Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Anti-Atherosclerotic Nanoparticles

Evidence Summary

rHDL based approaches have been clinically unsuccessful. More research *in vivo* is needed on how the nanoparticle composition affects biodistribution, clearance, and long-term safety.

Neuroprotective Benefit: The effects of anti-atherosclerotic nanoparticles on cognition or dementia prevention has not been studied.

Aging and related health concerns: Nanoparticles alone have been unsuccessful for atherosclerosis, but modifications that facilitate macrophage targeting and encapsulation of anti-inflammatory agents may increase efficacy.

Safety: Nanoparticles accumulate in the liver and the long-term safety is unknown. Nanoparticle and drug combinations need to be optimized to mitigate toxicities. There is a risk for immunogenic reactions of varying severity.

Availability: In clinical trials (CSL-112), others are research use only.	**Dose**: Not established	**Chemical formula:** Varied **MW**: Varied
Half-life: Varied	**BBB**: N/A	
Clinical trials: Trials conducted for rHDL-based therapies (ETC-216, MDCO-216, CER-001) in coronary diseases largely failed. Phase 3 trial for CSL-112 is ongoing.	**Observational studies**: None	

What is it?

Nanoparticles are generally less than 100 nM in at least one dimension and can be comprised of a variety of different materials [1]. The most commonly used materials are metals, lipids, and polymers. Nanoparticles vary in terms of their size, charge, and hydrophobicity/hydrophilicity. **Anti-atherosclerotic nanoparticles are designed to prevent and remove the buildup of plaque- causing lipids from arterial walls, and are primarily targeted towards macrophages**. While most nanoparticles traffic to clearance organs such as the kidney, liver, and spleen, the nanoparticles can be modified to promote their preferential uptake by highly phagocytic cells, such as macrophages. The goal is for these nanoparticles to enhance lipid removal and reduce inflammation within atherosclerotic vessels. There are a variety of approaches being developed for imaging, theranostics, and therapeutics. Many of the imaging approaches use paramagnetic particles, such as iron oxide-based nanoparticles, while therapeutic nanoparticles typically use a combination of lipids and polymers. Nanoparticles can also be used to deliver encapsulated therapeutic agents to target organs, and have the potential to minimize systemic toxicities. Some anti-atherosclerotic nanoparticles based on high density lipoproteins (HDL) have been clinically tested, but the majority have failed to produce clinical benefits. Further preclinical testing on biodistribution, *in vivo* modification, clearance, and long-term safety is needed.

Neuroprotective Benefit: The effects of anti-atherosclerotic nanoparticles on cognition or dementia prevention has not been studied.

Types of evidence: None

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function? N/A

Human research to suggest benefits to patients with dementia: N/A

Mechanisms of action for neuroprotection identified from laboratory and clinical research: N/A

APOE4 interactions: Unknown

Aging and related health concerns: Nanoparticles alone have been unsuccessful for atherosclerosis, but modifications that facilitate macrophage targeting and encapsulation of anti-inflammatory agents may increase efficacy.

Types of evidence:

- 7 RCTs [ETC-216 (2), MDCO-216 (2), CER-001 (2), CSL-112 (1)]
- Numerous laboratory studies

One of the major challenges with nanoparticle-based therapies is ensuring that the nanoparticles accumulate in the target tissues in high enough concentration to exert clinically meaningful therapeutic effects. The primary mechanism by which nanoparticles reach non-clearance tissues is via the enhanced permeation and retention (EPR) effect, which relies on the leakiness of the vasculature. It is still controversial whether this method is sufficient for tumor targeting, as entry is likely dependent on the vascular microenvironment of a given tumor [2]. The available evidence suggests that EPR driven nanoparticle entry will be insufficient to elicit a therapeutic response in the context of atherosclerosis [3]. Rodent models indicate that the gaps in the vessels, which allow extravasation of the nanoparticles, open in a dynamic manner, leading to heterogeneous accumulation and the potential for the particles to get trapped within these entry pores [4]. In the ApoE-/- atherosclerosis mouse model, it was found that early in the disease course there was prominent disruption of the vasculature endothelial barrier, which facilitated the entry of hyaluronan-coated nanoparticles, but with advancement of atherosclerosis, there was stabilization of endothelial junctions, which inhibited entry via EPR [5]. This suggests nanoparticle-based therapies may be more effective earlier in disease course, and may have contributed to the clinical failures in patients with established coronary disease.

