

Review

Focus on the Lymphatic Route to Optimize Drug Delivery in Cardiovascular Medicine

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Abstract: While oral agents have been the gold standard for cardiovascular disease therapy, the new generation of treatments is switching to other administration options that offer reduced dosing frequency and more efficacy. The lymphatic network is a unidirectional and low-pressure vascular system that is responsible for the absorption of interstitial fluids, molecules, and cells from the peripheral tissue, including the skin and the intestines. Targeting the lymphatic route for drug delivery employing traditional or new technologies and drug formulations is exponentially gaining attention in the quest to avoid the hepatic first-pass effect. The present review will give an overview of the current knowledge on the involvement of the lymphatic vessels in drug delivery in the context of cardiovascular disease.

Keywords: lymphatics; cardiovascular diseases; drug delivery route; nanotechnology

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1. Introduction

Cardiovascular diseases (CVD) are one of the leading causes of death worldwide [1]. CVD include coronary heart disease, myocardial infarction (MI), heart failure (HF), stroke, and artery diseases [2]. Treatments for cardiovascular diseases are numerous, and the routes of administration are diverse. The chosen drug delivery route is a key determinant of the pharmacodynamics, pharmacokinetics, as well as toxicity of the delivered compounds. Yet, side effects or therapeutic failures are raising concerns, highlighting the need for new administration routes and improved formulation of molecules that reduce their degradation by hepatic metabolism. Drug delivery refers to the methods, approaches, or strategies employed for the transport of pharmaceutical compounds to an organism to achieve a desired therapeutic outcome. With this intent, various routes of administration are used to manage CVD and their risk factors, including parenteral (intravenous (IV), intradermal (ID), intramuscular (IM), subcutaneous (SC), and intraperitoneal (IP)), and transmucosal (oral, nasal, pulmonary, ocular, and genital) and transdermal route [3]. Drug absorption and transport through the lymphatic system makes it possible to avoid hepatic metabolism and is a privileged target in pathologies, such as particular types of cancer (chemotherapeutics [4]) or vaccines [5,6] (HIV [7]), but also for macromolecules [8], and the extensively hepatic-metabolized compounds [9,10].

Discovered in the 17th century [11], the lymphatic system is composed of lymphatic vessels (LV), lymph nodes (LN), and lymphoid organs. LV are organized as initial lymphatics, also called capillaries, localized in the interstitium (i.e., such as the skin, the intestines, and peripheral tissues), pre-collecting and collecting LV. Lymphatic capillaries take

up the interstitial fluid that normally consists of immune cells, cellular debris, proteins, electrolytes, chylomicrons, and high-density lipoproteins (HDL) [12,13]. Then, lymph circulates in a unidirectional way due to the presence of valves and lymphatic muscle cells (LMC) surrounding a lymphatic endothelial cell (LEC) monolayer. Lymph passes through the LN and the efferent LV until it reaches the thoracic lymphatic duct and, finally, the bloodstream via the subclavian vein [12]. Given its role in the absorption of interstitial fluids, molecules, and cells from the peripheral tissue, a dual interplay of the lymphatic system in CVD was first brought up in the early 1980s [14] and has gained growing attention in the past few decades [13,15–22]. Atherosclerosis, the main cause of CVD, is characterized by lipid and immune cell accumulation within the artery wall [23]. LV present in the blood vessel walls are involved in the removal of cholesterol from the arterial intima [15]. Further, after an MI, the lymphatic system is involved in the reduction of edema, improvement of cardiac function, and reduction of hypertrophy and fibrosis [24–27]. Whereas there is an interest in improving lymphatic function to further control CVD onset and progression, targeting the lymphatic system for cardiovascular drug delivery is a promising strategy to limit CVD outcomes. Preferential uptake into the lymphatics is highly dependent on the route of administration and physicochemical properties of the compounds, including size, molecular weight, surface charge, and lipophilicity [28]. This review focuses on the potential key involvement of the lymphatic system in the optimization of drug delivery in CVD.

2. Conventional and Novel Therapies to Treat CVD

Historically, small molecules have been used for the treatment of CVD. However, these molecules improve the symptoms and slow down the disease progression without having an actual regenerative effect on the affected tissues or organs [29]. Thus, the remaining unmet clinical needs necessitated the urgent seek for other potential therapeutic options.

Gene therapy is one of the most promising treatment strategies for CVD [30–34], inherited or acquired, through targeting the causative genes engaged in the induction and progression of the disease. It works through replacing defective genes, silencing overexpressed ones or providing functional copies of specific therapeutic genes, thanks to DNA, RNA (siRNA, microRNA, mRNA), and antisense oligonucleotides (ASO) [35]. Back in the 1950s and 1960s, several attempts were made to directly transfect cells with DNA and RNA. Nevertheless, *in vivo* studies failed to show a noticeable success. Thus, selecting a suitable vector to deliver gene therapy is as important as selecting the agent itself [36,37]. Generally, vectors can be divided into viral and non-viral. The most commonly used viral vectors are retrovirus (RV), adenovirus (AV), adeno-associated virus (AAV), and lentivirus [38]. The most commonly used non-viral vectors include lipid-based vectors using cationic lipids and polymer-based vectors using cationic polymers [39]. Cationic lipids complex with the genetic materials to form lipoplexes or lipid nanoparticles (LNP), while cationic polymers form polyplexes [40]. In 2012, cardiovascular gene therapy was the third most common application for gene therapy (8.4% of the total gene therapy trials). However, clinically, it is still in the infancy stage, and a lot of effort is yet to be expended to correct the underlying basal molecular mechanisms behind different cardiovascular disorders [41,42].

On the other hand, vaccines, developed to produce a long-term immune response against specific antigens, are considered the most economic life-saving medical intervention so far. Previous studies have demonstrated that antigens are trafficked by immune cells from the administration site through the lymphatic network to activate the residing lymphocytes in the secondary lymphoid tissues to produce antibodies [6], a fundamental step for a successful vaccination process. Vaccination against infectious diseases depends on activating T cells and inducing antibody production, following antigen presentation by antigen presenting cells (APC) to T cells through major histocompatibility complex

(MHC) molecules. This binding produces cytotoxic T cells that may trigger an autoimmune response. However, the success of vaccination against self-antigen in life-long diseases, such as hypertension, depends on the ability of the vaccine to produce antibodies without triggering a cytotoxic immune response, for safety issues [43]. For example, vaccination against influenza and pneumococcal infection, a potential risk factor for CVD, could have a protective role in populations with a high risk of CVD, including MI and HF [44,45].

Moreover, conventional orally-administered molecules failed to reach satisfaction owing to their poor aqueous solubility, lack of specificity, short half-life, and, hence, low therapeutic outcome and high systemic adverse events [46]. Driven by these facts, researchers have made great efforts to deliver these molecules in a more effective and safer way. One of the most important strategies used for this purpose is nanotechnology. Indeed, using nanotechnology in the chronic management of CVD could revolutionize the cardiovascular healthcare sector. Nanotechnology can serve as a drug delivery platform to improve the characteristics of the free drugs, e.g., solubility, stability, biodistribution, pharmacokinetics, and toxicity profile. Hence, the choice of a suitable carrier has a great impact on the therapeutic outcome [47].

Related to their administration route, the use of conventional cardiovascular therapy, gene-based therapy, vaccines, and nanotechnology to treat CVD is discussed in this review.

