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BIOCHEMICAL BASIS OF ATHEROSCLEROSIS IN ACUTE CORONARY SYNDROME:

CLINICAL TRIALS INVOLVING SECONDARY PREVENTION

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Index

Abstract

Atherosclerosis is a determinant cause of cardiovascular disease (CVD), which remains the top cause of morbidity and mortality worldwide. Ischemic heart disease, especially acute coronary syndromes (ACS), accounts for the majority of CVD deaths. Despite improvements in risk factor management and declines in mortality in high-income countries, global trends remain heterogenous, as the interplay between inflammation, thrombosis and lipid assimilation is yet to be explored. Therefore, investigating the impact of atherosclerosis on major adverse cardiovascular events is crucial for refining prevention strategies. This study aimed to identify key biochemical mechanisms of atherosclerosis targeted by ongoing Phase III clinical trials on secondary prevention of ACS, specifically focusing on therapies addressing inflammation, thrombosis and lipid metabolism.

The project involved a comprehensive literature review combined with a systematic search of ClinicalTrials.gov and the European Union Clinical Trials Register to discern relevant clinical trials according to the criteria established prior.

This work identified 17 new Phase III clinical trials focusing on secondary prevention strategies after ACS. These included 2 trials dampening key molecules in the inflammatory pathways, 7 antiplatelet and antithrombotic trials exploring innovative therapeutic approaches, focusing on the coagulation cascade and platelet receptors, and 8 trials investigating lipid-lowering strategies, 5 of which involved entirely new drugs not previously tested in this population. While eligibility criteria varied widely in these trials, results may give a greater assortment of applications in different situations and pathologies.

As a conclusion, there is a need for integrated therapeutic approaches that address the interconnected pathways. The review of ongoing Phase III clinical trials demonstrates that targeting these mechanisms is an emerging priority in secondary prevention after ACS, offering alternative treatments that may provide greater results. Therefore, this work emphasizes the importance of continuing research efforts to refine and personalize therapies, aiming to improve outcomes in high-risk patients.

1. Introduction

Atherosclerosis is a chronic, progressive and multifactorial disease of the arterial wall, widely recognized as the underlying pathological substrate of acute coronary syndromes (ACS). It is characterized by the accumulation of lipids, inflammatory cells and fibrous tissue, culminating in the formation of atherosclerotic plaques that compromise vascular integrity. Far from being a passive buildup of lipids, atherosclerosis is now understood as a complex interplay of biochemical and cellular processes, involving endothelial dysfunction, lipid metabolism dysregulation, oxidative stress, immune activation and vascular smooth muscle cell (VSMCs) proliferation (1,2).

These interconnected mechanisms not only initiate and sustain the atherosclerotic process but also drive its progression and contribute to plaque destabilization, leading to clinical consequences such as rupture or erosion. These events, in turn, trigger platelet activation and thrombus formation, ultimately resulting in acute ischemic manifestations (1,3).

Among the various clinical outcomes of advanced atherosclerosis, acute coronary syndrome (ACS) is one of the most severe and life threatening. ACS encompasses a spectrum of conditions including unstable angina, non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI), all of which result from the acute disruption of a vulnerable atherosclerotic plaque followed by thrombus formation that partially or completely occludes the coronary artery. Although ACS presents as a clinical emergency, its origins lie in the chronic, inflammatory nature of atherosclerosis and the associated biochemical deterioration of the arterial wall (4).

The understanding of these molecular foundations is critical not only for prevention but also for the development of effective secondary prevention strategies. It is known that patients who have experienced an ACS event are at an elevated risk for subsequent cardiovascular events, needing interventions that go beyond symptom management to address the underlying pathophysiology. While primary prevention focuses on controlling risk factors before clinical disease, secondary prevention aims to mitigate the risk of future events in patients with established atherosclerosis. This has led to a growing number of clinical trials investigating pharmacologic therapies that target key biochemical pathways involved in inflammation, thrombosis and lipid metabolism demonstrating varied degrees of efficacy in reducing morbidity and mortality (5,6).

A comprehensive review of these trials is therefore essential for understanding the current landscape of secondary prevention and for identifying areas where our knowledge remains incomplete. For instance, the relative contribution of inflammation versus thrombosis in plaque destabilization is still debated. Emerging therapies targeting inflammatory pathways, thrombosis and cholesterol metabolism offer promising avenues, but their long-term effects and optimal combinations are areas of ongoing investigation.

This thesis aims to bridge the gap between molecular understanding and clinical application by reviewing the major clinical trials that have tested therapies targeting inflammation, thrombosis and lipid pathways in the context of secondary prevention after ACS. By examining these interventions through the lens of their molecular targets and trial outcomes, this work seeks to provide a comprehensive and critical perspective on the current therapeutic landscape and identify potential directions for future research.

2. Pathophysiology of Atherosclerosis

2.1. General concept of Atherosclerosis

As we mentioned before, atherosclerosis is a chronic, systemic disease characterized by the progressive accumulation of lipids, fibrous elements and inflammatory cells within the intimal layer of large- and medium-sized arteries. This vascular pathology is the underlying cause of the majority of major cardiovascular events such as myocardial infarction (heart attack), stroke, unstable angina (ischemic heart pain), sudden cardiac death, and peripheral arterial disease. The atherogenic process is initiated by endothelial dysfunction or injury, which disrupts vascular homeostasis and increases endothelial permeability. This dysfunction is frequently triggered by well-established cardiovascular risk factors including hyperlipidemia, hypertension, smoking and diabetes mellitus (7).

Consequently, lipoproteins – particularly low-density lipoprotein (LDL) – penetrate the arterial wall, where they undergo biochemical changes, such as oxidative modification, that initiate an inflammatory response. This promotes the recruitment of immune cells and the development of early lesions known as fatty streaks. As the disease progresses, additional cellular and molecular mechanisms contribute to plaque expansion and structural remodeling within the vessel wall. Over time, these plaques may become unstable and prone to rupture or erosion, which may lead to thrombus formation and acute clinical events. Although it begins as a localized vascular condition, atherosclerosis reflects broader systemic alterations, including chronic inflammation and pro-thrombotic state, and remains a major contributor to cardiovascular morbidity and mortality worldwide (6).

2.2. Role of the endothelium and mechanisms of injury

The vascular endothelium is a monolayer of endothelial cells (ECs) lining the luminal surface of all blood vessels. It plays a pivotal role in maintaining vascular homeostasis through the regulation of vascular tone, permeability, leukocyte adhesion and thrombosis. As the interface between circulating blood and the vessel wall, endothelium represents the first barrier for molecules, cells or pathogens present in the bloodstream (7,8).

Located between circulating blood and underlying tissues, the endothelium functions as an integrator and transducer of both humoral and mechanical stimuli. It mediates vascular

responses through the release of biologically active molecules – including nitric oxide (NO), prostacyclin, endothelin-1 – that then act in an autocrine and paracrine manner to preserve vascular integrity. These responses are modulated by mechanical forces (e.g., elongation and wall shear stress [WSS]), as well as variations in the concentration of metabolic signals. Under physiological conditions, NO exerts vasodilatory, anti-inflammatory and antithrombotic effects, and its reduced bioavailability marks the onset of endothelial dysfunction (7,9).

This dysfunction is characterized by impaired vasodilation, increased oxidative stress and a pro-inflammatory state, often driven by conventional cardiovascular risk factors such as hyperlipidemia, hypertension, smoking and diabetes mellitus. Under these conditions, ECs upregulate the expression of adhesion molecules (e.g., VCAM-Q, ICAM-1) and reduce their barrier integrity, facilitating the adhesion and transmigration of leukocytes into the subendothelial space. Concurrently, increased endothelial permeability allows low-density lipoprotein (LDL) particles to infiltrate the intima as seen in *Figure 1*, where they undergo oxidative modification, forming oxidized LDL (oxLDL), which further exacerbated endothelial dysfunction and promotes the recruitment of monocytes. These monocytes differentiate into macrophages, ingest oxLDL and become foam cells, contributing to the formation of fatty streaks – the earliest visible lesions in atherosclerosis. This phase of lesion formation marks a shift from a merely metabolic imbalance to an active, immune-mediated process (6,10,11).

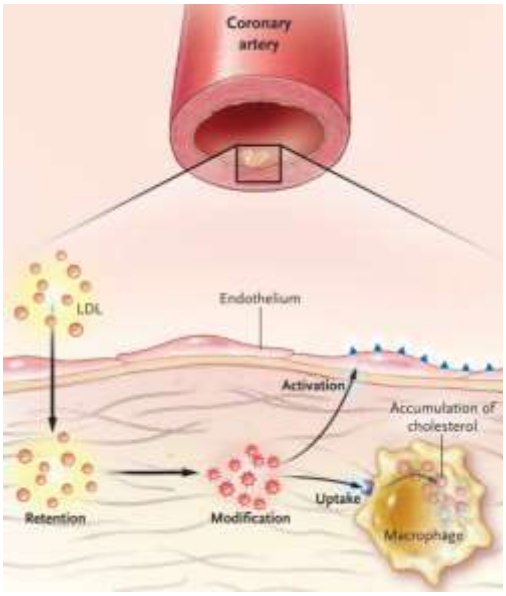

Figure 1. *Infiltration of LDL into a coronary artery and its effect. Adapted from (Inflammation, Atherosclerosis, and Coronary Artery Disease, 2005).*

Recent insights also implicate mitochondrial dysfunction in ECs as a key contributor to oxidative stress, endothelial apoptosis and inflammation. Mitochondria-derived reactive oxygen species (ROS) impair NO signaling and promote further oxidative damage, thereby exacerbating endothelial injury (12,13).

Atherosclerotic lesions (atheroma) mainly occur in regions characterized by low WSS and flow separation and most frequently involve branch points and bifurcations. These regions induce a pro-atherogenic endothelial response via mechanosensitive pathways involving molecules like NF-κβ. Substantial experimental and clinical evidence correlates disturbed flow patterns with the spatial distribution of atherosclerotic plaques, underscoring the importance of evaluating arterial bifurcations and branch points in the diagnosis and assessment of lesion development and progression (9).

In summary, these molecular and biomechanical alterations highlight the endothelium's central role in the initiation and progression of atherosclerosis. Endothelial dysfunction is not

merely a passive consequence of risk exposure but an active, multifactorial process that orchestrates the inflammatory and structural changes underlying plaque development, progression and rupture.

2.3. Atherosclerotic plaque formation

As the disease progresses beyond the early formation of fatty streaks, atherosclerosis develops into a more complex and chronic inflammatory condition. At this stage, the persistent buildup of oxLDL and apoptotic foam cells in the intima draws in additional immune cells, particularly macrophages and T cells. These cells fuel ongoing inflammation by releasing signaling molecules such as interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), which further amplify leukocyte recruitment and keep the endothelium activated and inflamed.

Meanwhile, VSMCs from the middle layer are stimulated by growth factors like PDGF and TGF-β to migrate into the intima. Once there, they proliferate and begin synthesizing structural proteins, including collagen and proteoglycans, which form the fibrous cap that characterizes mature atherosclerotic plaques. This cap is essential because it forms a barrier that separates the soft, lipid-rich necrotic core from the bloodstream, providing structural stability to the lesion. As VSMCs are also able to internalize oxLDL in a non-regulated way, they make their contribution to the total foam cell population within the plaque significant (7,14).

Even though, as the plaque expands, the local system for eliminating apoptotic cells can become compromised. When the recruited immune cells can no longer efficiently clear out dead and dying cells (efferocytosis), those cells begin to disintegrate uncontrollably, leading to the synthesis of a necrotic core, which keeps the inflammation going and causes more tissue damage.

In addition, the excess of lipid uptake by macrophages perpetuates the inflammatory response and could be considered a protective mechanism, as they remove cytotoxic elements from the intima. Still, the increased migration to the intima of monocytes and the upcoming differentiation into macrophages lead to a large number of foam cells causing the atherosclerotic lesion to grow. Hence, cholesterol accumulation is considered an indicator of atherosclerotic lesions. Cholesterol buildup within the subendothelial space can lead to the formation of cholesterol crystals, both intracellularly and extracellularly, which contributes to the progression of atherosclerotic plaque development.

In essence, plaque formation in atherosclerosis is much more than just an accumulation of fat in the arteries; it is a complicated process that involves immune system malfunction, issues with dead cell clearance and continuous tissue remodeling. Over time, these factors transform what starts as a stable lesion into a weak, rupture-prone plaque that can lead to serious, life-threatening cardiovascular complications (7,14,15).

Understanding these mechanisms provides valuable insight into the pathophysiology of plaque rupture and offers potential therapeutic strategies targeting inflammation without compromising host defense.

2.4. Plaque rupture and acute coronary syndrome

As previously mentioned, the presence of a necrotic core and a fibrous cap represents a characteristic sign of advanced atherosclerotic lesions, and atheromatous plaque regression becomes highly improbable in this stage. Moreover, the excessive accumulation of leukocytes, a highly pro-inflammatory cytokine setting, expansion of the necrotic core due to cell death and protease-mediated destruction of the extracellular matrix (ECM) leading to the fibrous cap-thinning are features of unstable atherosclerotic plaques.

As stated, the fibrous cap functions as a barrier to avoid exposure of prothrombotic material of the necrotic core to the bloodstream that otherwise would trigger thrombosis. Under physiological conditions, VSMCs regulate the blood vessel diameter and blood flow. However, in response to lesions, VSMCs are activated to perform their activities of migration and proliferation, initiating the synthesis of various growth factors. As VSMCs continue to proliferate, they help stabilize the plaque by surrounding the lesion and reinforcing the fibrous cap, reducing the risk of rupture. Although, in a sustained environment of inflammation, oxidative stress and thrombogenicity caused by macrophage death and impaired efferocytosis, these VSMCs can end up dying. Their loss weakens the fibrous cap and increases the plaque's vulnerability to rupture (7,15).

As the injury progresses, phagocytic cells fail to clear the accumulating cholesterol crystals, which continue to grow and persist within the subendothelial space. This process activates the complement system and further amplifies the plaque's pro-inflammatory environment (14,16).

Therefore, a plaque is defined as vulnerable when it exhibits a large necrotic core, a thin fibrous cap and heightened inflammatory activity driven by persistent exposure to a pro-atherogenic milieu.

As aforementioned, the fibrous cap separates the thrombogenic necrotic core and circulating coagulation factors and platelets, with its thickness closely linked to plaque vulnerability. As VSMCs undergo apoptosis, ECM synthesis decreases, while the activity of matrix metalloproteinases (MMPs) increases, collectively weakening the structural integrity of the cap. A mismatch between MMPs and their inhibitors weakens the fibrous cap, making it more vulnerable to rupture. In this stage of plaque rupture, inflammation has its relevance as it promotes the instability of the fibrous cap too.

When the plaque ruptures, the subendothelial space is exposed to the bloodstream, triggering a coagulation response to cover the wound. Platelets are the first to react by

adhering to subendothelial collagen and becoming activated. This activation leads to the recruitment and aggregation of more platelets in the region, starting the wound-healing process. At the same time, thrombogenic substances from the necrotic core – including oxLDL, tissue factor and cellular debris – are liberated and interact with the growth factors located in the plasma. In particular, the tissue factor from the core binds to factor VII of the plasma, initiating the coagulation cascade that induces thrombin synthesis. Thrombin is key for transforming fibrinogen into fibrin, an insoluble protein that forms mesh-like structures. Altogether, fibrin and platelets create a stable clot, which is known as the thrombus, sealing the damaged area (6,7,16).