Vascular inflammation is a primary driver of atherosclerosis, and the accumulation of macrophages/monocytes in vessels is associated with disease progression. Therefore, **targeting**

nanoparticle-based therapies directly to macrophages, particularly plaque-associated macrophages, is thought to be the most promising approach. This is an extremely active area of research, and it remains to be seen whether any of the new approaches that have shown promise in preclinical models will translate into the clinic.

Approaches to target nanoparticles to vascular macrophages:

CLINICAL STUDIES

Recombinant HDL based nanoparticles: LACK OF BENEFIT IN CLINCAL TRIALS

Recombinant (or reconstituted) HDL (rHDL) based nanoparticles are the only type of anti-atherosclerotic nanoparticles that have undergo clinical testing thus far. This has historically been considered the best approach based on expected biocompatibility and endogenous anti-atherosclerotic activity stemming from apolipoprotein-A1 (Apo-A1) mediated reverse cholesterol transport [6]. rHDLs encompass a wide range of nanoparticles comprised of different lipid/polymer cores as well as different forms and sources of Apo-A1. Apo-A1 can be purified from HDL in human plasma, generated in a recombinant form, or mimicked by Apo-A1 mimetic peptides, which exhibit functional, but not structural similarity to Apo-A1. The clinically tested rHDLs have primarily used purified or recombinant Apo-A1. ETC-642, which used the 22A mimetic peptide in combination with sphingomyelin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), was successfully tested in a Phase 1 study, but clinical development was terminated when it was acquired by Pfizer [7]. With the exception of CSL-112, which is still undergoing clinical testing, all other clinically tested rHDL nanotherapies have failed to improve residual cardiovascular risk in statin treated patients [6]. **It is unclear whether the lack of efficacy stems from non-optimal composition and/or dosage of the rHDL, inefficient targeting to plaques, low endogenous reverse cholesterol transport activity, treating too late in the disease course**, or a combination of all these factors. The poor efficacy of rHDL may also stem from *in vivo* modification of the rHDLs, since an inflammatory and oxidative environment can induce modifications on HDL, which modify its function, and reduce its anti-atherosclerotic capacity [8]. A better understanding of how rHDL are modified *in vivo* is needed to optimize therapeutic efficacy.

ApoA-1 Milano (ETC-216/MDCO-216): These rHDLs contain a recombinant form of Apo-A1 that includes the R173C substitution found in carriers of the Apo-A1 Milano mutation, which is associated with lower levels of atherosclerosis [9]. They have phospholipid 1-palmitoyl-2-oleoyl phosphatidylcholine complex (POPC) based cores. ETC-216 was originally developed by Esperion Therapeutics. While it was associated with a 4.2% decrease in atheroma volume in patients with acute coronary syndrome treated for 5 weeks

at 15 or 45 mg/kg/week [10], higher doses (100 mg/kg in men and 50 mg/kg in women) were associated with adverse changes to the blood immune cell profile [11]. Development was subsequently taken over by the Medicines Company, and the manufacturing processes were changed to eliminate the immunostimulatory effects [12]. MDCO-216 (5 to 40 mg/kg IV) was found to increase free plasma cholesterol in a dose dependent manner in healthy volunteers and patients with stable coronary artery disease, which was associated with a decrease in cholesterol esterification and increase in SR-B1 mediated cholesterol efflux [13]. However, in the MILANO-PILOT trial (NCT02678923), MDCO-216 (20 mg/kg/ week IV) **failed to induce plaque regression** in patients (n=122) with acute coronary syndromes concomitantly treated with statins [14]. Development was discontinued due to lack of efficacy.

