclerotic lesions. The size of nanoparticles also has an important role in their accumulation in plaques. For example, Tang and colleagues investigated how the size of HDL nanoparticles affects their accumulation in aortic plaques⁶². Using ex vivo near-infrared fluorescence imaging, the researchers discovered that the accumulation of nanoparticles with a diameter of 7–30 nm exceeded that of nanoparticles with a diameter of >70 nm. This size-dependent accumulation of nanoparticles is due to the fact that, when the nanoparticle size is >6 nm in diameter (the size threshold for renal clearance), smaller HDL nanoparticles have longer blood circulation times than larger HDL nanoparticles. Interestingly, a study published in 2020 showed that single-walled carbon nanotubes (SWNTs) accumulate in atherosclerotic lesion areas because they are taken up by circulating monocytes that subsequently migrate to the plaques⁶³. Conversely, this monocyte-targeting approach could be used to prevent monocyte recruitment to inflammatory lesions by silencing *Ccr2*, which encodes a chemokine receptor, in pro-inflammatory monocytes

through administration of siRNA-encapsulated lipid nanoparticles as shown in mice⁶⁴.

After circulation in the blood, efficient accumulation of nanoparticles in lesional macrophages relies on extravasation through the leaky vasculature of atherosclerotic lesions, similar to nanoparticle accumulation in tumour tissues^{65,66}. Specifically, circulating nanoparticles should first penetrate through the openings in the impaired vascular endothelium (on the lumen side) or in those of neovessels (on the adventitial side) to reach subendothelial atherosclerotic plaques¹³. In this area, attempts have been made to modify the nanoparticle surface with subendothelial collagen type IV-targeted peptides that can direct the nanoparticles to the plaque^{48,67,68}. Interestingly, a 2017 study indicated that, after the nanoparticles infiltrate the leaky endothelium, restricted diffusion inside the atherosclerotic plaques makes the nanoparticles deposit only in the peripheral areas around the endothelial openings, achieving heterogeneous distribution⁶⁹. Given this paradigm, nanoparticles with optimal physicochemical properties for deeper plaque penetration might improve their distribution profiles in plaques and improve the diagnostic and therapeutic efficacy. However, how the nanoparticle size, surface charge, shape, stiffness, chemical composition, and surface functional groups and ligands affect their distribution in the plaque microenvironment

Box 1 | Diversity of nanoparticles

Although the International Union of Pure and Applied Chemistry (IUPAC) defines nanoparticles (NPs) as particles of any shape in the range of 1–100 nm in size, the term NPs extensively refers to materials that are up to several hundred nanometres in size. The essence of applications of NPs for atherosclerosis management is their capacity to improve treatment and diagnosis efficacy as well as to provide a deeper insight into plaque biology and pathophysiology. The overview below shows the diversity of nanomaterials described in this Review and provides the readers with background information.

Polymeric nanoparticles

Polymeric NPs, typically in the range of tens to hundreds of nanometres in size, are composed of polymers. Among them, poly(D,L-lactic-co-glycolic acid) NPs are the most extensively studied polymeric NPs for atherosclerosis management owing to their excellent biocompatibility and biodegradability^{48,52,67,121,142,145,165}. Other types of polymeric NPs (for example, polyglucose¹⁰⁷) described in the Review also show great promise in imaging and treating plaque macrophages. The surface of polymeric NPs can be derivatized with a wide range of targeting ligands (such as S2P peptide⁵² and RGD peptide¹⁴¹) or biomimetic materials (such as erythrocyte membrane⁵³, exosomes⁵⁷ and extracellular vesicles⁵⁵) for the targeting of and/or preferential accumulation in plaque macrophages or another type of polymer (for example, polyethylene glycol (PEG))^{52,132,152} to prolong their circulation in the blood.

HDL-like nanoparticles

HDL, an endogenous lipidic NP ranging from 7 nm to 13 nm in size, is composed of phospholipids and apolipoprotein A-I (apoA-I). HDL can move cholesterol from lipid-laden plaque macrophages to the liver through the process of reverse cholesterol transport. As extraction of apoA-I from human plasma is time consuming, various genetic variants of apoA-I^{192,196} or recombinant apoA-I^{194,195} were used to replace human plasma apoA-I to obtain reconstituted HDL or HDL-like NPs with similar atheroprotective effects to those of HDL. HDL-like NPs have therefore

been widely studied as nanocarriers to deliver therapeutics or imaging agents for atherosclerosis management^{58,59,62,108,120,128,129,163,197}.

LDL-like nanoparticles

LDL-like NPs, ranging from 18 nm to 25 nm in size, are lipid structures that mimic protein-free LDL composition. Because LDL-like NPs can be avidly taken up by plaque macrophages through cell membrane receptors, they have been extensively used to deliver anti-inflammatory and anti-proliferative agents for atherosclerosis treatment¹⁴⁰.

Inorganic nanoparticles

Inorganic NPs described in the Review include superparamagnetic iron oxide NPs^{60,86–92,198–201}, Gd complex-containing NPs^{54,94}, Gd inorganic nanocrystals⁹⁵, gold NPs^{104,151,153,193}, upconversion NPs^{78,109} and quantum dots⁷⁹, which are composed of metal or metal derivatives and have sizes approximately ranging from 6 nm to 100 nm. The surface of these NPs could be derivatized with macrophage-targeting ligands (such as dextran^{86–88}, osteopontin antibody^{78,89,111}, macrophage receptor with collagenous structure antibody¹⁰⁹ and annexin V⁹⁰) or the biomimetic materials described above for the targeting of and/or preferential accumulation in plaque macrophages^{54,60}. These inorganic NPs can serve as excellent imaging agents for the identification of macrophage-rich plaques at risk of rupture, as described in the section on macrophage-targeted nanoplatforms for diagnostic imaging of atherosclerosis.

Liposomes

Liposomes are spherical-shaped vesicles formed by lipid bilayers of phospholipids and cholesterol. The size of liposomes described in this Review is about 100 nm. Liposomes have both hydrophobic and hydrophilic compartments, which can be used to carry a wide range of imaging agents or therapeutic cargos to plaque macrophages^{132,144,169}. Similar to other NP platforms, the surface of liposomes can be derivatized with PEG and targeting ligands to increase their circulation time in the blood and their accumulation in plaque macrophages.

remains largely unexplored and warrants future systematic investigation. Nevertheless, evidence from a study in 2019 demonstrated that atherosclerotic plaque progression supports endothelial junction stabilization, thereby reducing nanoparticle infiltration⁷⁰. This nanoparticle-aided characterization suggested that the accumulation of nanoparticles in advanced plaques might not be as efficient as in earlier-stage plaques and that the severity of atherosclerosis might not be characterized solely by infiltration-dominant nanoparticle accumulation because the imaging intensity produced by accumulated nanoparticles might decrease as atherosclerosis progresses. In addition to extravasation through the leaky endothelium mentioned above, accelerated dynamin-dependent transcytosis through endothelial cells that overlay the atherosclerotic lesions could improve the accumulation of ultrasmall iron oxide nanoparticles in plaques⁷¹, similar to discoveries of nanoparticle accumulation in tumour tissues⁷². The finding might provide a new strategy to design nanoparticles that can target endothelial cells and finally reach lesional macrophages by transcytosis.

After nanoparticles accumulate in atherosclerotic lesions, they can be taken up by various cell types in the microenvironment of the plaque, such as vascular smooth muscle cells^{63,69} and neutrophils^{58,73}. Therefore, surface-targeted proteins or other ligands that can specifically recognize the epitopes overexpressed on macrophages^{52,61,74–80} have pivotal roles in

increasing nanoparticle internalization by lesional macrophages and delivering therapeutics that specifically manipulate macrophages. The administration of macrophage-targeted nanoparticles is particularly crucial to identifying and modulating the functions of pro-inflammatory macrophages. In addition, efficient nanoparticle internalization has a key role in promoting therapeutic efficacy because many therapeutics carried by nanoparticles act on intracellular targets and compartments. This point is particularly crucial for biomolecular payloads, such as siRNA and miRNA, which are involved in the RNA-interference pathway and require endosomal escape and cytosolic delivery following nanoparticle uptake^{52,74,81–84}. Polymer-based and ionizable lipid-based nanoparticles that can escape from endolysosomes have shown great promise in delivering those biomolecules to lesional macrophages^{52,74,81,82}.

Nanoparticles for diagnostic imaging

Molecular imaging technology facilitates the visualization of atherosclerotic plaques at high risk of rupture or erosion with high spatiotemporal resolution, addressing the pressing need for robust and reliable imaging agents to assist in the development of drugs for atherosclerosis. To this end, the rational design of nanomaterial-based imaging contrast agents that can specifically target macrophages in plaques can offer pivotal insights into plaque biology as well as help to quantify atherosclerosis burden and to evaluate the efficacy of therapies at the molecular,

Transcytosis

Transcellular process through which nanoparticles are transported across the interior of a cell.

Endosomal escape

Process through which nanoparticles escape from endosomes and enter the cytoplasm, either by crossing the endosome membrane or as a result of endosome rupture.

Plaque burden

A measure of atherosclerotic plaque volume relative to the volume of the arterial wall.

cellular and functional levels. In this section, we review and discuss state-of-the-art non-invasive imaging technologies, including MRI, CT, PET, fluorescence imaging, photoacoustic imaging (PAI) and combined imaging modalities, and the corresponding nanomaterial-based contrast agents for the identification of macrophage-rich atherosclerotic plaques at risk of rupture or erosion and to assess the efficacy of macrophage-specific anti-atherosclerotic therapies (FIG. 2).

