Lymphatic Route in Cardiovascular Medicine

Subjects: Pharmacology & Pharmacy

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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.

lymphatics cardiovascular diseases drug delivery route nanotechnology

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].

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 [11]. 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 [12][13][14][15][16], 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) [17]. 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 [18][19]. 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 [20]. The most commonly used non-viral vectors include lipid-based vectors using cationic lipids and polymer-based vectors using cationic polymers [21]. Cationic lipids complex with the genetic materials to form lipoplexes or lipid nanoparticles (LNP), while cationic polymers form polyplexes [22]. 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 [23][24].

3. Treating CVD through Various Administration Routes

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 [25] (**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 [26][27][28].

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 [29]
Diabetes	Sulfonylureas Meglitinide		Dosage is very different from one class of medication to another [30]
Diabetes	Acarbose, Miglitol	Carbohydrate digesting enzymes in the brush border	50 mg three times daily (up to 100 mg) [31]

Condition	Intervention and Identifier	Target	Dose and Outcome
	Voglibose		
Diabetes	Rosiglitazone Pioglitazone	PPAR-α	Rosiglitazone: 4 mg per day (up to 8 mg) Pioglitazone: 15–30 mg per day [32]
Diabetes	Sitaglipin Vildaglipin Saxaglipin Linaglipin Aloglipin	DPP4	2.5–100 mg once daily depending on the inhibitor used [33]
Diabetes	Dapagliflozin Canagliflozin Empagliflozin	SGLTP2	Dapagliflozin: 2.5–10 mg daily Canagliflozin: 100–300 mg Empagliflozin: 5–25 mg daily [34]
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

Condition	Intervention and Identifier	Target	Dose and Outcome
			bioavailability [17]
Diabetes	Electrostatically-complexed insulin with partially uncapped cationic liposomes		Improved insulin pharmacokinetic profile [35]
Diabetes	Insulin-loaded PLGA		Improved bioavailability and sustained hypoglycemic effect [36]
Diabetes	Exenatide combined to phase-changeable nanoemulsion with medium-chain fatty acid		Enhancement of intestinal absorption and lymphatic transport [37]
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 [38]
HTN	Clonidine Methyldopa	Alpha-adrenergic receptor (agonists)	Clonidine: 0.1 mg twice daily [39] Methydopa: 250 mg two to three times daily [40]
HTN	Carvedilol into nanoemulsion	Beta-adrenergic receptors	Significant improvement in its absorption, permeability, and bioavailability [41][42]
HTN	Valsartan, Ramipril and Amlodipine into nanoemulsion		Enhanced solubility, oral bioavailability, and

Condition	Intervention and Identifier	Target	Dose and Outcome
			pharmacological outcome [43]
HTN	Felodipine-loaded PLGA nanoparticles	Calcium-channel	Sustained drug release both in vitro and ex vivo [44]
MI HF HTN Arrhythmia	ß-blocker	Beta-adrenergic receptors	Acebutol: 200 mg twice daily [45]
MI HF HTN	Conversion enzyme inhibitors	Conversion enzyme	Captopril: 100 mg per day [46]
MI HF HTN	Valsartan Losartan	Angiotensin II	20 mg twice a day, up to 160 mg [47]
HF HTN	Hydrochlorothiazide Bumetanide	Angiotensin/neprilysin receptor	49 mg/51 mg twice daily and doubled after 2–4 weeks [48]
HF HTN	Sacubitril Valsartan	Calcium channel	5–10 mg daily [49] 60 mg three times daily [50]
HTN	Amlodipine	Calcium channel	5–10 mg daily [49]