CER-001: CER-001 is a negatively charged pre-β rHDL containing recombinant Apo-A1 with a phospholipid core containing sphingomyelin and dipalmitoylphosphatidylglycerol (DPPG) that was developed by Cerenis Therapeutics. In a double-blind, placebo controlled RCT (NCT01201837), CER-001 (3, 6, or 12 mg/kg IV for 6 weeks) **failed to reduce atheroma volume** or cumulative coronary steatosis score relative to placebo in patients (n=507) with acute coronary syndrome treated with statins [15]. CER-001 (3 mg/kg IV for 10 weeks) also failed to reduce atheroma volume relative to placebo in a separate RCT (NCT2484378) in acute coronary syndrome patients treated with statins (n=293) [16]. Clinical development of CER-001 for atherosclerosis has been discontinued due to lack of efficacy.

CSL-112: CSL-112 is a formulation of Apo-A1 purified from human plasma and reconstituted with phosphatidylcholine to form rHDL (See CSL112 Report). In the Phase 2b AEGIS-I trial in patients with acute myocardial infarction (n=1258), it was found to be well-tolerated, and increased cholesterol efflux capacity by up to four-fold, although the risk for major adverse cardiac events (MACE) did not differ across treatment groups [17]. CSL Behring is currently testing CSL-112 in the large Phase 3 RCT AEGIS-II (NCT03473223) on its ability to reduce the risk for MACE in patients with acute coronary syndrome.

PRECLINICAL STUDIES

Influence of nanoparticle composition on efficacy and safety

Many studies have found that the composition of the nanoparticles controls their *in vivo* behavior, which in turn determines the success or failure of a given therapeutic nanoparticle. The size and composition of the nanoparticles influences their biodistribution, which directly affects their safety and ability to exert therapeutic effects in target tissues.

The fluidity of the membrane impacts the uptake of nanoparticles by different phagocytic cells, which in liposomes can be influenced by cholesterol content [18]. The fluidity of the lipid membrane negatively

correlates with the degree of opsonin binding and uptake. However**, the desired functionality of the nanoparticle may dictate the optimal rigidity**. One studying assessing the optimal composition of rHDL found that phospholipid (POPC) based nanoparticles had superior Apo-A1 mediated cholesterol efflux capacity compared to those with a polymer core (PLGA) because the polymer core limited the conformational flexibility of Apo-A1, whereas the phospholipid core was less rigid and thus more similar to natural HDL [19].

The composition of the particles greatly influences their pharmacokinetics and pharmacodynamics. In the above study, phospholipid-based rHDL also exhibited a longer half-life and greater accumulation in the aortic macrophages relative to the spleen and liver, compared to polymer-based rHDL [19]. A separate study showed that both the phospholipid content and Apo-A1 can be further optimized to enhance cholesterol efflux capacity. In this study, distearoyl phosphatidylcholine (DMPC)-based rHDL had the longest half-life of 6 hours, compared to only 1 hour for POPC-based rHDL, which translated to a 6.5-fold AUC increase in mobilized cholesterol in rats [20]. The 22A-P Apo-A1 mimetic peptide has an added proline after a labile lysine to make it more proteolytically stable, which also extends the half-life of rHDL. Furthermore, mouse studies indicate that there are **distinct pharmacokinetics and clearance pathways for Apo-A1 and the phospholipid components of rHDL**, in which case their biology *in vivo* may be distinct from the mechanisms identified by characterizing the activity of the intact rHDL particles in *in vitro* models. The inclusion of cargo within the core of the nanoparticles may also influence the efficiency of Apo-A1 mediated cholesterol efflux [21].

Targeting macrophages

There are a variety of approaches being developed to preferentially target nanoparticles to macrophages within atherosclerotic vessels. Several methods have shown promise in preclinical models, but further validation is needed to determine whether they are clinically viable.

