RNA THERAPEUTICS IN CARDIOVASCULAR TREATMENT: A SYSTEMATIC REVIEW ON CLINICAL OUTCOMES

By

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Bachelor of pharmacy(Hons.)

School Of Pharmacy
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8th July 2025

Declaration

It is hereby declared that

1. The project is my original work.

2. The thesis is not previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been submitted or published at a university or other institution.

4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

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Approval

The thesis titled " RNA Therapeutics in Cardiovascular Treatment: A systematic Review on Clinical Outcomes" submitted by Adiba Ayesha Khan (20146022) in Spring, 2020 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy (B.Pharm.) on 7th July 2025.

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Ethics Statement

Through this project, no animal is used or harmed, and no human trials have been conducted.

Abstract

Cardiovascular diseases (CVDs) are the number one cause of death around the world, and there is a critical demand for new and efficient therapeutic strategies. In the last decade, RNA-based drugs, such as mRNA, miRNA, siRNA, have become an effective therapeutic approach. These treatments are directed at the root cause of disease or molecular origins of disease and are a more personalized and precise form of treatment than conventional medicines.

The objective of this systematic review was to assess the clinical use of RNA-based treatments for cardiovascular disease in current studies. It also reviews the methods applied in this field of study, highlights areas that have gaps or in need of research and describes future research directions that would benefit from the RNA based therapies.

We searched the studies in the PubMed, ScienceDirect, and Google Scholar databases for RCTs mentioning the use of RNA-based treatments for cardiovascular diseases and their publication year was between 2015 and 2025. Selection criteria Based on the PRISMA guide, studies were considered for inclusion. The RCTs involved hypertension, atherosclerosis, myocardial infarction, and heart failure. We collected data on treatment methods, RNA delivery systems, results, and side effects from each study. Cochrane Risk of Bias 2.0 was used to evaluate the risk of bias.

This systematic review includes six RCT studies that were done on humans. The findings highlight the therapeutic potential of RNA-based treatments, especially the siRNA drug Inclisiran, which effectively lowers cholesterol by targeting the PCSK9 protein. miRNA therapies were found to influence cardiac changes in hearts in a way that modulates fibrosis and inflammation, while mRNA therapies showed promise in regenerating damaged heart

tissue. Among these, Inclisiran stands out for its clinical efficacy and improved patient adherence due to its dosing regimen.

But a number of included studies were at moderate to high risk of bias in terms primarily of insufficient randomisation, lack of blinding, incomplete data reporting and small size. These hold limit the integrity and long-term stability of current results. Future developments in the field of RNA therapy should also focus on the specific disease-related genes or proteins as a more effective treatment for cardiovascular diseases. Although the results are encouraging, limited data, well-designed RCTs, ideal delivery systems, and long-term follow-up evaluations.RNA therapeutics merged with personal genomic information could revolutionize the future of cardiovascular therapy opportunity to target CVD gene mutations directly and gene regulatory analyses, coupled to a patient's genomic profile can offer a more comprehensive understanding of an individual patient's phenotype and how it responds to therapy.

Keywords: RNA Therapy ,Cardiovascular disease ,SiRNA, miRNA, mRNA,Treatment

Dedication

I would like to dedicate this to my wonderful parents, whose love and support have shaped me into who I am today. Forever grateful for your unwavering guidance and endless sacrifices

Acknowledgement

Firstly, I would like to thank Almighty Allah; the journey would have been impossible without his mercy.

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List of Acronyms

ACE inhibitors	Angiotensin-Converting Enzyme inhibitors
CCBs	Calcium Channel Blockers
ARBs	Angiotensin II Receptor Blockers
GalNAc	N-Acetyl galactosamine
RISC	RNA-Induced Silencing Complex
APOC3	Apolipoprotein C3 (a gene/protein involved in lipid metabolism)
AZD8601	A modified mRNA encoding VEGF-A, developed by AstraZeneca for therapeutic angiogenesis
PICO	Patient/Population, Intervention, Comparator, Outcome
LDL	Low-Density Lipoprotein
NT–proBNP	N-terminal pro–B-type Natriuretic Peptide
siRNA	Small-interfering Ribonucleic Acid
miRNA	Micro Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
CVD	Cardiovascular Disease
PCSK9	Proprotein Convertase Subtilisin Kexin Type 9

Chapter 1

Introduction

Cardiovascular disease implies a group of diseases that affect many parts of the heart and blood vessels, starting up atherosclerosis by means of depositing fatty substances on the blood vessels, by making the blood vessels narrower by building plaque(Soppert et al., 2020). This can end up with less oxygen rich blood flow to pass through (heart blockage) resulting in chest pain or angina which can also lead to heart attack(*Pathogenesis of Angina Pectoris*, 1982). Most common cardiovascular diseases are coronary artery disease, heart failure, angina, arrhythmias and stroke(Zhao et al., 2018). Additionally, in most cases these diseases are linked with other health conditions like diabetes, hypertension, smoking, unhealthy lifestyle, less to no physical activities and some genetic factors(Fuchs & Whelton, 2019 & Rees et al., 2019). According to WHO, around 20.5 million people die every year suffering from cardiovascular disease globally. This reports a significant rate of mortality and morbidity of cardiovascular diseases(Di Cesare et al., 2024). The chance of getting heart disease increases with age, people who are in their or over 40s are at risk of getting them if they are kept away from physical activities, have diabetes, chronic hypertension, have a saturated fat rich diet or by leading an unhealthy lifestyle(Ortega et al., 2016).

Even though there are already significant advancements in medications and surgery procedures, these only help to manage the symptoms but do not cure the heart condition(Nielsen et al., 2024). Conventional drugs like ACE inhibitors and ARBs lowers blood pressure effectively but sometimes it makes blood pressure drop dangerously which also