3. Treating CVD through Various Administration Routes

The following sections describe several routes of administration, with an overview on the lymphatic involvement and several conventional and novel therapies used to treat CVD (Tables 1–6).

3.1. Oral Administration

Among the various routes of administration, the oral route is the most commonly employed. It exhibits many advantages, including pain avoidance, ease of administration, patient compliance, reduced care cost, and low incidence of cross-infection. Furthermore, it is amenable to various types and forms of pharmaceuticals [48] (Table 1). While some drugs are intended to target the gastrointestinal tract (GIT), the majority are employed to exert a systemic therapeutic effect. Nevertheless, the oral bioavailability of most pharmaceutical compounds depends mainly on their solubility, permeability, and stability in the GIT environment [49–51].

Orally-administered compounds enter the systemic circulation through either the portal vein or the intestinal lymphatics after being absorbed, respectively, by the blood or lymphatic vasculature, draining the interstitium. However, these compounds should initially be able to pass through the intestinal epithelium and reach the interstitium to be amenable for transport [10]. Once in the interstitium, two essential factors determine the predominant way of transport: size and lipophilicity. Small molecules/particles are predominantly transported by the blood due to the higher portal blood flow rate as compared to the intestinal lymph flow rate, although they have relatively free access to both blood and lymphatic capillaries. Conversely, large (>500 g/mole) [52] and highly lipophilic molecules ($\log P > 5$, long-chain triglyceride (TG) solubility > 50 mg/g) and particles (up to 10 μm) [53] preferentially enter the highly permeable lymphatics to different extents [10,53,54]. Thus, in order to avoid the hepatic metabolism, the use of a lipophilic molecule is essential, allowing the drug to reach the mesenteric lymphatic vessels permitting the absorption of lipids [55]. The lymphatic system in the intestines is composed of capillaries in the microvilli (lacteals) and collecting lymphatic vessels in the mesentery [56].

- **Diabetes**

Cardiovascular diseases are known as the leading cause of mortality in diabetes mellitus (DM) [57], a chronic debilitating metabolic disorder that spreads worldwide. As

presented in Table 1, diabetes is commonly treated by conventional therapy (i.e., metformin, sulfonylureas), while gene therapy, vaccines, and nanotechnology are under intensive investigation [35,58–66].

Nanotechnology has attracted an increasing interest in the treatment and management of diabetes mellitus. Oral insulin delivery as a convenient and pain-free alternative to SC injection has been the focus of researchers over the last century [67]. Nanocarriers can be used to protect insulin and other hypoglycemic medications from enzymatic degradation [35]. Indeed, encapsulating insulin in nanoparticles in different studies has shown improved oral bioavailability [68–70]. However, the clinical translation of these studies has been so far negative [64]. Several approaches have been employed to improve the encapsulated insulin bioavailability through enhancing its absorption from the intestinal lumen. One of them is via promoting its lymphatic absorption. Indeed, complexing insulin with cationic liposomes has shown a gradual increase in insulin concentration in the lymphatic system over time, upon investigating the absorption pathway in lymph fistula- rat model, indicating a major involvement of the lymphatic system in the transport of these nanoparticles [64]. Similarly, lymphatic transport of insulin-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles was believed to be the reason, in part, beyond its improved bioavailability and sustained hypoglycemic effect [65]. The same approach was used to improve the oral bioavailability of exenatide, the glucagon-like peptide-1 (GLP-1) analogue that is primarily administered SC. Exenatide is injected frequently in high doses to overcome its short plasma half-life. Hence, oral delivery seems a favorable alternative; however, it is limited by the poor bioavailability of the peptide drug. Nevertheless, using a phase-changeable nanoemulsion with medium-chain fatty acid has increased the relative bioavailability of exenatide by enhancing its intestinal absorption and lymphatic transport, bypassing the hepatic first-pass effect. Lymphatic involvement in exenatide transport was confirmed by inhibiting the lymphatic pathway using cycloheximide. Results confirmed the absence of nanoparticles in epithelial villi, Peyer's patches, and major organs [66].

- **Hypertension**

Hypertension is a major risk factor for several CVD. Conventional antihypertensive medications (Table 1) mostly suffer from certain challenges that limit their therapeutic outcome, including short half-life, poor water solubility, low bioavailability, and serious side effects, such as angioedema, bronchospasm, asthma, male breast hyperplasia, reflex tachycardia, and others [46,71–87]. Formulating drugs, such as carvedilol, into nanoemulsion demonstrated a significant improvement in its absorption, permeability, and bioavailability following oral administration [88–92]. The enhanced bioavailability of these medications in nanoemulsion was attributed to their transport via lymphatics, in accordance with the previously established role of nanoemulsion in promoting lymphatic absorption of lipophilic molecules [88,89]. Besides nanoemulsion, other nanocarriers have been widely tested to improve the characteristics of the antihypertensive medications. For instance, felodipine-loaded PLGA were able to bypass the hepatic metabolism by absorption via M-cells of the Peyer's patches and transport through the lymphatics, facilitated by the negative charges on the particles [93]. Other nanosystems that improved the oral bioavailability and efficacy of antihypertensive medications through promoting lymphatic uptake in preclinical studies include: solid lipid nanoparticles [94], nanostructured lipid carriers [95], proliposomes [96], and Eudragit nanoparticles [97].

- **Hypercholesterolemia and hyperlipidemia**

Hyperlipidemia is a family of disorders characterized by elevated level of lipids in the blood and is a major risk factor for atherosclerosis and CVD. One of the most common hyperlipidemia is hypercholesterolemia [98].

Several medications intended for the treatment of hyperlipidemia and hypercholesterolemia have various constraints that affect their efficacy [99,100]. Nanoparticles promoting lymphatic uptake seems a good strategy to resolve these constraints. For instance, atorvastatin, the widely prescribed statin for the treatment of hyperlipidemia and hypercholesterolemia, has been formulated into several polymeric and lipid-based nanocarriers that enhanced its lymphatic absorption and its oral bioavailability, in consequence (Table 1) [101–105].

The same strategy has been used with other statins to improve their bioavailability through enhancing lymphatic uptake, such as simvastatin [106], rosuvastatin [107], and fluvastatin [108], as well as other agents, such as fibrates [109–111] and the cholesterol absorption inhibitor ezetimibe [112–114].

Table 1. Oral delivery of various treatments for CVD.