Single walled carbon nanotubules (SWCNTs): Carbon nanotubules may provide a mechanism for preferential uptake of nanoparticles by macrophages through a mechanism more efficient than EPR. Some types of carbon nanotubules are implicated in promoting tissue inflammation, fibrosis, and malignancy [22]. However, coating the carbon nanotubules, such as through PEGylation, improves biocompatibility and reduces potential toxicities. **PEG (polyethylene glycol) coated SWCNTs were found to be preferentially taken up by Ly-6C^{hi} monocytes**, which have a pro-inflammatory phenotype, where they can act as a 'Trojan horse' to deliver encapsulated drugs directly to the relevant immune cells [23]. In mice, the SWCNTs distribute to the organs of the reticuloendothelial system, with initial uptake in macrophage rich clearance organs such as the spleen and liver, and they are then taken up by circulating

monocytes to sites of vascular inflammation, such as atherosclerotic vessels. SWCNTs appear to have higher specificity for inflammatory monocytes/macrophages than other nanocarriers, such as iron oxide nanoparticles [24]. In mice, SWCNT injections were taken up by 70% of Ly-6C^{hi} monocytes, 60% of macrophages, 15% of neutrophils, and only 5% of fibroblasts and endothelial cells [23]. SWCNTs have not yet been tested as therapeutic nanocarriers in clinical trials.

CD36 Targeted: 1-(Palmitoyl)-2-(5-keto-6-octene-dioyl) phosphatidylcholine (KOdiA-PC), a major type of oxidized phosphatidylcholine found on oxidized LDL (oxLDL), has a high binding affinity to the class B macrophage scavenger receptor CD36 [25]. CD36 is important for lipid homeostasis, and its upregulation on macrophages promotes inflammation and progression in atherosclerosis. These CD36 targeted nanoparticles bound to macrophages *in vitro*. Atherosclerotic mice (LDLr-/-) treated with EGCG loaded CD36-targeted particles showed a decrease in macrophage production of pro-inflammatory cytokines and lesion load [25].

CCR2 Targeted: The chemokine CCL2, also known as MCP-1, acts as a chemoattractant for monocytes and some leukocytes to sites of inflammation. A truncated form of CCL2 can act as an antagonist at its cognate receptor, CCR2, and the 4-mer peptide CCTV was found to exert similar antagonistic activity at CCR2 [26]. CCTV conjugated nanoparticles synthesized from PEG tagged phospholipids (DSPE-PEG-CO2H or DSPE-PEG-MAL) were selectively taken up by CCR2+ macrophages in ApoE-/- mice and accumulated in macrophage-rich tissues, including the atherosclerotic aorta. In addition to macrophage targeting, the CCTV peptide may also confer anti-inflammatory activity, through inactivation of the NLRP3 inflammasome.

MMP12 Targeted: The matrix metallopeptidase MMP12, also called macrophage metalloelastase, is highly expressed in the macrophages in atherosclerotic plaques. MMP12 inhibitors have been shown to reduce plaque development in atherosclerosis rodent models [27]. Conjugation of the MMP12 inhibitor RXP470 (as RXP470.1-PEG2-NH2-Mal) to lipid-based (Suppocire™NB and soybean oil) nanoparticles did not improve targeting to arterial atherosclerotic plaques in ApoE-/- mice [28].

Hyaluronan: Hyaluronan, also known as hyaluronic acid, is a polysaccharide based linear polymer that is a major component of the extracellular matrix. Hyaluronan interacts with CD44, which is a receptor highly expressed on the surface of activated macrophages and implicated in the pathogenesis of atherosclerosis [29]. A pilot study in mice found that hyaluronan coated nanoparticles had preferential uptake by aortic macrophages relative to normal tissue macrophages, which was dependent on the expression of CD44 [30]. However, a subsequent study found that EPR was the predominant mechanism by which these particles were taken up into the aorta, such that only a limited amount of nanoparticles

were capable of accumulating at the needed sites of action within the plaques [5]. This suggests that hyaluronan alone is not sufficient for *in vivo* aortic macrophage targeting, and additional modifications are likely needed.