comes up with dizziness, high potassium level and kidney damage(Cutrell et al., 2023). Beta blockers may reduce heart rates but end up with getting tiredness, shortness of breath and depression as side effects(Roston et al., 2019). Statins are remarkable at lowering cholesterol levels in blood. However, the increasing risk of diabetes or metabolism abnormalities and muscle pain(Attardo et al., 2022). Anti platelets might prevent the clotting problem, however, can cause excessive bleeding(Guthrie, 2011). Angioplasty cannot provide a guarantee of not re narrowing the arteries by plaque formation over time, which might require getting done with the procedures over again(Burzotta et al., 2020). In the same way coronary artery bypass grafting surgery comes with infection, clot ,stroke, graft failure and needs a long recovery time(Weir, 2006). Heart transplantation is also expensive for most patients and has drawbacks like severe infections, finding donors and organ rejections(Hwang & Sivathasan, 2022). Besides, all of the treatments mentioned above need to be carried out throughout the whole life ,for a patient(Zhang et al., 2024). Not to mention, current drug choices can be different based on a patient's profile as well(Chen et al., 2024). Also, conventional drugs are far away from treating patients in terms of genetic or gene pathways like, RNA based therapies can target a specific gene or gene pathway which is involved in that specific heart disease. This indicates a revolutionary shift of a treatment for cardiovascular diseases(Wang et al., 2023). One of the RNA based therapies, mRNA therapy (AZD8601) has shown that the areas in heart muscles become damaged, which later leads to heart failure(Anttila et al., 2022). Because due to the irreversible stress on heart muscle, an area of the heart muscle’s blood circulation is cut off. The absence of blood circulation leads to tissue death, because the tissue death to that particular site of the heart puts extra workload on the rest of the heart muscles, this is what leads to heart failure eventually(Mosterd & Hoes, 2007). With the help of conventional drugs that damaged tissues cannot be reversed in the first place(Greyson, 2012). However, the damaged heart tissue repair is possible ,by stimulating angiogenesis and cell growth to that particular damaged area

of heart. This cellular regenerative trait is absent in conventional drugs(Magadum et al., 2018 & Cooke & Youker, 2022). AZD8601 or its mRNA is translated inside the cytoplasm directly, which makes this therapy safe from using any viral vector and its mutagenesis. Its delivery system only uses lipid nanoparticles to encapsulate the mRNA to make sure it reaches the desired site inside the body to show its therapeutic effect and prevent its further modification before reaching that damaged or targeted heart muscle(Cooke & Youker, 2022). When they reach the targeted damaged heart calls, the lipid nanoparticles merge or fuse with the cell membrane and the mRNA then enters the cell. This is how AZD8601 or the mRNA therapy is injected directly into the cardiac muscle, the mRNA helps the cardiac cells to use them and to produce VEGF-A protein. VEGF-A protein promotes more new blood vessels growth which leads to more oxygen and nutrients supplied to that damaged area(Cooke & Youker, 2022). By means the damaged heart tissue becomes functional enough to return to its normal and functional state as it was before by making heart muscle strong by regenerating the blood supply. This AZD8601 therapy is even effective against implants or surgeries(Evers et al., 2022).

Another type of RNA based drugs that have shown remarkable ability in lowering cholesterol and triglycerides level effectively are Inclisiran and Plozasiran(siRNA)(Kosmas et al., 2025). This therapy needs to be delivered through a system which is called GalNAc conjugation, because RNA breaks down really fast when it’s in contact with other enzymes and in the blood stream while carrying on its function(Springer & Dowdy, 2018). The liver has special receptors called ASGPR or Asialoglycoprotein receptors, which can only recognize these GalNAc(N-Acetylgalactosamine) sugar-like molecules and bind to them effectively(Ahn et al., 2021). Plozasiran and Inclisiran are attached to the GalNAc molecules, after recognizing it binds to the receptor and the receptors absorb the Plozasiran or Inclisiran directly into the liver cells.

Inside the liver cell, siRNA binds to a protein complex called RISC(RNA induced silencing complex),this protein complex cuts the desired mRNA(Iwakawa & Tomari, 2021). .siRNA helps RISC and then goes and finds the specific mRNA which is responsible for producing the targeted harmful proteins and cuts it so that it can no longer produce that targeted protein, either PCSK9 or APOC3(Ballantyne et al., 2024). This RNA based drug significantly lowers LDL levels in patients and is highly selective to only the protein they are targeting which makes them a precise medication choice(M. M. Zhang et al., 2021). Inclisiran targets PCSK9 protein which is responsible for destroying LDL receptors on the liver cells. When Inclisiran stops the liver from producing PCSK9(Leiter et al., 2018). Additionally, the less PCSK9 protein the more active LDL receptors stay active on liver cells, which removes all the excess LDL cholesterol from the blood by 6 months up to 50 percent. Also, only two- or one-time administration a year ensures patient compliance, and it has very few side effects, which is unlike conventional drugs(Ray et al., 2023). .Now, about Plozasiran, it mainly targets APOC3 protein in the liver which is responsible for the breakdown of fat components.(Chebli et al., 2024). Too much APOC3 slows the breakdown of triglycerides or fat components, which leads to a situation where triglycerides stay in the bloodstream for a longer time and this starts causing several heart diseases and pancreatitis. This is how Plozasiran helps to clear out excess fat components from the blood(Gaudet et al., 2024).

Moreover, Antisense Oligonucleotide (CDR132L)targets miRNA ,which is responsible for reducing heart function by suppressing some good heart proteins like SERCA2a(Zhou et al., 2021). Too much miRNA-132 causes the heart to become bigger in its shape with weak muscles(Foinquinos et al., 2020). CDR132L is chemically modified in a way that it can be administered directly into the bloodstream without getting altered by enzymes and show its therapeutic effects well(Bauersachs et al., 2024). In patients with heart failure,miRNA-132

molecules stay overactive and eventually damage the heart. However,CDR132L blocks miRNA-132L,which helps to pump out blood better and prevents heart from its further damage .CDR132L is administered through intravenous infusion ,by directly injecting to the bloodstream it reaches to heart as itself without any other type of vector. Just like the previous two RNA based drugs ,this one also deals with the root cause of the heart disease by blocking miRNA-132,unlike managing disease symptoms like the conventional drugs(Kamrul-Hasan et al., 2024). Additionally, only a few administrations of CDE132L are enough for providing its efficacy and therapeutic effect. Three of these RNA based drug's randomized controlled trial studies showed significant results in patients' condition improvement and both safety and efficacy(Bauersachs et al., 2024). Furthermore, these interventions ensure a personalized medicine approach as well(Anttila et al., 2022 & Ray et al., 2024)

RNA based drugs show promising results in delivering precise and gene targeted treatment which works with the root cause of a disease. This makes the treatment an innovative approach and personalized and, their long-lasting therapeutic effect eliminates frequent administering of doses(Dzau & Hodgkinson, 2024). This systematic review will help to establish this RNA based drug for the treatment of cardiovascular diseases as a game changer to physicians and researchers by wearing them. This fact also highlights the need for more studies on these RNA based drugs for future research development. By comparing the RNA based drugs with conventional drugs help to understand that RNA based drugs have better efficacy and also, by targeting precise disease pathways, which definitely brings up their clinical advantages. Additionally, Plozasiran and Inclisiran, these drugs showed some promising effects to prevent some major heart diseases like heart failure, myocardial infarction ,also,CDR132L and