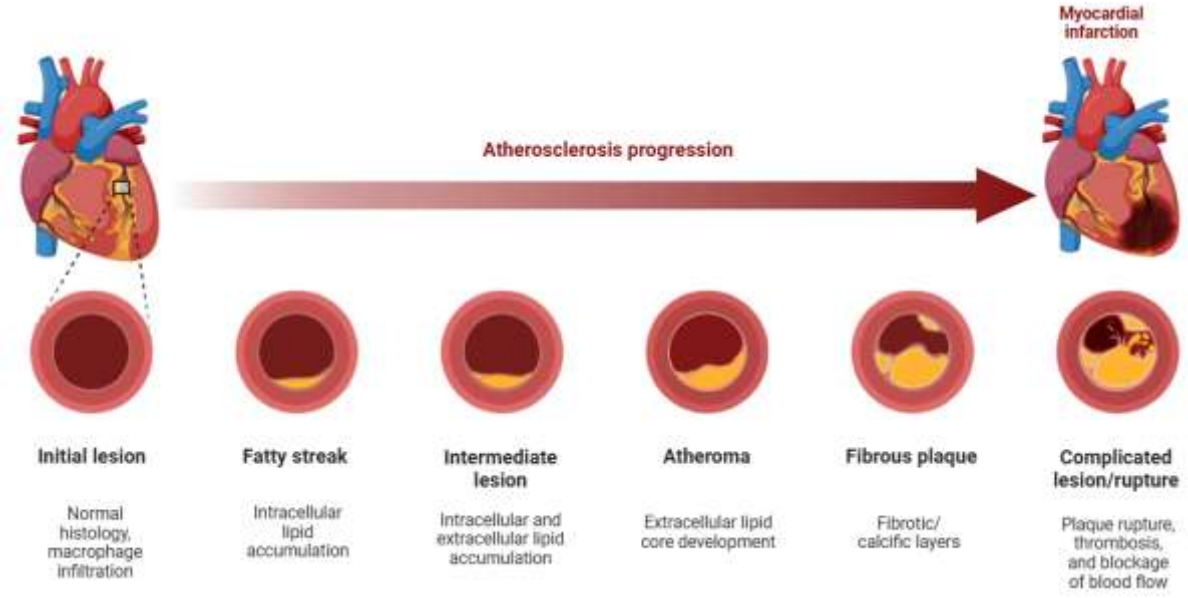

Figure 2. *Schematic illustration of atheroma plaque development, depicting the progression from a healthy artery to plaque rupture and thrombosis.*

As thrombus develops (*Figure 2*), it sets off a range of reactions that make the lesion less prone to rupture. However, due to the growing of the plaque, the risk of obstruction in the vessel increases, blood flow in coronary arteries is reduced, generating ischemic cardiopathies or acute coronary syndromes (ACS) such as cardiac insufficiency or angina, transient ischemia without myocardial injury, characterized by new or worsening chest pain without biomarker elevation. Additionally, if the obstruction in the vessel is complete or almost complete, it can develop into myocardial infarction (MI), whether it being NSTEMI or STEMI, based on electrocardiographic findings and levels of cardiac biomarkers such as troponins, or stroke (6,7,10). An MI is one of the most serious complications of atherosclerosis, being the leading cause of mortality and disability worldwide (6).

If the thrombus detaches from the arterial wall, it becomes a circulating clot known as embolus, which may eventually lodge in smaller, distal arteries where it can block blood flow. This obstruction can result in localized ischemia, impair organ function or potentially lead to infarction.

Importantly, plaque rupture is not the only mechanism that can cause ACS, plaque erosion – where endothelial cells are lost without cap rupture – can also start thrombosis, especially in younger patients, women and smokers. Unlike rupture, eroded plaques typically have less lipid core and less inflammation, but a disrupted endothelial lining allows thrombus formation. Nevertheless, plaque rupture remains the predominant cause, accounting for up to 75% of all ACS, whether some studies show that up to 25% ACS cases are caused by plaque erosion (10,17).

Additionally, prompt diagnosis relies on a combination of patient history, physical examination, ECG and serial measurements of cardiac biomarkers. Elevated high-sensitivity cardiac troponins are pivotal in identifying myocardial necrosis and differentiating between unstable angina and MI. Imaging modalities, such as coronary angiography, may be employed to confirm the presence and severity of occlusion. In high-risk patients, early invasive interventions like percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) are considered. Long-term therapy focuses on secondary prevention, including lipid-lowering agents, blood pressure control, smoking cessation, and lifestyle modifications to reduce the risk of future events.

To summarize, plaque rupture represents the critical transition from chronic atherosclerotic disease to acute thrombotic complications. The resulting coronary thrombosis underpins the pathogenesis of ACS and remains a major cause of morbidity and mortality worldwide (18–20).

3. Biochemical Basis of the Atherothrombotic Processes

3.1. Inflammatory processes involved

As previously mentioned, there are several inflammatory processes in atherosclerosis with many signaling pathways involved in this disease.

Firstly, there is endothelial dysfunction and activation where the initial step involves the activation of endothelial cells by oxLDL, disturbed shear stress and pro-inflammatory cytokines. A key signaling pathway is the **NF-κβ** cascade where TNF-α binds to TNFR1, recruiting adaptor proteins **TRADD** and **TRAF2**, which activate the **IKK complex** (IKKα, IKKβ and NEMO, also named, IKKγ). This complex phosphorylates the inhibitor protein Iκβα, which normally binds and retains NF-κβ in the cytoplasm, marking phosphorylated Iκβα for proteasomal degradation. Freed NF-κβ translocates to the nucleus and binds to promoters of pro-inflammatory genes, attracting immune cells. This process has downstream effects such as increased **VCAM-1**, **ICAM-1**, **E-selectin** and **chemokines** increased expression, thus promoting leukocyte adhesion (21,22).

Following endothelial activation, circulating monocytes adhere to the endothelium and transmigrate into the intima. This action is driven by the chemokine **CCL2** (also known as MCP-

1) binding to **CCR2** on monocytes. This receptor is coupled to Gi proteins that trigger **PI3K/Akt** and **PLCβ** pathways, which rearrange the cytoskeleton to facilitate chemotaxis. Integrins, such as **LFA-1 (αLβ2)**, are activated and bind to **ICAM-1** on the endothelial surface, stabilizing adhesion and enabling transendothelial transmigration (21,22).

Within the intima, monocytes differentiate into macrophages under the influence of **macrophage colony-stimulating factor (M-CSF)** and ingest modified LP like oxLDL through scavenger receptors including **SR-A**, **CD36** and **LOX-1** independently of LDLR regulation. This uptake is not subject to feedback regulation, meaning intracellular cholesterol levels do not affect this process, resulting in the formation of lipid-laden foam cells. The accumulation of cholesterol crystals or oxLDL can trigger lysosomal destabilization and activate the **NLRP3 inflammasome** through the release of cathepsins. The NLRP3 complex assembles with **ASC** (apoptosis-associated speck-like protein containing a CARD) and **procaspase-1**, leading to **caspase-1** activation and cleavage of **pro-IL-1β** and **pro-IL-18** into their mature forms. These potent pro-inflammatory cytokines are secreted, perpetuating inflammation (21,22).

This inflammatory environment is amplified by cytokine networks. **IL-1β** binds to its receptor **(IL-1R1)** on endothelial and immune cells, activating the **MyD88-IRAK-TRAF6** pathway, which further stimulates NF-κβ and reinforces pro-inflammatory gene expression. **IL-6** signals through its receptor complex **(IL-6Rα and gp130)**, activating **JAK kinases** and phosphorylating **STAT3**, which then dimerizes and translocates to the nucleus to induce transcription of acute-phase proteins like **CRP** and additional cytokines. **IFN-γ**, secreted by Th1 cells, binds to its receptor on macrophages and smooth muscle cells, activating **JAK1/JAK2** and **STAT1** to enhance antigen presentation by increasing MHC class II receptor and perpetuate inflammation (21,22).

VSMCs, as explained prior, also contribute to plaque formation. Under the influence of platelet-derived growth factor (**PDGF**), these cells migrate into the intima and transition from a contractile to a synthetic phenotype. PDGF binding to **PDGFRβ** activates **Ras/Raf/MEK/ERK** and **PI3K/Akt** pathways, promoting proliferation, migration and matrix metalloproteinase (MMP) production, thus enabling extracellular matrix remodeling. **TGF-β** further signals via **Smad2/3** transcription factors and also activates **MAPK** signaling **(p38, JNK, ERK)**, enhancing collagen synthesis and matrix production. MMPs (**MMP-2** and **MMP-9**) produced by VSMCs and macrophages degrade the extracellular matrix, thinning the fibrous cap and increasing the risk of rupture (21,22).

Under normal circumstances, resolution of inflammation through specialized mediators is expected, but in an atherosclerotic milieu often fails. **Specialized pro-resolving mediators (SPMs)** such as lipoxins, resolvins and protectins bind to **G-protein-coupled receptors** (like **ALX/FPR2**) on macrophages to suppress NF- κβ signaling, reduce neutrophil recruitment and promote **efferocytosis**, which is apoptotic cell clearance. When efferocytosis is defective, secondary necrosis ensues, releasing **damage-associated molecular patterns (DAMPs)** that further sustain inflammation (21,22).

Ultimately, chronic inflammation leads to plaque destabilization. Both **NF-κβ** and **AP-1** transcription factors **increase MMP gene expression** (including **MMP-1** and **MMP-9**), contributing to **fibrous cap degradation and plaque rupture**. This process underlies the thrombotic complications of atherosclerosis, such as myocardial infarction and stroke (21,22).

Understanding these detailed signaling pathways highlights multiple potential therapeutic targets, which will be illustrated in later sections. A thorough gasp of these pathways offers a framework for developing targeted interventions that could transform the management of atherosclerotic CV disease.

Table 1. *Summary of key inflammation pathways involved in atherosclerosis.*

Process	Key Pathways	Mechanistic insight
Endothelial activation	TNF-α → TNFR1 → TRADD/TRAF2 → IKK → NF- κβ	Induces adhesion molecules and chemokines
Monocyte recruitment	MCP-1/CCL2 → CCR2 → PI3K/Akt, PLCβ	Promotes monocyte migration
Foam cell formation	SR-A, CD36 → oxLDL uptake; NLRP3 → Caspase-1 → IL-1β	Drives chronic inflammation
Amplification	IL-1β → My88 → IRAK/TRAF6 → NF-κβ; IL-6 → JAK/STAT3; IFN-γ → JAK/STAT1	Sustains inflammation
SMCs activation	PDGFRβ → Ras/ERK; TGF-β → Smad2/3 + MAPKs	Drives migration, matrix remodeling
Resolution failure	SPMs → ALX/FPR2; defective efferocytosis → DAMPs	Fails to resolve inflammation
Plaque destabilization	NF-κβ/AP-1 → MMPs	Leads to plaque rupture

3.2. Platelet activation and coagulation

Platelet activation and coagulation are central to the pathogenesis of atherosclerosis and its thrombotic complications, including ACS. Under normal conditions, platelets circulate in an inactive state, maintaining vascular integrity without initiating clot formation. However, in the context of atherosclerotic plaque rupture or erosion, subendothelial matrix components such

as **collagen** or **von Willebrand factor (vWF)** become exposed, triggering a complex biochemical cascade that leads to platelet activation and aggregation, while tissue factor and damage activate the coagulation cascade simultaneously (17,23,24).

The process begins when circulating platelets encounter exposed collagen at the site of endothelial disruption. The initial adhesion is mediated primarily by the interaction between platelet **glycoprotein Ib-IX-V complex (GPIb-IX-V) receptor** and immobilized **vWF**, which acts as a bridge between subendothelial matrix and the platelet surface. This interaction slows the platelets, allowing for form adhesion through direct binding of collagen to **glycoprotein VI (GPVI)** and **integrin α2β1** (17,23,24).

GPVI-collagen engagement triggers a tyrosine kinase signaling cascade involving the **Src family kinases (Fyn and Lyn)**, which phosphorylate the **immunoreceptor tyrosine-based activation motif (ITAM)** in the **GPVI-associated Fc receptor γ-chain**. This process recruits and activates **Syk kinase**, leading to the phosphorylation of **phospholipase C gamma 2 (PLCγ2)**. PLCγ2 hydrolyzes **phosphatidylinositol 4,5-biphosphate (PIP2)** into **inositol triphosphate (IP3)** and **diacylglycerol (DAG)**, guiding to the mobilization of intracellular calcium and activation of **protein kinase C (PKC)**. These pathways converge to promote granule secretion, integrin activation (αIIbβ3) and cytoskeletal rearrangement (17,23,24).

In addition, activated platelets release secondary antagonists, notably **adenosine diphosphate (ADP)**, **thromboxane A2 (TXA2)** and serotonin, which amplify platelet activation through autocrine and paracrine mechanisms. **ADP** activates two G-protein receptors: **P2Y1** (coupled to Gq) and **P2Y12** (coupled to Gi). **P2Y1** triggers shape change and initial aggregation through **PLCβ** activation and **calcium** release, while **P2Y12** inhibits adenylyl cyclase, decreasing **cAMP** levels and reducing **PKA**'s inhibitory effects, thus promoting platelet activation. On the other hand, **TXA2** is produced through the **arachidonic acid** pathway. Arachidonic acid, released from membrane phospholipids by **cytosolic phospholipase A2 (cPLA2)**, is converted by **COX-1** into **prostaglandin H2 (PGH2)**, then by **thromboxane synthase** to TXA2. TXA2 activates the **Gq-coupled TP receptor**, which promotes calcium release and **PKC** activation sustaining platelet activation (17,23,24).

A pivotal step in platelet aggregation is the activation of the integrin **αIIbβ3 (GPIIb/IIIa)**. Convergence of GPVI, P2Y1/P2Y12 and TXA2 pathways induces a conformational change in αIIbβ3 via **Rap1** activation, which is activated by calcium and DAG, increasing its affinity for **fibrinogen** and vWF. Fibrinogen bridges between adjacent platelets, facilitating aggregation and thrombus growth.

Concurrently, exposure of **tissue factor (TF)** on macrophages, SMCs and activated endothelium at the site of plaque rupture initiates the extrinsic coagulation cascade. TF binds factor **VIIa**, forming a complex that activates factor **X** to **Xa**. **FXa**, together with **Va**, forms the prothrombinase complex, which converts prothrombin to thrombin. Thrombin generation accelerates exponentially in a positive feedback loop, and thrombin acts on platelets via **protease-activated receptors (PAR-1 and PAR-4)**. PAR-1 (Gq and G12/13-coupled), leading to

PLCβ activation, calcium mobilization, **PKC** and **Rho GTPase** activation. These signaling pathways reinforce platelet activation, clot retraction and aggregation (17,23,24).

Thrombin also cleaves soluble **fibrinogen** into insoluble **fibrin monomers**, which spontaneously polymerize into fibrin strands. Thrombin activates **FXIII** to **FXIIIa**, which cross-links these fibrin monomers, stabilizing the clot structure and trapping platelets and RBCs within the thrombus (17,23,24).

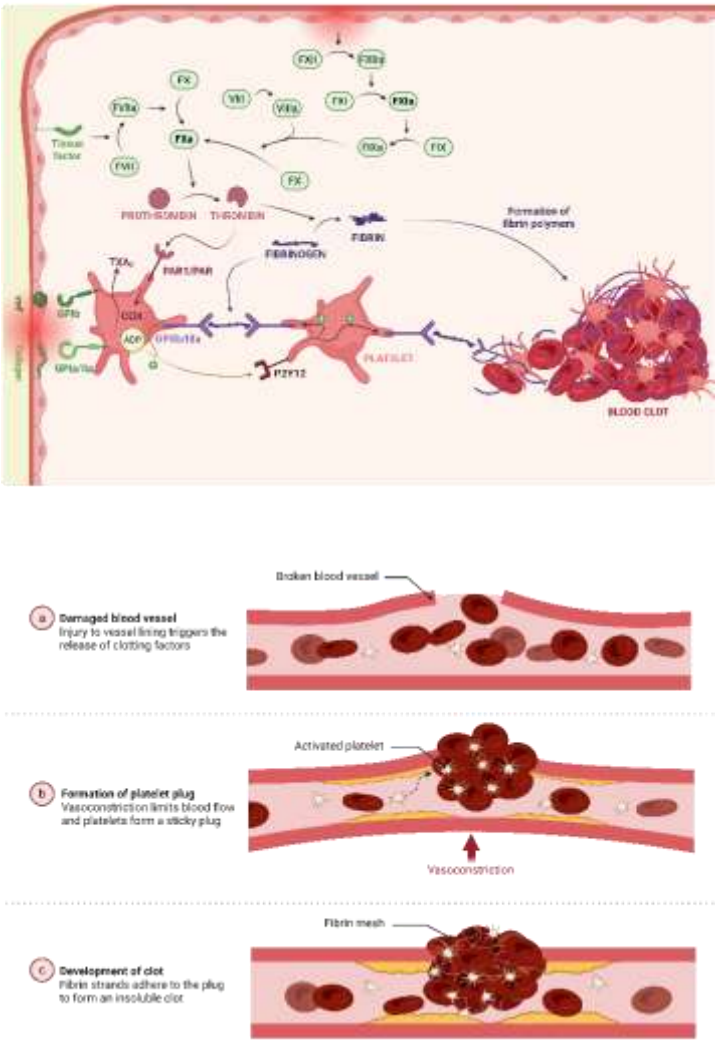

Figure 3. *Simplified platelet aggregation and coagulation processes in atherosclerosis. Adapted from G. Martinez Bravo et al. (2024).*

These biochemical mechanisms not only initiate the formation of a platelet-rich thrombus but also sustain its growth and stability, ultimately contributing to the acute clinical manifestation of ACS.