Condition	Intervention and Identifier	Target	Dose and Outcome
Arrhythmia	Diltiazem		60 mg three times daily [50]
HF	Ivabradine		Bradycardic 5–7.5 mg twice a day [51]
HF MI	Eplerenone Spironolactone	Aldosterone	50 mg once a day [52] and 12.5–25 mg with each intake [53]
HF Arrhythmia	Digoxin		0.25 mg once daily [54]
HF MI HCL	Statin	HMG-CoA	10 mg once daily [55]
MI	Aspirin	Platelets	325 mg, then 81 mg per day [56]
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 [57][58]
HCL	Ezetimibe	Intestinal cholesterol absorption	10 mg once daily [59]

Condition	Intervention and Identifier	Target	Dose and Outcome
HLD	Tricor Triglide		Fenofibrates 100–300 mg per day [60]
HCL HLD	Atorvastatin formulated into ethylcellulose nanoparticles		Enhanced atorvastatin's lymphatic absorption and oral bioavailability [61]
HCL HLD	Atorvastatin formulated into nanocrystals prepared with poloxamer 188		Improved atorvastatin's gastric solubility and bioavailability [62] Reduced circulating cholesterol, TG and LDL
HCL HLD	Atorvastatin formulated into polycaprolactone nanoparticles		Enhanced atorvastatin's bioavailability [63]
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 [64]
HCL HLD	Nanoemulsion		Increased the bioavailability of atorvastatin compared to the commercial tablet ozovas™ [65]
HCL HLD	Simvastatin Rosuvastatin		Improved bioavailability via lymphatic uptake [66][67][68][69][70][71][72][73][74]

241–250.

5. Maisel, K.; Sasso, M.S.; Potin, L.; Swartz, M.A. Exploiting lymphatic vessels for immunomodulation: Rationale, opportunities, and challenges. Adv. Drug Deliv. Rev. 2017, 114,

Condition	Intervention and Identifier	Target	Dose and Outcome
	Fluvastatin Fibrates Ezetimibe lipid-based nanoparticles		

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 [75]. 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 [76][77][78][79][80][81][82][83][84][85][86][87][88][89][90][91][92][93][94][95][96][97][98][99][100][101][102][103].

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 [76]

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10. Yáñez, J.A.; Wang, S.W.; Knemeyer, I.W.; Wirth, M.A.; Alton, K.B. Intestinal lymphatic transport for drug delivery. Adv. Drug Deliv. Rev. 2011, 63, 923–942.
11. Li, T.; Liang, W.; Xiao, X.; Qian, Y.J. Nanotechnology, an alternative with promising prospects and advantages for the treatment of cardiovascular diseases. Int. J. Nanomed. 2018, 13, 7349.
12. Wong, M.S.; Hawthorne, W.J.; Manolios, N. Gene therapy in diabetes. Self Nonself 2010, 1, 165–178.
13. Phillips, M.I. Gene therapy for hypertension: Sense and antisense strategies. Expert Opin. Biol. Ther. 2001, 1, 655–662.
14. Tromp, T.R.; Stroes, E.S.; Hovingh, G.K. Gene-based therapy in lipid management: The winding road from promise to practice. Expert Opin. Investig. Drugs 2020, 29, 483–493.
15. Kieserman, J.M.; Myers, V.D.; Dubey, P.; Cheung, J.Y.; Feldman, A.M. Current landscape of heart failure gene therapy. J. Am. Heart Assoc. 2019, 8, e012239.
16. Shimamura, M.; Nakagami, H.; Taniyama, Y.; Morishita, R. Gene therapy for peripheral arterial disease. Expert Opin. Biol. Ther. 2014, 14, 1175–1184.
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Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
Diabetes	Exenatide Lixisenatide Liraglutide Exenatide LAR Albiglutide Dulaglutide				GLP-1 analogues [77] Exenatide: 5–10 µg twice a day Lixisenatide: 10–20 µg 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 µg 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-1β, and reprieved the onset of amyloid-induced hyperglycemia [78]