AZD8601 shown to improve hearts function gradually(Dzau & Hodgkinson, 2024). Which clearly indicates that the preventive measure of this drug is really strong, which is solely unlike the conventional medicines. However, these treatments fail to provide a comprehensive assessment for long term cardiovascular events. On the contrary, any cardiovascular disease progression requires minimum 10 years ,by means, to justify the effectiveness of the intervention and as a long treatment RNA based therapies must go through a minimum 10 year follow up period. Although all the randomized controlled trial studies on RNA based therapies assess only short-term outcomes of the intervention. Hence, this gives an unclear efficacy and safety profile for each RNA based drug. This is why, until this day, there has been no systematic review done on RNA based therapies for the treatment of cardiovascular diseases, I conducted this systematic review study of the RNA based therapies as a treatment option among patients who have been suffering from cardiovascular disease worldwide to establish this intervention's safety, efficacy for a long run. This systematic review aims to analyze the safety, efficacy and potential of the advanced RNA based therapies in the treatment of cardiovascular diseases, collecting evidence from randomized controlled trial studies for future line research and clinical practice.

Chapter 2

Methodology

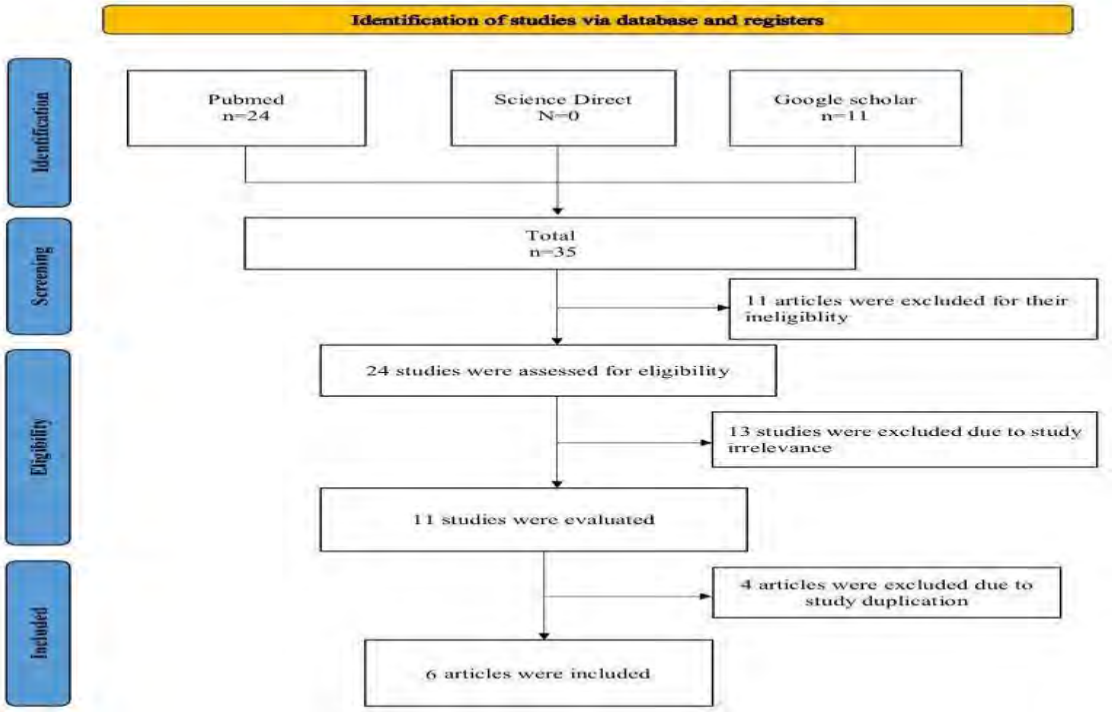

Figure 1. PRISMA 2020 flow diagram which includes searches of the databases

2.1 Eligibility Criteria (Study design and selection of inclusion-exclusion criteria)

A systematic method was followed to find the relevant randomized controlled trial studies of RNA based therapies for treating cardiovascular diseases following the PRISMA guidelines. Only articles that were peer reviewed and focused on randomized controlled trials of RNA based treatments for cardiovascular patients were included in the study. The inclusion and exclusion criteria for this systematic review were determined using the Population, Intervention, Comparison, and Outcomes (PICO) framework. The systematic review included

studies published between 2015 and 2024. For inclusion criteria, the population is targeted were adults (≥18 years) diagnosed with cardiovascular diseases (CVD) or high-risk conditions such as hyperlipidemia, myocardial infarction (MI), or hypertension. As intervention for this study ,was targeted only, RNA-based therapies which also include small interfering RNA (siRNA), messenger RNA (mRNA), antisense oligonucleotides, RNA aptamers, and microRNA (miRNA) modulators. For comparison, conventional medicines were selected such as statins, anticoagulants, ACE inhibitors, ARBs ,CCBs and diuretics. Outcomes were focused on therapeutic efficacy, improvement in cardiovascular events such as lipid profile improvement .The subject study design was only randomized controlled trials that were available in English. In terms of exclusion criteria, studies that were focused on patients under 18 years old or pediatrics, non-cardiovascular cohorts or preclinical studies those involved in vitro, or animal model's experiments were considered to be excluded. In intervention, studies were cut out of non-RNA based therapies. In case of study design, non-randomized studies, case reports, observational studies, systematic reviews, conference abstracts and narrative reviews, were kept out. Outcomes of studies reporting endpoints without direct clinical relevance to cardiovascular health were rejected.

2.2 Search strategy and study inclusion

A search strategy was developed or designed to narrow down all the relevant studies to carry out a good screening process. We used three online databases like PubMed, Google Scholar, and ScienceDirect by using multiple MeSH terms e.g., “RNA therapy,” “treatment,” “CVD,” along with their similar terms or full forms e.g., “cardiovascular disease” along with similar terms like “myocardial infarction,” “heart attack,” “hyperlipidemia,” and “cardiac dysfunction” in place of “CVD.” And “RNA therapy” along with “RNA based therapy,” “mRNA therapy,” “siRNA therapy,” “microRNA therapy,” “antisense oligonucleotides,” and “circRNA therapy.” MeSH words were then connected to each other by using (AND/OR) while searching in those three databases mentioned.

The PubMed string used was:

((‘‘RNA therapy” OR “RNA based therapy” OR “siRNA therapy” OR “mRNA therapy” OR “microRNA therapy” OR “antisense oligonucleotides” OR “cirRNA therapy”) AND (“cardiovascular disease” OR “CVD” OR “heart attack” OR “myocardial infraction” OR “hyperlipidemia” OR “cardiac dysfunction”)) AND (randomized controlled trial).