Mitochondria Targeted: Since cholesterol metabolism is initiated in the mitochondria of hepatic cells, mitochondria-targeted rHDL nanoparticles are hypothesized to increase lipid removal efficacy. A proof-of-concept study found that nanoparticles with a PLGA polymer core, DSPE-PEG lipid layer, apo-A1 mimetic peptide L-4F, along with stearyl triphenylphosphonium (stearyl-TPP) bromide for mitochondria targeting and stearyl mannose for macrophage targeting, had improved targeting to the aorta and reduced triglyceride levels in wild type mice [21]. Further testing is needed to validate this approach.

Anti-inflammatory drugs delivered via nanoparticles to target atherosclerosis

Due to the lack of efficacy of empty rHDL nanoparticles thus far, there are many efforts underway to determine whether the delivery of anti-atherosclerotic and anti-inflammatory drugs via nanoparticles with or without intrinsic bioactivity could be a more effective approach. **A major challenge in this area is the optimization of the drug-nanoparticle combination**, as is highlighted in the studies below, the composition of the nanocarrier can greatly influence the therapeutic profile of the encapsulated drug. Similarly, for nanoparticles with intrinsic bioactivity, such as rHDL, the inclusion of the drug cargo may limit or alter its bioactivity.

CD47 Inhibitor: CD47, also known as integrin-associated protein, is a cell surface receptor that acts as an anti-phagocytic or 'don't eat me' signal. It is best known for its role in helping tumor cells to evade the immune system in the context of cancer, and anti-CD47 therapies are being developed for this indication [31]. CD47 is also upregulated on atherosclerotic plaques, where it acts as a ligand for S1RPα on macrophages to activate SHP-1 mediated intracellular signaling which suppresses phagocytic clearance [23]. Anti-CD47 therapeutic antibodies have been shown to reduce plaque expansion by promoting efferocytosis, however, systemic administration of anti-CD47 is not a viable therapeutic approach for atherosclerosis due to the severe anemia stemming from the elimination of red blood cells in the spleen. Encapsulation of an SHP-1 inhibitor within PEG functionalized SWCNTs prevented hematological side effects seen with traditional systemic anti-CD47 approaches [23]. Treatment of dyslipidemic ApoE-/- mice with these nanoparticles improved efferocytosis, reduced the necrotic cores of plaques, and decreased arterial inflammation based on FDG-PET measures.

CD40 Inhibitor: CD40 is a co-stimulatory protein involved in the activation of antigen presenting cells. The CD40-CD40L interaction is an important driver of inflammation in atherosclerosis and other chronic

inflammatory diseases. Inhibition of CD40-mediated signaling can reduce atherosclerosis in mouse models, however, due to its important role in adaptive immunity through the activation of lymphocytes, inhibiting CD40 also leads to immune suppression. The signaling downstream of CD40-CD40L is mediated by TRAF proteins, and it was found that TRAF6 is key to mediating inflammation in atherosclerosis, but has minimal impact on lymphocyte (B and T cell) activation [32]. A small molecule inhibitor of the CD40-TRAF6 interaction, 6877002, was shown to delay the onset of atherosclerotic disease when administered early, and to slow progression when administered in the context of established disease in ApoE-/- mice [32; 33]. rHDL with encapsulated CD40-TRAF6 inhibitor were preferentially taken up by Ly6-C^{hi} monocytes and macrophages along with neutrophils and dendritic cells to a lesser extent [32]. Treatment led to a decrease in recruitment of macrophages (by 67%) and T-cells (by 50%) into the aorta, which was associated with a reduction in plaque area in ApoE-/- mice [33]. Although, most particles trafficked to the spleen and liver, toxicology studies in mice and non-human primates found no evidence of immunogenicity or toxicity [33].