MRI. Atherosclerotic plaque burden and the accumulation of pro-inflammatory macrophages have been extensively characterized by MRI, which can generate images with high spatial resolution and excellent soft-tissue contrast. Additionally, systemic administration of

nanoparticle-based imaging contrast agents, such as superparamagnetic iron oxide nanoparticles (SPIONs), which have superior safety profiles and can readily be phagocytosed by plaque macrophages, can induce signal loss in T2-weighted images and thereby improve the image contrast of plaques in the arterial walls⁸⁵. More importantly, surface coating of SPIONs with targeting moieties, such as dextran^{86–88}, human ferritin protein cage⁶⁰, osteopontin (OPN)⁸⁹ or annexin V⁹⁰, can specifically target the SPIONs to the corresponding epitopes on the macrophage surface and increase their accumulation in vulnerable plaques in aortic or carotid arteries. In clinical settings, SPION-enhanced MRI has already been used to assess macrophage-associated inflammatory burdens^{87,91} and therapeutic outcomes of lipid-lowering

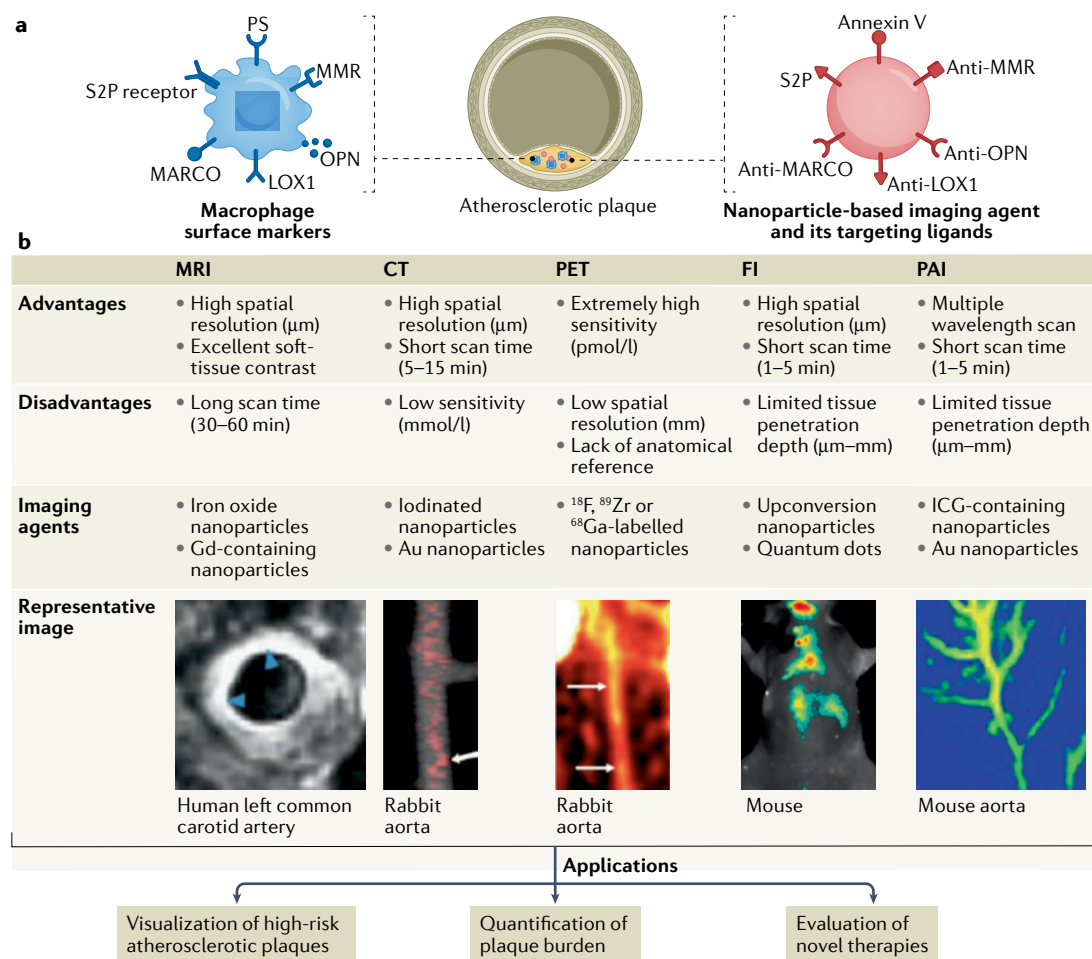


Fig. 2 | Macrophage-targeted nanoplatforms and non-invasive diagnostic imaging of atherosclerosis. Non-invasive bioimaging technologies facilitate the visualization of high-risk atherosclerotic plaques with high spatiotemporal resolution. **a** | The unique epitopes expressed on the macrophage surface can be recognized with the use of targeted nanoparticle-based imaging agents. Targeted nanoparticles have been designed to improve the delivery of imaging agents to inflammatory macrophages in atherosclerotic plaques, thereby improving imaging contrast. **b** | Various non-invasive bioimaging modalities, advantages, disadvantages and the associated nanoparticle-based imaging contrast agents for plaque visualization. The arrows and arrowheads show areas of high agent uptake. Nanoparticle-facilitated non-invasive bioimaging can provide insights into atherosclerotic plaque biology as well as help to quantify atherosclerosis burden and evaluate the efficacy of therapies at the molecular, cellular and functional levels. FI, fluorescence imaging; ICG, indocyanine green; LOX1, lectin-like oxidized LDL receptor 1; MARCO, macrophage receptor MARCO; MMR, macrophage mannose receptor; OPN, osteopontin; PAI, photoacoustic imaging; PS, phosphatidylserine. MRI image adapted with permission from REF.⁹², Elsevier. CT image adapted from REF.¹⁰⁰, Springer Nature Limited. PET image adapted from REF.¹⁰⁷, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>). FI image adapted with permission from REF.⁷⁹, American Chemical Society. PAI image adapted with permission from REF.¹¹², American Chemical Society.

therapy (ATHEROMA study⁹²). Of note, the newly developed ultra-high-field MRI scanner (10.5 T), which can generate images with an exceptionally high signal-to-noise ratio, might provide more detailed data at the cellular and molecular levels for clinical cardiovascular research⁹³.

Macrophage burden can also be characterized by systemic administration of Gd complexes, such as Gd-diethylenetriaminepentaacetic acid (Gd-DTPA)-containing nanoparticles^{54,94} or Gd inorganic nanocrystals⁹⁵, which can increase the signal and improve the contrast of T1-weighted images. For instance, macrophage scavenger receptor-targeted immunomicelles⁹⁴ and platelet membrane biomimetic nanoparticles⁵⁴ that contain Gd-DTPA were used to study macrophage burden; the resulting increase in image contrast was proportional to the macrophage content of the plaques. Of note, the platelet membrane-coated nanoparticles can also target pre-atherosclerotic areas and thereby provide deeper mechanistic insights into atherogenesis beyond simply aiding in imaging the regions with clinically significant plaque formation. However, although these Gd-containing imaging agents seem to be promising, they might cause nephrogenic systemic fibrosis⁹⁶, thereby hindering their clinical use.

In addition to conventional MRI, which detects the relaxation process of ¹H, the use of ¹⁹F MRI in conjunction with the corresponding perfluoro-nanoemulsion contrast agents has been considered to be an attractive modality for the characterization of plaque inflammation owing to the minimal endogenous ¹⁹F signal in living organisms⁹⁷. More intriguingly, after administration of perfluoro-nanoemulsions with distinctive chemical shifts⁹⁸, ¹⁹F MRI-generated multiplexed images might be used to probe various pathological biomarkers of lesional macrophages and to study the pathophysiological processes of atherogenesis.

CT imaging. In clinical settings, the high spatial resolution and short acquisition time make CT a powerful tool to monitor atherosclerotic macrophages in coronary arteries⁹⁹. Iodine-containing nanoparticles with a high X-ray attenuation coefficient are particularly advantageous in identifying pro-inflammatory macrophages in vulnerable plaques in coronary arteries and the abdominal aorta^{100,101}. However, owing to the low sensitivity of CT, the administration of a high dose of nanoparticles (100 mg of iodine per kg for mice¹⁰¹ and 250 mg of iodine per kg for rabbits¹⁰⁰) is required to achieve detectable image contrast enhancement. The potential toxicity of such a high dose of nanoparticles must be systematically investigated before future clinical translation. Despite this issue, the use of CT for atherosclerosis diagnosis is expanding because findings obtained through PET–CT dual-modality imaging have been extensively adopted as surrogate markers in several clinical trials^{102,103}. Remarkably, the spectral multicolour CT, developed in the past decade, has shown great promise because this technology can distinguish between CT imaging agents with different X-ray attenuation coefficients and can be used to characterize the composition of advanced atherosclerotic plaques prone to rupture^{104,105}.

PET. PET is particularly suitable for the non-invasive and quantitative characterization of atherosclerotic macrophage-mediated inflammation owing to the high tissue penetration and superior sensitivity (in the picomolar range) of PET. Because PET does not provide anatomical information, this imaging modality is usually coupled with MRI or CT for anatomical reference. ¹⁸F-fluoro-2-deoxyglucose (¹⁸F-FDG) radioactive tracers, which can be avidly taken up by macrophages and reflect the metabolic activity of inflamed plaques, have been extensively used to study the progression of atherosclerosis and the effectiveness of medical interventions in both animal models and patients^{58,106}. However, imaging macrophages in coronary arteries with the use of ¹⁸F-FDG is particularly challenging because ¹⁸F-FDG lacks specificity for macrophages and can also be avidly taken up by cardiomyocytes, yielding false-positive signals¹³. Nanoparticle-based PET imaging agents can provide a solution to this problem.

PET radioactive tracer-labelled nanoparticles have favourable pharmacokinetics and can specifically and quantitatively monitor pro-inflammatory macrophages in the cardiovascular system. For example, Keliher and colleagues used ¹⁸F-labelled polyglucose nanoparticles (termed ¹⁸F-Macroflor) to non-invasively visualize atherosclerotic plaques and infarcted myocardial tissue in mouse and rabbit models because lesional macrophages take up these glucose-based nanoprobe with high efficiency¹⁰⁷. Owing to this favourable macrophage uptake profile, ¹⁸F-Macroflor produced imaging patterns distinct from those of ¹⁸F-FDG, facilitating imaging in ischaemic heart disease in rabbits. In addition to ¹⁸F radionuclide, ⁸⁹Zr-labelled nanoparticles have also been widely used to monitor pro-inflammatory macrophages and characterize the severity of atherosclerosis in animal models owing to their slower radioactive decay (⁸⁹Zr, $t_{1/2}$ = 78.4 h; ¹⁸F, $t_{1/2}$ = 1.8 h), which can match the in vivo kinetics of long-circulating and macrophage-targeting nanoparticles such as those incorporating dextran⁷³, hyaluronan⁷⁷ or HDL¹⁰⁸. In addition, owing to the long half-life of ⁸⁹Zr, advantageous prolonged monitoring of macrophages becomes feasible¹⁰⁸. Senders and colleagues studied atherosclerosis progression in a rabbit model by using ⁶⁸Ga-labelled nanobody radiotracers that have an extremely short blood circulation time (<2 min) and can specifically target atherosclerosis-associated biomarkers such as VCAM1, macrophage mannose receptor (MMR) and lectin-like oxidized LDL receptor 1 (LOX1)⁷⁵. These clinically translatable multiparametric nano-radiotracers might aid in the identification of high-risk atherosclerotic plaques and in anti-atherosclerosis drug development.