Table 2. *Summary of key platelet and coagulation-related pathways involved in atherosclerosis.*

Process	Key pathways	Mechanistic insight
Adhesion and activation	Vwf-GPIb-IX-V; GPVI-collagen → Src/Syk/PLCγ2	Initiates platelet activation via Ca^{2+} and PKC
Secretion and amplification	ADP → P2Y1/P2Y12 → PLCβ, ↓cAMP; TXA2 → TP → PLCβ	Amplifies platelet recruitment and granule release
Aggregation	αIIbβ3 → Rap1 → Talin/Kindlin	Mediates stable platelet-platelet interactions
Thrombin generation	TF-FVIIa → FX/Xa → Thrombin; PAR-1/PAR-4 → PLCβ/Rho	Drives thrombin burst and clot stability
Fibrin formation	Thrombin → Fibrinogen → Fibrin; FXIIIa	Forms a stable fibrin mesh

3.3. Lipid metabolism and dyslipidemia

Lipid metabolism encompasses complex biochemical pathways that regulate synthesis, transport and clearance of LP. The exogenous pathway begins with the absorption of dietary fats in the small intestine, where they are emulsified by bile acids and incorporated into **chylomicrons (CM)** alongside **apolipoprotein B-48**. These triglyceride-rich particles reach the bloodstream where **LPL**, activated by **apolipoprotein C-II**, hydrolyzes their triglycerides into free fatty acids for uptake by muscle and adipose tissue. CM remnants, enriched in cholesterol, are then cleared by the liver via the **LDL receptor-related protein-1 (LRP1)** (25).

In parallel, the endogenous pathway involves the hepatic synthesis of **VLDL**, which carries triglycerides and cholesterol to peripheral tissues. VLDL particles, containing **apolipoprotein B-100**, are hydrolyzed by **LPL** to form **intermediate-density lipoproteins (IDL)**. **Cholesteryl ester transfer protein (CETP)** mediates the exchange of triglycerides and cholesterol esters between VLDL, LDL and HDL, influencing LP remodeling and composition.

LDL particles, especially **small dense LDL (sdLDL)**, are particularly atherogenic. These particles infiltrate the subendothelial space of the arterial wall, where they are susceptible to oxidative modification. Unlike native LDL, **oxLDL** are not recognized by the classical LDLRs but instead bind to scavenger receptors such as **SR-A**, **CD36** and **LOX-1** on macrophages. This leads to the formation of **foam cells**, which are a hallmark of early atherosclerotic lesions. Unlike LDLR-mediated uptake, scavenger receptor pathways lack feedback inhibition by intracellular cholesterol, promoting continuous lipid accumulation.

HDL play a protective role through reverse cholesterol transport, wherein it mediates the efflux of excess cholesterol from macrophages back to the liver. This process is initiated by the interaction of **apolipoprotein A-I (apoA-I)** with **ATP-binding cassette transporter A1 (ABCA1)**. **Lecithin-cholesterol acyltransferase (LCAT)**, activated by apoA-I, esterifies free cholesterol within HDL, converting it into mature HDL. Mature HDL particles deliver cholesterol esters to the liver via **scavenger receptors class B type I (SR-BI)** or transfer them indirectly to apoB-containing lipoproteins through **CETP** (25).

In dyslipidemia, an imbalance between lipoprotein synthesis, clearance, and remodeling drives the progression of atherosclerosis. Elevated plasma triglycerides, increased VLDL production, and CETP-mediated remodeling generate triglyceride-rich LDL, which is subsequently hydrolyzed by **hepatic lipase** into sdLDL. These particles penetrate the arterial intima more easily and are more prone to oxidative modification, increasing their atherogenicity. Concurrently, HDL dysfunction is exacerbated by systemic inflammation. Cytokines such as **TNF-α** and **IL-1β** modify HDL composition by promoting the incorporation of **serum amyloid A (SAA)** at the expense of apoA-I, impairing HDL's capacity for cholesterol efflux and its antioxidant properties (25).

To conclude, these biochemical pathways promote the accumulation of atherogenic LP, formation of foam cells and the dysfunction of protective mechanisms like reverse cholesterol transport, thereby fueling the development and progression of atherosclerotic lesions in ACS.

Table 3. *Summary of key lipid pathways involved in atherosclerosis.*

Process	Key pathways	Mechanistic insight
LP assembly	MTP, ApoB, LPL	Facilitates dietary and hepatic lipid transport
LDL uptake	LDLR → SREBP-2; SR-A/CD36	LDLR: regulated uptake; Scavenger receptors: unregulated foam cells formation
HDL metabolism	ABCA1 → ApoA-I; LCAT; SR-BI	Promotes cholesterol efflux and reverse transport
sdLDL formation	CETP → Hepatic lipase	Generates highly atherogenic particles
HDL dysfunction	SAA, TNF-α, IL-6	Reduces reverse cholesterol transport

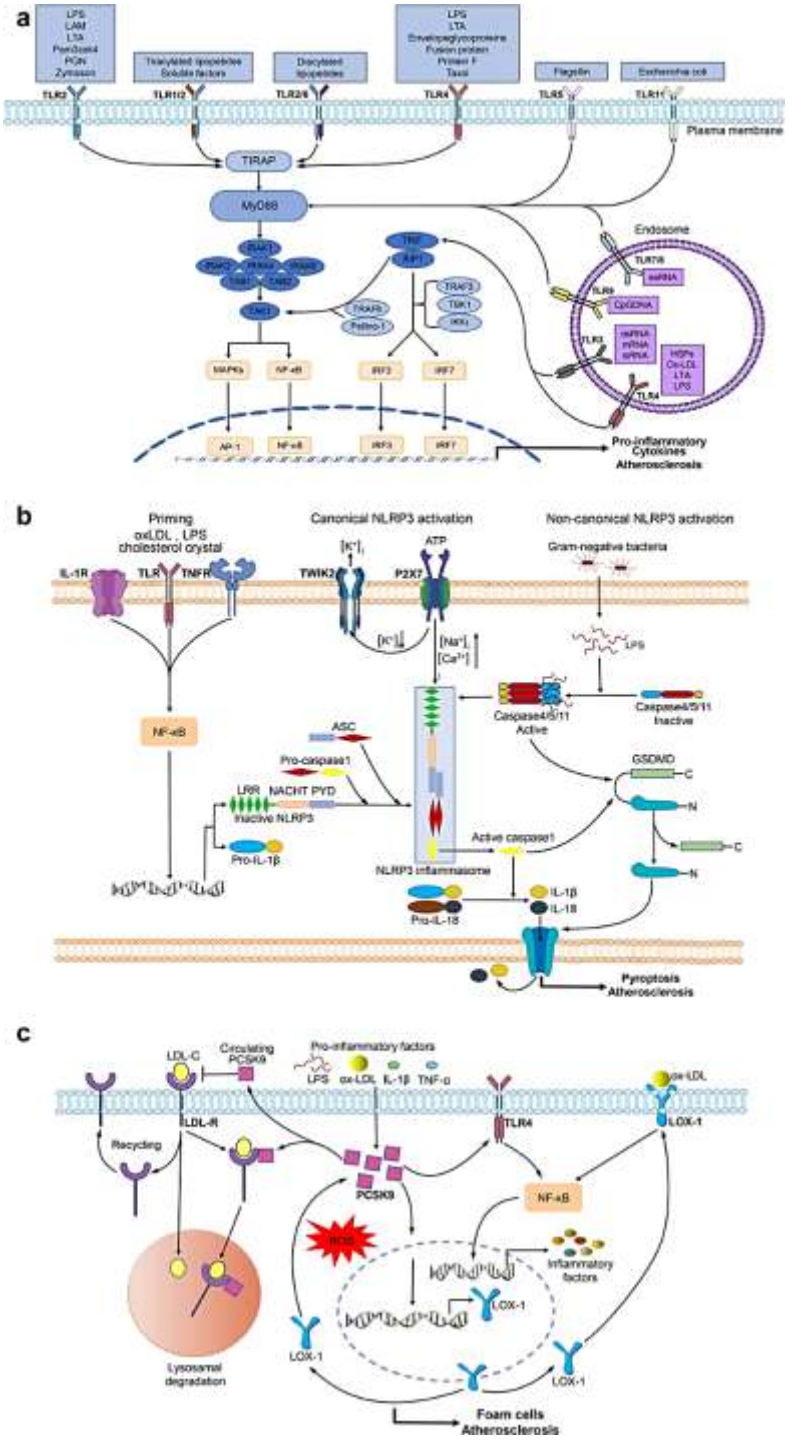

Figure 4. *Simplified summary of key inflammatory and cholesterol signaling pathways in atherosclerosis from P. Kong et al. (2022).*

4. Interaction Between Inflammation, Thrombosis and Cholesterol: An Integrated Perspective

Atherosclerosis is no longer considered a mere lipid storage disease but a complex interplay of lipid metabolism, inflammation and thrombosis. Emerging evidence highlights that

cholesterol accumulation, especially in the form of oxLDL, not only contributes to plaque formation but also amplifies inflammatory signaling pathways within the vascular wall. Concurrently, inflammation promotes pro-thrombotic states by upregulating tissue factor expression and impairing endogenous anticoagulant mechanisms. Thrombosis, in turn, exacerbates inflammation by releasing DAMPs that further activate immune pathways. This dynamic crosstalk among cholesterol metabolism, inflammatory processes, and coagulation cascades forms a vicious cycle that accelerates plaque progression and destabilization, ultimately culminating in thrombotic events. An integrated biochemical perspective on these interactions is critical for understanding the pathogenesis of atherosclerosis and for developing targeted therapies aimed at secondary prevention (7,23).

4.1. Inflammation-thrombosis axis

The interplay between inflammation and thrombosis forms a self-perpetuating loop central to the pathogenesis of ACS. Inflammatory stimuli, including cytokines such as IL-1β, TNF-α and IL-6, activate endothelial cells to express pro-coagulant molecules such as TF and P-selectin. Tissue factor, through its binding with FVIIa, initiates the extrinsic coagulation pathway, resulting in thrombin generation and fibrin deposition. Additionally, inflammatory cytokines upregulate the expression of adhesion molecules (ICAM-1, VCAM-1) on endothelial cells and platelets, facilitating leukocyte-platelet interactions that promote thrombus stability. Thrombin itself is not merely a coagulation factor but also a potent inflammatory mediator that activates PARs on endothelial cells, platelets and leukocytes, amplifying NF-κβ and MAPKs pathways and further enhancing cytokine release.

Moreover, neutrophil extracellular traps (NETs), formed in response to inflammatory stimuli, consist of decondensed chromatin and granule proteins that provide a scaffold for platelet adhesion and fibrin deposition, linking innate immunity with thrombogenesis. NETs also bind and activate FXII, contributing to the contact activation pathway of coagulation, thus, not only promoting thrombosis, but sustaining local inflammation as well by activating the NLRP3 inflammasome in macrophages, leading to additional IL-1β and IL-18 secretion (7,23).

This bidirectional axis ensures that once initiated, inflammation and thrombosis sustain each other, creating a vicious cycle of cytokine release, coagulation activation, and immune cell recruitment. As a result, plaque disruption and the risk of occlusive thrombotic events are exacerbated, representing a key pathophysiological mechanism underlying ACS (7,23).

4.2. Dyslipidemia as an inflammatory modulator and prothrombotic regulator

Dyslipidemia, particularly elevated levels of LDL-C and its oxidized derivates (oxLDL), acts as a potent modulator of vascular inflammation, reinforcing the progression of atherosclerosis. LDL particles infiltrate the subendothelial space and undergo oxidative modifications that

render them immunogenic, stimulating pattern recognition receptors (e.g., TLR4, LOX-1) on macrophages and endothelial cells. This triggers the activation of NF-κβ and other pro-inflammatory transcription factors, leading to the secretion of cytokines (IL-1β, IL-6) and chemokines (MCP-1/CCL2) that recruit monocytes and amplify local inflammation. Additionally, oxLDL uptake by macrophage scavenger receptors (CD36, SR-A) initiates foam cell formation and NLRP3 inflammasome activation, releasing IL-1β and promoting further inflammatory signaling. High circulating LDL-C levels also impair endothelial function by reducing nitric oxide bioavailability and enhancing the expression of adhesion molecules, thereby facilitating leukocyte adhesion and platelet activation. Conversely, HDL exert anti-inflammatory effects by promoting cholesterol efflux via ABCA1 and ABCG1 transporters and by neutralizing oxLDL through paraoxonase-1 (PON1) activity. Moreover, oxLDL and pro-inflammatory mediators downregulate thrombomodulin expression on endothelial cells, diminishing protein C activation and reducing the anticoagulant capacity of the endothelium. As a consequence, the regulatory balance tilts toward a prothrombotic state, further predisposing to occlusive thrombus formation following plaque rupture.

Thus, dyslipidemia establishes a pro-inflammatory vascular environment that synergizes with the coagulation system, accelerating plaque progression and destabilization (18,25).

5. Secondary Prevention in Acute Coronary Syndrome

5.1. Definition of secondary prevention and objectives

Secondary prevention following an ACS represents a backbone in the management of patients at high risk of recurrent atherothrombotic events. From a medical standpoint, it should begin as soon as possible after the index event. A comprehensive approach to secondary prevention integrates risk factors control, the promotion of a healthy lifestyle, and pharmacological treatment. It includes lifestyles modifications such as cardiac rehabilitation, dietary and exercise counseling, and smoking cessation, in addition to intensive pharmacological therapy as seen in *Figure 5*.

Figure 5. *Key factors to consider for secondary prevention after an ACS. Adapted from R. Byrne et al. (2023).*

The main goal of secondary prevention is to increase quality of life and reduce morbidity and mortality in post-ACS patients, which is crucial because these individuals continue to have an extremely high risk of additional atherothrombotic events and mortality, even with optimal therapy. Reducing ischemic events is a key benefit. Long-term treatment targets within secondary prevention include achieving specific objectives for blood pressure (systolic < 130 mmHg and diastolic < 80 mmHg), LDL-C (< 1.4 mmol/L), and HbA1c in diabetic patients (< 53 mmol/mol [< 7%]) (5,6,20).

5.2. Biomarkers used in risk stratification

According to current guidelines and supporting literature, biomarkers play a pivotal role in both diagnosis and risk stratification of patients with suspected ACS.

In the context of NSTEM-ACS, which encompasses unstable angina and NSTEMI, the **cardiac troponins (cTn)** represent the reference biomarker. More specifically, high-sensitivity cardiac troponin (hs-cTn) analyses are preferred over earlier markers such as CK-MB due to their superior diagnostic characteristics and their capacity to detect myocardial injury at very low concentrations, even in apparently healthy individuals.

For the diagnosis of MI, according to the 4th Universal Definition of Myocardial Infarction (UDMI), an increase or decrease of **cTn** levels (or other biomarkers in the absence of cTn), or both, in conjunction with clinical evidence of myocardial ischemia, such as characteristic symptoms, ECG changes, or imaging evidence of new regional wall motion abnormality or intracoronary thrombus, is required. Importantly, with hs-cTn assays, cTn levels usually rise rapidly, typically within an hour from symptom onset, enabling immediate diagnosis (20,26).

The development of hs-cTn assays has facilitated the implementation of "**rule-out**" algorithms, such as the European Society of Cardiology (ESC) 0h/1h and 0h/2h. These protocols rely on measuring hs-cTn at presentation (0h) and at a subsequent timepoint (1-3 hours later), incorporating both the absolute concentrations and the magnitude of change:

- Patients with very low baseline hs-cTn values or low baseline values with no significant change ate 1h/2h are assigned to the "rule-out" route.
- Patients with high baseline hs-cTn values or significant changes at 1h/2h are assigned to the "rule-in" route (confirm quickly).
- Patients who do not meet either criteria enter the "observe" category, which requires further investigation.