In ScienceDirect, the same search terms were entered using quotation marks and meshwords. In Google Scholar, the search was performed using the command: allintitle: "RNA therapy" "cardiovascular disease" "randomized controlled trial"

Filters applied included publication year (2015–2025), article type (randomized controlled trials only), and human subjects. The final search was conducted on May 13, 2024.

Publication date filters were applied for 10 years during the searching or screening process. Duplications in studies were checked carefully.

2.3 Data extraction

Extracted data sets consist of study design, age, number of patients, test parameters involved in each therapy, baseline and endpoint of the study, p values and outcome of the study. Inclusion and exclusion criteria of the study were carefully resolved. Data that were not available or reported were listed as NA and NR respectively.

Chapter 3

3.1 Result

The tables given below are summarizing the key clinical outcomes of the included RCT studies such as changes in triglycerides ,LDL-C, and other parameters or cardiovascular markers . Additionally, the Risk of Bias (RoB) assessment figure is included to visually represent the methodological quality of the selected studies based on the Cochrane RoB 2.0 tool. These tables highlight the therapeutic effectiveness, dosage patterns, and safety profiles of RNA based drugs such as Inclisiran, Plozasiran, CDR132L, and AZD8601. Most trials demonstrated statistically significant improvements in lipid profiles and cardiovascular function, especially when compared to placebo or conventional therapies. The RoB figure supports the reliability of findings in well conducted studies, while also identifying methodological gaps in others that require cautious interpretation.

Inclisiran(siRNA based therapy) for participants with atherosclerotic cardiovascular disease and elevated low density lipoprotein cholesterol ORION 9, Patients with heterozygous familial hypercholesterolemia (HeFH)

Study Design	No. of Patients (Placebo/ Intervention)	Age	Intervention (Inclisiran/siRNA)	Conventional drugs	Parameters	Endpoint (Intervention)	Endpoint (Placebo /inert saline based solution)	P-Value	Comment	Outcome (at 510 day)
Koenig et al., 2020 Randomized, Double blinded, Placebo controlled	482 (Placebo: 240 / Intervention: 242)	65.3 (Mean)	284 mg Inclisiran sodium (300 mg) subcutaneously at Day 1, Day 90, then every 6 months	Statins (99%) High Intensity Statins(79.1 %) Ezetimibe (29.9%)	LDL-C (mg/dL)	-50.2%	-2.0%	P<0.0001	Significant reduction in LDL-C levels	Effective LDL-C lowering
					PCSK9	-69.4%	+3.6%	P<0.0001	Strong inhibition of PCSK9	Enhanced lipid regulation
					Total Cholesterol	-32.8%	-2.4%	P<0.0001	Reduced overall cholesterol	Cardiovascular risk reduction
					Apolipoprotein B (ApoB)	-41.1%	-1.0%	P<0.0001	Lower ApoB, better lipid profile	Cardiovascular benefits
					Non-HDL-C	-44.3%	-2.5%	P<0.0001	Significant non-HDL-C reduction	Reduced atherosclerotic risk
					Triglycerides	-12.6%	-0.3%	P<0.0001	Small but positive effect	Beneficial for metabolic health
					Lipoprotein(a)	-26.5%	-1.5%	P<0.0001	Meaningful reduction in Lp(a)	Lowered cardiovascular risk

Patients with heterozygous familial hypercholesterolemia (HeFH),Inclisiran(siRNA-based therapy) for Participants With Atherosclerotic Cardiovascular Disease and Elevated Low-density Lipoprotein Cholesterol ORION 10

Study (Year)	No. of Patients	Age	Intervention	Conventional Drugs	Parameters	Baseline for Intervention	Endpoint for Intervention	Baseline for Placebo(inert saline based solution)	Endpoint for Placebo(inert saline based solution)	P Value	Comments	Outcome
Ray et al., 2020 Randomized, Double-blind, Placebo-controlled, Phase III Trial ORION-10 (Phase III)	1561	66.4 (Mean)	Inclisiran Sodium 300 mg SC (Day 1, Day 90, then every 6 months)	Statins (89.2%) High Intensity Statins (68.0%) Ezetimibe (9.9%)	LDL-C	104.7±38.3 mg/dL	-52.3%	104.8±37.0 mg/dL	1.0%	P<0.001	Effective in lowering LDL-C	Significant LDL-C reduction compared to placebo
					PCSK9	422.1±176.9 µg/	-69.8%	414.9±145.7 µg/L	13.5%	P<0.001	PCSK9 reduction	Strong PCSK9 inhibition
					ApoB	94.1±25.6 mg/dL	-49.2%	94.6±25.1 mg/dL	1.0%	P<0.001	ApoB reduction associated with LDL-C lowering	Potential cardiovascular benefit
					Non-HDL-C	134.0±44.5 mg/dL	-50.5%	134.7±43.5 mg/dL	3.4%	P<0.001	Non-HDL-C reduction	ASCVD risk reduction

Patients with atherosclerotic cardiovascular disease (ASCVD) Inclisiran for Participants With Atherosclerotic Cardiovascular Disease and Elevated Low-density Lipoprotein Cholesterol ORION 11(Patients with ASCVD)

Study (Year)	No. of Patients	Age	Intervention	Conventional Drugs	Parameters	Baseline for Intervention	Endpoint for Intervention	Baseline for Placebo(inert saline based solution)	Endpoint for Placebo(inert saline based solution)	P-Value	Comments	Outcome
Ray et al., 2020 Randomized, Double-blind, Placebo-controlled, Phase III Trial ORION-11 (Phase III, 2020)	1617	64.8 (Mean)	Inclisiran 284 mg SC on Day 1, Day 90, and then every 6 months (Subcutaneously)	Statins (94.7%) High Intensity Statins (78.6%) Ezetimibe (7.1%)	LDL-C	105.5 ± 39.1 mg/dL	-49.9%	105.5 ± 39.1 mg/dL	4.0%	P<0.001	Significant LDL-C reduction with inclisiran compared to placebo	Effective LDL-C lowering therapy
					Time-Adjusted LDL-C	105.5 ± 39.1 mg/dL	-49.2%	105.5 ± 39.1 mg/dL	3.4%	P<0.001	Sustained LDL-C reduction over 540 days	Supports long-term LDL-C management
					PCSK9	NA	-63.6%	NA	15.6%	P<0.001	Strong inhibition of PCSK9 with Inclisiran	Key mechanism for LDL-C reduction
					Total Cholesterol	187.3 ± 48.2 mg/dL	NA	183.3 ± 42.8 mg/dL	NA	P<0.001	Significant improvement in total cholesterol levels	Cardiovascular benefit potential
					ApoB	97.1 ± 28.0 mg/dL	NA	95.1 ± 5.2 mg/dL	NA	P<0.001	Effective ApoB lowering	ApoB reduction correlates with LDL-C lowering
					Non-HDL-C	137.6 ± 46.9 mg/dL	NA	133.9 ± 41.0 mg/dL	NA	P<0.001	Non-HDL-C reduction suggests cardiovascular benefit	Supports broader lipid management