Statins: Statins, also known as HMG-CoA reductase inhibitors, inhibit the synthesis of cholesterol and are the most commonly used treatment available to prevent cardiovascular disease in people with high cholesterol. Statins also have anti-inflammatory properties, but due to low systemic oral bioavailability, these anti-inflammatory effects are often minimal with standard treatment [34]. Preclinical studies suggest that nanoparticle (rHDL)-mediated delivery of statins may promote both their lipid lowering and anti-inflammatory properties. *In vitro* studies in macrophage cell lines show that statin-loaded rHDL nanoparticles reduce pro-inflammatory cytokine production, likely through inhibition of the mevalonate pathway [35]. The combination of rHDL and statin also appears to have a synergistic effect on cholesterol efflux and the inhibition of oxLDL uptake [36; 37]. In ApoE-/- atherosclerotic mice, statin-rHDL treatment (15 mg/kg statin, 10 mg/kg ApoA1) decreased aortic vessel wall thickness and plaque area relative to statin or rHDL alone [35].

Dexamethasone: Glucocorticoids are commonly used anti-inflammatory agents. A preclinical study in atherosclerotic rabbits (balloon injury with high cholesterol diet model) found that treatment with dexamethasone-conjugated nanoparticles exacerbated inflammatory burden in plaques at early stages and offered no benefits at late stages [38]. This study used lauric acid/human serum albumin coated superparamagnetic iron oxide nanoparticles (SPIONs) containing 300 ug of dexamethasone. It is not clear if these effects are nanocarrier type specific.

IL-10: IL-10 is a major anti-inflammatory cytokine, but has a short half-life when administered systemically. Proof-of-principle studies suggest that nanoparticle-mediated delivery can extend the half-life and targeting of IL-10 to atherosclerotic vessels to reduce inflammation. Col-IV IL-10 nanoparticles

(Polymer 1: NH2-PLGA5K-NH2; Polymer 2: PLA18K-PEG2K-OMe; Polymer 3: PLGA7.5K-PEG3.4K-Col IV; IL-10 + Glucosamine) improved fibrous cap thickness and decreased the necrotic core area of plaques in LDLr-/- atherosclerotic mice [39]. IL-10 containing pluronic-based nanoparticles conjugated with the cRGD peptide (αvβ3 antagonist) to target plaque macrophages, reduced the production of pro-inflammatory cytokines (IL-1β) within plaques and reduced the progression of atherosclerotic plaques in ApoE-/- mice [40].

LXR agonist: The liver X receptor (LXR) plays key roles in cholesterol metabolism and inflammation. LXR agonists have been found to delay the progression of atherosclerosis in animal models; however, they are poor candidates for clinical use due to severe liver toxicity concerns. One study found that the LXR agonist, GW3965, could be safely administered through rHDL-based nanoparticles and reduce lipid content in aortic monocytes/macrophages in ApoE-/- mice [19]. However, **the beneficial therapeutic profile was nanoparticle type dependent,** as the same concentration of GW3965 administered via polymer core (PLGA)-based nanoparticles lacked therapeutic efficacy and exacerbated toxicity, due to enhanced accumulation in the liver.

Safety: Nanoparticles accumulate in the liver and the long-term safety is unknown. Nanoparticle and drug combinations need to be optimized to mitigate toxicities. There is a risk for immunogenic reactions of varying severity.
Types of evidence:

- 9 RCTs [ETC-216 (3), MDCO-216 (2), CER-001 (2), ETC-642 (1), CSL-112 (1)]
- Numerous laboratory studies

Nanoparticles accumulate in clearance organs such as the lungs, kidneys, spleen, and liver. **The composition of the nanoparticle dictates its pattern of accumulation in the body; therefore, each nanoparticle will have a distinct safety profile**. An important safety feature of the nanoparticles, is that they are composed of materials with non-toxic metabolites. PLGA is the most commonly used polymer, because its degradation products are the natural metabolites, lactic acid and hydroxyacetic acid [1]. Due to its established biocompatibility, PLGA has been approved by the FDA and EMA for medical use. However, in the case of nanoparticle mediated drug delivery, each encapsulated drug has its own safety profile, which also needs to be taken into account. As was highlighted in a study testing the encapsulation of the LXR agonist GW3965 in different types of nanoparticles, **it is critical to optimize the combination of the nanoparticle with the drug, as some nanoparticles could increase drug toxicity by**

preferentially delivering it to the organs where it exerts its toxic side effects [19]. For example, while PLGA based nanoparticles alone are generally considered non-toxic, the encapsulation of GW3965 within PLGA based nanoparticles increased the toxicity of this drug [19]. Each nanoparticle-drug combination is going to have a unique safety profile, and will have to undergo its own safety testing, thus it is not possible to characterize the safety of nanoparticle-based therapies as a general class [3].