Fluorescence imaging. Owing to its high spatial resolution, fast acquisition, lack of radiation-associated risk and cost-effectiveness, non-invasive fluorescence imaging has been widely used to image macrophage-rich vascular lesions in preclinical studies. To achieve deep tissue penetration, current fluorophores, such as organic dyes or nanoparticle-based probes mainly emitting in the near-infrared window (700–900 nm), have been used to probe macrophages in atherosclerotic lesions⁶¹.

For example, NaGdF₄:Yb,Er@NaGdF₄ upconversion nanoparticles modified with antibodies against OPN or macrophage receptor MARCO were used to fluorescently image pro-inflammatory foamy macrophages⁷⁸ and M1-like macrophages¹⁰⁹, respectively. This fluorescence imaging-facilitated diagnosis readily distinguished atherosclerotic plaques at risk of rupture from stable plaques, suggesting a possible solution to some of the current challenges in atherosclerosis management. Additionally, the early, developmental and late stages of atherosclerosis could be identified with fluorescence imaging with near-infrared-emissive, quantum dot-encapsulated, virus-like nanoparticles whose surfaces were modified with the corresponding targeting peptides⁷⁹. In addition to quantitatively characterizing macrophage burden, macrophage-associated enzyme-activatable nanoparticles that can be switched from fluorescent off to on have also shown great promise in imaging vulnerable plaques⁷⁶.

Photoacoustic imaging. As documented by findings from a clinical trial¹¹⁰ reported in 2020, non-invasive PAI, which combines the sensitivity of optical imaging with the acoustic resolution and imaging depth of ultrasonography, is an excellent imaging modality for the study of plaque composition in carotid arteries. Similar to other imaging modalities, the use of macrophage-targeting PAI nanoprobables could substantially improve the regional PAI intensity for vulnerable plaque visualization. For instance, intravenous administration of anti-OPN antibody-conjugated Ti₃C₂ nanosheet–indocyanine green nanocomposites that specifically target macrophage-derived foam cells markedly improved the PAI contrast of vulnerable plaques in the aortic arch in mice¹¹¹. In addition, self-assembled bovine serum albumin-based nanoprobables that have distinct PAI responses to glutathione and H₂O₂ allowed the study of inflammation-related processes of macrophages in rupture-prone plaques in mice¹¹². These imaging nanoprobables could help differentiate advanced vulnerable plaques from early-stage lesions on the basis of the image pattern and degree of contrast enhancement.

Dual-modality imaging. Each imaging modality has unique diagnostic characteristics and the combination of two or more imaging modalities provides complementary information from different angles and deepens our understanding of the complex pathophysiology of atherosclerosis. For example, the lack of anatomical reference of PET necessitates the integration of CT or MRI to synergistically characterize inflammation in atherosclerotic plaques and evaluate the therapeutic efficacy of medical interventions^{58,59,63,107,113,114}. With a fully integrated MRI–PET scanner, the high sensitivity and specificity of PET, in combination with the superior soft-tissue contrast of MRI, yields functional, molecular and anatomical information useful to the study of plaque burden in the clinical setting⁷⁵. In addition, the combination of MRI and fluorescence imaging enables the acquisition of images with both high spatial resolution and high sensitivity, which is particularly advantageous in monitoring inflammatory macrophages in carotid plaques^{78,109}.

As imaging technology continues to evolve, more powerful, combined-modality imaging will accelerate and improve the diagnosis of atherosclerosis.

Nanotherapeutics in atherosclerosis

The activation and suppression of specific signalling pathways (pro-inflammatory or anti-inflammatory) in macrophages has important roles in the progression of atherosclerosis. Consequently, the manipulation of macrophage functions with the use of exogenous small molecules or biomolecules (such as siRNA, miRNA, peptides and proteins) has therapeutic potential in atherosclerosis via the activation or silencing of signalling pathways in macrophages, including manipulations that promote the inflammation resolution roles of macrophages. In this section, we review and discuss the latest progress and developments in using macrophage-targeted nanotherapeutics for atherosclerosis treatment. Specifically, we discuss design and strategy considerations relevant to how nanomaterials could efficiently carry and stabilize encapsulated therapeutics. In addition, we focus on how to substantially improve the delivery efficiency and bioavailability of cargo molecules to macrophages with the use of rationally designed nanomaterials. Specific targeting moieties that could substantially increase the uptake efficiency of nanomaterials by macrophages are also discussed. The primary pathological processes involved in atherosclerosis include monocyte recruitment, macrophage accumulation and proliferation, defective efferocytosis, plaque inflammation, and cholesterol and oxidized LDL accumulation (FIG. 3). These processes have been considered compelling therapeutic targets for atherosclerotic plaque regression and stabilization (TABLE 1).

Inhibition of monocyte recruitment and macrophage proliferation. Experimental data indicate that monocyte recruitment to the arterial wall is one of the earliest processes that support the progression of atherosclerotic plaques^{6,115,116}. The suppression of monocyte recruitment has been considered a promising therapeutic target because it would prevent not only the ensuing macrophage accumulation and proliferation in the plaques but also the destabilization and rupture of atherosclerotic plaques^{117–119} (FIG. 3a). For instance, Lameijer and colleagues used reconstituted HDL nanoparticles to deliver a small-molecule inhibitor of the CD40–TRAF6 (tumour necrosis factor receptor-associated factor 6) interaction to circulating Ly6C^{high} monocytes⁵⁹, which are particularly pro-inflammatory. Short-term infusion (four infusions over a period of 1 week) of HDL nanoparticles loaded with the CD40–TRAF6 inhibitor rapidly decreased plaque inflammation by reducing Ly6C^{high} monocyte recruitment and macrophage content in the lesion areas in atheroprone *Apoe*^{-/-} mice as confirmed by transcriptome analysis⁵⁹. Another study using the same mouse model showed that long-term administration (biweekly treatment for 6 weeks) of this nanoformulation increased plaque stability by increasing collagen cap areas and decreased plaque size while preserving the innate immunity of the mice¹²⁰. Importantly, the safety of these HDL-based nanoimmunotherapeutics has been proven in non-human primates⁵⁹, highlighting

Plaque regression

In humans, plaque regression refers to atherosclerotic plaques becoming smaller and more stable, which is associated with a lower risk of coronary artery disease.

their potential for clinical translation. Furthermore, another study showed that intravenous administration of pitavastatin-encapsulated poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles can reduce plaque destabilization by inhibiting monocyte recruitment in *ApoE*^{-/-} mice¹²¹. Collectively, these findings demonstrate that targeted nanoparticles can effectively stabilize atherosclerotic plaques by acting on circulating monocytes and diminishing their recruitment to the vessel wall.

Aside from leukocytes, vascular endothelial cells that express VCAM1 and promote monocyte recruitment can also serve as therapeutic targets for atherosclerosis management. For instance, Sager and colleagues used siRNA-encapsulated polymeric nanoparticles to inhibit monocyte recruitment to atherosclerotic lesions in *ApoE*^{-/-} mice after myocardial infarction and to reduce matrix-degrading protease activity in plaques by simultaneously silencing five endothelial cell adhesion

molecules in the vascular endothelium¹⁸. In addition, the same group employed siRNA-encapsulated lipid-polymer nanoparticles to inhibit leukocyte release from the haematopoietic stem cell niche by silencing *Ccl2* (also known as *Mcp1*) in bone-marrow endothelial cells, thereby reducing the supply of monocytes to the atherosclerotic lesions after myocardial infarction in mice¹²². These types of nanotherapeutics might provide numerous opportunities for the treatment of atherosclerosis.

Macrophage proliferation in the plaque after monocyte trafficking across the vessel walls and monocyte-to-macrophage differentiation specifically contributes to inflammation in advanced atherosclerotic plaques¹²³ and, therefore, suppression of pro-inflammatory macrophage proliferation with the use of nanotherapeutics could stabilize vulnerable plaques (FIG. 3b). For example, targeted delivery of rapamycin