Inclisiran for Participants With Atherosclerotic Cardiovascular Disease and Elevated Low-density Lipoprotein Cholesterol ORION-8 (ASCVD and ASCVD-risk equivalent patients from ORION-9, ORION-10, and ORION 11)

Study	No. of Patients	Age	Intervention	Conventional Drugs	Parameters	Baseline for Intervention	Endpoint for Intervention	Baseline for Placebo	Endpoint for Placebo	P-Value	Comments	Outcomes
Wright et al., 2024 (ORION-8) Open-label extension study following placebo-controlled Phase 2 and Phase 3 trials	3274	64 years (Mean)	Inclisiran 300 mg (Day 1, Day 90, then every 6 months) Subcutaneously	Statins (88.6%) High Intensity Statins (68.5%) Ezetimibe (16.6%)	LDL-C Reduction (%)	2.9 ± 1.2 mmol/L	-49.4% (95% CI: -50.4 to -48.3)	2.9 ± 1.2 mmol/L	NA	P < 0.001	Inclisiran consistently reduced LDL-C levels over a follow-up period of up to 6.8 years	Significant and sustained LDL-C lowering

Novel antisense therapy targeting microRNA 132 in patients with heart failure

Study (Year)	No. of Patients	Age	Intervention	Conventional Drugs	Parameters	Endpoint for Intervention	Endpoint for Placebo(0.9 % Saline)	P-Value	Comments	Outcome of the Patients
Phase 1b Study of CDR132L (2021) Randomized, Double-Blind, Placebo-Controlled	28 patients (20 CDR132L, 8 placebo)	Mean: 65.5 years	CDR132L (0.32, 1, 3, and 10 mg/kg) vs placebo (IV)	ACE Inhibitors (60%) Angiotensin II Receptor Blockers(25%) Aldosterone Antagonists(38%) Beta Blockers(63%) Platelet Aggregation Inhibitors(88%)	NT-proBNP (pg/mL)	+23.3%	0.9% increase	P = 0.2519	No statistical significance, but trend suggests potential benefit	Well-tolerated, dose-dependent miR-132 reduction, possible cardiac function improvement
					QRS Narrowing (%)	-5%	+2%	P = 0.0333	Statistically significant improvement in cardiac function	
					Plasma miR-132 levels	NA	NA	P < 0.0061	Strong evidence of target engagement with miR-132 inhibition(higher doses)	

Study on VEGF mRNA Injection in CABG Patients (AZD8601/mRNA)

Study	No.of patients	Age	Intervention(AZD8601/mRNA)	Conventional Drug	Parameters	Endpoint for Intervention	Endpoint for placebo(Citrate buffer)	P value	Comments	Outcome
Anittila et al., 2023 Randomized ,Double Blind ,Placebo Controlled	24	69 (Mean)	3 mg (injected directly into the myocardium)	Angiotensin-Converting Enzyme (ACE) Inhibitors Angiotensin Receptor Blockers (ARBs) Statins Beta-Blockers	LVEF	4.8	-6.0	0.054	Small improvement	AZD8601 showed safety and some improvements
					NT–proBNP	0.990	1.735	0.108	No significant change	
					SAQ Parameters					
					KCCQ	17.04	-5.55	0.044	KCCQ improvement is significant, but LVEF is not statistically significant	KCCQ shows significant improvement
					SAQ Physical Limitation Score	18.39	10.18			
					SAQ Angina Stability Score	6.91	6.66			
					SAQ Angina Frequency Score	26.73	18.22			
					SAQ Treatment Satisfaction Score	7.47	-4.21			
					SAQ Quality Of Life Score	31.11	14.31			

Plozasiran Study in Mixed Hyperlipidemia Patients(siRNA)

Study (Year)	No. of Patients	Age	Intervention(Plozasiran/siRNA)	Conventional Drug	Parameters	Baseline for Intervention	Endpoint for Intervention	Baseline for Placebo (volume based placebo/ saline)	Endpoint for Placebo	P value	Comments	Outcome
Ballantyne et al., 2024 (Phase 2b) Randomized, Double-Blind, Placebo-Controlled	353	61 (Mean)	10 mg, 25 mg, 50 mg quarterly, 50 mg half-yearly (Subcutaneous injection)	Statins (92%) PCSK9 inhibitors (2%) Fibrates (14%) Icosapent ethyl (3%)	Fasting Triglyceride Level (%)	244 mg/dL	-49.8% (10 mg)	237 mg/dL	-1.7%	P<0.001	Significant triglyceride reduction in all treatment groups	triglyceride reduction
					APOC3 Level (%)	14.6 mg/L	-57.3% (10 mg), -72.5% (25 mg), -78.5% (50 mg quarterly)	14.6 mg/L	NA	P<0.001	APOC3 reduction correlated with triglyceride lowering	Strong target engagement, effective APOC3 inhibition
					Non-HDL Cholesterol (%)	151 mg/dL	-16.7%	148 mg/dL	NA	P<0.001	Non-HDL cholesterol reductions	Potential cardiovascular benefit