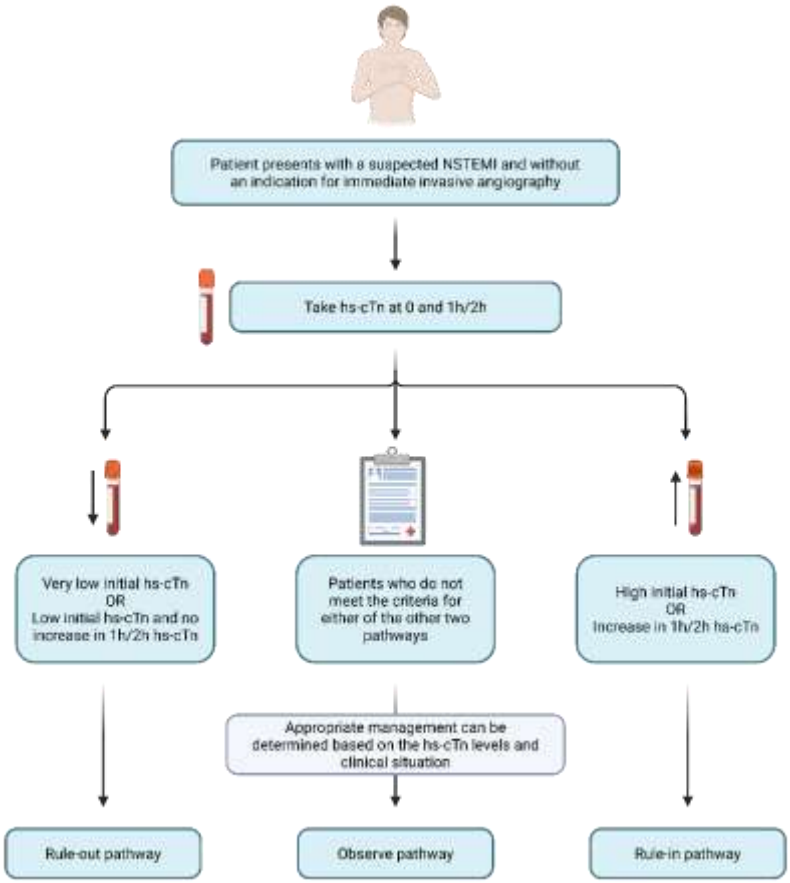

Figure 6. *Visual representation of the "rule-out" algorithm. Adapted from R. Byrne et al. (2023).*

It is essential to note that elevated cTn can occur in alternative clinical conditions apart from MI type 1 (caused by atherothrombosis), such as myocarditis, sepsis, Takotsubo cardiomyopathy, heart valve disease, arrythmias, and heart failure. Myocardial injury (release of troponin due to non-ischemic mechanisms) represents a distinct clinical entity that must be differentiated from MI. Hence, differential diagnosis is a critical step in clinical interpretation (20,26).

As for other biomarkers, their use in ACS diagnosis is not recommended unless cTn assays are unavailable. Biomarkers such as **CK-MB**, **cardiac myosin-binding protein C**, and **copeptin** may have clinical relevance when used in combination with cTn but offer minimal additional diagnostic value in most clinical scenarios.

Beyond diagnosis, biomarkers are also valuable for risk stratification. Initial cTn levels provide prognostic information that refines risk assessment for both short- and long-term mortality, complementing clinical variables and ECG findings. Higher hs-cTn concentrations are associated with increased risk of death. Serial measures of cTn aid in identifying peak levels, which inform risk stratification in patients with confirmed MI.

Other biomarkers, including **natriuretic peptides (BNP and NT-pro BNP)**, add prognostic insights regarding mortality, acute heart failure, and the development of atrial fibrillation,

supplementing the information provided by cTn. Serum creatinine levels and estimated glomerular filtration rate (eGFR) should also be measured in all patients with ACS, as they influence prognosis and are integral components of risk models such as the **Global Registry of Acute Coronary Events score** (20,26).

The GRACE score is a validated risk stratification tool that estimates the risk of adverse outcomes in patients with ACS, including NSTE-ACS and STEMI. It combines key clinical and biochemical variables, such as age, heart rate, systolic blood pressure, serum creatinine, cardiac arrest on admission, ST-segment changes on the ECG, and elevated cTn levels, to calculate both in-hospital and long-term mortality risk. Its continuous scoring system allows for tailored therapeutic decision-making, including urgency of invasive management and the intensity of medical therapy. As a result, the GRACE score is considered a backbone of evidence-based risk assessment in ACS management (27).

5.3. Currently available therapies

The cornerstone therapies actually available that aim to reduce the risk of recurrent CV events include antithrombotic therapy, generally **dual antiplatelet therapy (DAPT)**, which is usually **Aspirin** plus a **P2Y12 inhibitor** such as Ticagrelor or Prasugrel for at least 12 months after index event, which significantly lowers the risk of stent thrombosis and recurrent ischemic events compared to Clopidogrel in most patients. **High-intensity statins**, such as Atorvastatin (40-80 mg daily) or Rosuvastatin (20-40 mg daily), are recommended to achieve aggressive LDL-C lowering, often with the possible addition of **ezetimibe** (which block intestinal cholesterol absorption) or **PCSK9 inhibitors** (e.g., evolocumab, alirocumab) for patients who do not reach desirable LDL-C targets (< 1.4 mmol/L) (5,20,28).

Beta-blockers (e.g., metoprolol, carvedilol, bisoprolol) to lower myocardial oxygen demand and prevent arrythmias, as well as control heart rate, especially in the setting of reduced left ventricular function. **Renin-angiotensin-aldosterone system (RAAS) inhibitors**, including **ACE inhibitors** (e.g., ramipril, perindopril) or **angiotensin receptor blockers (ARBs)** (e.g., valsartan, losartan), are indicated to reduce adverse ventricular remodeling, reduce afterload, and lower mortality. In patients with heart failure or left ventricular dysfunction (LVEF ≤ 40%), **mineralocorticoid receptor antagonists (MRAs)** may be added such as eplerenone or spironolactone, which provide additional mortality benefit by antagonizing the pro-fibrotic and pro-arrhythmic effects aldosterone (5,20,28).

Lifestyle modifications, as aforementioned, complement pharmacologic therapy. This integrated approach addresses multiple risk factors simultaneously, aiming to lower long-term CV risk, improve survival chances, and enhance overall quality of life in patients with ACS.

6. Hypothesis and Objectives of the Study

6.1. Hypothesis

The interplay between inflammation, thrombosis, and lipid metabolism plays a central role in the development and progression of the atherosclerotic plaque and acute coronary syndrome (ACS). Targeting these interconnected pathways through personalized pharmacological interventions can improve effectiveness of secondary prevention strategies after an ACS event. A systematic review of Phase III interventional clinical trials will reveal emerging strategies with specific mechanisms, reflecting a shift toward personalized and integrative therapies in CVD addressing said biochemical pathways following an event of such characteristics.

6.2. Objectives

This study aims to provide an integrative overview of the pathophysiological mechanisms linking inflammation, thrombosis and lipid metabolism in the development and clinical progression of atherosclerosis and ACS, with particular emphasis on secondary prevention strategies. To support this, the study also involves a systematic search and analysis of ongoing Phase III interventional clinical trials that target the three processes previously named – inflammations, thrombosis and lipid metabolism – in the context of secondary prevention following an ACS event.

To achieve this, the study fist explores the current evidence on the molecular and cellular mechanisms involved in plaque formation, destabilization and rupture in atherosclerosis, analyzing how chronic inflammation, pro-thrombotic states and lipid dysregulation contribute to the clinical manifestations of ACS – how they influence plaque rupture, thrombosis formation and downstream ischemic complications. Building on this understanding, the project then seeks to identify and evaluate Phase III interventional clinical trials registered in major databases such as ClinicalTrials.gov and PubMed that investigate therapies targeting these key pathways in the setting of secondary prevention after ACS. By evaluating the design and pharmacological targets of these trials, this work aims to assess the current landscape of secondary prevention and provide insight into future directions for personalized, mechanism-based therapeutic strategies based on current clinical evidence and ongoing research in cardiovascular diseases.

7. Bibliographic Search Methodology

A comprehensive literature search was conducted for this research using tools including PubMed, ClinicalTrials.gov, the European Union Clinical Trials Register and SciSpace databases to gather peer-reviewed articles, clinical trial data and relevant reviews.

For PubMed – which is a publicly accessible biomedical literature database maintained by the U.S. National Library of Medicine (NLM) at the National Institutes of Health (NIH), offering

access to citations, abstracts and links to full-text articles across medicine, biology and healthcare fields – combinations of Medical Subject Headings (MeSH) terms and free-text keywords such as “acute coronary syndrome”, “secondary prevention”, “acute myocardial infarction” and “atherosclerosis” were used, alone and in combination. Boolean operators (“AND”, “OR”) helped refine the search.

In each search, the results were filtered by time, from 2018 to present, and by article type, it being Clinical Trial, Phase III or Clinical Trial, having found a total of 7 articles. Those whose title and/or abstract did not seem fit to the project’s objective were excluded from the search. Finally, 4 articles were appropriate for this research project, but only 3 could be used due to a restriction access in the remaining article.

On the other hand, ClinicalTrials.gov was consulted to identify ongoing and completed clinical trials focusing on interventions targeting inflammation, thrombosis, and lipid metabolism in acute coronary syndromes. This database is a public registry of privately and publicly funded clinical studies conducted worldwide, maintained by the U.S. NLM at the NIH. It provides detailed information about clinical trials, including study design, interventions, eligibility criteria, locations, and results, thus promoting transparency and accessibility in clinical research.

For the condition requirement, keywords such as "acute coronary syndrome," "acute myocardial infarction," and "atherosclerosis" were employed as keywords in separate queries. Simultaneously, words including “inflammation”, “thrombosis” and “lipid” were also added as to refine the search in each query. Studies were filtered to include only those that were ongoing (either recruiting or not yet recruiting) and classified as interventional Phase III trials. A total of 60 results in all searches altogether were obtained and examined; only 11 of which were used for this project as they mentioned topics of relevance and were studying interventions for secondary prevention.

To search for more specific interventions which could not be found with the previous databases, the European Union (EU) Clinical Trials Register was used. It is a publicly accessible online register that provides information on interventional clinical trials involving medicines conducted in the EU and European Economic Area (EEA). It also includes certain trials conducted outside the EU/EEA, particularly those related to pediatric medicine development. This database offers details on trial protocols, objectives, design, participant criteria and outcomes and, when available – for those that are completed – it includes results.

In order to identify clinical trials relevant to this research, comprehensive searches were conducted using specific keywords such as “acute myocardial infarction” in combination with the name of the intervention under investigation, particularly those not previously reviewed. All retrieved trials from each individual query were carefully screened and reviewed one by one, evaluating the trial’s objective, design, eligibility criteria and intervention type to determine its relevance. Only those studies that aligned with the aims of the current research

– specifically those assessing secondary prevention interventions after an ACS – were selected for further analysis, while those not meeting these criteria were excluded.

Lastly, SciSpace, an AI-powered research platform designed to ease literature discovery, summarization and citation management, was employed to complement and validate the search strategy. This tool was used to verify the relevance of previously selected articles and to identify additional studies aligned with the search objectives. A specific query was formulated to retrieve studies related to ongoing Phase III interventional clinical trials focused on secondary prevention after an ACS and atherosclerosis, targeting inflammation, lipid-lowering therapies and antiplatelet and antithrombotic agents. The search used the following keywords: “secondary prevention”, “ACS”, “atherosclerosis”, “inflammation”, “lipid-lowering drugs” and “antiplatelet/antithrombotic agents”.

The platform submitted 311 relevant results, which were systematically reviewed. SciSpace AI’s capabilities provided concise and accessible summaries of the top 20 most relevant papers offering interpretations of each study’s design, outcomes and relevance to the topic, which facilitated efficient screening and literature management.

8. Emerging Pharmacological Strategies in Secondary Prevention (Ongoing Studies)

To contextualize current therapeutic strategies in secondary prevention following ACS, this section offers a focused overview of selected Phase III interventional clinical trials identified through systematic searches. This work will be focused on discussing the most relevant aspects of each trial for understanding their biochemical context and approach, particularly those related to inclusion and exclusion criteria, the drug they are working with and the target in which they focus, which are highlighted to illustrate their alignment with the objectives of this thesis. This approach allows for a broader understanding of the ongoing clinical efforts without deviating from the central themes of the search.

8.1. Anti-inflammatory Agents

As previously mentioned, chronic inflammation plays a pivotal role in the development and progression of atherosclerosis. Recent trials have demonstrated that targeting inflammatory pathways can significantly reduce cardiovascular events in high-risk patients or patients who have suffered an ACS event before.

CANTOS Trial (Canakinumab)

The **Canakinumab** Anti-inflammatory Thrombosis Outcome Study (CANTOS) investigated the use of Canakinumab, a monoclonal antibody targeting interleukin-1β (IL-1β).

IL-1β is a pro-inflammatory cytokine produced by activated macrophages in response to stimuli and is a major component of the NLRP3 inflammasome pathway, which promotes vascular inflammation in atherosclerosis. This molecule also stimulates IL-6 production, which, in turn, drives CRP and other acute-phase proteins. Canakinumab binds selectively and with high affinity to IL-1β, neutralizing its bioactivity, and prevents it from binding to the IL-1 receptor on endothelial cells, VSMC and immune cells, blocking the signaling pathway and halting the downstream activation of NF-κB and MAPK pathways. Additionally, Canakinumab was found to not necessarily impair host immune function as it did not impact signaling via IL-1α.

The trial showed a 15% reduction in major adverse cardiovascular events (MACE) compared to placebo, reducing hsCRP independent of lipid-lowering effects. Subjects in the pooled Canakinumab group had more neutropenia with significantly more deaths due to infection or sepsis, as well as increased thrombocytopenia, but with no significant increase in hemorrhage compared with the placebo group.

Although the CANTOS trial is not ongoing as the other trials included in this work, it remains highly relevant as a landmark study that first provided clinical evidence supporting the inflammatory hypothesis of atherosclerosis. Its findings demonstrated that targeting inflammation can reduce the risk of recurrent cardiovascular events and laid the groundwork for subsequent and ongoing trials investigating anti-inflammatory strategies in secondary prevention after ACS. Therefore, it was deemed appropriate to reference it.

To better understand the trial's applicability to the current analysis, the following outlines the key inclusion and exclusion criteria used in CANTOS.

Inclusion criteria

- Age ≥ 18 years.
- Written informed consent
- Male or Female of non-child-bearing potential.
- Spontaneous MI at least 30 days before randomization.
- hsCRP ≥ 2 mg/L.

Exclusion criteria

- Pregnant or nursing (lactating) women.
- Women of child-bearing potential.
- Any of the following concomitant diseases.
- Planned coronary revascularization (PCI or CABG).
- Major non-cardiac surgical or endoscopic procedure within past 6 months.
- Multi-vessel CABG surgery within the past 3 years.
- Symptomatic patients with Class IV heart failure (HF) (New York Heart Association [NYHA]).

- Uncontrolled hypertension.
- Uncontrolled diabetes.
- History or evidence of active
- tuberculosis (TB) infection.

With these criteria in mind, the CANTOS trial serves as a foundational reference for evaluating subsequent interventions (22,29).

Ziltivekimab (ARTEMIS)

The ARTEMIS trial studies the Effects of **Ziltivekimab** Versus Placebo on Cardiovascular Outcomes in Patients With AMI, which translates to the usage of a human IgG1 monoclonal antibody (Ziltivekimab) designed so that it specifically targets interleukin-6 (IL-6).

This molecule is a pivotal pro-inflammatory cytokine implicated in the development and progression of atherosclerosis and cardiovascular disease, it stimulates the hepatic production of acute-phase proteins like CRP, fibrinogen and serum amyloid A, all of which are linked to CV risk. By binding soluble IL-6, it prevents its interaction with both membrane-bound and soluble IL-6 receptors. This altogether inhibits downstream JAK/STAT signaling, particularly the STAT3 pathway which is responsible for transcription of many inflammatory genes. Therefore, Ziltivekimab reduces hepatic CRP production, fibrinogen and serum amyloid A, among others. It also interferes with multiple pro-atherogenic inflammatory pathways, particularly those mediated by cells of the myeloid lineage implicated in the progression of atherosclerosis and CV events (30,31).

Research on this antibody is being led by several prominent institutions and pharmaceutical companies, most notably by Novo Nordisk, which has been at the forefront of its development.

It is a Phase III, randomized, double-blind, placebo-controlled clinical trial that focuses on patients who have recently been hospitalized for an AMI, including both STEMI and NSTEMI presentations. Participants also exhibit hsCRP levels and may have additional risk factors such as chronic kidney disease (CKD), diabetes mellitus or a history of atherosclerotic cardiovascular disease. The primary aim is to determine whether inhibiting IL-6-mediated inflammation can reduce MACE in this population as a secondary prevention strategy after atherosclerosis-related events like MI. The drug is administered subcutaneously through an injection into a flat skin surface once every month.

The following inclusion and exclusion criteria were applied in the ARTEMIS trial to ensure the selection of a well-defined population.

Inclusion criteria

- Age ≥ 18 years at the time of signing the informed consent.

- Hospitalization of AMI with evidence by invasive angiography performed at site with PCI capabilities.
- STEMI with: relevant onset of symptoms suggestive of cardiac ischemia within 12H before hospitalization and electrocardiogram (ECG)-change: ST-segment elevation at the J point in at least two contiguous leads greater than or equal 0.25 (millivolt) mV in men less than 40 years, greater than or equal 0.2 mV in men greater than or equal 40 years, or greater than or equal 0.15 mV in women in leads V2-V3; and/or greater than or equal 0.1 mV in all other leads.