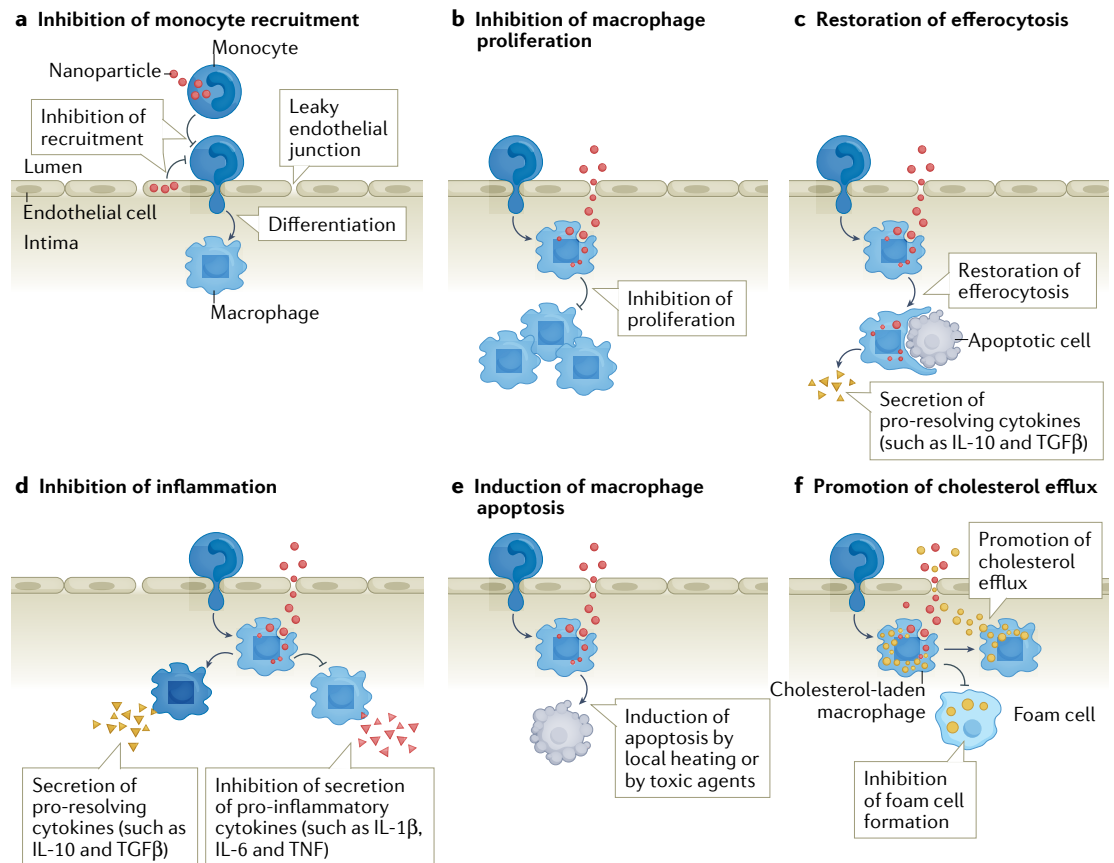


Fig. 3 | Approaches for atherosclerosis treatment with macrophage-targeting nanotherapeutics. a | Monocyte recruitment to the atherosclerotic lesion areas can be reduced by delivering therapeutics to monocytes or to vascular endothelial cells by nanoparticles. **b** | The proliferation of inflammatory macrophages can be inhibited by nanoparticle-assisted delivery of therapeutics to the lesional macrophages. **c** | The restoration of efferocytosis in macrophages by nanotherapeutics can help to remove dead cells from atherosclerotic plaques, prevent secondary necrosis and elicit the production of anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGFβ). **d** | Inflammation can be ameliorated by modulating the functions of lesional macrophages via nanotherapeutics, increasing the secretion of pro-resolving cytokines (such as IL-10 and TGFβ) or inhibiting the secretion of pro-inflammatory cytokines (such as IL-6, IL-1β and TNF) from lesional macrophages. **e** | Induction of macrophage apoptosis by local heating or by toxic agents can reduce macrophage burden in atherosclerotic lesions. However, this strategy is suitable only for early lesions. In late atherosclerotic lesions, the impaired phagocytic clearance of apoptotic macrophages might lead to secondary necrosis of these cells and a pro-inflammatory response. **f** | Promotion of cholesterol efflux from the cholesterol-laden macrophage by nanotherapeutics can reduce foam cell formation.

Table 1 | Examples of preclinical-stage macrophage-targeted nanomedicine for atherosclerosis treatment

Nanoparticle platform	Active pharmaceutical ingredients	Animal model	Treatment frequency/method	Therapeutic outcomes	Ref.
Inhibition of monocyte recruitment					
Reconstituted HDL nanoparticles	Small-molecule inhibitor of CD40–TRAF6 signalling	<i>Apoe</i> ^{-/-} mice fed a high-fat diet	Four treatments for 1 week	Decreased macrophage burden and inflammation in atherosclerotic plaques	59
			Twice per week for 6 weeks	Reduced plaque sizes, increased collagen cap areas and attenuated the initiation of atherosclerosis	120
PLGA nanoparticles	Pitavastatin	<i>Apoe</i> ^{-/-} mice infused with angiotensin II and fed a high-fat diet	Once per week for 4 weeks	Reduced macrophage burden and MMP9 and CCL2 levels in atherosclerotic plaques, decreased plaque area and plaque destabilization and rupture, and increased fibrous cap area in the brachiocephalic artery	121
Polymeric nanoparticles	Five siRNAs targeting key adhesion molecules	<i>Apoe</i> ^{-/-} mice fed a high-fat diet followed by permanent carotid ligation for MI induction	Once per week for 3 weeks	Reduced macrophage and neutrophil burden and necrotic core area in atherosclerotic plaques, decreased plaque area, increased fibrous cap area and decreased protease activity in the aortic root	18
Inhibition of macrophage proliferation					
Reconstituted HDL nanoparticles	Simvastatin	<i>Apoe</i> ^{-/-} mice fed a high-fat diet	Four treatments for 1 week	Decreased macrophage burden in atherosclerotic plaques	128
			Twice per week for 12 weeks	Reduced macrophage burden in atherosclerotic plaques and reduced plaque sizes	129
Cyclodextrin-based phospholipid nanoparticles	Simvastatin	<i>Apoe</i> ^{-/-} mice fed a high-fat diet	Twice per week for 4 weeks	Decreased atherosclerotic plaque size in aortic roots, aortic arches and brachiocephalic arteries	133
Restoration of efferocytic capacity of macrophages					
SWNTs	Small-molecule inhibitor of antiphagocytic CD47–SIRPα	<i>Apoe</i> ^{-/-} mice infused with angiotensin II and fed a high-fat diet	Once per week for 9 weeks	Increased macrophage-associated apoptotic cells and reduced inflammation burden in atherosclerotic plaques and decreased plaque area and necrotic core area	63
S2P receptor-targeted PLGA-PEG nanoparticles	<i>Camk2g</i> -targeting siRNA	<i>Ldlr</i> ^{-/-} mice fed a high-fat diet	Twice per week for 4 weeks	Increased fibrous cap thickness and macrophage-associated apoptotic cells in atherosclerotic plaques and decreased necrotic core area	52
Anti-inflammatory therapies					
Collagen IV-targeted PLGA nanoparticles	The inflammation-resolving protein IL-10	<i>Ldlr</i> ^{-/-} mice fed a high-fat diet	Once per week for 4 weeks	Increased fibrous cap thickness and lesional efferocytosis and decreased necrotic core area and ROS levels in advanced atherosclerotic lesions	67
Collagen IV-targeted PLGA nanoparticles	Ac2-26 pro-resolving peptide	<i>Ldlr</i> ^{-/-} mice fed a high-fat diet	Once per week for 5 weeks	Increased fibrous cap thickness and decreased necrotic core area and ROS levels in advanced atherosclerotic lesions	48
Platelet-derived extracellular vesicles	NLRP3 inflammasome inhibitor	<i>Apoe</i> ^{-/-} mice fed a high-fat diet	Nine treatments during 3 weeks	Decreased local inflammation and macrophage and T cell burdens in atherosclerotic plaques and reduced plaque size	55
PLGA nanoparticles	Pioglitazone	<i>Apoe</i> ^{-/-} mice infused with angiotensin II and fed a high-fat diet	Once per week for 4 weeks	Decreased circulating inflammatory Ly6C ^{high} monocytes, reduced activity of MMP and cathepsins in brachiocephalic arteries, and increased fibrous cap area	145
Cyclodextrin-based polysaccharide nanoparticles	Superoxide dismutase mimetic agent and hydrogen peroxide-scavenging compound	<i>Apoe</i> ^{-/-} mice fed a high-fat diet	Twice per week for 8 weeks	Decreased systemic and plaque oxidative stress and inflammation, reduced cholesterol crystal levels, necrotic core area and plaque size, and increased fibrous cap area in aortic roots, aortic arches and brachiocephalic arteries	147
Macrophage apoptosis					
Crosslinked dextran-coated iron oxide nanoparticles	Photosensitizer	<i>Apoe</i> ^{-/-} mice fed a high-fat diet	Carotid artery was stimulated with 650-nm laser light 24 h after one injection	Increased macrophage apoptosis and decreased macrophage burden in the carotid artery	149

Table 1 (cont.) | Examples of preclinical-stage macrophage-targeted nanomedicine for atherosclerosis treatment

Nanoparticle platform	Active pharmaceutical ingredients	Animal model	Treatment frequency/method	Therapeutic outcomes	Ref.
<i>Macrophage apoptosis (cont.)</i>					
PEG-SWNTs	Photothermal agent	Mice fed a high-fat diet and treated with streptozotocin, followed by carotid artery ligation	Carotid artery was excised and photothermally ablated with 808-nm laser light 48 h after one injection	Increased macrophage apoptosis and decreased macrophage burden in the carotid artery	152
<i>Promotion of cholesterol efflux from macrophages</i>					
Cyclodextrin-derived and integrin-targeting nanoparticles	Anti-miR-33	<i>Apoe</i> ^{-/-} mice fed a high-fat diet	Two treatments in the first week and once per week thereafter for 2 months	Decreased cholesterol crystal levels, MMP expression and macrophage burden in atherosclerotic plaques, reduced plaque size in aortic roots, aortic arches and brachiocephalic arteries, and increased fibrous cap area	74
Collagen IV-targeted PLA nanoparticles	Liver X receptor agonist (GW3965)	<i>Ldlr</i> ^{-/-} mice fed a high-fat diet	Twice per week for 5 weeks	Decreased macrophage burden and increased <i>Abca1</i> expression in atherosclerotic plaques and no adverse effect on hepatic lipid metabolism	68

MI, myocardial infarction; MMP, matrix metalloproteinase; PEG, polyethylene glycol; PLA, polylactic acid; PLGA, poly(D,L-lactic-co-glycolic acid); ROS, reactive oxygen species; siRNA, small interfering RNA; SIRPα, signal-regulatory protein-α; SWNT, single-walled carbon nanotube.

(which is extensively used clinically as an immunosuppressant) with the use of biomimetic nanoparticles, such as leukosomes¹²⁴ or erythrocyte membrane⁵³, or with H₂O₂-responsive¹²⁵ or dual-responsive¹²⁶ cyclodextrin-based nanoparticles suppresses the proliferation of macrophages in atherosclerotic plaques and reduces the levels of key pro-inflammatory cytokines via rapamycin-mediated inhibition of the NF-κB pathway in mice and is associated with reduced toxicity compared with free rapamycin. Additionally, nanoparticle-assisted delivery of simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor historically regarded as a blood LDL-cholesterol-lowering drug for atherosclerosis treatment¹²⁷, can inhibit lesional macrophage proliferation and resolve local inflammation in atherosclerotic *Apoe*^{-/-} mice^{128–130}. A regimen of 1 week of high-dose (four infusions of 60 mg/kg simvastatin) and 12 weeks of low-dose (24 infusions of 15 mg/kg simvastatin) simvastatin-loaded reconstituted HDL (rHDL) nanoparticles reduced the local macrophage burden in the aorta and aortic roots and reduced the plaque area in the aortic sinus of atherosclerotic *Apoe*^{-/-} mice, respectively^{128,129}. In an effort to demonstrate the clinical translatability of this regimen, the same group used a microfluidizer-based high-pressure homogenization approach for the large-scale production of simvastatin-rHDL nanoimmunotherapeutics in a controlled and reproducible manner for anti-atherogenesis studies in larger animals, including rabbit and pig models of atherosclerosis^{58,131}. Interestingly, a systematic comparison showed that the simvastatin-encapsulated PEGylated polymeric micelles have higher anti-atherosclerosis efficacy in *Apoe*^{-/-} mice than simvastatin delivery by rHDL nanoparticles or liposomes¹³². These findings are probably due to the higher stability of the polymeric drug nanoformulation as well as the increased efficiency of targeted drug delivery to plaque macrophages¹³².