The long-term safety of nanoparticle-based therapy has not been established, and thus represents one of the primary areas of potential concern. Additionally, more preclinical studies are needed to track the biodistribution, clearance routes, and immunoreactivity of the various types of nanoparticles *in vivo*.

rHDL: Clinical trials testing rHDL based therapies (MDCO-216, CER-001, CSL-112) have generally found them to be well tolerated in patients with coronary artery syndromes [34]. **The primary safety concern is the risk for infusion related reactions.** However, safety concerns associated with ETC-216 highlight how changes to the manufacturing process can influence nanoparticle safety. In a Phase 1 study in healthy volunteers, doses about 100 mg/kg in men and 50 mg/kg in women were not well-tolerated, and led to adverse immune stimulation involving neutrophil leukocytosis and lymphopenia [11]. A later trial in patients with coronary syndrome was halted due to a severe systemic inflammatory reaction leading to multi-organ failure in one patient [12]. An analysis of the manufacturing process revealed that ETC-216 contained small quantities of residual host proteins (primarily flagellin, OppA, DppA, MalE), which provoked an immune response [12]. The manufacturing process was altered to eliminate the immunostimulatory effect, including deleting the genes encoding the contaminating proteins, to produce MDCO-216, which had an improved safety profile in subsequent clinical trials.

Manufacturing is one of the major challenges for nanoparticle-based therapy. Because the exact size, composition, and modifications of the nanoparticles are critical for its safety and efficacy, it will be necessary to develop better methods to produce large medical grade batches of uniform nanoparticles for therapeutic use specifically tailored for different indications.

SWCNTs: The safety of carbon nanotubules is a controversial topic. Potential toxicities are likely dependent on the formulation and route of administration [41]. Their toxicity stems largely from their small size, which allows them to accumulate in the liver, lungs, and spleen. In mice, intratracheal administration of SWCNTs triggered epithelial granulomas, interstitial inflammation, and increased mortality [22]. *In vitro* studies have also demonstrated their cytotoxic and genotoxic potential. The coating of SWCNTs with recombinant globular heads, such as PEG attachment, has been shown to significantly reduce toxicity in mice. However, more studies are needed to determine how these

modifications influence processing, clearance, and long-term safety before they can be considered good candidates for clinical testing.

Sources and dosing:

No nanoparticle-based therapies have yet been approved for the treatment or prevention of atherosclerosis. The optimal dose and clinical stage of intervention have not yet been established, and may have contributed to the clinical failures seen with this approach thus far. Preclinical studies suggest that the atherosclerotic vessels may have greater capacity to accumulate nanoparticles at early stages of disease [24], but it remains to be seen if this is a clinically translatable finding.

Research underway:

Nanoparticle-based therapy is an extremely active area of research, but the majority of research is still at the preclinical stage. There are extensive efforts underway to develop biocompatible nanoparticles, and optimize them for different conditions through modifications which target them to the organs and cell types of interest.

A large Phase 3 trial for CSL-112 (NCT03473223) in acute coronary syndrome is currently underway, and has an estimated completion date of June 2022.

Search terms:

Pubmed, Google: Nanoparticles, rHDL

- Atherosclerosis, macrophage targeting, anti-inflammatory, safety, clinical trial, biodistribution

Websites visited for Anti-atherosclerotic nanoparticles:

- Clinicaltrials.gov (MDCO-216, CER-001, CSL-112)
- Clinicaltrialsregister.eu (MDCO-216, CER-001)

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