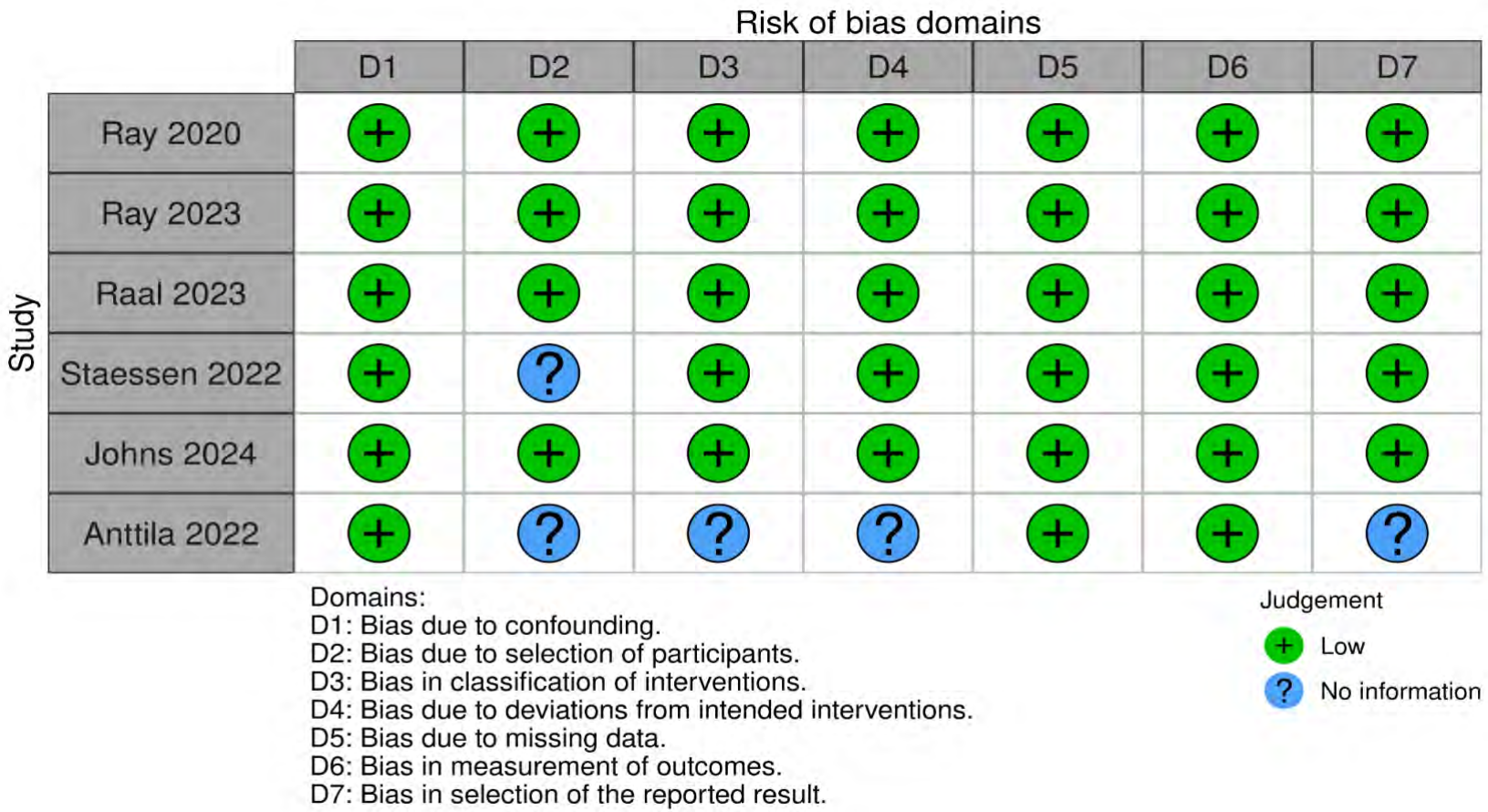

Figure : Risk Of Bias Assessment

3.2 Discussion

RNA based drugs are showing promising results in improving health conditions in patients with cardiovascular diseases. These RNA therapies target at molecular and genetic levels which are behind causing heart diseases like stroke and heart attacks' therapies like siRNA, MiRNA, mRNA target specific proteins or enzymes which are responsible for initiating cardiovascular diseases, either by blocking or boosting the production that protein or enzyme. These therapies are more targeted therapies for which they deliver precision in treating heart patients than the conventional drugs do.RNA based drugs may direct at innovating a whole new way in managing elevated cholesterol levels, atherosclerosis and heart failure(Burnett & Rossi, 2012b). Ray et al. (2020) initiated two clinical trials called ORION-10 and ORION-11 for the intervention which is Inclisiran. Inclisiran targets a protein which is produced by the liver called PCSK9. By targeting this protein, this RNA based drug reduces the LDL-C levels in blood. Patients who were only involved in the study had uncontrolled levels of cholesterol or heart diseases. They were taking statins on stable doses for a long time, however, their cholesterol levels still remained at their higher levels. The RNA based drug, Inclisiran was administered only two times a year, hence it showed promising results by reducing the LDL cholesterol up to 50% within 18 months. This is significant because of its patient compliance which made it easier for patients to take this drug with only in fewer doses. The study was well defined and designed well. It included so many patients to bring out the accuracy of the study and used a placebo for

picking up the comparison, and the study was double blinded ,which means neither the doctors nor patients knew which group is taking Inclisiran and which group is taking placebo, which made the study results precise and reliable. However, this study had some setbacks. It did not concern whether Inclisiran is able to prevent second heart attacks or further strokes or deaths.

It is only concerned with lowering cholesterol levels. Lowering the cholesterol levels in blood is useful to prevent the development of other heart diseases. Moreover, it fails to show the drugs performance as in its long-term result to monitor this drug delivers a better therapeutic effect in the long run. Also, the study was recently held and lasted for only 18 months, which makes this study impossible to fully understand the medicine's long-term effects and safety. Some of the patients were excluded in the trials, especially those with kidney or liver impairment. This left us unclear about whether Inclisiran is safe or effective for these patients or not .Although, side effects were none and very similar to the conventional drug or placebo group, this study did not add up to the possible major or minor cardiac events for taking Inclisiran. Overall, this study shows that Inclisiran shows a promising result for reducing LDL cholesterol levels in blood. Especially for those patients ,for those ,only statins are not working in the management of cholesterol

levels and needs a more effective drug like Inclisiran . Inclisiran needs only two doses a year to give therapeutic effect, which is long lasting as well ,which makes it easier for patients to continue the treatment well. However, further studies on Inclisiran are mandatory to make sure that it helps to reduce the chances of getting second heart attacks and other serious cardiovascular events in future. Ballantyne et al. (2024) highlights the promising result of Plozasiran which is another RNA based drug(RNA interference therapy) for treating patients who are suffering from mixed hyperlipidemia. This RNA based drug targets a special protein called apolipoprotein C-III or APOC3.This protein is responsible for increasing the levels of triglycerides, which is a type of blood lipid which is harmful and responsible for developing other cardiovascular diseases. With the help of this RNA based drug, Plozasiran, it targeted APOC3 protein, which eventually lowered triglyceride levels and other harmful blood lipids as well in patients who are at higher risk of getting cardiovascular diseases. Compared to the placebo, Plozasiran showed significant improvement in lowering non-HDL cholesterol,