Condition	Intervention and Identifier	Target	Dose and Outcome
Diabetes	Metformin		From 500 to 850 mg, 2–3 times a day, during the meal [58]
Diabetes	Sulfonylureas Meglitinide		Dosage is very different from one class of medication to another [59]
Diabetes	Acarbose, Miglitol Voglibose	Carbohydrate digesting enzymes in the brush border	50 mg three times daily (up to 100 mg) [60]
Diabetes	Rosiglitazone Pioglitazone	PPAR- α	Rosiglitazone: 4 mg per day (up to 8 mg) Pioglitazone: 15–30 mg per day [61]
Diabetes	Sitagliptin Vildagliptin Saxagliptin Linagliptin Alogliptin	DPP4	2.5–100 mg once daily depending on the inhibitor used [62]
Diabetes	Dapagliflozin Canagliflozin Empagliflozin	SGLTP2	Dapagliflozin: 2.5–10 mg daily Canagliflozin: 100–300 mg Empagliflozin: 5–25 mg daily [63]
Diabetes	AG019 (NCT03751007) or in combination with the anti-CD3 monoclonal antibody teplizumab		2 or 6 capsules per day for 8 weeks (repeated dose) or for one day (single dose)
Diabetes	Insulin nanocarriers		Protection of insulin from enzymatic degradation Enhancement of stability, intestinal permeability, and bioavailability [35]
Diabetes	Electrostatically-complexed insulin with partially uncapped cationic liposomes		Improved insulin pharmacokinetic profile [64]
Diabetes	Insulin-loaded PLGA		Improved bioavailability and sustained hypoglycemic effect [65]
Diabetes	Exenatide combined to phase-changeable nanoemulsion with medium-chain fatty acid		Enhancement of intestinal absorption and lymphatic transport [66]
HTN	Prazosine Terazosine Doxazosine	Alpha-adrenergic receptor	Prazosine: 3–7.5 mg per day in two doses Terazosine: 1–9 mg per day in the evening at bedtime

			Doxazosine: 4 mg per day [71]
HTN	Clonidine Methyl dopa	Alpha-adrenergic receptor (agonists)	Clonidine: 0.1 mg twice daily [72] Methy dopa: 250 mg two to three times daily [73]
HTN	Carvedilol into nanoemulsion	Beta-adrenergic receptors	Significant improvement in its absorption, permeability, and bioavailability [88,89]
HTN	Valsartan, Ramipril and Amlodipine into nanoemulsion		Enhanced solubility, oral bioavailability, and pharmacological outcome [90]
HTN	Felodipine-loaded PLGA nanoparticles	Calcium-channel	Sustained drug release both in vitro and ex vivo [93]
MI HF HTN Arrhythmia	β -blocker	Beta-adrenergic receptors	Acebutol: 200 mg twice daily [74]
MI HF HTN	Conversion enzyme inhibitors	Conversion enzyme	Captopril: 100 mg per day [75]
MI HF HTN	Valsartan Losartan	Angiotensin II	20 mg twice a day, up to 160 mg [76]
HF HTN	Hydrochlorothiazide Bumetanide	Angiotensin/neprilysin receptor	49 mg/51 mg twice daily and doubled after 2–4 weeks [77]
HF HTN	Sacubitril Valsartan	Calcium channel	5–10 mg daily [78] 60 mg three times daily [79]
HTN Arrhythmia	Amlodipine Diltiazem	Calcium channel	5–10 mg daily [78] 60 mg three times daily [79]
HF	Ivabradine		Bradycardic 5–7.5 mg twice a day [80]
HF MI	Eplerenone Spironolactone	Aldosterone	50 mg once a day [81] and 12.5–25 mg with each intake [82]
HF Arrhythmia	Digoxin		0.25 mg once daily [83]
HF MI HCL	Statin	HMG-CoA	10 mg once daily [84]
MI	Aspirin	Platelets	325 mg, then 81 mg per day [85]
MI	Clopidogrel Prasugrel Ticagrelor	Platelets	300 mg, then 75 mg daily with aspirin 60 mg, then 10 mg daily 180 mg, then 90 mg twice a day [86,87]
HCL	Ezetimibe	Intestinal cholesterol absorption	10 mg once daily [99]
HLD	Tricor Triglide		Fenofibrates 100–300 mg per day [100]
HCL HLD	Atorvastatin formulated into ethylcellulose nanoparticles		Enhanced atorvastatin's lymphatic absorption and oral bioavailability [101]
HCL HLD	Atorvastatin formulated into nanocrystals prepared with poloxamer 188		Improved atorvastatin's gastric solubility and bioavailability [102] Reduced circulating cholesterol, TG and LDL

HCL HLD	Atorvastatin formulated into polycaprolactone nanoparticles	Enhanced atorvastatin's bioavailability [103]
HCL HLD	Nanostructured lipid carriers	Enhanced atorvastatin bioavailability by 2.1 fold compared to the commercial product: lipitor® Reduced the serum level of cholesterol, TG and LDL [104]
HCL HLD	Nanoemulsion	Increased the bioavailability of atorvastatin compared to the commercial tablet ozovas™ [105]
HCL HLD	Simvastatin Rosuvastatin Fluvastatin Fibrates Ezetimibe lipid-based nanoparticles	Improved bioavailability via lymphatic uptake [106–114]

PPAR- α : peroxisome proliferator-activated receptor- α ; DPP4: dipeptidyl peptidase-4; SGLTP2: Sodium glucose co-transporter-2; PLGA: Poly lactic-co-glycolic acid; HTN: Hypertension; MI: Myocardial infarction; HF: Heart failure; HCL: Hypercholesterolemia; HMG-CoA reductase: Hydroxymethyl glutaryl coenzyme A reductase; HLD: Hyperlipidemia; TG: Triglycerides; LDL: Low density lipoprotein.

3.2. Subcutaneous Injection

Subcutaneous injections consist of injecting a molecule under the dermis, in the SC cell layer (interstitial space), and slightly before the muscle, mostly in the abdomen or thigh. The injected molecules will, therefore, either be degraded or phagocytized at the site of injection and join the lymphatic system or the bloodstream [115]. To target the lymphatic system exclusively, this type of injection must be combined with the use of macromolecules. As described in Table 2, subcutaneous injections are used as treatment for various conditions [116–143].

Table 2. Therapies targeting CVD using subcutaneous injection.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
Diabetes	Insulin				Different types of insulin At least 3 injections per day Dosage adapted to the patient [116]
Diabetes	Exenatide Lixisenatide Liraglutide Exenatide LAR Albiglutide Dulaglutide				GLP-1 analogues [117] Exenatide: 5–10 μ g twice a day Lixisenatide: 10–20 μ g once daily Liraglutide: 0.6–1.8 mg once daily Exenatide LAR: 2 mg once a week Albiglutide: 30–50 mg once a week Dulaglutide: 0.75–1.5 mg once a week
Diabetes	Vaccine formed of virus-like particles coupled to IAPP		Against the insoluble IAPP-derived amyloid aggregates		Three doses—10 μ g Strong immune response against these aggregates and restored insulin production Diminished the amyloid deposits in the pancreatic islets, reduced the level of the pro-inflammatory cytokine IL-1 β , and

			retrieved the onset of amyloid-induced hyperglycemia [118]
Diabetes	IL-1 β epitope peptide	Against IL-1 β	Three doses—50 μ g Enhancement glucose tolerance, improved insulin sensitivity, restored β -cell mass, reduced β -cell apoptosis, and enhanced β -cell proliferation, as well as downregulation of IL-1 β expression and inhibition of the inflammatory activity [119,120]
Diabetes	hIL1 β Qb vaccine (NCT00924105)	Against IL-1 β	Six doses—300 μ g Mediated a dose-dependent IL-1 β -specific antibody response More studies are required to precisely investigate the clinical efficiency of this vaccine [121]
Diabetes	Neutralizing antibodies against DPP4	The GLP-1 and GIP inhibitor, DPP4	Five doses—2–20 μ g Increased pancreatic and plasma insulin level and improved postprandial blood glucose level [122]
HTN	hR32 vaccine	Renin-derived peptide	Five doses—500 μ g Reduced systolic blood pressure by 15 mmHg [123]
HTN	Angiotensin I vaccine (PMD3117)		Three or four doses—100 μ g The vaccine failed to reduce the blood pressure [124]
HTN	AngI-R vaccine	Modified endogenous angiotensin I peptide	Four doses—50 μ g 15 mmHg reduction in systolic blood pressure and reduced angiotensin I/II level [125]
HTN	ATRQ β -001	Angiotensin II type I receptors	Two doses—100 μ g Protective role against target organ damage induced by hypertension [126]
HTN	ATR12181 vaccine	Angiotensin II type I receptors	Nine doses—0.1 mg Attenuated the development of hemodynamic alterations of hypertension [127]
HTN	CYT006-AngQb vaccine	Against angiotensin II	100 or 300 μ g Reduction in blood pressure and reduced ambulatory daytime blood pressure [128]
HF HTN	Ang II-KLH vaccine	Angiotensin II	Three doses—5 μ g Suppressed post-MI cardiac remodeling and improved cardiac function [129]
MI	Celecoxib loaded in nanoparticles		Promoted vascularization in the ischemic myocardium and delayed HF progression [130]
MI	Chitosan-hyaluronic acid based hydrogel containing deferoxamine-PLGA		Persistent neovascularization in mice [131]