Removing inflammation-triggering cholesterol from the plaque microenvironment by the same type

of nanoparticles might also yield therapeutic benefits. For instance, Kim and colleagues designed cargo-switching nanoparticles that can scavenge intracellular cholesterol while releasing simvastatin from the methyl-β-cyclodextrin phospholipid core-shell nanoparticles¹³³. This cargo-switching strategy enabled not only the local delivery of simvastatin to lesional macrophages but also the depletion of pre-existing cholesterol in plaques, thereby achieving synergistic anti-atherogenic efficacy in *Apoe*^{-/-} mice¹³³. Of note, the cargo-switching nanoparticles did not reduce plasma cholesterol levels, indicating that the anti-atherogenic effects were not mediated by a systemic cholesterol-lowering effect¹³³. Similar to cargo-switching nanoparticles, the administration of atorvastatin-encapsulated macrophage-biomimetic nanoparticles that can inhibit local macrophage proliferation and sequester pro-inflammatory cytokines in the lesions yielded synergistic therapeutic efficacy⁵⁶. Targeted delivery of dual therapeutics by the same type of nanoparticle showed great promise in improving the therapeutic efficacy in mice⁵⁶ owing to their capacity to act on complementary anti-atherogenic pathways.

Restoration of the efferocytic capacity of macrophages.

Restoring the capacity of macrophages to carry out efferocytosis (the phagocytic clearance of apoptotic cells) has the potential to stabilize vulnerable plaques and reduce inflammation^{7,49} (FIG. 3c). A 2016 study reported that the upregulation of CD47, a ‘do-not-eat-me’ signal, in atherosclerotic plaques is one cause of defective efferocytosis by plaque macrophages, allowing the local accumulation of apoptotic cells and the promotion of atherosclerosis¹³⁴. Indeed, although the first trial in humans of an anti-CD47 antibody therapy efficiently restored efferocytosis by macrophages, the antibody also resulted in anaemia owing to the removal of red blood cells from the spleen¹³⁵. To reactivate efferocytosis while reducing adverse effects, Flores and colleagues

developed a macrophage-specific nanotherapy, namely SWNTs that encapsulate a small-molecule inhibitor of signal-regulatory protein- α (SIRP α), the antiphagocytic target of CD47 (REF.⁶³). The researchers showed that these nanoparticles reactivated macrophage efferocytosis in atherosclerotic plaques and substantially reduced plaque burden in *Apoe*^{-/-} mice without inducing off-target adverse effects⁶³.

Our findings reveal that the calcium-activated kinase CaMKII γ is activated in lesional macrophages during atherosclerosis progression and this process was shown to reduce the expression of the efferocytosis receptor MERTK and to further impair efferocytosis and exacerbate plaque necrosis in aortic root lesions of *Ldlr*^{-/-} mice fed a high-fat diet^{44,136}. To restore efferocytosis in lesional macrophages, we employed macrophage-targeted siCamk2g nanoparticles (termed S2P-siCamk2g), which consist of a PLGA core with encapsulated siRNA-cationic lipid complex and surface-derivatized S2P targeting peptides⁵². Treatment of atherosclerotic *Ldlr*^{-/-} mice with S2P-siCamk2g nanoparticles not only effectively restored efferocytosis in atherosclerotic lesional macrophages but also promoted plaque stabilization by increasing fibrous cap thickness and decreasing the necrotic core area.

Anti-inflammatory therapies. Given that chronic inflammation is a central pathological feature throughout all stages of atherosclerosis, inhibition of inflammation has been considered an essential therapeutic strategy^{137,138} (FIG. 3d). However, as revealed by the CIRT trial¹³⁹, systemic treatment with a low dose of the anti-inflammatory drug methotrexate did not lower the plasma levels of pro-inflammatory cytokines or reduce cardiovascular events compared with placebo. These findings, combined with the results of the CANTOS³⁶ and COLCOT³⁷ studies, suggest that the targeted delivery of anti-inflammatory nanotherapeutics to lesional macrophages might be preferred over a systemic approach to effectively resolve inflammation while reducing adverse effects. Therefore, an ongoing phase II clinical trial¹⁴⁰ has been designed to treat patients with stable coronary disease with methotrexate-loaded LDL-like nanoparticles, which can specifically target LDL receptors on the inflammatory macrophage surface. This approach might provide more therapeutic benefits by the targeted delivery of this anti-inflammatory drug to lesional macrophages.

Great efforts have been made in the past 10 years towards the targeted delivery of anti-inflammatory drugs in animal models of atherosclerosis. For example, attempts have been made to deliver IL-10, an inflammation-resolving cytokine that can temper inflammation, by long-circulating collagen type IV-targeted PLGA⁶⁷ and cRGD peptide-conjugated pluronic-based¹⁴¹ nanocarriers. The targeted delivery of IL-10 not only significantly increased its systemic half-life but also substantially improved delivery to lesional macrophages compared with free IL-10, effectively improving atherosclerotic plaque stability in *Ldlr*^{-/-} mice⁶⁷ or *Apoe*^{-/-} mice¹⁴¹ fed a high-fat diet. In addition, the same type of collagen type IV-targeted PLGA

nanoparticles has been used to deliver a pro-resolving annexin A1 biomimetic Ac2-26 peptide to atherosclerotic macrophages in mice^{48,142}. The targeted delivery of the Ac2-26 peptide by nanoparticles significantly increased its atheroprotective effects in *Ldlr*^{-/-} mice fed a high-fat diet compared with those of free Ac2-26 peptide treatment⁴⁸. In 2020, M2-like macrophage-derived exosomes with inflammation tropism were used to deliver hexyl 5-aminolevulinate hydrochloride, an FDA-approved drug that can promote the intracellular generation of anti-inflammatory carbon monoxide and bilirubin via the intrinsic biosynthesis pathway⁵⁷. The anti-inflammatory cytokines released from M2-like macrophages and the anti-inflammatory bio-products synergistically promoted regression of atherosclerotic plaques in *Apoe*^{-/-} mice. Interestingly, unlike findings with conventional drug-loaded nanotherapeutics, a study showed that hyaluronan nanoparticles can selectively target inflammatory macrophages in atherosclerotic mice and function as an anti-inflammatory agent on their own (without any payload) by directly interacting with macrophages to downregulate the inflammatory response⁷⁷. The therapeutic effects were demonstrated by the reduced production of nitric oxide and tumour necrosis factor, two key pro-inflammatory mediators in atherosclerosis, by inflammatory macrophages⁷⁷. This therapeutic strategy might simplify the preparation of nanotherapeutics without the concerns about drug loading and release behaviour.

The targeted delivery of inhibitors that can reduce the secretion of pro-inflammatory cytokines from plaque macrophages is a particularly promising approach for atherosclerosis treatment. For example, a study published in 2021 showed that inflammatory cell-targeted platelet-derived extracellular vesicles efficiently delivered an NLRP3 inflammasome inhibitor to macrophage-derived foam cells in atherosclerotic plaques in atherosclerotic mice⁵⁵. This approach significantly reduced local inflammation (reflected by decreased IL-1 β levels), atherosclerotic plaque area, and macrophage and T cell burdens in these mice⁵⁵. Similar therapeutic effects were reported in mice after the targeted delivery of methotrexate with the use of polymeric nanoparticles^{143,144} and liposomes¹⁴⁴. Remarkably, owing to the improved pharmacokinetic profile, bioavailability and drug accumulation in the lesional macrophages, the nanoparticle-facilitated targeted delivery approach significantly increased anti-inflammatory and anti-atherogenic efficacy and reduced systemic toxicity compared with the free drugs alone. Nakashiro and colleagues used PLGA nanoparticles to deliver pioglitazone, a potent agonist of PPAR γ that can polarize macrophages towards a less-inflammatory, M2-like subtype in angiotensin II-infused *Apoe*^{-/-} mice fed a high-fat diet¹⁴⁵. This therapeutic strategy is particularly advantageous in advanced atherosclerotic plaques given that macrophages that differentiate from recruited monocytes in advanced lesions might favour the pro-inflammatory M1-like subtype over the M2-like subtype that participates in inflammation resolution¹³⁸.

Lastly, efficient scavenging of overproduced ROS from lesional macrophages to attenuate

ROS-induced inflammation and reduce the secretion of pro-inflammatory cytokines is of considerable interest for atherosclerosis treatment¹⁴⁶. Indeed, a series of superoxide dismutase mimetic cyclodextrin-based nanoparticles that efficiently scavenge a broad spectrum of ROS and significantly reduce the serum and atherosclerotic plaque levels of ROS-induced pro-inflammatory cytokines and decreased cholesterol crystal formation in the lesions in *Apoe*^{-/-} mice were developed^{147,148}.