34. Neuen, B.L.; Cherney, D.Z.; Jardine, M.J.; Perkovic, V. Sodium-glucose cotransporter inhibitors in type 2 diabetes: Thinking beyond glucose lowering. CMAJ 2019, 191, E1128–E1135.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
Diabetes	IL-1β epitope peptide		Against IL-1β		Three doses—50 µg 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-1β expression and inhibition of the inflammatory activity [79][80]
Diabetes	hIL1bQb vaccine (NCT00924105)		Against IL-1β		Six doses—300 µg Mediated a dose-dependent IL-1β-specific antibody response More studies are required to precisely investigate the clinical efficiency of this vaccine [81]
Diabetes	Neutralizing antibodies against DPP4		The GLP-1 and GIP inhibitor, DPP4		Five doses—2–20 µg Increased pancreatic and plasma insulin level and improved postprandial blood glucose level [82]

outpatients with and without coronary artery disease. JAMA 2012, 308, 1340–1349.

46. Lazar, H.L. Role of angiotensin-converting enzyme inhibitors in the coronary artery bypass patient. Ann. Thorac. Surg. 2005, 79, 1081–1089.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
HTN	hR32 vaccine		Renin-derived peptide		Five doses—500 µg Reduced systolic blood pressure by 15 mmHg [83]
HTN	Angiotensin I vaccine (PMD3117)				Three or four doses—100 µg The vaccine failed to reduce the blood pressure [84]
HTN	AngI-R vaccine		Modifiedendogenous angiotensin I peptide		Four doses—50 µg 15 mmHg reduction in systolic blood pressure and reduced angiotensin I/II level [85]
HTN	ATRQβ-001		Angiotensin II type I receptors		Two doses—100 µg Protective role against target organ damage induced by hypertension [86]
HTN	ATR12181 vaccine		Angiotensin II type I receptors		Nine doses—0.1 mg Attenuated the development of hemodynamic

59. Vavlukis, M.; Vavlukis, A. Adding ezetimibe to statin therapy: Latest evidence and clinical implications. Drugs Context 2018, 7, 212534.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
					alterations of hypertension [87]
HTN	CYT006-AngQb vaccine		Against angiotensin II		100 or 300 µg Reduction in blood pressure and reduced ambulatory daytime blood pressure [88]
HF HTN	Ang II-KLH vaccine		Angiotensin II		Three doses—5 µg Suppressed post-MI cardiac remodeling and improved cardiac function [89]
MI	Celecoxib loaded in nanoparticles				Promoted vascularization in the ischemic myocardium and delayed HF progression [90]
MI	Chitosan-hyaluronic acid based hydrogel containing deferoxamine-PLGA nanoparticles				Persistent neovascularization in mice [91]
HCL	Alirocumab		PCSK9		One dose every two weeks [92][93]

72. Agrawal, Y.O.; Mahajan, U.B.; Agrimoth, V.V.; Nilange, M.S.; Mahajan, H.S.; Sharma, C.; Ojha, S.; Patil, C.R.; Goyal, S.N. Ezetimibe-Loaded Nanostructured Lipid Carrier Based Formulation

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
	Evolocumab				
HCL	Inclisiran		PCSK9		Two doses per year [94]
HoFH HeFH severe HCL	Mipomersen (NCT00607373) (NCT00706849) (NCT00770146) (NCT00794664)	ASO	ApoB	Approved	200 mg once/week. Phase III: reduction in LDL-C [95]
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 [94][96]
FCS	Volanesorsen (NCT02211209)	ASO	ApoC3	Approved	300 mg once/week Phase III: reduction in mean plasma APOC3 and TG level [97]
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

double-blind placebo-controlled study of an angiotensin immunotherapeutic vaccine (PMD3117) in hypertensive subjects. Clin. Sci. 2004, 107, 167–173.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
					Phase I/II: reduction in plasma Lp(a) concentration [98]
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) [98]
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 [99]
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 [100]
HCL	Neutralizing antibodies against PCSK9		PCSK9		Three doses—5–50 µg Long-lasting reduction in the level of total cholesterol, VLDL and