nanoparticles					
HCL	Alirocumab Evolocumab		PCSK9		One dose every two weeks [132,133]
HCL	Inclisiran		PCSK9		Two doses per year [134]
HoFH HeFH severe HCL	Mipomersen (NCT00607373) (NCT00706849) (NCT00770146) (NCT00794664)	ASO	ApoB	Approved	200 mg once/week. Phase III: reduction in LDL-C [135]
ASCVD HCL HeFH	Inclisiran (NCT03399370) (NCT03400800) (NCT03397121)	siRNA	PCSK9	Approved	284 mg inclisiran, injected on day 1, day 90 and then twice/year Phase III: reduction in LDL-C level [134,136]
FCS	Volanesorsen (NCT02211209)	ASO	ApoC3	Approved	300 mg once/week Phase III: reduction in mean plasma APOC3 and TG level [137]
Elevated LP(a)	ISIS-APO(a)Rx (NCT02160899)	ASO	APO(a)	Phase II (Complete)	Multiple escalating (100–300 mg) doses, injected on a weekly interval for 4 weeks each Phase I/II: reduction in plasma Lp(a) concentration [138]
Elevated LP(a) CVD	AKCEA-APO(a)- LRx (NCT03070782) (NCT02414594) (NCT04023552)	GalNAc3 conjugated- ASO	APO(a)	Phase III (Recruiting)	80 mg administered monthly Phase I/II: reduction in plasma Lp(a) [138]
HTG CVD FCS	AKCEA- APOCIII-LRx (NCT02900027) (NCT03385239) (NCT04568434)	GalNAc3 conjugated- ASO	APOC3	Phase III (Recruiting)	Multiple dosing injected as once/4 weeks for up to 49 weeks Phase II: reduction in ApoC3 and TG levels [139]
HTG FH HLP	Vupanorsen (NCT02709850) (NCT04459767) (NCT04516291)	ASO	ANGPTL3	Phase IIb (Active, Not recruiting)	Multiple escalating dosing (60–160 mg, once/2 or 4 weeks) Phase I: reduction in TG and LDL-C levels [140]
HCL	Neutralizing antibodies against PCSK9		PCSK9		Three doses—5–50 µg Long-lasting reduction in the level of total cholesterol, VLDL and chylomicron [141]
HCL	AT04A		PCSK9		Five doses Strong and persistent anti-PCSK9 antibody production, reduced plasma cholesterol level, attenuated progression of atherosclerosis and reduced vascular and systemic inflammation [142]
HCL	AT04A		PCSK9		Four doses—15 µg and 75 µg Reduced serum LDL-C level and elevated anti-PCSK9 antibody titer [143]
HCL	A peptide representing the		Angiopoieti n-like		Three doses—5 µg Reduced steady-state plasma TGs and promoted LPL activity

mouse ANGPTL3	proteins 3 (ANGPTL3)
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GLP-1: glucagon-like peptide-1; IAPP: Islet amyloid polypeptide; DPP4: dipeptidyl peptidase-4; GIP: glucose-dependent insulinotropic polypeptide; HTN: Hypertension; HF: Heart failure; MI: Myocardial infarction; HCL: Hypercholesterolemia; HoFH: Homozygous familial hypercholesterolemia; HeFH: Heterozygous familial hypercholesterolemia; AngII-KLH: Angiotensin II—keyhole-limpet hemocyanin; PCSK9: Proprotein convertase subtilisin/kexin type 9; ASO: Antisense oligonucleotides; ApoB: Apolipoprotein B; LDL-C: low density lipoprotein cholesterol; ASCVD: Atherosclerotic cardiovascular disease; FCS: Familial chylomicronemia syndrome; TG: Triglycerides; LP(a): Lipoprotein(a); APO(a): Apolipoprotein (a); CVD: Cardiovascular diseases; GalNAc3: Triantennary N-acetyl galactosamine; HTG: Hypertriglyceridemia; FH: Familial hypercholesterolemia; HLP: Hyperlipoproteinemia; ANGPTL3: Angiopoietin-like proteins 3; VLDL: Very low density lipoprotein; LPL: Lipoprotein lipase.

- **Diabetes**

The commonly used treatment for diabetes is insulin injections at least three times per day, and the dosage is adapted to the patient [116]. In addition, many vaccines showed significant protection against type 2 diabetes mellitus (T2DM) (Table 2).

Islet amyloid polypeptide (IAPP) is a normally secreted product by β -cells in the pancreas. However, their accumulation in the extracellular space as insoluble aggregates mediates β -cell toxicity and inflammation. Moreover, interleukin 1 β (IL-1 β) is a key proinflammatory cytokine implicated in pancreatic islets inflammation and insulin resistance in T2DM.

- **Hypertension**

Angiotensinogen (AGT) represents an important target for gene therapy. Indeed, recent studies based on siRNA targeting hepatic AGT, through SC or IV administration, resulted in stable and durable antihypertensive and cardioprotective effect in spontaneously hypertensive rats [144].

On the other hand, three targets in the renin-angiotensin system that have been extensively studied in the preclinical and clinical studies for the treatment of hypertension with vaccines include: renin, angiotensin I, and angiotensin II and its receptors. Renin, as an initiator of the renin-angiotensin system, was the first reported target for vaccination. Furthermore, angiotensin I converts into angiotensin II, which causes dramatic changes in blood pressure. Not only does angiotensin II exert a direct effect on blood pressure, but it also induces the release of the potent vasoconstrictor aldosterone. Direct angiotensin II effect on blood pressure is mediated through stimulating angiotensin II type I receptors that enhance sodium retention and raise blood pressure [145,146]. Thus, blocking the effect of the renin-angiotensin system would have a beneficial role in the management of hypertension.

- **Myocardial infarction**

The high mortality rate associated with MI requires urgent intervention and repair of the affected zones. Advances in this area mainly depend on restoring the blood supply. Angiogenesis is one of the most exciting therapeutic strategies in the management of CVD. Nanotechnology plays an important role in initiating and promoting angiogenesis (or lymphangiogenesis, thanks to VEGF receptor 3), as described in Table 2 [130,131].