OR

- NSTEMI with: relevant onset of symptoms suggestive of cardiac ischemia within 24 hours before hospitalization and rise and/or fall in cardiac troponin I or T with at least one value above the 99th percentile upper reference limit.
- Possibility for both randomization and administration of the loading dose of study intervention as early as possible after invasive procedure, and latest within 36 hours of hospitalization (time 0) for STEMI, and latest within 72 hours of hospitalization (time 0) for NSTEMI.
- Presence of at least one of the following criteria confirmed based on the participant's medical records and/or medical history interview:
    - Any prior MI.
    - Prior coronary revascularization.
    - Diabetes mellitus treated with ongoing glucose-lowering agent(s).
    - Known CKD (estimated glomerular filtration rate (eGFR) greater than or equal to 15 and less than 60 mL/min/1.73 m^2).
    - Prior ischemic stroke.
    - Known carotid disease or peripheral artery disease in the lower extremities.
    - Multivessel coronary artery disease (current/prior).
    - For STEMI patients only: anterior MI at index AMI.

Exclusion criteria

- Use of fibrinolytic therapy for treatment of the current AMI.
- Chronic heart failure classified as being in NYHA Class IV.
- Ongoing hemodynamic instability defined as any of the following:
    - Killip Class III or IV.
    - Sustained and/or symptomatic hypotension (systolic blood pressure less than 90 millimeters of mercury (mmHg)).
- Severe kidney impairment defined as any of the following:
    - eGFR less than 15 milliliter per minute per 1.73 m^2.
    - Chronic haemodialysis or peritoneal dialysis.
- Known alanine aminotransferase (ALT) greater than 8 x upper limit of normal (reference range) (ULN).

- Severe hepatic disease defined as at least one of the following:
    - Previously known or current hepatic encephalopathy (clinical evaluation).
    - Previously known or current ascites (clinical evaluation).
    - Jaundice (clinical evaluation).
    - Previous esophageal/gastric variceal bleeding.
    - Known hepatic cirrhosis.
- Major cardiac surgical (including but not restricted to coronary artery bypass graft surgery (CABG)), non-cardiac surgical, or major endoscopic procedure (thoracoscopic or laparoscopic) within the past 60 days or any major surgical procedure planned at the time of randomization or as treatment for the current AMI (CABG). Deferred (staged)percutaneous coronary intervention for a non-culprit vessel identified during the current AMI is allowed.
- Clinical evidence of, or suspicion of, active infection at the discretion of the investigator.
- Known (acute or chronic) hepatitis B or hepatitis C.
- History or evidence of untreated latent tuberculosis (TB) such as (but not limited to):
    - History of a positive TB test or chest X-ray compatible with latent TB; and TB treatment initiated less than 28 days prior to randomization.
    - Participants with TB risk factors but unwilling to undergo TB treatment if confirmed positive for latent TB based on central laboratory test at baseline (V2).

Once initiated, the study is expected to last approximately two years. During an initial recruitment period, eligible patients are to be enrolled. After the enrollment phase concludes, the remaining duration focuses on patient follow-up, data collections and analysis to fulfill the study objectives. Patient follow-up consists of presential and telephonic visits in the span of two years and in an interval of 3 months between visits.

Colchicine (CADENCE)

The CADENCE trial focuses on evaluating the effect of oral **colchicine** versus placebo on arterial inflammation in patients with diabetes who have currently experienced a cardiovascular event, such as an ACS, MI or a stroke/transient ischemic attack (TIA).

Colchicine is an anti-inflammatory drug that has shown potential to reduce cardiovascular events through microtubule disruption forming complexes with tubulin and disturbs the cytoskeletal structure necessary for the motility and function of leukocytes; it inhibits inflammatory cell activation and migration of neutrophils and monocytes at sites of vascular inflammation, reducing the inflammatory response within atherosclerotic plaques; and it suppresses the activation and assembly of the NLRP3 inflammasome, reducing the levels of downstream inflammatory mediators such as IL-6, hsCRP and IL-1β (32).

CADENCE is a Phase III, multicenter, randomized, double-blind, placebo-controlled trial. Eligible participants are randomized to receive either oral colchicine 0.6 mg daily or placebo for 6 months. The study's primary objective is to determine colchicine's effect on arterial inflammations, assessed by 18F-fluorodeoxyglucose (FDG) PET-CT imaging of the carotid arteries and thoracic aorta, as well as changes in inflammatory biomarkers such as hsCRP and IL-6. Secondary and exploratory objectives include identifying biomarkers or imaging features that predict response to therapy and evaluating the relationship between imaging, biomarkers and clinical outcomes.

Following this information, inclusion and exclusion criteria are listed to know how the CADENCE trail selected appropriate participants, helping define the study population, maintain participant safety and ensure accuracy and validity of the research findings.

Inclusion criteria

- Age ≥ 18 years.
- Type 2 Diabetes or pre-diabetes.
- Suffered a recent cardiovascular event (STEMI or NSTEMI) or TIA/stroke with associated large vessel atherosclerotic disease confirmed.
- Stable symptoms and hemodynamics.
- Given informed consent

Exclusion criteria

- Planned revascularization more than 120 days after index event.
- Recent CV event likely to have been embolic or secondary to MI with non-obstructive coronary arteries.
- Severe LV dysfunction (EF<30%)
- Severe valve disease requiring intervention.
- Decompensated heart failure
- Active infection
- Chronic diarrhea.
- Immune compromise
- History of cancer within the last 3 years.
- Active inflammatory conditions.
- Pregnancy and breastfeeding.
- Women of childbearing potential who refuse to use two forms of contraception throughout the study OR men capable of fathering a child who refuse to use contraception.
- Glomerular filtration rate (GFR) <50 ml/min/1.72m^2.
- Use of potent p-glycoprotein inhibitors or a strong CYP3A4 inhibitor.
- Hemoglobin < 105 (women), < 110 (men) g/L; WBC < 3.0$x10^9$/L, platelet count < 110 $x10^9$/L.

- History of cirrhosis, chronic active hepatitis or severe hepatic disease or with ALT levels greater than 3 times the upper limit of normal.
- Unable to give informed consent.
- TIA/stroke patients with atrial fibrillation.

With this eligibility criteria in mind, patients are to be recruited to one of two arms: colchicine or placebo. At baseline and 6 months patients are to have FDG PET-CT of carotids and aorta; at 0, 3 and 6 months a clinical evaluation and blood sampling for inflammation biomarkers. Taking this into account, each patient is to be participating in the study for about 6 months in total.

8.2. Antithrombotic and Antiplatelet Agents

As previously outlined, thrombotic processes – driven by platelet activation and coagulation – play a pivotal role in the pathogenesis of ACS and recurrent ischemic events. Over recent decades, numerous clinical trials have demonstrated that targeting these pathways with antithrombotic and antiplatelet agents can significantly reduce the risk of subsequent CV events in patients who have experienced an ACS. Building upon these advances, recent and ongoing clinical trials are now exploring novel strategies and therapeutic agents aimed at enhancing secondary prevention through more targeted and personalized modulation of thrombosis-related pathways.

Aspirin/Clopidogrel (ADEN)

The ADEN trial investigates the impact of genotype-guided de-escalation of antiplatelet therapy in patients at high bleeding risk following an ACS. Standard care for ACS typically involves long-term dual antiplatelet therapy (DAPT) with **Aspirin** (AAS) and a P2Y12 inhibitor to reduce risk of stent thrombosis and recurrent ischemic events. However, prolonged use of the high-potency P2Y12 inhibitors such as Ticagrelor or Prasugrel is associated with a substantial increase in bleeding risk, which carries significant prognostic implications, especially in high bleeding risk patients.

On the other hand, the benefits of these potent P2Y12 inhibitors over **Clopidogrel** or AAS (low potency antiplatelet) mostly occur in patients with a polymorphism in the enzyme CYP2Y19, which metabolizes Clopidogrel to its active form.

Recent data has shown that short-term DAPT followed by early transition to single antiplatelet therapy (SAPT) can maintain efficacy in preventing ischemic events while reducing bleeding. Therefore, this trial aims to determine whether a systematic and rapid genetic screening for CYP2Y19*2 or *17 polymorphisms can guide the selection of early SAPT with low-potency agents (AAS or Clopidogrel), potentially reducing bleeding events without compromising

protection against recurrent cardiac events, compared to ongoing high-potency antiplatelet therapy in high bleeding risk patients post-ACS.

Aspirin acts irreversibly inhibiting the enzyme COX-1, which is required to convert arachidonic acid into prostaglandin H2 (PGH2), a precursor to thromboxane A2 (TXA2) in platelets. TXA2 is a potent vasoconstrictor and promoter of platelet aggregation thus, by blocking this molecule synthesis, AAS reduces platelet activation and aggregation, producing an antithrombotic effect. As platelets lack a nucleus, they cannot synthesize new COX-1 once it's inhibited, therefore, a single low dose of Aspirin can have a lasting antiplatelet effect for an entire lifespan of the platelet (33).

The ADEN trial is a Phase III multicenter, randomized, open-label study using a PROBE (Prospective Randomized Open, Blinded Endpoint) design. Patients are randomized 1 to 3 months after an ACS event (preferably at 1 month as they are considered high bleeding risk patients) into two parallel treatment arms, stratified by revascularization status, genotype (loss-of-function, fast metabolizer or none) and site. In the control arm, patients discontinue aspirin and continue SAPT with high-potency P2Y12 inhibitor (Ticagrelor or Prasugrel). In the intervention arm, patients undergo rapid genetic screening for CYP2C19*2 or *17 polymorphisms. Those without loss-of-function alleles are treated with SAPT by Clopidogrel and those with various polymorphisms that lead to loss of function to metabolize Clopidogrel or to fast metabolization of this antiplatelet agent switch to SAPT by aspirin.

With all this in mind, the trial focuses on selecting a specific population (high bleeding risk patients) to align it with the goal of the study and focus on patients with the polymorphisms mentioned previously.

Inclusion criteria

- Age ≥ 18 years.
- Admission for type I AMI (STEMI or NSTEMI).
- Bedside genetic testing for Clopidogrel resistance that can be performed during hospital stay for ACS.
- Treated with AAS and Ticagrelor, or AAS and Prasugrel at the screening phase and at the randomization visit.
- High bleeding risk as defined by the Consensus Document From the Academic Research Consortium for High Bleeding Risk (at least one criterion):
    - Age ≥ 75 years.
    - Baseline hemoglobin < 11 g/dL (or anemia requiring transfusion during the 4 weeks prior to randomization).
    - Chronic Kidney Disease with estimated glomerular filtration rate ≤ 30 mL/min.
    - Thrombocytopenia with platelet count < 100 x 109 /L.
    - Chronic bleeding diatheses: inherited or acquired conditions known to be associated with increased bleeding risk such as platelet dysfunction, von

Willebrand disease, inherited or acquired clotting factor deficiencies (including factors VII, VIII, IX and XI), or acquired antibodies to clotting factors, among others.
- Cirrhosis with portal hypertension.
- PCI after major traumatism or surgery.
- Any documented stroke in the last 12 months.
- Hospital admission for bleeding or transfusion within the last 6 months.
- Nonskin cancer diagnosed or treated ≤ 3 years.
- Planned daily nonsteroidal anti-inflammatory drugs (other than AAS) or steroids for ≥ 30 days after PCI.

- Patient affiliated to a social security system.
- Signed informed consent form.
- For women of childbearing potential, an effective contraception method must be used up to V3.

Exclusion criteria

- Enrolled in another clinical trial except non interventional studies.
- Any prior documented intracerebral bleed.
- Contra-indication, known allergy or expected interactions with Clopidogrel. Baseline treatment (at screening) should not include an antiplatelet therapy for which a contra-indication, known allergy or expected interactions is known.
- Patients on concomitant treatment with an anticoagulant agent (Vitamin-K antagonists or novel oral anticoagulants such as rivaroxaban, dabigatran or apixaban).
- Planned surgery within 12 coming months.
- Patient under guardianship or curatorship.
- Pregnancy or breastfeeding.
- Inability to sign the informed consent form.

Finally, every patient may undergo a one year of follow-up visits after randomization to monitor for any MACE as well as major and minor bleeding events – individually or combined – classified according to BARC types 2 to 5, that may occur during the treatment phase of the clinical trial.

P2Y12 inhibitors – Clopidogrel/Ticagrelor (TADCLOT)

The TADCLOT trial investigates the safety and efficacy of a twice daily 90mg **Clopidogrel** regimen compared to **Ticagrelor** in reducing MACE in patients undergoing primary PCI following a STEMI event. Therefore, the trial focuses on whether a modified dosing strategy for Clopidogrel can enhance platelet inhibition in high-risk populations, altogether comparing it to a twice-daily regimen of Ticagrelor. It is a comparative study on two established drugs to evaluate which is the better and more affordable option.

While Ticagrelor, a potent P2Y12 inhibitor, has demonstrated superiority over standard-dose Clopidogrel in reducing MACE in ACS populations, its higher cost and limited accessibility pose significant challenges. Conversely, Clopidogrel remains the most widely used P2Y12 inhibitor globally due to its affordability and availability, despite concerns regarding variable patients' response and reduced efficacy in certain genetic subgroups.

Clopidogrel, like Ticagrelor, Prasugrel and Cangrelor is a P2Y12 receptor inhibitor. It is a thienopyridine prodrug that requires hepatic bioactivation via cytochrome P450 enzymes (primarily CYP2C19). Once metabolized to its active form, it irreversibly binds to the P2Y12 receptor on platelets, preventing ADP-mediated activation of the GPIIb/IIIa receptor complex, which is crucial for platelet aggregation. This inhibition reduces the risk of thrombus formation over atherosclerotic plaques or implanted stents (34).

It is a Phase III, double-blind, randomized, controlled clinical trial, designed specifically to evaluate treatment strategies in South Asian populations. This demographic is disproportionately affected by coronary artery disease (CAD) and has a higher prevalence of CYP2C19 loss-of-function mutations, leading to Clopidogrel non-responsiveness and threefold higher odds of recurrent MI. The trial addresses this gap by assessing whether higher Clopidogrel dosing may offer a more effective alternative in these genetically predisposed patients in comparison to a potent P2Y12 inhibitor, which, in this case, is Ticagrelor.

The eligibility criteria for this trial were carefully defined to ensure the inclusion of patients at high risk for adverse CV outcomes and to address the genetic variability in drug metabolism considering the CYP2C19 enzyme. The inclusion and exclusion criteria are as follows:

Inclusion criteria

- Age ≥ 18 years.
- ST-segment elevation ≥ 1 mm in ≥ 2 contiguous ECG leads or new or presumably new left bundle branch block.
- Written informed consent.

Exclusion criteria

- Patients with STEMI secondary to stent thrombosis or index event being a complication of PCI within 30 days.
- Thrombolytic therapy < 24H.
- Platelet count < 100.000 and Hemoglobin < 10gm/dL.
- Pregnancy or lactation.
- Moderate to severe hepatic impairment.
- Patients with advanced CKD and those on hemodialysis.
- Recent ICH or major bleed transfusion.
- Inability to give informed consent
- Participation in another study.

- Inability to fulfill protocol (living outside the city etc.)

With this in mind, this study's objective is to treat patients in a 30-day period post-PCI with this antiplatelet agents, a group is to receive Ticagrelor 180 mg as a loading dose and 90 mg twice daily as a maintenance dose for this period, whether the other group is to receive Clopidogrel 600 mg as a loading dose and 75 mg twice daily as a maintenance dose for the 30 days period. A month after the first dosage, patients are to receive doses according to standards clinical practice guidelines.

P2Y12 inhibitors – Vicagrel (VCP1-III-01)

This trial aims to test the efficacy and safety of **Vicagrel** in patients with ACS undergoing PCI and investigates whether is non-inferior or superior to Clopidogrel in preventing MACE and assesses its safety profile, especially in relation to bleeding and genetic variability in drug metabolism.

Vicagrel is a novel antiplatelet prodrug designed to overcome limitations associated with Clopidogrel, especially in individuals carrying CYP2C19 polymorphisms, as mentioned in previous trials. It is intended to inhibit platelet aggregation by blocking the P2Y12 ADP receptor on platelets and, unlike Clopidogrel, is activated via esterase-mediated hydrolysis rather than CYP2C19-dependent pathways. Its activation mechanism is in the intestine by carboxylesterase-2 (CES2) and arylacetamide deacetylase (AADAC), which convert Vicagrel into 2-oxo-clopidogrel, that is then further metabolized by hepatic cytochrome P450 enzymes into its active thiol metabolite. The active compound irreversibly binds to and inhibits the P2Y12 receptor, preventing ADP-induced platelet activation and aggregation. Because Vicagrel's activation bypasses the CYP2C19-dependent step, it allows for a more consistent and potent antiplatelet effect across different genetic backgrounds, overcoming the variability and resistance seen with Clopidogrel (35).