96. Raal, F.J.; Kallend, D.; Ray, K.K.; Turner, T.; Koenig, W.; Wright, R.S.; Wijngaard, P.L.; Curcio, D.; Jaros, M.J.; Leiter, L.A. Inclisiran for the treatment of heterozygous familial hypercholesterolemia. N. Engl. J. Med. 2020, 382, 1520–1530.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
					chylomicron [101]
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 [102]
HCL	AT04A		PCSK9		Four doses—15 µg and 75 µg Reduced serum LDL-C level and elevated anti-PCSK9 antibody titer [103]
HCL	A peptide representing the mouse ANGPTL3		Angiopoietin-like proteins 3 (ANGPTL3)		Three doses—5 µg Reduced steady-state plasma TGs and promoted LPL activity

107. Hirano, K.; Hunt, C.A. Lymphatic transport of liposome-encapsulated agents: Effects of liposome size following intraperitoneal administration. J. Pharm. Sci. 1985, 74, 915–921.

108. Flessner, M.; Bedrick, R.; Schultz, J.S. Exchange of macromolecules between peritoneal cavity and plasma. Am. J. Physiol. Heart Circ. Physiol. 1985, 248, H19–H25.

109. Lim, H.Y.; Thiam, C.H.; Yeo, K.P.; Bisoendial, R.; Hii, C.S.; McGrath, K.C.; Tan, K.W.; Heather, A.; Alexander, J.S.J.; Angeli, V. Lymphatic vessels are essential for the removal of cholesterol from peripheral tissues by SR-BI-mediated transport of HDL. Cell Metab. 2013, 17, 671–684.

GLP-1: glucagon-like peptide-1; IDL: Intermediate density lipoprotein; 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); ApoC: Apolipoprotein C; 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.

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 [104], leading to capillaries that have inter-endothelial gaps with size ranges from a few nanometers to several microns [4][105]. Small particles (<10 nm) [4] and medium-sized macromolecules (up to 16 kDa) [106] are mainly transported away from the interstitial spaces by blood capillaries, thanks to mass transport [107][108]. 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 [106] show preferential uptake into the highly permeable lymphatic capillaries either passively (paracellular) or actively (transcellular) through the lymphatic endothelial cells [109]. Indeed, it has been shown that the optimal diameter to target the lymphatic vessels in the dermis is 5 to 50 nm in mice [110].

Table 3 presents several vaccines used for diabetes through intradermal injection [111][112][113].

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 µg Vaccine was well tolerated [111]
Diabetes	C19-A3 (NCT02837094)	CD4 T cells	Three doses—10 ug 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 [112]

110. Reddy, S.T.; Rehor, A.; Schmoekel, H.G.; Hubbell, J.A.; Swartz, M.A. In vivo targeting of dendritic cells in lymph nodes with poly (propylene sulfide) nanoparticles. J. Control. Release 2006, 112, 26–34.
111. Thrower, S.L.; James, L.; Hall, W.; Green, K.M.; Arif, S.; Allen, J.S.; Van-Krinks, C.; Lozanoska-Ochser, B.; Marquesini, L.; Brown, S.; et al. Proinsulin peptide immunotherapy in type 1 diabetes: Report of a first-in-man Phase I safety study. Clin. Exp. Immunol. 2009, 155, 156–165.
112. Dul, M.; Nikolic, T.; Stefanidou, M.; McAteer, M.; Williams, P.; Mous, J.; Roep, B.; Kochba, E.; Levin, Y.; Peakman, M. Conjugation of a peptide autoantigen to gold nanoparticles for intradermally administered antigen specific immunotherapy. Int. J. Pharm. 2019, 562, 303–312.
113. Nikolic, T.; Zwaginga, J.J.; Uitbeijerse, B.S.; Woittiez, N.J.; de Koning, E.J.; Aanstoot, H.-J.; Roep, B.O. Safety and feasibility of intradermal injection with tolerogenic dendritic cells pulsed with proinsulin peptide—For type 1 diabetes. Lancet Diabetes Endocrinol. 2020, 8, 470–472.
114. Nicoll, L.H.; Hesby, A. Intramuscular injection: An integrative research review and guideline for evidence-based practice. Appl. Nurs. Res. 2002, 15, 149–162.
115. Nakajima, Y.; Mukai, K.; Takaoka, K.; Hirose, T.; Morishita, K.; Yamamoto, T.; Yoshida, Y.; Urai, T.; Nakatani, T. Establishing a new appropriate intramuscular injection site in the deltoid muscle. Hum. Vaccin. Immunother. 2017, 13, 2123–2129.
116. Ogston-Tuck, S. Intramuscular injection technique: An evidence-based approach. Nurs. Stand. 2014, 29, 52–59.
122. Vlachopoulos, C.V.; Terentes-Printzios, D.G.; Aznaouridis, K.A.; Pietri, P.G.; Stefanadis, C.I. Association between pneumococcal vaccination and cardiovascular outcomes: A systematic review and meta-analysis of cohort studies. Eur. J. Prev. Cardiol. 2015, 22, 1185–1199.