- **Hypercholesterolemia and hyperlipidemia**

The use of gene-therapy has also been investigated for the treatment of hypercholesterolemia and hyperlipidemia. Indeed, mipomersen (Kynamro; Kastle Therapeutics) is a second-generation antisense oligonucleotides (ASO) that targets apolipoprotein-B 100 (ApoB-100) and was approved for the treatment of Homozygous familial hypercholesterolemia (HoFH) [147]. It reduces lipoproteins containing the ApoB-100 (e.g., lipoprotein(a) and LDL) through targeting ApoB-100 mRNA in the liver [148]. HoFH is an inherited disorder characterized by low density lipoprotein-cholesterol (LDL-C) uptake and caused by mutations in the *ldl-receptor* (*ldlr*), *apolipoprotein-b* (*apob*), and *proprotein convertase subtilisin/kexin type 9* (*pcsk9*). Following SC administration, mipomersen is rapidly absorbed and

distributed, compared to a 2 h IV infusion. One of the major accumulation sites of the drug in animals is mesenteric lymph nodes, assuming a considerable absorption by the lymphatics [149]. Kynamro has been withdrawn from the market in 2019 due to safety issues represented in liver toxicity, injection-site reaction, and flu-like symptoms [32,150].

Likewise and for safety issues, represented in thrombocytopenia and bleeding risks, volanesorsen (Waylivra®; Akcea Therapeutics, Cambridge, MA, USA) has been denied FDA approval for the treatment of familial chylomicronemia syndrome (FCS), a rare genetic disorder caused mainly by mutations in *lpl* gene or encoding genes required for LPL function [151]. However, in 2019, the European Medicines Agency (EMA) granted it a conditional marketing authorization for genetically confirmed FCS cases with a high risk of pancreatitis and patients with inadequate response to TG-lowering therapy and low-fat diet [152]. FCS is characterized by accumulation of TG-rich lipoproteins in the blood. Apolipoprotein-C3 (ApoC3) plays a regulatory role in the determination of plasma TG level through inhibiting lipoprotein clearance via both LPL-dependent/independent pathways [153]. Thus, inhibiting ApoC3 activity could have a favorable impact on the lipid profile in FCS. Volanesorsen is an ASO that targets and inhibits ApoC3 production in the liver and enhances TG metabolism via the LPL-independent pathway. In rats, one of the main accumulation sites of volanesorsen following SC administration are mesenteric lymph nodes, indicating a reasonable uptake by the lymphatics [154].

Furthermore, inclisiran (Leqvio®; Novartis, Schaftanau, Austria), a short-chain, double-stranded siRNA, conjugated to triantennary N-acetylgalactosamine carbohydrates to target the hepatocytes, has been approved in the European Union for the treatment of primary hypercholesterolemia or mixed dyslipidemia in adults, as an adjunct to low-fat diet [155,156]. Inclisiran is injected SC twice a year to produce a long-term anti-hypercholesterolemic effect through targeting PCSK9 [155]. Following SC injection of ¹⁴C labeled inclisiran in monkeys, lymph nodes showed the third-highest exposure organ, after liver and kidney [157].

On the other hand, several studies reported beneficial effects of active vaccination on hypercholesterolemia. For instance, vaccines targeting angiopoietin-like proteins 3 (ANGPTL3) in mice has reduced the steady-state plasma TGs and promoted LPL activity (Table 2). ANGPTL3 has a key role in TG metabolism and plasma levels through inhibiting LPL activity. People with loss-of-function mutations in ANGPTL3-encoding genes exhibit reduced TG level and a low risk of CVD. Thus, vaccination against ANGPTL3 could serve as a promising strategy for protection against hypertriglyceridemia and relative CVD [158].

3.3. Intradermal Injection

Lymphatic capillaries are present in the dermis and, thus, preferentially take up the injected molecules. Unlike the blood capillaries, initial lymphatics lack the basement membrane underlying the endothelial layer. The distal part of initial LV is exclusively composed of LECs with button-like junctions [159], leading to capillaries that have inter-endothelial gaps with size ranges from a few nanometers to several microns [4,160]. Small particles (<10 nm) [4] and medium-sized macromolecules (up to 16 kDa) [161] are mainly transported away from the interstitial spaces by blood capillaries, thanks to mass transport [162,163]. In contrast, lymphatic access of large particles with diameters exceeding 100 nm is hindered by their restricted movement through the interstitium, via diffusion and convection [4]. In between, particles with a size of 10–100 nm [4] and macromolecules with a size of 20–30 kDa [161] show preferential uptake into the highly permeable lymphatic capillaries either passively (paracellular) or actively (transcellular) through the lymphatic endothelial cells [164]. Indeed, it has been shown that the optimal diameter to target the lymphatic vessels in the dermis is 5 to 50 nm in mice [165]. Macromolecules can enter into lymphatics through transcytosis via receptors localized on the surface of LECs, the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), and scavenger receptor class B member 1 (SRB1) or p32 [28].

Intradermal therapy, also known as mesotherapy, is used in dermatology and consists of a series of micro-injections of drugs that will slowly diffuse into the peripheral tissues. For cardiovascular diseases or to target the immune system, ID injection would be a privileged pathway to target the lymphatic system. In fact, the skin has a higher lymph flow rate, higher interstitial pressure, and an abundance of APCs (e.g., macrophages, dendritic cells (DCs), and T cells) compared to the other interstitial spaces [166,167]. Then, drugs will be found directly in the lymph and, ultimately, reach the lymph nodes [168]. The antigens present in the lymph will then activate the immune response of T and B lymphocytes [169]. This method is used to treat, for example, alopecia, cystic acne, and psoriasis [170], or for certain vaccines, such as Bacille Calmette-Guérin (BCG) for tuberculosis disease [171].

- **Diabetes**

In type 1 diabetes mellitus (T1DM), the main goal of vaccination is to induce diabetes-specific immune tolerance via vaccination against autoantigens that induce β -cells destruction, through antigen-specific and non-antigen-specific pathways, to restore β cell tolerance and suppress disease progression at early stages [172]. Non-antigen-specific pathway include using antibodies to suppress T cell immune response. However, the limited specificity of this approach can result in serious systemic side effects.

Antigen-specific modulation has proved efficacy in T1DM murine models and, recently, in clinical studies. Vaccination leads to the establishment of a tolerance of effector T cells (Teff) to diabetes-specific autoantigens and induce/expand immunoregulatory T cells (Treg). Teff has an essential role in the development of β cells- specific immune response, while Treg help maintain peripheral tolerance [173,174]. Tolerizing Teff is usually attained via delivery of a high amount of autoantigen to induce exhaustion, clonal anergy or clonal deletion [175]. Proinsulin, among others, is a major B cell autoantigen peptide targeted by CD4⁺ and CD8⁺ Teff at the early stages of the disease. Thus, proinsulin vaccines seem a good option in recently diagnosed cases [176].

Table 3 presents several vaccines used for diabetes through intradermal injection [177–179].

Table 3. Intradermal administration as treatment for diabetes.

Condition	Intervention and Identifier	Target	Dose and Outcome
Diabetes	Proinsulin peptide vaccine C19-A3	CD4 T cells	Three equal doses—10–100 μ g Vaccine was well tolerated [177]
Diabetes	C19-A3 (NCT02837094)	CD4 T cells	Three doses—10 μ g In vitro and ex vivo studies of in human skin reported rapid diffusion of the injected particles through the skin layers and preferential uptake by Langerhans cells in the epidermis, which have a primary role in the tolerance mechanism [178]
Diabetes	PIpepTolDC vaccine (NCT04590872)	Tolerogenic DC Vaccine	One dose and another after 28 days No results yet, but, it is believed to be able to produce proinsulin-specific Treg [179]

DC: Dendritic cells; Treg: immunoregulatory T cells.