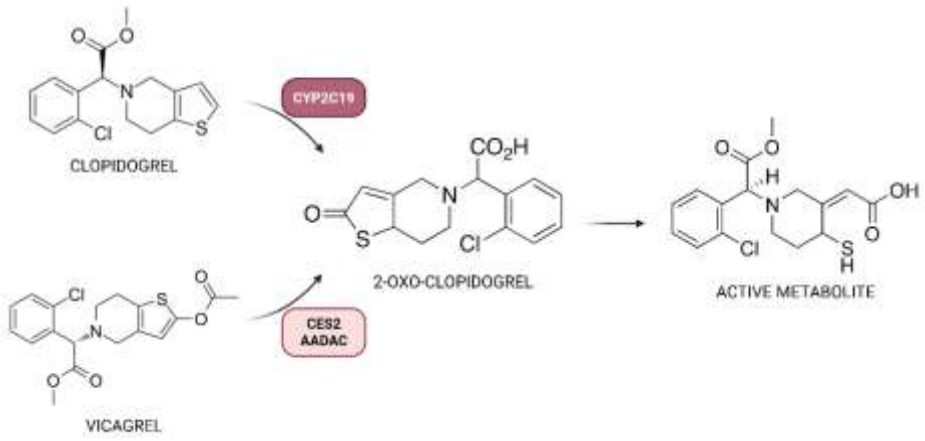

Figure 7. *Vicagrel's metabolism in comparison with Clopidogrel's, resulting in the same active metabolite. Adapted from Li H et al. (2020)*

This is a Phase III, multicenter, randomized, double-blind, double-dummy, parallel-controlled clinical trial conducted in China. It focuses on patients with ACS, whether it is unstable angina or even NSTEMI, who are undergoing PCI. Patients are randomized to either receive doses of Vicagrel or Clopidogrel, both in combination with AAS as part of DAPT.

To better understand the targeted patient population and ensure the applicability of its findings, the trial defines the eligibility criteria that guide participant selection. These criteria are outlined below to highlight the specific clinical and genetic characteristics deemed relevant for enrollment.

Inclusion criteria

- Age between 18 and 80 years, with no gender restrictions.
- Diagnostic with ACS and scheduled for PCI, including STEMI, NSTEMI and unstable angina.
- Voluntarily sign the informed consent form and be able to follow the visit arrangements specified in the protocol during the trial period.

Exclusion criteria

- Expected survival time < 12 months.
- Severe liver dysfunction (non-heart disease induced ALT or AST > 3x ULN) and cirrhosis.
- Pregnant or lactating women, or participants and their partners who plan to become pregnant during the trial period.
- The researchers own criteria to determine a participant is not suitable for this experiment.

With all this in mind, the patient follow-up is estimated to last for 6 months to assess the incidence of MACE in each patient included in the study.

Zalunfiban (CELEBRATE)

The CELEBRATE trial aims to study the safety and efficacy of a single subcutaneous dose of **Zalunfiban** versus placebo on clinical outcomes in patients with STEMI enrolled in the ambulance if they meet all eligibility criteria. These patients are to be evaluated by paramedics who transport the subjects to the participating hospitals in Europe and North America. In comparison to the other trials discussed in this present work, this is a particular case where antiplatelet agents are administered early on for secondary prevention, as patients are enrolled on the same day as their CV event, then monitored as late as a year after drug administration.

Zalunfiban, or also named RUC-4, is a novel, small-molecule inhibitor of the platelet glycoprotein IIb/IIIa (αIIbβ3) receptor (GPIIb/IIIa receptor), specifically designed for early prehospital antiplatelet therapy at the time of first medical contact in STEMI patients. Unlike traditional GPIIb/IIIa inhibitors, it exhibits a unique mechanism of action: Zalunifban displaces

the Mg^{2+} ion in the metal ion dependent adhesion site (MIDAS) of the GPIIIa, which is essential for fibrinogen binding and platelet aggregation. This displacement locks the receptor in an inactive conformation, preventing ligand binding without inducing the conformational changes typically caused by other GPIs like eptifibatide or tirofiban. These conformational alterations are thought to contribute to drug-induced thrombocytopenia through immune-mediated mechanisms, hence by avoiding this, Zalunfiban is postulated to reduce the risk of thrombocytopenia. Notably, as a GPI, it exhibits significantly greater in vitro potency than the P2Y12 antagonist Ticagrelor in blocking platelet-fibrinogen interactions, particularly when platelets are activated by peptides targeting one or both thrombin receptors (36).

This is a Phase III, prospective, randomized, double-blind, placebo-controlled, international, multicenter clinical trial. Eligible patients are screened and enrolled directly in the ambulance, using the information available; those fulfilling the eligibility criteria who have provided verbal/witnessed/short written/Exception from Informed Consent Requirements (EFIC) process informed consent are to be randomized and included in the study. Participants are randomized to receive one of two weight-based doses of Zalunfiban (0.110 or 0.1130 mg/kg) or placebo, administered subcutaneously before hospital arrival. Later, the patient is to be transferred to the clinical site PCI center for angiography and intervention. The study focuses on early inhibition of platelet aggregation to reduce thrombotic burden and improve outcomes in the pre-PCI window, addressing a key vulnerability in acute STEMI management.

The following eligibility criteria are established to ensure appropriate patient selection for the trial and to maintain both the safety of participants and the integrity of the study results.

Inclusion criteria

- Males aged ≥ 18 years or post-menopausal or surgically sterile females ≥ 50 years or ≥ 55 years (for Czech Republic study sites only).
- Weight between 52 and 130 kg.
- Subjects with STEMI, presenting with persistent ischemic chest pain (> 10 minutes) and new ≥ 2 mm ST-segment elevation in two adjacent ECG leads, in whom the total duration of symptoms is 4 hours maximum. If the time of symptom onset is uncertain, the cardiologist may be contacted to confirm inclusion criteria.
- Exception from Informed Consent Requirements (EFIC) process, with verbal witnessed/short written informed consent, or written informed consent signed by subject or legally authorized representative/independent witness is to be obtained in the acute phase by paramedics, according to local applicable legal regulations. Subject is willing and able to give informed consent. Written informed consent is to be obtained as soon as the subject's clinical condition allows it.

Exclusion criteria

- Cardio Pulmonary Resuscitation (CPR) for current Out of Hospital Cardiac Arrest (OHCA).

- Presenting with systolic blood pressure < 90 mmHg (confirmed on repeat assessment) and heart rate > 100 beats per minute (bpm).
- Current known active COVID-19 infection.
- Currently treated with renal dialysis.
- Current treatment with oral anticoagulation (Vitamin K antagonists (VKA), direct oral anticoagulants (DOACs), or thrombotic agents).
- Major surgery or trauma or bleeding leading to hospitalization within the past month.
- Known severe anemia (regular transfusion needed).
- Previously enrolled in this study.
- Participation in another clinical trial study with an investigational product or device within the past month.
- Life expectancy less than one year.

Once patients are stabilized, full written informed consent is to be obtained, as well as additional blood samples for safety are to be collected during the patient's hospital admission. The trial includes 12 months of follow-up, with key endpoints involving adverse events (AEs), bleeding complications, injection site reactions, hospitalizations for atrial fibrillation or heart failure and stroke disability (assessed at 90 days if applicable). The duration of participation for each subject is 12 months, including enrollment, study drug administration, hospitalization and phone contact follow-up at 30 days and at 12 months.

DOAC Factor Xa inhibitor – Apixaban (POTAMI)

The POTAMI trial focuses on testing the safety and efficacy of low-dose **Apixaban** (2.5 mg twice daily) in addition to guideline-directed medical therapy (GDMT), compared to placebo and guideline-directed medical therapy alone, in preventing left ventricular (LV) thrombus formation 30 days following primary PCI in patients presenting with AMI and severe LV dysfunction. This study also evaluated clinical outcomes such as bleeding risk and composite CV events during early post-infarction care.

Apixaban is a direct oral anticoagulant (DOAC) that selectively inhibits Factor Xa, a key enzyme in the coagulation cascade responsible for converting prothrombin into thrombin. It inhibits both free and clot-bound Factor Xa, as well as prothrombinase activity, offering broad and consistent anticoagulant effects. Whilst inhibiting Factor Xa, Apixaban reduces the generation of thrombin, thereby preventing the consequent conversion of fibrinogen to fibrin, the protein strands that form the structural framework of a blood clot, thus halting the development of a blood clot. While it does not directly act on platelet aggregation, it indirectly reduces it by inhibiting thrombin generation, simultaneously providing more predictable pharmacokinetics, fewer food and drug interactions, and not requiring routine coagulation monitoring compared to more traditional anticoagulants (37,38).

This targeted mechanism makes Apixaban particularly effective and safer in preventing thromboembolic events in various clinical settings, including atrial fibrillation and ACS.

The POTAMI trial is a Phase III randomized, open-label, controlled clinical study enrolling patients aged 18 to 65, presenting with anterior STEMI and a reduced ejection fraction (<35%) and who underwent primary PCI. Once randomized, participants either receive Apixaban 2.5 mg twice daily with DAPT (with AAS and a P2Y12 inhibitor) or standard GDMT alone. The intervention arm receives low-dose Apixaban plus DAPT for two weeks, followed by Apixaban with a single antiplatelet agent until week four, dropping AAS until the end of the study. After four weeks, the treatment group is to be switched to DAPT, just like control arm.

To include patients in this trial, the following eligibility criteria must be considered to ensure patient safety and optimal applicability of the study's outcomes, to better understand targeted population and guide participant selection.

Inclusion criteria

- Age between 18 and 65 years.
- Presenting with acute anterior STEMI.
- Severe LV dysfunction (EF <35%) with antero-apical akinesia, dyskinesia or aneurysm.
- Without evidence of LV thrombus.

Exclusion criteria

- Patients with previous AMI or LAD revascularization procedures.
- With cardiogenic shock.
- With LV thrombus.
- With advanced CKD (Cr >2 and those on hemodialysis).
- Recent ICH or major bleed requiring transfusion, low platelet count < 100.000.
- History of recent CVA (within the past three months).
- With atrial fibrillation or other indications for chronic anticoagulation.
- Pregnant patients and those with hematological disorders.

All primary and secondary outcomes are to be assessed within the first month post-PCI, as the effect of Apixaban is measured only during this period. Patients are scheduled to discontinue the anticoagulant four weeks after enrollment in the trial. Follow-up is to be done via phone at two weeks to assess medication compliance, side effects and clinical status, and in-person at four weeks with ECG to assess endpoints.

DOAC Factor XIa inhibitor – Milvexian (LIBREXIA-ACS)

The LIBREXIA-ACS trial finds its purpose in evaluating whether **Milvexian**, a direct oral anticoagulant (DOAC) inhibitor of Factor XIa, has superior effects compared to placebo, in addition to standard-of-care, in reducing the risk of MACE – CV death, AMI and ischemic stroke. It is a study focused on patients enrolled within 7 days of an ACS, have undergone cardiac catheterization with PCI or are being managed conservatively with or without

catheterization, and who are receiving antiplatelet therapy standard-of-care (whether it be DAPT or SAPT).

Milvexian is a direct, high-affinity, reversible inhibitor of human activated coagulation Factor XI (Factor XIa), a serine protease that plays a central role in the amplification phase of the intrinsic coagulation cascade. It is one of the first FXIa inhibitors to be developed as a new potential orally administered antithrombotic drug under a co-development agreement between Bristol Myers Squibb Company and Jansen Pharmaceuticals Inc.

By targeting FXIa, Milvexian attenuates thrombin generation through inhibiting, in complex with Factor VIIIa, Factor X's conversion to Factor Xa, thereby reducing thrombus formation while preserving hemostasis. Regardless of which pathway activated the common pathway of the coagulation cascade, thrombin, as a downstream product, reinforces the intrinsic pathway through positive feedback to FXI. Therefore, inhibition of Factor XI interrupts the thrombin-factor XI feedback loop and prevents pathological formation of a thrombus. As FXIa is dispensable for hemostasis, its inhibition allows physiological hemostasis while preventing pathological thrombosis. This selective suppression allows for antithrombotic effects and offers a promising therapeutic approach for anticoagulation with a lower risk of bleeding compared to inhibitors of thrombin or FXa (like apixaban or dabigatran) (39,40).

This is a Phase III, multicenter, randomized, double-blind, placebo-controlled, parallel group, event-driven, superiority, sequential study to evaluate the efficacy and safety of Milvexian in participants who have recently had an ACS. Patients enrolled within 7 days of the event are to either be randomized into the interventional arm or the placebo one, receiving Milvexian 25 mg or the same dose of placebo, both in the form of tablets and twice daily.

The next eligibility criteria are designed to identify patients at heightened risk for thromboembolic events while minimizing confounding risks associated with bleeding or comorbidities.

Inclusion criteria

- Must have an index event that meets all three of the following criteria within 7 days prior to randomization:
    - Clinical syndrome consistent with spontaneous cardiac ischemia.
    - Diagnosis of ACS (STEMI, NSTEMI or UA)
    - Cardiac biomarker elevation above the upper limit of normal as determined by the local laboratory.
- Must have at least two of the following risk factors:
    - Age ≥ 65 years.
    - Diabetes mellitus.
    - History of previous MI, other than index ACS.
    - Multivessel CAD.
    - History of coronary artery bypass graft (CABG) surgery prior to index ACS event.
    - History of PAD or cerebrovascular disease.

  - Conservative management (no PCI or CABG after index ACS event).
  - Any one or more of the following high-risk angiographic features: a) total stent length of > 30 mm, b) thrombotic target lesion, c) bifurcation lesion treated with more than one stent, d) calcified target lesion treated with atherectomy, e) treatment of obstructive left main or proximal left anterior descending artery for index ACS.
- All female participants of childbearing potential must have a negative highly sensitive serum beta-human chorionic gonadotropin (hCG) or urine test at screening.
- Female participant must not be pregnant, breastfeeding or planning to become pregnant until 4 days (5 half-lives) after the last dose of study intervention.

Exclusion criteria

- MI secondary to ischemia due to either increased oxygen demand or decreased supply (Type 2 MI) or periprocedural MI as the index ACS event.
- Planned CABG or staged PCI after randomization.
- Any condition that requires chronic anticoagulation at the discretion of the investigator and/or local guidelines.
- Conditions with a significant increased risk of bleeding.

The study is designed to evaluate the incidence of key clinical events – namely CV death, MI, ischemic stroke, major adverse limb event and symptomatic venous thromboembolism – in each participant from the time of enrollment until the study's conclusion, which is anticipated to occur approximately three years and six months after initiation.

Xuesaitong (2024XLA098-3)

The **Xuesaitong** (XST) trial is destined to assess the effects of this drug on platelet function, clinical efficacy, prognosis and safety in the treatment of ACS in patients who underwent PCI previously to Xuesaitong administration. This study is only being conducted in China, as it is the only country where XST is approved as a prescription medication for the treatment of CV diseases, while in other countries it may be available as a dietary supplement.

Xuesaitong literally translates to "blood stasis relief", reflecting its primary function in traditional Chinese medicine. It is composed of multiple herbal ingredients, mainly consisting of *Panax notoginseng saponins* (PNS), which are known for their neuroprotective properties, including anti-inflammatory and antioxidative effects. These saponins are believed to improve cerebral blood flow, reduce blood viscosity, thus improving blood circulation, and inhibit platelet aggregation, which collectively contribute to their therapeutic effects in cardiovascular events. By enhancing nitric oxide production and reducing oxidative stress, XST helps to relax blood vessels and improve microcirculation. This medication inhibits the NF-Kb and JAK2/STAT3 pathways and down-regulates NLRP3 inflammasome activity, mitigating inflammation after an atherosclerotic event. It also serves as an ADP receptor antagonist,

inhibiting ADP-induced platelet aggregation just as Clopidogrel, and reduces thromboxane A2 synthesis by inhibiting COX activity, thus reducing thrombus formation and platelet activation (41,42).

This is a Phase III, randomized, double-blind, placebo-controlled, parallel-group study designed for eligible participants diagnosed with STEMI, NSTEMI or UA to take soft capsules of 0.33g/tablet Xuesaitong or matching placebo tablets orally twice a day.

In order to ensure the safety of participants and the validity of the study outcomes, specific eligibility criteria have been established. Designed to select a specific population for secondary prevention when administering Xuesaitong, only individuals who meet all the following outlined inclusion criteria are to be enrolled in the trial.

Inclusion criteria

- Aged between 18 and 80 years, with no gender restrictions.
- Patients diagnosed with AMI, whether it be STEMI, NSTEMI or UA according to western medical standards.
- Within 4 weeks post-PCI.
- Voluntarily participating in the clinical trial and having signed the informed consent form.