Condition	Intervention and Identifier	Target	Dose and Outcome
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 [113]

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 [114] (hepatitis, flu virus, tetanus) or with specific pathologies, such as rheumatoid arthritis and multiple sclerosis. It is frequently performed in the upper arm [115] but also in the hip or thigh [116]. It is possible to administer up to 5 mL via this route, based on the site of injection [117]. As lymphatic vessels are present in the skeletal muscle and the connective tissue [118], 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 [119][120][121][122].

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 [119]
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 [120]

placebo-controlled, phase 2b trial. Lancet 2016, 387, 1178–1186.

126. Hammond, H.K.; Penny, W.F.; Traverse, J.H.; Henry, T.D.; Watkins, M.W.; Yancy, C.W.; Swels, R.N.; Adler, E.D.; Patel, A.N.; Murray, D.R. Intracoronary gene transfer of adenylyl cyclase 6 in patients with heart failure: A randomized clinical trial. JAMA Cardiol. 2016, 1, 163–171.

127. Jessup, M.; Greenberg, B.; Mancini, D.; Cappola, T.; Pauly, D.F.; Jaski, B.; Yaroshinsky, A.; Zsebo, K.M.; Dittrich, H.; Hajjar, R.J. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID) a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure. Circulation 2011, 124, 304–313.

128. Hulot, J.S.; Salem, J.E.; Redheuil, A.; Collet, J.P.; Varnous, S.; Jourdain, P.; Logeart, D.;

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Condition	Intervention and Identifier	Target	Dose and Outcome
MI	Influenza vaccine		Risk of cardiovascular-related death was significantly lower [121]
CVD MI	Pneumococcal vaccines		Reduced incidence of cardiovascular events and mortality Reduced risk of MI in the elderly [122]
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.

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 [123]. It is a preferential route to directly target lymphatic vessels due to their high density in the myocardium [104][124], various CVD and their treatments via intramyocardial injection are presented in **Table 5** [125][126][127][128][129][130][131][132][133][134][135][136][137][138][139][140][141].

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)

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
					Phase II: Reduced HF admission rate. Enhanced left ventricular function beyond the optimal HF therapy following a single administration [126]
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 [127]
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 [125]
HF	MYDICAR (NCT01966887)	AAVI	SERCA2a	Phase II (Terminated)	1×10^{13} DRP of AAV1/SERCA2a as a single intracoronary infusion

152. Onwordi, E.N.; Gamal, A.; Zaman, A. Anticoagulant Therapy for Acute Coronary Syndromes. Interv. Cardiol. 2018, 13, 87–92.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
					Phase II: no improvement observed in the ventricular remodeling.The study terminated driven by the CUPID-2 trial neutral outcome [128]
HF	SRD-001 (NCT04703842)	AAVI	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-1α, 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 [129]
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 [130]
HF	JVS-100	Plasmid DNA	SDF-1	Phase II	Single injection of escalating doses (15 and 30 mg)

it Be Used in Experimental Animal Studies? Pharm. Res. 2020, 37, 12.