3.4. Intramuscular Injection

Intramuscular injections are used to target the deeper muscle tissue that is highly irrigated. This route of injection allows a rapid absorption and prolonged action. The medication would enter the bloodstream directly and, thus, allow the “bypass” of the hepatic metabolism. It is mainly used for the administration of vaccines [180] (hepatitis, flu virus, tetanus) or with specific pathologies, such as rheumatoid arthritis and multiple sclerosis.

It is frequently performed in the upper arm [181] but also in the hip or thigh [182]. It is possible to administer up to 5 mL via this route, based on the site of injection [183]. As lymphatic vessels are present in the skeletal muscle and the connective tissue [184], this leads to the assumption the lymphatic system might be involved in the drug absorption following intramuscular administration. As presented in Table 4, several conditions are treated with this type of injection [185–188].

Table 4. CVD therapies using intramuscular administration.

Condition	Intervention and Identifier	Target	Dose and Outcome
Diabetes	Preproinsulin-encoding plasmid DNA	Pancreatic islets	40% higher survival rate as compared to the control group [185]
HTN	CoVaccine HT (NCT00702221)	Against angiotensin II	Three doses Terminated in 2016 due to dose-limiting adverse effects
HTN	AGMG0201 vaccine	Against angiotensin II	High or low dose (0.2 mg plasmid DNA and 0.5 or 0.25 mg Ang II-KLH conjugate) Ongoing
ACS HF CVD	Inactivated influenza vaccine		Less frequent hospitalization from ACS, hospitalization from HF and stroke [186]
MI	Influenza vaccine		Risk of cardiovascular-related death was significantly lower [187]
CVD MI	Pneumococcal vaccines		Reduced incidence of cardiovascular events and mortality Reduced risk of MI in the elderly [188]
MI HF Stroke	Influenza vaccine (NCT02831608)		The primary endpoints: death, new MI and stent thrombosis Secondary endpoints: patients with hospitalization for HF

HTN: Hypertension; AngII-KLH: Angiotensin II—keyhole-limpet hemocyanin; ACS: Acute coronary syndrome; CVD: cardiovascular disease; HF: Heart failure; MI: Myocardial infarction.

- **Diabetes**

In a study carried out by Abai et al., they found that streptozotocin (STZ)-induced diabetic mice injected IM with preproinsulin-encoding plasmid DNA showed a 40% higher survival rate as compared to the control group. Muscles of the treated group were able to produce insulin, to which the increased survival was attributed [185].

In addition, patients with T2DM could benefit from vaccination against obesity, being a leading risk factor for T2DM. The main targets for obesity vaccines include GIP, adipose tissue antigen, somatostatin, and ghrelin [189]. Other vaccines that showed considerable benefits and are recommended for patients with DM are influenza, pneumococcal, hepatitis A and B, varicella vaccines, and others [190].

- **Heart failure**

The last few decades have witnessed several studies that target the major pathogenic pathways implicated in HF. Among these targets: cardiac muscle contractility, angiogenesis, cytoprotection, and stem cell homing [191]. For instance, the β -adrenergic system plays a major role in the regulation of cardiac contractility. Up-regulation of the myocardial G-protein-coupled receptor kinase 2 (GRK2) protein in failing hearts was found to desensitize and down-regulate β -adrenergic receptors by up to 50% [192], which lead to the hypothesis that cardiac function could be improved with the GRK2-inhibitor peptide beta adrenergic receptor kinase carboxyl-terminus (β ARKct). Several studies in mice, rats, rabbits, and pigs showed that overexpressing β ARKct helped to prevent the development

and progression of HF, restored β -adrenergic receptor sensitization and prolonged the survival [193–197], and improved and sustained contractile function [198].

Furthermore, disrupted calcium (Ca^{2+}) homeostasis in the cardiomyocytes was found to be a consistent feature in HF, which is linked to down-regulation in the expression of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) protein pump and reduced cardiac contractility. The prominent success of SERCA2a gene transfer in enhancing cardiac contractility has paved the way to launch the clinical trial CUPID “ Ca^{2+} Up-regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease” [199,200]. CUPID was the first gene therapy clinical trial for HF and used AAV1 vectors for SERCA2a gene delivery [201].

- **Hypercholesterolemia and hyperlipidemia**

An AAV1-based therapy encoding LPL gene has been commercialized and approved since 2012 for the treatment of lipoprotein lipase deficiency (LPLD), alipogene tiparvovec (Glybera; uniQure Biopharma) [202]. LPLD, otherwise identified as chylomicronaemia syndrome, is a rare, hereditary autosomal disease resulting from loss-of-function mutations in LPL gene, which has a central role in the breakdown and clearance of triglyceride (TG)-rich lipoproteins (chylomicron and very low-density lipoprotein (VLDL)). As a result, patients with LPLD are prone to severe, recurrent attacks of pancreatitis, and they are at high risk of developing diabetes mellitus [203]. Although the highest level of vector DNA was observed at the injection site following IM injection of the drug in mice, a considerable amount was detected in the draining lymph nodes indicating the involvement of the lymphatic system in drug uptake [204]. Clinical trials reported immediate LPL expression following AAV1 injection and an associated long-term enhancement in chylomicron clearance. In addition, a retrospective study has documented a potential benefit of Glybera in reducing the frequency of pancreatitis episodes [205]. However, in 2017, the company declined Glybera’s marketing authorization renewal due to the unprofitability driven by the disease rarity [206].

3.5. Intramyocardial Injection

Direct intramyocardial injection is the most effective and commonly used way for gene delivery to the heart owing to its ability to achieve a high concentration of the injected compound at the injection site [207]. It is a preferential route to directly target lymphatic vessels due to their high density in the myocardium [159,208]. Various CVD and their treatments via intramyocardial injection are presented in Table 5 [201,209–224].

- **Myocardial infarction**

The use of nanotechnology as treatment demonstrated significant protection against post-reperfusion myocardial injury in ischemic hearts via reducing the elevated level of reactive oxygen species (ROS) and the intracellular Ca^{2+} (Table 5). These nanoparticles protected the cardiomyocytes in isolated hearts from damage and oxidative stress, an approach that could be very useful upon in vivo application [223]. Although two nanoparticle systems have received FDA approval for post-MI imaging, none was approved for therapeutic purposes [225].

On the other hand, modulating one or more of the following growth factors, vascular endothelial growth factor (VEGF) [226], fibroblast growth factor (FGF) [227], hepatocyte growth factor (HGF) [228], and platelet-derived growth factor (PDGF) [229] could have a repairing effect on the affected zones within the myocardium (Table 5).