triglycerides and apoB levels. Those are all directly related to the development of cardiovascular diseases, when their levels are elevated. In higher doses, Plozasiran provides an effective therapeutic effect which lasts up to 48 weeks even in fewer doses of injections in a year by lowering triglyceride ,LDL cholesterol and apoB levels significantly. This study is significant enough because it's not only just lowering APOC3,but also it's quite safe since all the side effects were mild and similar for both placebo groups' and intervention groups' side effects. There are common problems with lipid lowering drugs that are not liver and kidney friendly. However, there were no major adverse events identified. Although, there are few limitations. This study did not focus on a long-term result, it was held for 24–48 weeks and the results we got are not enough as evidence whether this treatment is actually enough alone to reduce the risk of strokes ,heart attacks and deaths . More studies on Plozasiran required to see if this RNA based drug is able to reduce all these risks of cardiovascular diseases. Additionally, the study was

done on patients with mixed hyperlipidemia ,although it would give us a clearer view if the patient population was selected in broader and more diverse groups like diabetes and lipid disorders. In the end, this study implies that Plozasiran is really helpful in managing lipid problems in blood by targeting APOC3 .This intervention is great help for patients who do not respond to conventional drug treatments and those who prefer long term therapeutic effects with a better targeted option. Koenig et al. (2024) found that Inclisiran, which is a small interfering RNA ,is a safe and effective treatment option for patients with polyvascular disease,which is a serious health condition where multiple blood vessels from different locations of the body get affected for patients who are at high risk for cardiovascular events. Inclisiran blocks the production of a protein called PCSK9, which increases the levels of LDL cholesterol . By targeting PCSK9,Inclisiran reduces the LDL levels in blood which is necessary in managing lowering cholesterol. In comparison to placebo, the combined randomized

controlled trials of these three respectively,ORION-9, ORION-10, and ORION-11 trials showed promising results in lowering LDL-C in blood significantly. This therapeutic effect lasted for a longer time than expected and significantly improved cholesterol levels were noticed in patients with different types of cardiovascular diseases, even applicable for patients with polyvascular disease,which comes with higher risk of getting heart attacks and strokes. This finding of this study is a phenomenon because this polyvascular disease affects more than one blood vessel at a time. Inclisiran helps to lower the risk of heart attacks and strokes by keeping the LDL-C at lower levels. The best thing about Inclisiran is it requires only two doses a year to give therapeutic effect after the first two doses, which ensures patient compliance since the patients didn't have to take medicines every day or every week. This makes it easier for them to stay on this treatment til the end of each trial. The graph on this study showed that after taking each shot, the LDL-C levels got reduced and stayed at a normal range in the interval of doses, which implies that Inclisiran provides long term of therapeutic effects even in by taking fewer injections. Safety is a concrete value and strength for this Inclisiran treatment. Also, this study found that all adverse effects of the study were mild and similar as the placebo groups .In drugs like this type of lipid lowering agents, they usually cause liver or kidney or muscle related conditions which were absent in Inclisiran. However, these are some limitations to be considered.Since,this study is post hoc and pooled. Which means that the researchers collected the results after the trials were finished and combined the results from each trial to find a pattern in the study which might not be the main focus of the study. This can often lead to bias. Also, even though Inclisiran is successful in lowering LDL-C levels but couldn't show clearly whether it can prevent other cardiovascular diseases from occurring. Further studies are needed to fully understand whether Inclisiran can benefit people directly or not, especially in patients with polyvascular disease. In conclusion, this study provides concrete evidence that Inclisiran works well for patients who are at high risk with polyvascular disease. It effectively

lowers LDL-C levels, has shown that its safety profile is quite favorable, requires it to be taken twice a year and patient compliance make this Inclisiran is a promising treatment option for patients with cardiovascular diseases. Anttila et al. (2023) highlights the potential benefit of using VEGF-A mRNA therapy for patients with end stage coronary artery disease injected directly into the heart muscle for those patients who do not have the physical capability due to their end stage coronary artery disease to go through bypass surgery and stenting.VEGF-A mainly promotes angiogenesis which actually promotes new blood vessels to improve heart's blood supply. This study showed that patients who received direct myocardial injections of VEGF-A mRNA noticed some improvements in symptoms such as chest pain or angina and quality of life. Most importantly, this VEGF-A mRNA showed no complication during this trial with its good safety profile and this injection was well tolerated as well. Imaging test results also showed blood flow improvement in areas where enough oxygen couldn't reach earlier because of the damaged cardiac tissues. This new approach is a gamechanger for patients who have severely damaged cardiac tissue which leaves them with very few treatment options. This VEGF-A mRNA injection promotes the healing process of that particular damaged area. This new treatment also helps to regenerate and repair heart muscles. Normal cell-based therapies usually come with issues like immune rejection. However, this is unlike cell-based therapies which means it avoids immune rejection. Also, the study showed that this injection is safe, well tolerated and targeted therapy. However, some of the limitations of this treatment approach are present. The patient number was really small to come to any conclusion, since the patient number should be large enough to be confirmed with a more designed study. Also, this study provided us many improvements in patients but as in the short term therapeutic effects.But this study is way recent and new to explore the long-term therapeutic effects in patients. This is why further research is needed on this drug to evaluate its safety for the long run and to see whether it prevents second heart attacks or improves the quality of life. In the

end, this study highlights promising evidence that direct intramyocardial injection of VEGF-A improves hearts blood flow and function as well as symptoms in patients who have very limited treatment options with severe coronary artery disease. Even so, more study on this intramuscular myocardial injection of VEGF-A mRNA is required to understand its long-term benefits. This treatment approach is valuable for patients who are at higher risk to go for any other treatments for their physical capability. Wright et al. (2024) brings out the strong potential of a randomized controlled trial ORION 8 where a small interfering RNA therapy was used which is Inclisiran ,in treating patients with homozygous familial hypercholesterolemia (HoFH),which is a serious medical condition where patients have uncontrolled and high LDL cholesterol levels in blood since birth and this is inherited or a genetic health condition. These patients do not respond to any other lipid lowering drugs or conventional drugs like statins. This makes them highly likely to get cardiovascular diseases at their early age. The study result showed that Inclisiran, after giving two initial doses, dropped the LDL cholesterol levels drastically in patients with HoFH. Even if the level of LDL cholesterol which dropped in these patients was still lower than the patients who have high levels of LDL cholesterol in blood but in less severe form ,but still, it is clinically meaningful for the patients with HoFH. This is significant because patients who already are suffering from HoFH have very few treatment options available. This is why even this modest change of the levels of LDL cholesterol will help to lower the risks of heart diseases. Importantly, even in this high-risk group of patients Inclisiran was found safe and well tolerated, since most of the side effects were really mild and short lived .For example reactions at the injection site but no severe side effects were noticed and reported during this trial. This new treatment approach for HoFH patients is quite beneficial because they already have to take more than one type of medication to lower the lipid levels in blood, which can lead to dangerous turns if the new drug is not safe enough to take. However, in the case of Inclisiran, this medication is quite safe for patients to take and showed results to