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
	(NCT01643590)			(Completed)	Phase II (STOP-HF): JVS-100 showed potential to improve cardiac function through reducing left ventricular remodeling and improving ejection fraction [131]
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 [132]
HF	AZD8601 (NCT02935712) (NCT03370887)	mRNA	VEGF-A165	Phase IIa (Active, not recruiting)	Single injection of escalating doses (3 mg and 30 mg) Preclinical studies: promoted angiogenesis, improved cardiac function and enhanced survival were reported [133] Phase I: ID injection of AZD8601 was well tolerated and enhanced the basal skin blood flow [134]
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 [135]

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
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 [136][137]
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 1/IIa: AdVEGF-D improved myocardial perfusion reserve in the injected region [137]
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 [138]; fractional repeated dosing significantly improved cardiac function compared with single injection [139]
MI	L-type Ca^{2+} channels' AID peptide and antioxidant molecule				Reduced the elevated level of ROS and the intracellular Ca^{2+} [140]

[45][54][56][147][148][149][150][151][152][153][154][155][156][157][158][159][160]

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 [147]
HF Arrhythmia	Digoxin				Dose: 0.25 mg once daily [54]
MI HF HTN Arrhythmia	ß-blocker		Beta-adrenergic receptors		Acebutol: 200 mg twice daily [45]
HF	Mesoporous silicon vector (Nanoconstruct)				Able to internalize, accumulate, and traffic within the cardiomyocytes [148]
HF	Combination of biocompatible magnetic nanoparticles and low-frequency magnetic stimulation		Cardio-myocytes		Managed the drug release by controlling the applied frequencies [149]
HF	S100A1-loaded nanoparticles, decorated with N-acetylglucosamine				Regulated Ca^{2+} release and restored contractile function in the isolated failing cardiomyocytes [150]

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
HF	Biodegradable nanoparticles conjugated with myocyte-targeting peptide and PDT-enabling photosensitizer	PDT	Cardio-myocytes		Induced cell-specific death upon application of laser light, leaving adjacent and surrounding cells completely intact [151]
MI	Unfractionated heparin				Anticoagulant 60 IU/kg for initial bolus 12 IU/kg/h for maintenance [152]
MI	Aspirin		Platelets		325 mg, then 81 mg per day [56]
MI	Human recombinant VEGF-165				Significant improvement in the infarcted zone perfusion and cardiac function for up to six weeks post-MI [153].
MI	Nanoparticles containing siRNA				Anti-inflammatory effect in the infarcted heart and reduction of the post-MI heart failure [154]
MI	Magnetic nanoparticles-loaded cells				Robust improvement in the left ventricular and cardiac function [155]

Condition	Intervention and Identifier	Therapy	Target	Stage and Status	Dose and Outcome
MI	Insulin-like growth factor electrostatically-complexed with PLGA nanoparticles				Higher incidence in preventing cardiomyocytes' apoptosis, reducing infarct size, and enhancing left ventricular function [156]
MI	Pitavastatin in PLGA nanoparticles				Cardioprotective effect against ischemia-reperfusion injury [157]
HoFH	AAV8.TBG.HldlR (NCT02651675)	AAV	hLDLR	Phase I/II (Completed)	Single dose Preclinical studies: reduction in total cholesterol [158][159]
Elevated LDL-C	ALN-PCS02 (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 [160]

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.

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 [161]. 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 [162]. Injected compounds can enter the circulatory system after IP injection via both blood and lymphatic capillaries draining the peritoneal submesothelial layer [162][163][164]. 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) [162]. Extensive experimental studies carried out on animals have revealed that the peritoneal cavity has favorable absorption of lipophilic and unionized compounds [165]. 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 [162][166]. 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 [167][168][169].