Table 5. Use of intramyocardial injections in several therapies targeting CVD.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
HF	Ad5.hAC6 (NCT007)	Ad5	AC6	Phase I/II (Completed)	Single administration of escalating doses (3.2×10^9 vp to 10^{12} vp) Phase II: Reduced HF admission rate. Enhanced left ventricular function beyond the optimal HF therapy following a single administration [209]
HF	Ad5.hAC6 (NCT03360448)	Ad5	AC6	Phase III (withdrawn)	Phase III: withdrawn for re-evaluation
HF	MYDICAR (NCT00454818)	AAV1	SERCA2a	Phase I/II (Completed)	Single administration of escalating doses (1.4×10^{11} – 1×10^{13} DRP of AAV1/SERCA2a) Phase I/II (CUPID): high-dose treatment resulted in increased time and reduced frequency of cardiovascular events within a year and reduced cardiovascular hospitalizations [210]
HF	MYDICAR (NCT01643330)	AAV1	SERCA2a	Phase IIb (completed)	Single infusion of 1×10^{13} DRP of AAV1/SERCA2a Phase IIb (CUPID-2b): no improvement was observed at the tested dose in patients with HF during the follow-up period [201]
HF	MYDICAR (NCT01966887)	AAV1	SERCA2a	Phase II (Terminated)	1×10^{13} DRP of AAV1/SERCA2a as a single intracoronary infusion Phase II: no improvement observed in the ventricular remodeling. The study terminated driven by the CUPID-2 trial neutral outcome [211]
HF	SRD-001 (NCT04703842)	AAV1	SERCA2a	Phase I/II (Active, not recruiting)	Single administration of 3×10^{13} vg CUPID-3: aims to investigate the safety and efficacy of SRD-001 in anti-AAV1 neutralizing antibody-negative subjects with HFrEF
HF CVD	INXN-4001 (NCT03409627)	Non-viral, triple effector plasmid	SDF-1 α , S100A1, VEGF-165	Phase I (Completed)	Single 80 mg dose, given in 40 mL or 80 mL at a rate of 20 mL/min Phase I: an improvement in the quality of life in 50% of patients was reported [212]
HF	ACRX-100 (NCT01082094)	Plasmid DNA	SDF-1	Phase I (Completed)	Single escalating doses, injected at multiple sites Preclinical studies: enhanced vasculogenesis and improved cardiac function reported with all doses [213]
HF	JVS-100 (NCT01643590)	Plasmid DNA	SDF-1	Phase II (Completed)	Single injection of escalating doses (15 and 30 mg) Phase II (STOP-HF): JVS-100 showed potential to improve cardiac function through reducing left ventricular remodeling and improving ejection fraction [214]
HF	JVS-100 (NCT01961726)	Plasmid DNA	SDF-1	Phase I/II (Unknown)	Single injection of escalating doses (30 and 45 mg) Phase I (RETRO-HF): JVS-100 showed promising signs of clinical efficacy [215]

					Single injection of escalating doses (3 mg and 30 mg)
HF	AZD8601 (NCT02935712) (NCT03370887)	mRNA	VEGF-A165	Phase IIa (Active, not recruiting)	Preclinical studies: promoted angiogenesis, improved cardiac function and enhanced survival were reported [216] Phase I: ID injection of AZD8601 was well tolerated and enhanced the basal skin blood flow [217]
HF	NAN-101 (NCT04179643)	AAV	I-1c	Phase I (Recruiting)	Single escalating doses (3×10^{13} vg– 3×10^{14} vg) of NAN-101 Preclinical studies: enhancement in left ventricular ejection fraction and improved cardiac performance [218]
AMI IHD	VM202RY (NCT01422772) (NCT03404024)	DNA plasmid	HGF-X7	Phase II (Recruiting)	Single escalating (0.5–3 mg) doses, administered into multiple sites Phase I: improved myocardial function and wall thickness [219,220]
MI Angina pectoris	AdVEGF-D (NCT01002430)	AV	VEGF-D	Phase I/IIa (Completed)	Single escalating (1×10^9 – 1×10^{11} Vpu) doses, injected into multiple sites in the endocardium Phase I/IIa: AdVEGF-D improved myocardial perfusion reserve in the injected region [220]
MI	Ad-HGF (NCT02844283)	AV	HGF	Phase I/II (Unknown)	Single dose Preclinical studies: Ad-HGF preserved cardiac function, reduced infarct size, and improved post-MI cardiac remodeling [221]; fractional repeated dosing significantly improved cardiac function compared with single injection [222]
MI	L-type Ca^{2+} channels' AID peptide and antioxidant molecule (curcumin) in poly nanoparticles				Reduced the elevated level of ROS and the intracellular Ca^{2+} [223]
LPLD	Alipogene tiparvovec (NCT00891306)	AAV	LPL	Approved	1×10^{12} GC/kg Phase II/III: reduction in mean total plasma and chylomicron TG level [224]

HF: Heart failure; hAC6: Human adenylyl cyclase type 6; vp: Virus particles; AAV: Adeno-associated virus; SERCA2a: Sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase; DRP: DNase-resistant particles; HFREF: HF with reduced ejection fraction; CVD: Cardiovascular diseases; SDF-1a: stromal cell-derived factor 1; VEGF: Vascular endothelial growth factor; I-1c: Constitutively active inhibitor-1; vg: Viral genomes; AMI: Acute myocardial infarction; IDH: Ischemic heart disease; HGF-X7: Hepatocyte growth factor-X7; AV: Adenovirus; Vpu: Viral protein U; HGF: Hepatocyte growth factor; AID: alpha-interacting domain; ROS: reactive oxygen species; LPL: Lipoprotein lipase; TG: Triglycerides; GC: Genome copies.

3.6. Intravenous Injection

Intravenous injections are often used for rehydration, nutrition, and therapeutic treatments (for example, blood transfusion or chemotherapy), as well as to avoid hepatic metabolism [230]. The interest of this route of administration is the continuous treatment,

or regular frequencies, by the installation of a catheter [231]. However, the lymphatic system is only scarcely involved following IV injections [232–234]. Table 6 presents several conditions treated with this type of injection [74,83,85,235–248].

Table 6. Intravenous administration of medication as treatment for CVD.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
HTN	NO-releasing nanoparticles				Reduction in the mean arterial blood pressure [235]
HF Arrhythmia	Digoxin				Dose: 0.25 mg once daily [83]
MI HF HTN Arrhythmia	β -blocker		Beta-adrenergic receptors		Acebutol: 200 mg twice daily [74]
HF	Mesoporous silicon vector (Nanoconstruct)				Able to internalize, accumulate, and traffic within the cardiomyocytes [236]
HF	Combination of biocompatible magnetic nanoparticles and low-frequency magnetic stimulation		Cardiomyocytes		Managed the drug release by controlling the applied frequencies [237]
HF	S100A1-loaded nanoparticles, decorated with N-acetylglucosamine				Regulated Ca ²⁺ release and restored contractile function in the isolated failing cardiomyocytes [238]
HF	Biodegradable nanoparticles conjugated with myocyte-targeting peptide and PDT-enabling photosensitizer	PDT	Cardiomyocytes		Induced cell-specific death upon application of laser light, leaving adjacent and surrounding cells completely intact [239]
MI	Unfractionated heparin				Anticoagulant 60 IU/kg for initial bolus 12 IU/kg/h for maintenance [240]
MI	Aspirin		Platelets		325 mg, then 81 mg per day [85]
MI	Human recombinant VEGF-165				Significant improvement in the infarcted zone perfusion and cardiac function for up to six weeks post-MI [241].
MI	Nanoparticles containing siRNA				Anti-inflammatory effect in the infarcted heart and reduction of the post-MI heart failure [242]
MI	Magnetic nanoparticles-loaded cells				Robust improvement in the left ventricular and cardiac function [243]