lower LDL cholesterol in blood in patients with HoFH. The greatest advantage of this treatment is after giving two initiate doses; it only needs to be taken twice a year. This long-term therapeutic effect of this drug made it easy for patients to stay on treatment and saved patients from taking injections on a regular basis or weekly. HoFh is a lifelong health condition, which is why it is easy to carry on treatment with Inclisiran because of its patient compliance. However, the study consists of a smaller patient number, as HoFH is a pretty rare genetic health condition. Also, the study only concerned with lowering LDL cholesterol levels in blood but long-term effects of the drug in conditions like heart attacks and strokes. Further studies are necessary to clarify

the benefits of Inclisiran in HoFH patients and the study needs to be done in a larger population for better follow up to confirm its therapeutic efficacy. In conclusion, this trial provides important early evidence on Inclisiran ,that it is a promising treatment for patients with homozygous familial hypercholesterolemia (HoFH). It offers a safe, long-acting option that can significantly lower LDL-C in people who need it most and have very few other options for their treatment.

Taubel et al. (2021) study targets one type of microRNA-132,which is mainly a small sized molecule ,which is responsible for worsening the condition of heart failure. The intervention ,which is CDR132L,an antisense oligonucleotide, blocks the microRNAs function to improve the heart function. The results of this study trial are very promising. Patients with heart failure who received CDR132L showed signs of improved heart function, as measured by NT-proBNP levels (a key marker of heart stress) and heart imaging. Additionally, the treatment was found to be safe and well-tolerated, with no serious side effects or safety concerns. These findings are especially important because heart failure is serious and growing health problems worldwide, and current treatments may not work for all patients. One of the

major strengths of this study is that it used a randomized, double-blind, placebo-controlled design, which helps make the results more reliable. The therapy also showed lasting effects, with benefits continuing several weeks after the last dose. This suggests that it may not need to be taken very often, which could be helpful for patient compliance. However, this trial was focused mainly on safety and early signs of effectiveness. The number of patients was limited, and the follow-up period was relatively short. As a result, more research is needed to confirm whether CDR132L can lead to long-term improvements in outcomes such as survival or reduced hospitalizations. In conclusion, this study introduces a new and promising approach to treat heart failure by targeting microRNA-132. The therapy appears safe and shows early signs of improving heart function. If future larger studies confirm these findings, CDR132L could become an important new tool in managing heart failure, especially in patients who do not respond well to current treatments.

RNA based medicines ,including mRNA, antisense oligonucleotides (ASOs), and siRNA have that ability to target the mechanism of that particular cardiovascular disease more precisely than the conventional drugs.siRNA therapies like Inclisiran and Plozasiran have shown promising results as an effective and a long term lipid lowering medicine by targeting specific genes like PCSK9 and APOC3 ,these genes are involved in the regulation of LDL-C and triglycerides . CDR132 , which is an antisense oligonucleotide, has a strategy for blocking miRNAs ,those are responsible for heart failure and this therapy effectively shows functional improvement in trials ,ensuring safety as well . Meanwhile , mRNA therapies like VEGF-A go by a regenerative method by inducing angiogenesis in patients ,those at the end stage of coronary artery disease ,have no other revascularization options . When it comes to comparing to gold standard drugs like PCSK9 inhibitors like alirocumab ,RNA based drugs have a competitive advantage in patient compliance ,it is because of its dosing frequency. Inclisiran

for example requires only biannual administration . However , alirocumab has extensive outcome data based on its cardiovascular events ,whereas ,all of the RNA based drugs still lack long term data on safety,mortality and recurrent events across the diverse population .Furthermore ,the inclusion of patients with comorbidities in these trials limits the generalizability . While these therapies signal a transformative shift in CVD treatment, further global-scale, long term randomized trials are critical to establish their place alongside or potentially beyond current gold-standard interventions.

The risk of bias assessment, provides that most of the studies which are included ,demonstrates a low risk of bias in all domains (D1-D7). This reflects strong methodological quality and increasing confidence in the findings of this systematic review . Ray (2020, 2023), Raal (2023), and Johns (2024) were evaluated in all seven domains and judged as low risk studies .This suggests that these studies had robust participation selection , clear protocols , proper intervention classification , and reliable outcome reporting . Inclusion of these studies ,strengthens the reliability and credibility of this systematic review .There were two studies with risk,respectively Staessen (2022) and Anttila (2022).These studies lacked information in several domains . In the case of Staessen(2022), this study lacked sufficient information on selection of participants which is on D2. This domain is considered as critical ,since unclear randomization might lead to the selection bias .Lastly, Anttila(2022) had no information in several domains like D2(selection of participants),D3(classification of interventions),D4(deviations from intended interventions), and D7(selection of the reported result).These uncertainties raise concerns regarding internal validity. Lack of detail on how the intervention was administered (D3–D4) or how outcomes were selectively reported (D7) could compromise the study's trustworthiness and influence the pooled outcomes.

Additionally, a large number of patients following up for at least 10 years is needed to assess

its long-term therapeutic effect and major cardiovascular events. These studies will then be able confirm the clinical benefits of RNA based drugs in treatment of cardiovascular diseases.

Chapter 4

Conclusion

This systematic review highlights the potential of RNA based therapies such as siRNA, mRNA, antisense oligonucleotides in targeting the disease mechanism directly of cardiovascular diseases,including heart failure,atherosclerosis and hyperlipidemia .For instance, siRNA therapy like Inclisiran lowers LDL-C in a way that is distinct from statins and provides patient compliance with fewer doses . Similarly, mRNA drugs promise in cardiac tissue repair .Despite these advancements of RNA drugs in the treatment for cardiovascular diseases, still several limitations are present .(Burnett & Rossi, 2012b) .As key challenges ,there are uncertainties about long term safety, lack of large scale randomized controlled trials , instability of RNA molecules in vivo and immunogenicity .There are still needs present for more biocompatible and targeted delivery systems like lipid nanoparticles, to enhance the specific tissue uptake and minimizing the immune responses. (Saw & Song, 2024). Future research must prioritize the development of robust delivery platforms, comprehensive safety evaluations, and clinical integration strategies that combine RNA therapeutics with standard treatments .(Paunovska et al., 2022).With ongoing innovation and validation, RNA-based drugs have the potential to transform cardiovascular care by enabling more precise and durable treatment options .(Burnett & Rossi, 2012b).

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