Macrophage apoptosis. Externally induced macrophage apoptosis can reduce macrophage burden and thereby reduce macrophage-associated inflammation and plaque burden in early atherosclerotic lesions (FIG. 3e). Owing to its high spatial resolution, light can be used to induce apoptosis specifically in lesional macrophages with the use of light-sensitive molecules and thereby avoid compromising the host defence. For instance, McCarthy and colleagues used macrophage-avid, crosslinked, dextran-coated iron oxide nanocrystals loaded with photosensitizers for targeted macrophage ablation¹⁴⁹. When activated by 650-nm light, these photosensitizers produce cytotoxic ROS to induce extensive macrophage apoptosis in the carotid artery atheroma in *Apoe*^{-/-} mice, reducing the macrophage burden and increasing plaque stability. The therapeutic effects shown in this study open new avenues for atherosclerosis treatment because externally modulated macrophage apoptosis could be used to promote plaque regression. Indeed, local hyperthermia generated by photo-activatable photothermal agents that induce macrophage apoptosis has also shown great promise for atherosclerosis treatment, as demonstrated in the Yucatan miniature swine model (PLASMONICS study)¹⁵⁰. Encouraged by the superior therapeutic efficacy of the approach used in this study, investigators in a first-in-human trial (NANOM-FIM)¹⁵¹ delivered the same type of silica-gold-based nanoparticles by using a bioengineered on-artery patch followed by local photothermal ablation. This photothermal therapy showed great promise, yielding an average 37.8% reduction in atherosclerotic plaque burden compared with baseline and significantly reducing cardiovascular mortality compared with patients who were implanted with a stent. In addition to gold nanoparticles, tremendous efforts have been made in the past 10 years in designing photothermal agents for photothermal-mediated macrophage apoptosis in preclinical studies such as SWNTs¹⁵², gold nanorods¹⁵³ and MoO₂ nanoclusters¹⁵⁴. The common advantages of these nanoparticles for efficient photothermal ablation are superior photothermal conversion efficiency, excellent biocompatibility and efficient phagocytic uptake by inflammatory macrophages. Of note, a non-invasive alternating magnetic field in conjunction with magnetic nanoparticles has been extensively studied for antitumour therapy¹⁵⁵ and we envision that such a spatiotemporally controllable approach might offer a promising alternative strategy for hyperthermia-induced macrophage apoptosis. Nevertheless, the therapeutic outcomes of this strategy might vary significantly in early versus late atherosclerotic lesions because defective phagocytic clearance of apoptotic macrophages can lead to secondary necrosis,

promote a pro-inflammatory response and exacerbate atherosclerosis in late lesions¹⁵⁶.

Promotion of cholesterol efflux from macrophages.

Lipid metabolism of macrophages is closely associated with the degree of macrophage foam cell formation and severity of atherosclerosis³⁴. Promoting cholesterol efflux from lipid-laden macrophages by increasing ABCA1 or ABCG1 expression has been considered a promising strategy to prevent excess lipid accumulation in atherosclerotic macrophages^{35,157,158} (FIG. 3f). miRNA-mediated oligonucleotide-based therapeutic approaches that specifically target predefined gene sequences have been shown to upregulate the expression of ABCA1 in macrophages and increase cholesterol efflux¹⁵⁹. Similar to siRNAs, this robust nanoparticle-mediated delivery approach is particularly practical to prevent the hydrolysis of delicate miRNA by nucleases in the circulation and peripheral tissues. For instance, chitosan nanoparticle-mediated delivery of efflux-promoting miR-206 or miR-223 markedly increased cholesterol efflux from macrophage-derived foam cells and reverse cholesterol transport in mice⁸¹. In addition, integrin-targeting and cyclodextrin-based nanoparticles were used to deliver an antisense oligonucleotide against miR-33, a miRNA that inhibits cholesterol efflux, to macrophages in atherosclerotic lesions and was shown to substantially increase the cholesterol efflux capacity of lesional macrophages and reduce plaque burden in atherosclerotic *Apoe*^{-/-} mice compared with free antisense miR-33 alone⁷⁴.

Another strategy that can upregulate ABCA1 and ABCG1 expression to promote cholesterol efflux from macrophages is the activation of LXRs³⁰. However, systemic LXR activation is known to induce liver toxicity and increase plasma and hepatic triglyceride levels, resulting in the failure of this approach in clinical translation¹⁶⁰. In the past decade, nanoparticle-mediated delivery of an LXR agonist has shown great promise in promoting ABCA1 expression and cholesterol efflux while reducing adverse liver toxicities^{62,161,162}. Yu and colleagues used biodegradable collagen type IV-targeted poly(D,L-lactide) polymeric nanoparticles to deliver the LXR agonist GW3965 to atherosclerotic lesions in *Ldlr*^{-/-} mice⁶⁸. The primary therapeutic effects of this nanoparticle-mediated delivery included increased cholesterol efflux from lesional macrophages and decreased macrophage burden and inflammatory cytokine levels in atherosclerotic mice, while plasma triglyceride and cholesterol levels and hepatic lipid metabolism remained unaffected. Both the improved therapeutic effects and reduced adverse effects generated by nanoparticle-mediated delivery of GW3965 are preferable to free GW3965 treatment⁶⁸. Additionally, cholesterol efflux was also promoted by synthetic HDL-mediated delivery of the LXR α agonist T1317 to plaque macrophages in *Apoe*^{-/-} mice¹⁶³.

In addition to cholesterol efflux, autophagy can prevent foam cell formation by using double membranes in macrophages to sequester and degrade lipid droplets through lysosomal acid lipase¹⁶⁴. The activation of autophagy to regress plaques has been

demonstrated in *ApoE*^{-/-} mice by inhibiting mechanistic target of rapamycin (mTOR) through targeted and H₂O₂-responsive delivery of siRNA with the use of cerium oxide nanowires⁸³ or by the targeted delivery of rapamycin through platelet membrane-coated PLGA nanoparticles¹⁶⁵. Of note, local inflammation in atherosclerosis might activate and stimulate platelet attachment to endothelial cells, which makes this biomimetic platelet membrane-coated delivery strategy promising for atherosclerosis management.

Different from the abovementioned cholesterol efflux and lipid-sequestering approaches, a library of sugar-based amphiphilic nanoparticles was designed to block and reduce the expression of macrophage scavenger receptor 1 and CD36, two macrophage surface receptors for oxidized lipid uptake¹⁶⁶. The competitive binding strategy and the downregulation of these two scavenger receptors by sugar-based amphiphilic nanoparticles synergistically reduced oxidized lipid accumulation in

the arterial wall and atherosclerotic plaque formation in *ApoE*^{-/-} mice¹⁶⁶.

Challenges and perspectives

Over the past two decades, our improved understanding of the pathophysiological processes of atherogenesis and rapid advances in nanotechnology and bioengineering have robustly fuelled research into novel nanotherapeutic strategies to treat cardiovascular diseases. Although clinically approved nanotherapeutics are still scarce¹⁶⁷, the shift in therapeutic target from lipid lowering to inflammation resolution has opened new avenues for nanomedical atherosclerosis management as highlighted in this Review (FIG. 4). Although modulation of the immunoprofile of lesional macrophages by nanoparticles has shown great promise in resolving inflammation in atherosclerosis, the concomitant hampering of the innate immune system (which forms the first line of defence against pathogens) needs to be carefully investigated before moving to clinical trials. In this regard, accurate modulation of the local immune responses is particularly crucial to reduce systemic adverse effects. On the basis of findings from the CANTOS³⁶, CIRT¹⁶⁸ and COLCOT³⁷ trials, which had large study cohorts, targeted delivery of anti-inflammatory therapeutics is superior to systemic delivery in reducing atherosclerotic plaque inflammation without compromising the host defence. Additionally, the targeted delivery of nanotherapeutics to lesional macrophages could also diminish systemic adverse effects, which are frequently the result of treatment with free drugs. Of note, the targeted delivery of dual therapeutics that synergistically act on different anti-atherogenic pathways might also increase the efficacy of the nanotherapeutics.

Nevertheless, despite the great promise already shown, we should note that the success of nanotherapeutics in preclinical studies cannot guarantee their efficacy in humans¹⁶⁹. This discrepancy might result from the substantial differences in atherosclerotic pathobiology between animal models and humans. In animal models, atherosclerosis is usually established in weeks, whereas atherosclerotic plaques in patients develop over a period of years or even decades and are therefore more complex and heterogeneous¹⁷⁰. Additionally, animal models are often genetically manipulated in a way that is not necessarily relevant to humans. For example, *ApoE*^{-/-} mice have a completely blunted clearance of apoE-containing lipoproteins and therefore develop atherosclerotic lesions independently of cholesterol feeding whereas, in humans, atherosclerosis usually develops in a multigenetic manner and in combination with environmental factors¹⁷¹. The use of mouse models, such as the *ApoE*^{*3}-*Leiden*.*CETP* mouse, with a lipoprotein metabolism closer to that of humans, might have advantages¹⁷². Mice are naturally deficient in cholesteryl-ester transfer protein (CETP) and therefore have high levels of HDL and low levels of LDL and VLDL. The *ApoE*^{*3}-*Leiden*.*CETP* mouse contains a human *CETP* transgene as well as a mutated human *APOE* gene, resulting in moderately reduced LDL receptor-mediated clearance of apoB-containing lipoproteins as well as a humanized lipoprotein cholesterol distribution. Although findings from *ApoE*^{*3}-*Leiden*.