Exclusion criteria

- Uncontrolled hypertension after medication.
- Increased bleeding risk: history of hemorrhagic stroke; intracranial aneurysm; major trauma or surgery within the past month; active bleeding disorders.
- History of gastrointestinal ulcers or significant gastrointestinal bleeding.
- Severe organic heart disease, such as LVEF < 35% or NYHA/Killip heart function grade IV.
- History of malignant arrythmias within the past year, congenital heart disease or malignant tumors.
- Severe liver or kidney dysfunction: ALT or AST ≥ 3×ULN, TBIL ≥ 2×ULN, or creatinine clearance < 30ml/min.
- Pregnant or lactating women.
- Recent blood donation or significant blood loss within the past 3 months (≥ 400mL).
- History of alcohol abuse (≥28 standard units/week for males, ≥21 standard units/week for females) or frequent alcohol consumption in the past 6 months (≥14 standard units/week).
- History of drug abuse or dependence within the past year. Participation in other clinical trials and taking trial drugs within the past 3 months.
- Allergy or intolerance to aspirin or P2Y12 receptor inhibitors.
- Allergy to any components of the trial drug.
- Other conditions deemed inappropriate for the participation by the investigator.

The intervention is to be administered over a 12-week period, in which the primary objective is to assess the efficacy of XST on platelet function, clinical efficacy, prognosis and safety in the treatment of ACS. Simultaneously, during this period, several ECG and other biochemical parameters such as serum creatinine, blood urea nitrogen, prothrombin time, platelet count and hemoglobin levels, among others, are to be collected to ensure the patient's correct evolution.

8.3. Lipid-lowering Therapies

Atorvastatin/Rosuvastatin (2009-012850-19)

The comparative trial studying the efficacy between **Atorvastatin** and **Rosuvastatin** in patients undergoing Percutaneous Transluminal Coronary Angioplasty (PTCA) through the monitoring of C-Reactive Protein (CRP) is designed to evaluate and compare the anti-inflammatory effects that exert these statins in patients with ACS. The primary objective is to assess the reduction in hsCRP levels, a biomarker of inflammation and predictor of CV events, over a specified treatment period.

Both Atorvastatin and Rosuvastatin are statins, selective and competitive inhibitors of the hydroxymethylglutaryl-CoA (HMG-CoA) reductase, enzyme responsible for converting HMG-CoA to mevalonate in the cholesterol synthesis pathway. By reducing hepatic cholesterol synthesis, an upregulation of LDL receptors and enhanced clearance of LDL-cholesterol from the bloodstream occurs. Statin therapy reduces the hepatic production of apo B100-containing lipoproteins, such as VLDL and LDL, resulting in decreased levels of both cholesterol and triglycerides. The effectiveness of statins can vary based on genetic factors, including variations in the ATP-binding cassette G2, lipoprotein(a) and apolipoprotein E (apoE) genes. From a general point of view, statins also reduce levels of CRP and cytokine production by inhibiting the mevalonate-derived synthesis of isoprenoid intermediates, which are critical for the function of intracellular or signaling proteins like Rho and Ras.

Atorvastatin is lipophilic and less selective, having low systemic bioavailability due to an extensive first pass effect at the liver, being metabolized by CYP3A4, but has high potency compared to other statins. On the contrary, Rosuvastatin is hydrophilic and it tends to be more potent, more hepatoselective and less prone to drug interactions due to its minimal metabolism via the CYP450 system (43,44).

This is a Phase III prospective, randomized, open-label, controlled clinical trial designed to compare the efficacy of both these statins in reducing inflammatory markers in patients with ACS undergoing PTCA. Patients diagnosed with ACS and scheduled for PTCA are to be randomized to either receive Atorvastatin 40 mg or Rosuvastatin 20 mg, both in the form of tablets and daily, in addition to standard therapy.

In order to ensure the population is both clinically appropriate and homogenous with respect to the condition being studied, while minimizing potential confounding factors, the following specific eligibility criteria have been established.

Inclusion criteria

- Age between 18 and 80 years.
- Diagnosis of non-ST-elevation acute coronary syndrome (NSTE-ACS).
- High-sensitivity C-reactive protein (hsCRP) > 2 mg/dL.

Exclusion criteria

- Diabetes mellitus.
- Active smokers or former smokers within the last ≤ 10 years.
- Dyslipidemia, defined as:
    - LDL cholesterol ≥ 160 mg/dL.
    - Triglycerides ≥ 150 mg/dL.
    - Currently undergoing lipid-lowering drug treatment.
- Known coronary artery disease (CAD).
- Pregnancy or breastfeeding.
- Chronic renal insufficiency, defined as:
    - Creatinine > 2.2 mg/dL.
    - GFR < 30 mL/min.
- Hepatic impairment.
- Ongoing or chronic infectious and/or inflammatory diseases.
- Active or past malignant neoplasia.
- Congenital or acquired immune deficiency.

Overall, the study is designed to monitor the reduction in hsCRP levels over a four-week treatment. Secondary outcomes include evaluation of lipid profiles and monitoring of AEs during this period of drug administration. This trial aims to determine which statin provides superior effects in the management of ACS patients.

PCSK9 inhibitors

PCSK9 is a proprotein convertase, which is involved in the degradation of LDL receptors in the liver, thus blocking its activity with monoclonal antibodies (mAb) reduces the degradation of LDL receptors, recycling more of them to the surface of the cell, and increases clearance of LDL cholesterol. Mutations in the PCSK9 gene cause familial hypercholesrolemia in a subset of patients by reducing the number of LDL receptors on the surface of hepatocytes. It is studied that blocking the activity of this molecule with injections of PCSK9-specific antibodies suppresses LDL cholesterol concentrations for several weeks.

Under physiological conditions, LDL receptors (LDLRs) on the hepatocyte surface bind circulating LDL-C particles and the whole complex is endocytosed into the liver cell, where LDL-C dissociates from LDLR and is directed to lysosomal degradation. There, LDLRs are recycled back to the cell surface for further uptake. However, when PCSK9 binds to the extracellular domain of LDLRs, it redirects the complex to lysosomal degradation, which

prevents receptor recycling, reducing the number of LDLRs on the hepatocyte surface. Consequently, LDL-C clearance from plasma is decreased, leading to elevated circulating LDL-C levels.

Currently, there are two FDA-approved medications being prescribed for treating high levels of LDL in the circulation: alirocumab (Praluent) and evolocumab (Repatha), which are shown to additionally reduce the risk of heart attack by 27% (45,46).

Recently, with hypercholesterolemia being of the outmost importance in ACS recurrence, there are new promising therapies being studied, with similar mechanisms of action as those of the monoclonal antibodies but with greater efficacy and potency. Accordingly, the ongoing clinical trials outlined below are designed to mitigate this substantial cardiovascular risk through the evaluation of emerging pharmacological interventions.

Injectable (LIBerate-HR)

The LIBerate-HR trial is developed to evaluate the long-term efficacy and safety of administration of **LIB003 (Lerodalcibep)** compared to placebo in reducing LDL cholesterol levels in patients with established CVD or those at high risk for CVD who remain inadequately controlled despite receiving a stable lipid-lowering regimen.

LIB003 is a small recombinant binding protein consisting of an 11kDA anti PCSK9 domain so that it inhibits the activity of PCSK9, a key regulator of LDL receptor degradation, thereby enhancing the liver's ability to clear circulating LDL-C. This molecule has a pharmacokinetic extender domain, in this case human serum albumin, in order to keep it the organism for a longer period of time, so it is a combination of fully human proteins. It works similarly to a mAb as it binds to PCSK9 in the bloodstream and prevents it from binding to the LDL receptor, which then recycles more frequently and is able to clear LDL cholesterol and deliver it to cells out of the circulation. By reducing PCSK9 activity, LIB003 offers a targeted therapeutic approach to achieving lower LDL-C concentrations in patients who require further risk reduction, patients with previous ACS for instance (47).

LIBerate-HR is a Phase III randomized, double-blind, placebo-controlled clinical trial in which participants are to be randomized in a 2:1 ratio to either receive LIB003 300 mg or a matching placebo dose, both administered once every four weeks (Q4W, ≤ 31 days) via subcutaneous injection.

The following specific eligibility criteria have been defined to identify individuals with persistent LDL-C elevation despite standard therapy to ensure the safety and scientific rigor of the study.

Inclusion criteria

- Age ≥ 18 years at the first screening visit, no gender restrictions.

- Provision of written and signed informed consent form prior to any study-specific procedure.
- Weight of ≥ 40 kg and body mass index ≥ 17 and ≤ 42 kg/m².
- History of CVD or very high risk for CVD (previous MI, angioplasty, CAD, cerebrovascular or peripheral arterial disease without a recent event within 3 months prior to screening), or high risk for CVD (including type 2 diabetes mellitus, FH, untreated LDL-C > 190 mg/dL, or a 10-year risk of a CVE of ≥ 10% as assessed by Risk Score for Cardiovascular Disease or equivalent).
- At screening or post washout/stabilization:
    - LDL-C ≥ 70 mg/dL and triglycerides ≤ 400 mg/dL while on stable oral lipid-lowering therapy (e.g., maximally tolerated statin with or without ezetimibe).
    - Patients unable to tolerate approved doses of a statin may take lower than approved doses and dose less frequently than daily as long as the dose and dosing frequency is consistent.
    - Patients with documentation of inability to tolerate any statin at any dose, or history of rhabdomyolysis, may also participate.
- Stable diet and oral lipid-lowering therapy (e.g., statins, ezetimibe, bile acid sequestrants, bempedoic acid) or combinations for ≥ 4 weeks.
- Patients on a PCSK9 mAb must undergo a washout period:
    - ≥ 4 weeks for those on 75 mg, 140 mg or 150 mg Q2W.
    - ≥ 8 weeks for those on 300 mg or 420 mg Q4W (≤ 31 days).
- Females of childbearing potential must be using a highly effective form of birth control if sexually active and have a negative urine pregnancy test at the last screening visit.
- Male patients will either be surgically sterile or agree to use highly effective forms of contraception and must refrain from sperm donation until 90 days following the last dose of study drug.

Exclusion criteria

- Use of prohibited oral lipid-lowering agents, including Mipomersen or Lomitapide within 6 months of screening, Gemfibrozil within 6 weeks of screening, apheresis within 2 months prior to randomization or received other investigational agent(s) such as PCSK9 or Lp(a) siRNA or locked nucleic acid-reducing agents within 12 months of the Screening Visit.
- History of Homozygous familial hypercholesterolemia (HoFH), defined clinically or genetically.
- Any active or prior clinical condition/systemic disease that compromises patient safety or data integrity including pulmonary, hematologic, endocrine (except diabetes), immunologic, dermatologic, neurologic or psychiatric diseases.
- Females of childbearing potential who are not using or unwilling to use highly effective contraception, are pregnant or breastfeeding or have a positive urine pregnancy test.
- Moderate to severe renal dysfunction with eGFR < 30 mL/min/1.73 m².

- Active liver disease or dysfunction, such as cirrhosis, alcoholic liver disease, HBV/HCV, autoimmune hepatitis, liver failure, liver cancer, history of liver transplant, AST or ALT > 2.5 × ULN (may repeat if ≤ 3 × ULN).
- Uncontrolled thyroid disease with TSH < LLN or > 1.5 × ULN at screening, unless FT3 is normal and treatment is stable ≥ 3 months.
- Uncontrolled diabetes with fasting glucose ≥ 200 mg/dL or HbA1c > 9%.
- Uncontrolled serious cardiac arrhythmia or CV event within 3 months.
- Planned cardiac surgery or revascularization procedures.
- NYHA class III-IV heart failure or LVEF < 30% in the past 12 months.
- Uncontrolled hypertension with reproducible BP ≥ 180/110 mmHg.
- Participation in another trial.
- Unexplained creatine kinase > 5 × ULN, unless clearly linked to recent physical activity.
- Inability to comply with protocol-required visits/procedures.
- Substance abuse within 6 months.
- Significant blood/plasma loss (> 500 mL) in the 30 days prior to randomization.
- Blood transfusion within 4 weeks of randomization or HIV diagnosis.
- Previous treatment with LIB003 or any adnectin-based product.

This trial is to be conducted over a 52-week treatment period with a total study duration of up to 63 weeks, including screening and follow-up, with the primary objective to assess the percentage reduction in LDL-C levels from baseline to W52 with monthly LIB003 300 mg SC compared to placebo.

Oral (CORALreef)

The CORALreef clinical trial is designed to evaluate the efficacy and safety on **Enlicitide Decanoate (MK-0616)**, a novel oral PCSK9 inhibitor, in reducing the incidence of major adverse CV events in adults with hypercholesterolemia at high risk for atherosclerotic CV disease and whether it may reduce cholesterol levels in these patients.

MK-0616 is a macrocyclic peptide designed to inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9), which, as mentioned before, is a crucial regulator of LDL receptor degradation, as it binds to these receptors and triggers their degradation. While current PCSK9 inhibitors are administered via injection, Enlicitide offers the same biological mechanism of action in an orally administered tablet taken once daily. MK-0616 binds to circulating PCSK9 in the bloodstream and prevents it from interacting with LDLRs, which preserves LDLR recycling and enhances LDL-C clearance. Its cyclic peptide conformation confers increased proteolytic stability, enhanced binding specificity to PCSK9 and improved membrane permeability for bioavailability (48).

CORALreef is a Phase III, randomized, placebo-controlled study that is part of a broader clinical development program investigating whether oral PCSK9 inhibition can provide an effective and safe alternative to injectable therapies. All participants continue standard lipid-lowering therapy, including statins, during the study to ensure consistency with clinical practice.

Participants are randomized to receive either Enlicitide Decanoate 20 mg or a matching placebo dose, both administered once daily.

The following eligibility criteria have been defined to ensure scientific validity and patient safety by enrolling individuals who exhibit persisting LDL-C elevation and remain at high CV risk despite receiving standard lipid-lowering therapy.

Inclusion criteria

- Age ≥ 18 years with a history of major atherosclerotic cardiovascular disease (ASCVD) event defined as at least one of the following:
    - ≥ 30 days post MI type 1.
    - ≥ 30 days post ischemic stroke.
    - ≥ 30 days post successful peripheral arterial revascularization or major amputation due to atherosclerosis.

OR

- High risk for first major ASCVD event defined as at least one of the following:
    - Age ≥ 50 years with evidence of CAD.
    - Age ≥ 50 years with evidence of atherosclerotic cerebrovascular disease.
    - Age ≥ 50 years with evidence of PAD.
    - Age ≥ 60 years with diabetes mellitus and at least one of the following: a) microvascular disease; b) urine albumin-creatinine ratio ≥ 30 mg/mmol within 6 months before V1; c) daily insulin use; d) diabetes for ≥ 10 years.
- Has fasted lipid values at V1 as follows:
    - History of major ASCVD Event: LDL-C ≥ 70 mg/dL (1.81 mmol/L) OR non-HDL-C ≥ 100 mg/dL (2.59 mmol/L)
    - High risk for first major ASCVD Event: LDL-C ≥ 90 mg/dL (2.33 mmol/L) OR non-HDL-C ≥ 120 mg/dL (3.11 mmol/L)
- Is treated with moderate- or high-intensity statin (± non-statin lipid-lowering therapy [LLT]) at V1.
- Is on a stable dose of all background LLTs for at least 30 days before V1 with no medication or dose changes planned during the participation in the study.

Exclusion criteria

- History of homozygous familial hypercholesterolemia (FH) based on genetic or clinical criteria, compound heterozygous FH or double heterozygous FH.
- Has NYHA Class IV heart failure, last known LVEF ≤ 25% by any imaging method, or had a heart failure hospitalization within 3 months before V1.
- Recurrent ventricular tachycardia within 3 months prior to randomization.
- Planned arterial revascularization procedure.
- Undergoing or previously underwent an LDL-C apheresis program.

- Previously treated/being treated with certain other cholesterol lowering medications, including PCSK9 inhibitors without adequate washout.
- Fasting triglyceride value ≥ 400 mg/dL (≥ 4.52 mmol/L) at V1.

This trial is expected to last approximately 6 years, including long-term follow-up to evaluate efficacy and safety. Participants are to be monitored periodic laboratory tests, clinical evaluations and AE reporting conducted throughout the study period to ensure robust data collection. MACE, including coronary heart disease death, MI, ischemic stroke, acute limb ischemia, major amputation of a vascular etiology or urgent arterial revascularization are primary outcomes to be measured from randomization until the end of the study.