MI	Insulin-like growth factor electrostatically-complexed with PLGA nanoparticles					Higher incidence in preventing cardiomyocytes' apoptosis, reducing infarct size, and enhancing left ventricular function [244]
MI	Pitavastatin in PLGA nanoparticles					Cardioprotective effect against ischemia-reperfusion injury [245]
HoFH	AAV8.TBG.HldLR (NCT02651675)	AAV	hLDLR	Phase I/II (Completed)		Single dose Preclinical studies: reduction in total cholesterol [246,247]
Elevated LDL-C	ALN-PCSK9 (NCT01437059)	siRNA	PCSK9	Phase I (Completed)		Single escalating (15 and 400 µg/kg) doses Phase I: reduction in the level of circulating PCSK9 protein and LDL-C [248]

HTN: Hypertension; NO: nitric oxide; HF: Heart failure; MI: Myocardial infarction; PDT: Photodynamic therapy; VEGF: Vascular endothelial growth factor; PLGA: Poly lactic-co-glycolic acid; AAV: Adeno-associated virus; HoFH: Homozygous familial hypercholesterolemia; hLDLR: Human low density lipoprotein receptor; TBG: Thyroxine-binding globulin; LDL-C: low density lipoprotein cholesterol.

- **Diabetes**

The first successful in vivo gene therapy study was completed in the early 1980s, when Nicolau et al. administered rats IV with liposome encapsulating preproinsulin 1-encoding plasmid DNA and demonstrated a significant hypoglycemic effect as well as elevated insulin level in the blood [249]. Ever since, several studies have been published in an attempt to control diabetes through targeting different tissues, such as pancreas [250], muscle [251,252], liver [253–255], and K-enteroendocrine cells (K cells) [256]. For effective hypoglycemic therapy, these tissues should be able to produce or secrete insulin in a glucose-dependent manner [257,258].

As for vaccination, several preclinical [259,260] and clinical [261–263] studies have demonstrated the efficacy of IV administration of anti-CD3 monoclonal antibodies in restoring β cell tolerance and reversing diabetes in recent-onset T1DM, via targeting the autoreactive T cells.

- **Hypertension**

Antisense oligonucleotides-mediated inhibition of β 1-receptors expression in hypertensive rats has resulted in a durable antihypertensive effect for more than two weeks after a single IV dose [264]. Moreover, Huang et al. showed that targeting renal G protein-coupled receptor kinase type 4 (GRK4) with siRNA helped to reduce the blood pressure significantly in spontaneously hypertensive rats [265].

To the best of our knowledge, none of the gene therapy studies targeting hypertension have reached the clinical trials so far [266].

From another perspective, nanoparticles can be used for controlling blood pressure by the generation/release of the vasodilator molecule nitric oxide (NO). Indeed, Cabrales et al. reported a reduction in the mean arterial blood pressure by using NO-releasing nanoparticles. Upon administration, hydration of these nanoparticles resulted in the release of NO at a therapeutic level into the circulation. NO acts by inducing vasodilatation and promoting microvascular perfusion [235]. In this study, NO-releasing nanoparticles were delivered via IV infusion; however, they could be injected IP or IM [235].

- **Heart failure**

So far, myocardial, intracoronary, and pericardial injections are the most commonly used to transduce the myocardium. IV injection offers a less invasive way; however, the

low transduction efficiency limits its application [191]. A nanoconstruct called mesoporous silicon vector was able to internalize, accumulate, and traffic within the cardiomyocytes following IV administration in HF murine model, without significant toxicity. This nanoconstruct could serve as a platform for therapeutic and diagnostic purposes in HF [236]. Indeed, as described in Table 6, the use of nanotechnology in this pathology seems promising.

- **Myocardial infarction**

Management of MI at the early stages is essential to avoid the development of irreversible HF, due to the poor cardiomyocytes turnover (Table 6) [267]. IV-injected human recombinant VEGF-165 (an important angiogenesis factor) has resulted in a significant improvement in the infarcted zone perfusion and cardiac function for up to six weeks post-MI [241]. Furthermore, post-MI extended inflammation is another potential target, being a risk factor for post-MI heart failure. Indeed, a study published in 2013 showed that IV administration of nanoparticles containing siRNA in atherosclerotic mice, with coronary ligation-induced MI, produced a significant anti-inflammatory effect in the infarcted heart and reduction of the post-MI heart failure. This effect reflected an improved inflammatory resolution and reduced monocyte numbers in the infarcted area [242].

On the other hand, liposomes, the most primitive form in nanomedicine, could have a great potential for targeting and accumulation in ischemic tissues, following the less invasive IV administration. This could be achieved through attaching targeting moieties and used for diagnosis or therapy [268]. These targeting nanoparticles were exploited to deliver different therapeutic products, e.g., proangiogenic factors [269], genetic materials [270], and cytokines [271]. Moreover, nanoparticles could be used in cell replacement therapy post-MI to restore cardiac function (Table 6).

3.7. Intraperitoneal Injection

Intraperitoneal administration, in which therapeutic compounds are injected directly into the peritoneal cavity, is another attractive approach of the parenteral extravascular strategies. It is used specifically for the local treatment of peritoneal cavity disorders, e.g., peritoneal malignancies and dialysis. The peritoneal cavity contains the abdominal organs and the peritoneal fluid, normally composed of water, proteins, electrolytes, immune cells, and other interstitial fluid substances [272]. The high absorption rate associated to IP administration is promoted by the vast blood supply to the peritoneal cavity, along with its large surface area, which is further increased by the microvilli covering the mesothelial layer [273]. Injected compounds can enter the circulatory system after IP injection via both blood and lymphatic capillaries draining the peritoneal submesothelial layer [273–275]. Besides, the peritoneal absorption of molecules is greatly affected by their physicochemical characteristics. This route of administration also allows for the injection of large volumes (up to 10 mL) [273]. Extensive experimental studies carried out on animals have revealed that the peritoneal cavity has favorable absorption of lipophilic and unionized compounds [276]. This type of injection is most exploited for preclinical studies, since it is the simplest to perform, especially in small animals and with little impact on the animals' stress [273,277]. IP use in humans is limited, despite showing many benefits in previous studies and even being recommended, for certain types of chemotherapy, by the National Cancer Institute [278–280].

- **Diabetes**

Cheung et al. were able to produce long-term protection against diabetes by using GIP promoters in transgenic mice treated with STZ to damage their pancreatic β cells [256].

- **Myocardial infarction**

IP injection of methotrexate-loaded lipid core nanoparticle in MI rat model has mediated angiogenesis through enhancing the expression of myocardial VEGF. Importantly, it reduced myocytes' hypertrophy, necrosis, and infarct size, effects that were not observed with the free methotrexate [281].

4. Conclusions

Treatments for cardiovascular diseases are numerous, and the routes of administration are diverse. However, side effects or therapeutic failures are also present. Therefore, improvement in therapeutic delivery is essential. This review highlights new administration routes and improved formulation of molecules that ameliorate their efficacy via lymphatic transport. Thus, ensuring an optimal lymphatic transport throughout the body would not only directly reduce CVD [16,25,164,217,220,282,283] but would also allow a proper drug delivery to the various targeted organs. Taken together, the combination of the use of nanotechnology, vaccination, and gene therapy, along with the appropriate administration route to target the lymphatic system, thus, seems to be a promising target for the prevention and treatment of cardiovascular diseases.

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