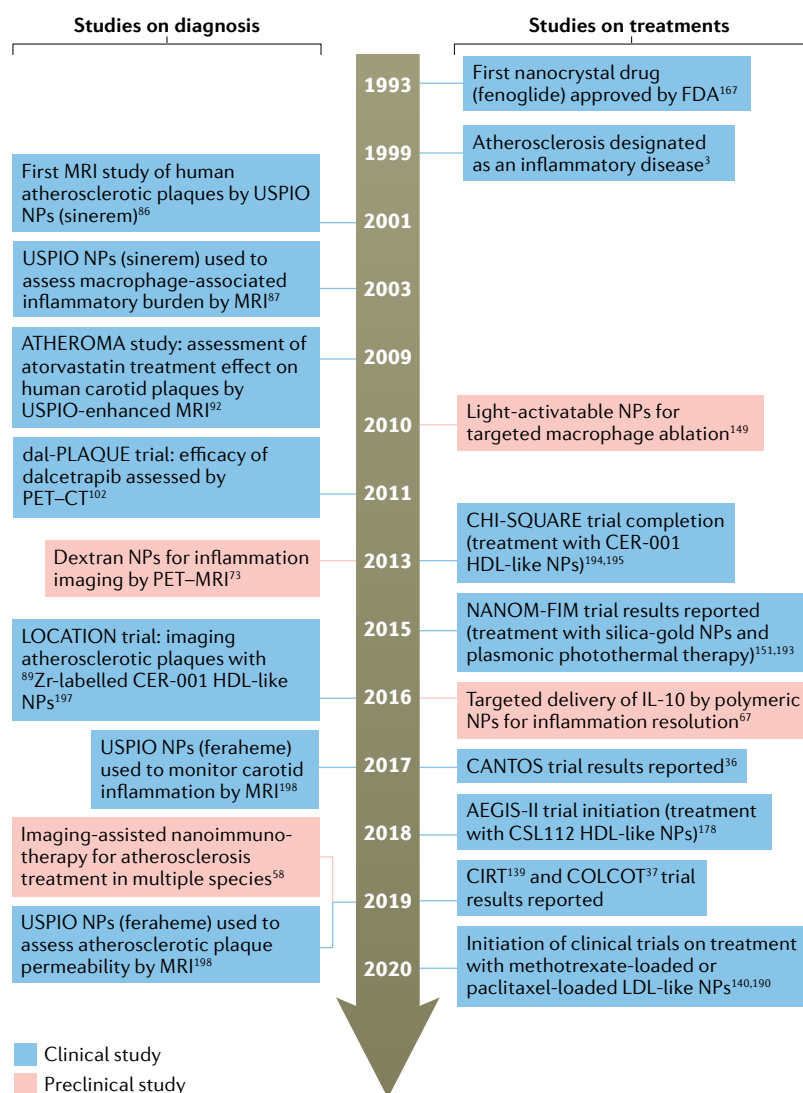
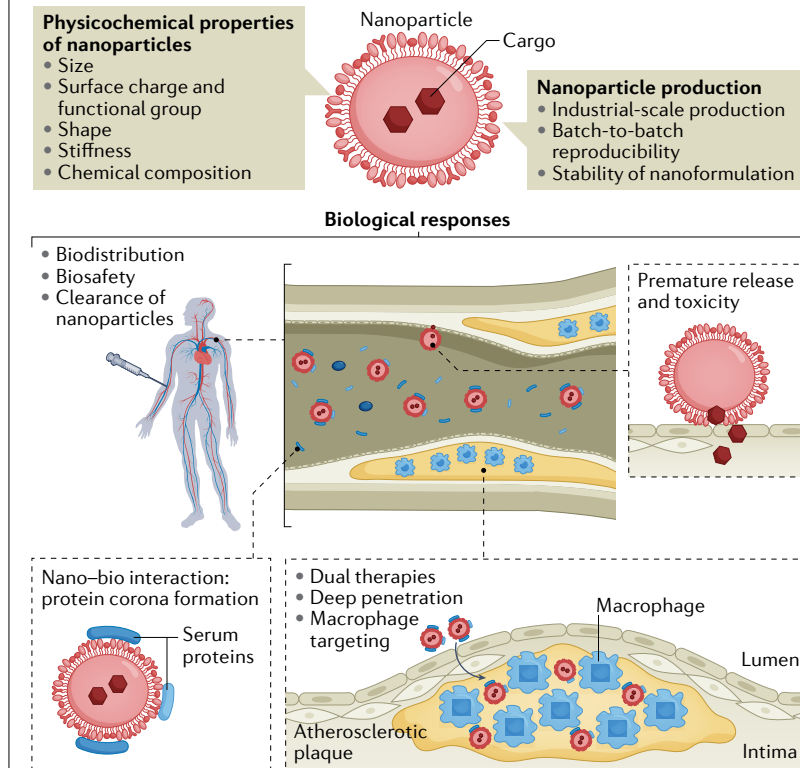


Fig. 4 | **Timeline of major developments in the field of atherosclerosis nanomedicine.** Milestones in the development of nanoparticle-based imaging contrast agents and therapeutics for atherosclerosis diagnosis and treatment. NP, nanoparticle; USPIO, ultrasmall superparamagnetic iron oxide.

Box 2 | Nanoparticle-associated challenges and considerations

- Exploration of the physicochemical interactions at the nano–bio interface, such as protein corona formation, and their effects on blood circulation, pharmacokinetics, biodistribution, intra-plaque distribution, drug-release profile, cellular uptake and intracellular trafficking of nanoparticles (see the figure).
- Evaluation of biosafety, toxicity, immunogenicity and clearance of nanotherapeutics and nanoparticle-based imaging agents.
- Optimization of the stability of nanoformulation and investigation of the pharmaceutical properties (such as therapeutic efficacy and toxicity) of the nanoformulation compared with those of the free drug alone.
- Examination of the effects of physicochemical properties of nanoparticles on the extravasation into atherosclerotic plaques, lesional macrophage targeting, plaque penetration, macrophage-specific uptake and subcellular distribution.
- Industrial-scale production of nanoparticles and the specific nanoformulation under good manufacturing practice (GMP) and chemistry, manufacturing and controls (CMC) for batch-to-batch reproducibility.
- Efficacy of targeted delivery of dual or multiple therapeutics on different anti-atherogenic pathways for synergistic anti-atherosclerosis therapies.



Nano–bio interface

The interface between nanoparticles and biological systems, such as serum proteins and extracellular matrix; interactions at the nano–bio interface determine the biological fates of nanoparticles in living organisms.

Bioimaging

Non-invasive imaging techniques to visualize biological processes.

CETP mice might translate better to humans compared with other mouse models, these models have not yet been used in preclinical studies on macrophage-targeted nanomedicine of atherosclerosis (TABLE 1). Furthermore, it is advisable to feed these mice a diet that only moderately increases plasma cholesterol levels and allow atherosclerosis to develop over a longer period of time, similar to atherosclerosis development in humans. Lastly, whenever possible, nanomedicine-mediated findings in mice should be validated by studies using human genetic banks and the identification of novel therapeutic targets in mice should be paralleled by human genetic studies. Moreover, macrophage subtypes and plaque composition vary from patient to patient and during different stages of atherosclerosis^{22,173}, making administration of

personalized nanotherapeutics particularly crucial to achieving the desirable therapeutic outcomes.

Nanoparticle-facilitated bioimaging technologies can non-invasively characterize plaque composition, monitor inflammatory macrophages and assess therapeutic outcomes. Current imaging modalities have rapidly evolved and state-of-the-art imaging technologies are now being transformed from tools for imaging existing atherosclerosis in symptomatic patients to sophisticated means of non-invasively characterizing early-stage subclinical abnormalities. The clinically available integrated imaging systems in combination with two or even more imaging modalities can synergize the strengths of each individual technique and thereby provide more insights into atherogenesis at the molecular and functional levels. This imaging-facilitated diagnosis would help physicians to provide appropriate early medical interventions to prevent further complications. In addition, the importance of accurate bioimaging has been demonstrated, for example, by providing surrogate end points for novel atherosclerosis therapeutics in clinical settings^{92,103,174}. Bioimaging-assisted characterization enables a dramatic reduction of the number of patients needed in studies and costs as well as shortening the observation period for assessing the efficacy of newly developed therapeutic interventions in clinical settings¹⁷⁵. In addition to clinical imaging modalities, the recently developed ‘shortwave infrared’ (SWIR, 1,000–2,000 nm) imaging, also called ‘second window of near-infrared’ (900–1700 nm) imaging, acquired using an InGaAs camera, might provide an alternative method to non-invasively monitor inflammatory burdens in real time. More specifically, the SWIR region of the electromagnetic spectrum has advantages over the traditional near-infrared window (700–900 nm) owing to deeper tissue penetration, higher spatial resolution, decreased autofluorescence, and minimized photon absorption and scattering by tissues at these wavelengths^{176,177}. In addition, the multiplexed SWIR images might provide more mechanistic insights into the interaction between different macrophage subsets (such as M1, M2a, M2b and M2c) and other cells (for example, T cells, B cells, neutrophils and vascular smooth muscle cells) or biomolecules in the microenvironments of atherosclerotic plaques when the target-specific nanoparticles are used.

If some nanoparticles for atherosclerosis diagnosis and therapy reached the clinic, it would be highly beneficial. However, before then, the many prerequisites include a detailed evaluation of the biosafety, biodistribution and clearance of the nanotherapeutics in preclinical studies, industrial-scale production of nanoparticles under good manufacturing practice (GMP) to guarantee reproducibility from batch to batch, and investigation of whether the nanotherapeutics show higher treatment efficacy and fewer adverse effects than those of free drugs alone. Nanoparticle-associated challenges and considerations are summarized in BOX 2. For example, several nanoparticle platforms (such as HDL-like and LDL-like nanoparticles) that can target lesional macrophages for atherosclerosis treatment and diagnosis have entered clinical trials, as highlighted in TABLE 2.

Table 2 | Examples of clinical trials on macrophage-targeted nanomedicine for atherosclerosis treatment and diagnosis

Study name	Design	Cohort	Number of patients	Nanoparticle platform	Active pharmaceutical ingredients	Study objectives	Status/outcomes	Refs; trial numbers
Treatment								
NA	Phase II, randomized, double-blind, placebo-controlled	Patients with stable CAD	40	LDL-like nanoparticles	Methotrexate	Safety and efficacy of LDL-like nanoparticles loaded with the anti-inflammatory agent methotrexate in reducing plaque volume and adverse characteristics	Ongoing, estimated completion in autumn 2023	¹⁴⁰ ; NCT04616872
PAC-MAN	Phase II, randomized, double-blind, placebo-controlled	Patients with stable CAD	40	LDL-like nanoparticles	Paclitaxel	Safety and efficacy of LDL-like nanoparticles loaded with the anti-proliferative agent paclitaxel in reducing plaque volume and adverse characteristics	Ongoing, estimated completion in summer 2021	¹⁹⁰ ; NCT04148833
AEGIS-II	Phase III, multicentre, randomized, double-blind, placebo-controlled	Patients with acute coronary syndrome	17,400	CSL112 HDL-like nanoparticles	HDL-like nanoparticles	Efficacy and safety of CSL112 in reducing the risk of major adverse cardiovascular events (cardiovascular death, myocardial infarction and stroke)	Ongoing, estimated completion in spring 2023	¹⁷⁸ ; NCT03473223
TANGO	Phase III, multicentre, randomized, double-blind, placebo-controlled	Patients with genetically determined very low levels of HDL cholesterol	30	CER-001 HDL-like nanoparticles	HDL-like nanoparticles	Efficacy of the HDL mimetic CER-001 in reducing vessel wall area and arterial wall inflammation	CER-001 treatment did not reduce carotid vessel wall dimensions or arterial wall inflammation	¹⁹¹ ; NCT02697136
MILANO-PILOT	Phase I, randomized, double-blind, placebo-controlled	Patients with recent acute coronary syndrome	122	MDCO-216 HDL-like nanoparticles	HDL-like nanoparticles	Efficacy of the HDL mimetic MDCO-216 in reducing coronary artery atherosclerosis progression	MDCO-216 treatment did not produce plaque regression in statin-treated patients following an acute coronary syndrome	¹⁹² ; NCT02678923

Table 2 (cont.) | Examples of clinical trials on macrophage-targeted nanomedicine for atherosclerosis treatment and diagnosis

Study name	Design	Cohort	Number of patients	Nanoparticle platform	Active pharmaceutical ingredients	Study objectives	Status/outcomes	Refs; trial numbers
<i>Treatment (cont.)</i>								
Gille et al.	Phase I, II and IIa, randomized, double-blind, placebo-controlled	Patients with stable atherosclerotic disease and healthy individuals	57, 36 and 44	CSL112 HDL-like nanoparticles	HDL-like nanoparticles	Efficacy of the HDL mimetic CSL112 in improving cholesterol efflux capacity	CSL112 treatment increased cholesterol efflux similarly in healthy individuals and in patients with stable atherosclerotic disease compared with untreated controls	¹⁷⁹ ; NCT01129661, NCT01281774, NCT01499420
NANOM-FIM	Multicentre, randomized, double-blind, observational	Patients with CAD and angiographic SYNTAX score ≤ 22	180	Silica-gold nanoparticles	Gold nanoparticles	Efficacy and safety of silica-gold nanoparticles in inducing plaque regression by plasmonic photothermal therapy	Reduction of plaque burden; 5-year follow-up study showed high safety with lower mortality, fewer major adverse cardiovascular events and less target-lesion revascularization compared with sirolimus-eluting stenting control	^{151,193} ; NCT01270139
CARAT	Phase II, multicentre, randomized, double-blind, placebo-controlled	Patients with acute coronary syndrome and high plaque burden receiving statin therapy	293	CER-001 HDL-like nanoparticles	HDL-like nanoparticles	Effect of the HDL mimetic CER-001 on coronary artery atherosclerosis progression	No regression of coronary atherosclerosis	¹⁹⁴ ; NCT02484378
CHI-SQUARE	Phase II, multicentre, randomized, double-blind, placebo-controlled	Patients with acute coronary syndrome	507	CER-001 HDL-like nanoparticles	HDL-like nanoparticles	Effect of the HDL mimetic CER-001 on coronary atherosclerosis progression	CER-001 infusions did not reduce coronary atherosclerosis measured by intravascular ultrasonography and quantitative coronary angiography	¹⁹⁵ ; NCT01201837
Nissen et al.	Pilot, multicentre, randomized, double-blind, placebo-controlled	Patients with acute coronary syndromes	123	ETC-216 HDL-like nanoparticles	HDL-like nanoparticles	Efficacy of the HDL mimetic ETC-216 in reducing atheroma burden	Regression of coronary artery atherosclerosis as measured by intravascular ultrasonography imaging	¹⁹⁶
SILENCE	Phase I/II, randomized, double-blind, placebo-controlled	Patients with severe inflamed carotid or aortic atherosclerosis	30	Liposomes	Prednisolone	Efficacy of long-circulating liposomal glucocorticoids in reducing vessel wall inflammation	Plaque-accumulated liposomal prednisolone had no anti-inflammatory effect	¹⁶⁹ ; NCT01601106

Table 2 (cont.) | Examples of clinical trials on macrophage-targeted nanomedicine for atherosclerosis treatment and diagnosis

Study name	Design	Cohort	Number of patients	Nanoparticle platform	Active pharmaceutical ingredients	Study objectives	Status/outcomes	Refs; trial numbers
Diagnosis								
LOCATION	Observational, single group	Patients with atherosclerotic carotid artery disease (>50% stenosis)	8	⁸⁹ Zr-labelled CER-001 HDL-like nanoparticles	⁸⁹ Zr	Evaluation of the delivery efficacy of the HDL mimetic CER-001 to carotid plaques, assessed by PET-CT imaging	Infusion of CER-001 increased plasma cholesterol efflux capacity; CER-001 targeted plaque regions	¹⁹⁷ ; NTR5178 (Netherlands trial register)
Zheng et al.	Observational	Patients with peripheral artery disease	18	Carboxymethyl-dextran-coated USPIO nanoparticles (ferumoxytol)	USPIO nanoparticles	Non-invasive assessment of whether delivery efficacy of nanoparticles depends on plaque permeability	Quantitative USPIO-enhanced MRI demonstrated that ferumoxytol accumulated in atherosclerotic plaques after systemic infusion	¹⁹⁸
Smits et al.	Observational	Patients with carotid artery stenosis >30%	9	Carboxymethyl-dextran-coated USPIO nanoparticles (ferumoxytol)	USPIO nanoparticles	Relationship between USPIO nanoparticle uptake and plaque burden in atherosclerotic plaques, assessed by MRI	Ferumoxytol was taken up by atherosclerotic plaques and might be used for carotid USPIO-enhanced MRI	¹⁹⁹
ATHEROMA	Observational, double-blind	Patients with carotid stenosis >40% assessed by duplex ultrasonography	47	Dextran-coated USPIO nanoparticles (ferumoxtran-10)	USPIO nanoparticles	Effect of atorvastatin therapy on carotid plaque inflammation as assessed by USPIO-enhanced MRI	Reduction of USPIO-defined inflammation after high-dose atorvastatin treatment	⁹² ; NCT00368589
Tang et al. and Howarth et al.	Observational	Patients with carotid stenosis	20	Dextran-coated USPIO nanoparticles (ferumoxtran-10)	USPIO nanoparticles	Differences in the degree of MRI-defined inflammation between patients with symptomatic carotid artery atherosclerosis and patients with asymptomatic carotid artery atherosclerosis	Plaques in symptomatic patients had a much greater degree of signal loss, indicating higher inflammation, than plaques from asymptomatic patients	^{200,201}
Schmitz et al.	Phase III, multicentre, observational	Patients with unenhanced and SPIO-enhanced MRI of the pelvis were included for retrospective analysis	20	Dextran-coated SPIO nanoparticles (ferumoxtran-10)	SPIO nanoparticles	Incidentally found SPIO nanoparticle uptake in aortic and arterial wall	Infusion of SPIO nanoparticles enhanced the contrast of atherosclerotic plaques in MRI	⁸⁶

CAD, coronary artery disease; NA, not applicable; SPIO, superparamagnetic iron oxide; USPIO, ultrasmall superparamagnetic iron oxide.

Of note, the ongoing AEGIS-II trial¹⁷⁸ will test whether HDL-like nanoparticles can reduce the risk of major adverse cardiovascular events in patients with acute coronary syndrome. This nanoformulation, previously demonstrated to improve cholesterol efflux efficacy of lesional macrophages in both healthy individuals and patients with coronary or peripheral artery disease¹⁷⁹, might provide therapeutic benefits for patients with acute coronary syndromes. In addition, an equally important but often overlooked consideration is that even though some nanocarriers have been proven to be biocompatible and to have superior safety profiles for drug delivery and bioimaging, they might not be suitable for studies on anti-atherosclerosis therapies. For example, the administration of amorphous silica nanoparticles would intensify macrophage infiltration, increase endoplasmic reticulum stress in atherosclerotic plaques, and increase the serum levels of LDL cholesterol and total triglycerides, thereby actually exacerbating atherosclerosis progression¹⁸⁰. In addition, systemic administration of the FDA-approved iron supplement ferumoxytol nanoparticles might increase iron accumulation and the presence of pro-inflammatory M1-like macrophages in atherosclerotic lesions^{181,182}. Consequently, the immune responses modulated by nanoparticles must be comprehensively investigated before the use of these strategies in clinical trials.

Thanks to the invention of advanced biological research techniques, such as single-cell RNA sequencing and flow cytometry, our understanding of the pathophysiological processes of atherosclerosis has evolved far beyond the former focus on lipid accumulation because more inflammation-associated pathological markers and the transcriptomic and epigenomic characteristics of lesional macrophages have been identified^{21,183}. This transcriptome-based landscape of atherosclerotic plaque progression could accelerate the discovery of more diagnostic and therapeutic targets and, consequently, more nanoparticle-based interventions can be tailor-made against those cardiovascular disease processes. In addition, vaccination against atherosclerosis by selectively modulating atheroprotective and pro-atherogenic

immunity has shown great promise over the past decade^{184–186}. Modulation of the immune response using a vaccine containing antigens relevant to atherogenesis offers the potential to elicit a specific immune response against the relevant antigens without affecting host immunity. For example, the administration of vaccines containing apoB-100 peptides derived from oxidized LDL has shown therapeutic efficacy in an animal model of atherosclerosis by increasing the activation of regulatory T lymphocytes in the plaques. Indeed, the emerging use of mRNA as a therapeutic agent has been applied to modulate innate and adaptive immunogenicity by increasing intracellular protein translation and activating specific signalling pathways¹⁸⁷. Indeed, such an effective nanoparticle-mediated mRNA delivery strategy has dramatically affected the landscape of vaccine development and mRNA vaccines have been applied to prevent coronavirus disease 2019 (COVID-19)^{188,189}. We envision that nanoparticle-mediated mRNA vaccine technology might also be used to prevent the initiation and even progression of atherosclerosis.

Conclusions

Although lipid-lowering therapies have been considered effective strategies for atherosclerosis treatment, cardiovascular disease remains the leading cause of death worldwide¹. Innovative nanotechnology and our improved understanding of atherosclerosis pathobiology have synergistically propelled the development of novel nanotherapeutics for atherosclerosis diagnosis and treatment. In this Review, we have highlighted progress in the past two decades in macrophage-targeted nanomedicine for atherosclerosis diagnosis and treatment and have addressed considerations for clinical translation. Despite the obvious challenges, we believe that the numerous successes of nanomedicine-based approaches in preclinical studies of atherosclerosis and their use in humans for cancer treatment bode well for their eventual use in the diagnosis and treatment of patients with cardiovascular disease.

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Author contributions

All the authors wrote the article and reviewed and edited the manuscript before submission. W.C. and M.S. designed the figures for initial submission.

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