Obicetrapib (PREVAIL)

The PREVAIL trial focuses on studying the effectiveness of **Obicetrapib**, a novel oral cholesteryl ester transfer protein (CETP) inhibitor, in participants with ASCVD who are not adequately controlled despite receiving maximally tolerated lipid-lowering therapy to assess whether it reduces the incidence of major adverse CV events.

Obicetrapib is a selective and highly potent investigational molecule that functions by inhibiting CETP, a plasma protein that facilitates the transfer of cholesteryl esters from high-density lipoprotein (HDL) to LDL and VLDL particles. This process may lead to cholesterol-poor LDL and an increase in production of ApoA-1, resulting in significant increases in pre-beta HDL, which translates to elevated circulating HDL-C and reduced levels of LDL-C. Potent CETP inhibition by Obicetrapib leads to reduction of hepatic intracellular cholesterol through multiple coordinated mechanisms. It enhances the excretion of cholesterol via the intestine and increases the formation of pre-beta HDL particles, which facilitate the removal of cholesterol from hepatocytes. This decrease in intracellular cholesterol triggers upregulation of LDLR, promoting more efficient clearance of LDL particles from the circulation and leading to a marked reduction in LDL-C and small dense LDL particles. Additionally, CETP inhibition facilitates the clearance of large HDL particles through both the LDLR and SR-B1 (scavenger receptor class B type 1) pathways. Obicetrapib may also contribute to a reduction in hepatic production of Lp(a), further supporting its lipid-lowering profile.

Unlike previous CETP inhibitors, Obicetrapib has demonstrated a favorable lipid-modifying profile with improved tolerability, offering a promising therapeutic strategy for further LDL-C reduction in high-risk patients already on standard therapy. Its mechanism of action complements statins and other LDL-lowering drugs by addressing residual risk through both LDL-C reduction and HDL-C enhancement (49,50).

PREVAIL is a Phase III interventional, randomized, placebo-controlled, double-blind, parallel clinical trial aiming to determine whether Obicetrapib 10 mg daily can significantly reduce the risk of CV death, MI, stroke or non-elective coronary revascularization compared to the same placebo dose, both in a tablet format. All participants remain on their existing lipid-lowering

regimen throughout the study with the additional of the trial medication, including statins and/or Ezetimibe to reflect real-world clinical practice.

The following eligibility criteria have been established to maintain scientific validity and safeguard patient safety by selecting individuals with confirmed ASCVD who continue to have elevated LDL-C levels despite receiving optimal medical treatment.

Inclusion criteria

- Age ≥ 18 years, no gender restriction.
- Established ASCVD including:
    - Coronary artery disease.
    - Cerebrovascular disease.
    - Peripheral artery disease.
- On maximally tolerated lipid-modifying therapy.
- Fasting LDL-C ≥ 55mg/dL.
- Fasting triglycerides < 400 mg/dL.
- eGFR ≥ 30 mL/min.

Exclusion criteria

- NYHA Class III or IV heart failure or LVEF < 30%.
- Hospitalization for heart failure within 5 years prior to screening.
- Had non-fatal MI, non-fatal stroke, non-elective coronary revascularization and/or hospitalization for unstable angina or chest pain within past 3 months prior to screening.
- Uncontrolled hypertension.
- Homozygous familial hypercholesterolemia.
- Active liver disease.
- HbA1c ≥ 10%.
- TSH > 1.5 times upper limit normal.
- Creatinine kinase > 3 times ULN.
- History of malignancy with surgery in the past 3 years.
- History of alcohol or drug abuse within the past 5 years.
- Received treatment with investigational product or device within the past 30 days excluding Coronavirus treatment or vaccine.
- Known allergy to study drug.
- Participated in previous Obicetrapib trial.
- Taking gemfibrozil within 30 days screening.

This trial is expected to span several years, including treatment and follow-up period designed to assess outcomes. Primary outcome measures are to be assessed up to 30 months after last participant is randomized. Patients are to be followed regularly throughout the study with

scheduled visits to monitor safety, adherence and lipid parameters, alongside event-driven surveillance to capture MACE.

Inclisiran (VICTORION-2 PREVENT)

The VICTORION-2 PREVENT trial aims to determine whether the administration of **Inclisiran sodium** could be beneficial taken in addition to well-tolerated high-intensity statin therapy in participants with established ASCVD to reduce the risk of MACE, altogether comparing it to placebo in adjunct to the same drug regimen.

Inclisiran sodium is a chemically modified small interfering RNA (siRNA) molecule, designed to lower LDL-C by targeting the mRNA that encodes PCSK9 in hepatocytes. It is chemically modified in order to avoid degradation in the bloodstream with 2'-O-methyl, 2'-fluoro nucleotides and phosphorothioate linkages. Conjugated to N-acetylgalactosamine (GalNAc) for targeted delivery to hepatocytes via the asialoglycopotein receptor, Inclisiran enters liver cells and engages the RNA interference (RNAi) pathway. Its antisense strand is incorporated into the RNA-induced silencing complex (RISC), guiding it to bind and degrade PCSK9 mRNA, thereby preventing its translation into the PCSK9 protein by cleavage. Since it has been established PCSK9 promotes degradation of LDLRs, its inhibition leads to increased receptor availability on hepatocytes, thus enhancing LDL particle clearance from circulation. This mechanism results in sustained reductions in circulating PCSK9 and LDL-C levels following administration (51,52).

The VICTORION-2 PREVENT is a Phase III, randomized, double-blind, placebo-controlled, multicenter clinical trial that focuses on evaluating whether Inclisiran sodium 300 mg administered subcutaneously on Day 1, month 3 and then every six months can significantly impact on MACE – defined as a composite of CV death, non-fatal MI and non-fatal ischemic stroke – compared with the same placebo dose, both given adjuncts to high-intensity statin therapy. The trial reflects real-world therapeutic conditions by allowing participants to conitune their background lipid-lowering treatments throughout the study duration.

The following eligibility criteria are designed to ensure specific population is enrolled in the study and results are of scientific rigor.

Inclusion criteria

- Age ≥ 40 years, no gender restrictions.
- Fasting LDL-C ≥ 70 mg/dL at randomization visit.
- Stable (≥ 4 weeks) and well-tolerated lipid-lowering regimen that must include a high-intensity statin therapy with either Atorvastatin ≥ 40 mg QD OR Rosuvastatin ≥ 20 mg QD.
- Established CV disease defined as any of the following:
    - Spontaneous MI ≥ 4 weeks from screening visit.
    - History of ischemic stroke occurred ≥ 4 weeks prior to screening visit.

- Symptomatic peripheral artery disease evidenced by either intermittent claudication with ankle brachial index < 0.85, prior peripheral arterial revascularization procedure, or amputation due to atherosclerotic disease.

Exclusion criteria

- ACS, stroke, peripheral arterial revascularization procedure or amputation due to atherosclerotic disease < 4 weeks before screening visit.
- Treatment with PCSK9 inhibitors within 90 days or planned use post first study visit.
- Planned or expected cardiac, cerebrovascular or peripheral artery surgery or revascularization within the 6 months after the first study visit.
- NYHA Class III or IV heart failure.
- Active liver disease defined as any known current infectious, neoplastic or metabolic pathology of the liver.
- Previous exposure to Inclisiran or any other non-mAb PCSK9-targeted therapy, either as an investigational or marketed drug within 2 years.
- Severe concomitant non-CV disease that is expected to reduce life expectancy to less than 5 years.
- History of malignancy that required surgery radiation therapy and/or systemic therapy during the 3 years prior to the first study visit.
- Pregnant or nursing women.

This study is expected to last from randomization of the first patient up to total follow-up time, which is up to 72 months. During this period, participants are to be monitored to assess whether they experienced any AEs and outcomes defined as CV death, non-fatal MI, non-fatal ischemic stroke, urgent coronary revascularization and acute limb amputation among others.

Bempedoic acid (HACOL-ACS)

The HACOL-ACS trial is developed to assess the effectiveness of **Bempedoic acid** in lowering LDL-C levels in patients following MI who are insufficiently treated with lipid-lowering medication such as Atorvastatin plus Ezetimibe.

Bempedoic acid is a liver-specific oral prodrug activated by ACSVL1 to bempedoyl-CoA, which inhibits ATP-citrate lyase, an enzyme upstream of HMG-CoA reductase in cholesterol synthesis. This inhibition reduces cytosolic acteyl-CoA levels, lowering hepatic cholesterol synthesis. The resulting drop in intracellular cholesterol activates SREBP-2, increasing LDL receptor expression and enhancing clearance of LDL-C from the bloodstream. Unlike statins, its activation is limited to the liver, minimizing muscle-related side effects. Bempedoic acid may also activate AMPK and offer anti-inflammatory benefits, though its role in LDL-C reduction in humans is yet to be determined (53,54).

This a Phase III open-label, prospective, interventional, single-center, single-arm clinical study designed to better navigate the benefits of Bempedoic acid 180 mg oral daily administration

in the form of film-coated tablets in patients following acute PCI for STEMI or NSTEMI insufficiently treated with high-intensity oral lipid-lowering therapy. Participants are not randomized as this trial does not use a placebo comparator, only the interventional arm is being studied.

To participate in this study, patients must meet all eligibility criteria listed below in order to maintain scientific rigor and patient safety, including diligent compliance with the protocol.

Inclusion criteria

- Age ≥ 18 years and ≤ 85 years, no gender restrictions.
- Signed written informed consent form.
- NSTEMI or STEMI with successful PCI within 7 days prior to screening.
- Therapy naïve LDL-C > 100 mg/dL.
- Patient must be able to cooperate with the study protocol and follow-up.
- Patients without childbearing potential defined as:
    - ≥ 6 weeks after surgical sterilization by bilateral tubal ligation of bilateral oophorectomy.
    - Hysterectomy or uterine agenesis
    - ≥ 50 years and in postmenopausal state for ≥ 1 year with serum FSH > 40 IU/L and serum estrogen < 30 ng/L or a negative estrogen test, both at screening.
- Patients with childbearing potential:
    - Practicing sexual abstinence.
    - Same sexual relationships only and/or have sexual relationships with sterile partners.
    - Sexually active with fertile partner, have a negative pregnancy test during screening and agree to use reliable methods of contraception – such as combined hormonal contraception associated with inhibition of ovulation, progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine device (IUD) or intrauterine hormone-releasing system (IUS) – from the time of screening until the end of the clinical trial and for a period of 4 days following the last administration of study medication.

Exclusion criteria

- History of gout.
- Scheduled surgery within the next 4 months.
- Patients who cannot come to visits.
- Participation in another clinical trial within 30 days before study start or during the trial.
- Hypersensitivity to any of the components of the medication used.
- Pregnancy/breastfeeding.

- Severe renal disorders defined as eGFR < 30 mL/min/1.73 m² or patients requiring dialysis with endstage renal disease.
- History of tendon disorders or tendon rupture.
- Person placed in a mental institution by court or official order.

This trial is expected to evaluate the following 8 weeks of treatment with the triple therapy of Atorvastatin plus Ezetimibe and additive Bempedoic acid, in which safety assessments will consist of monitoring and recording all AEs and serious AEs (SAEs) and safety labs. This is all conducted to determine the proportion of patients who successfully achieve the ESC LDL-C guideline targets (LDL-C < 55 mg/dL for patients with high CV risk) after 8 weeks of triple treatment.

Ezetimibe

Ezetimibe is an established lipid-lowering agent commonly prescribed to patients at high risk of CV recurrence, particularly following ACS events. Its efficacy in reducing LDL-C levels – either as monotherapy or in combination with statins – has been well documented. However, with the emergence of new lipid-lowering therapies, there is a growing need to evaluate potential new combination regimens. As such, Ezetimibe remains a key component in ongoing and future clinical trials aimed at determining whether these novel combinations offer improved safety and efficacy profiles or prove to be nonviable.

The trials listed below offer new combinatory strategies with different lipid-lowering medication, which are mentioned previously, plus ezetimibe, whether it be in combination or in comparison.

Ezetimibe/Obicetrapib (REMBRANDT)

The purpose of the REMBRANDT study is to evaluate how the combination of **Obicetrapib** and **Ezetimibe** affects the volume of non-calcified plaques in the coronary arteries in patients with ACS on coronary computed tomography angiography (CCTA). This imaging method provides detailed pictures of the heart's blood vessels, helping researchers understand how the treatment impacts the plaque buildup.

Ezetimibe works as a cholesterol absorption inhibitor that targets the NPC1L1 protein, primarily at the brush border of jejunal enterocytes, where it blocks dietary and biliary cholesterol uptake. It interferes with clathrin/AP2-mediated endocytosis of the NPC1L1-cholesterol complex by altering the shape of NPC1L1, preventing cholesterol internalization into enterocytes and hepatocytes. This leads to a reduction in hepatic cholesterol stores, which in turn activates a compensatory upregulation of LDLRs on hepatocyte surfaces. The increased expression of these receptors enhances LDL-C clearance from the bloodstream, effectively lowering serum LDL-C. Ezetimibe does not affect triglyceride or vitamin absorption and is metabolized via glucuronidation, undergoing enterohepatic circulation, which supports

its prolonged action. This medication also disrupts annexin 2/caveolin 1 complex, which, in turn, interferes with cholesterol transport (55,56).

The mechanism of action of Obicetrapib is described in the PREVAIL trial section above.

REMBRANDT is a Phase III, placebo-controlled, interventional, double-blind, randomized study being conducted in patients with high-risk atherosclerotic CV disease (ASCVD) who are not adequately controlled by their maximally tolerated lipid-modifying therapy, to determine the efficacy and safety of the Obicetrapib 10 mg plus Ezetimibe 10 mg daily, both taken as a single tablet. Participants are to be randomly assigned to receive either the combination treatment or placebo.

To ensure the selection of an appropriate and homogenous study population, the REMBRANDT trial outlines the following specific eligibility criteria.

Inclusion criteria

- Age ≥ 45 years, no gender restrictions.
- Willing and able to give written consent and follow study procedures.
- Body mass index between 18 and 40 kg/m².
- Women of childbearing potential must use effective birth control methods.
- Evidence of ASCVD.
- Evidence of specific type of plaque in the heart arteries shown by special heart scan.
- On medication to lower cholesterol, such as statins, or similar treatments.
- Fasting LDL-C ≥ 70 mg/dL.
- Fasting triglycerides < 400 mg/dL.
- Have kidney function eGFR ≥ 40 mL/min/1.73 m².

Exclusion criteria

- Pregnancy/plan to become pregnant during the study or breastfeeding.
- Presence of serious health condition that might interfere with the study.
- Recent heart attack or stroke.
- Severe liver or kidney disease.
- Currently participating in another clinical trial.
- Known allergy to the study medications.
- History of drug or alcohol abuse.
- Unable to follow study procedures or take study medication as directed.

This study is expected to last for 18 months, during which participants are to have regular check-ups and imaging tests to monitor their heart health and the effects of the treatment, as well as ensure adherence to the medication regimen. Participants are to undergo an initial assessment to establish baseline measurements, including a CCTA to evaluate coronary plaque characteristics, in addition to blood tests to determine LDL-C levels and other relevant biomarkers.

Rosuvastatin/Ezetimibe/Atorvastatin (REMEDY)

The REMEDY trial has its objective in evaluating whether high-dose **Rosuvastatin** administered immediately before PCI can reduce myocardial injury and systemic inflammation in patients with suspected coronary artery disease (CAD). It is a comparative study, which Rosuvastatin is being compared to the effects of **Ezetimibe**, **Atorvastatin** and placebo.

Both mechanisms of action of statins and Ezetimibe are described previously in different trial sections.

To summarize both metabolisms, Ezetimibe and statins lower LDL-C through complementary but distinct biochemical mechanisms, often used in combination to lower the risk of recurrence in patients with ASCVD. Statins inhibit HMG-CoA reductase and reduce intracellular cholesterol synthesis, which triggers upregulation of LDLRs on hepatocytes surfaces, enhancing clearance of circulating LDL particles. In contrast, Ezetimibe acts at the intestinal bush border, blocking the NPC1L1 transporter, which mediates cholesterol uptake from dietary and biliary sources into enterocytes. This reduces overall delivery of cholesterol to the liver, also stimulating LDL receptor expression. While both drugs increase LDL clearance, statins work by reducing endogenous synthesis, and Ezetimibe by limiting exogenous absorption – a mechanistic distinction relevant in ACS patients, where combined or alternative lipid-lowering strategies may be required when statin tolerance or response is suboptimal.

REMEDY is a Phase III, placebo-controlled, randomized, interventional, comparative, double-blind clinical study, designed to determine if high-dose Rosuvastatin administered immediately prior to PCI reduces peri-procedural MI in comparison to other medication. Participants are to be randomized to either receive Rosuvastatin40 mg, Ezetimibe10 mg, Atorvastatin 40 mg or placebo, all in the form of tablets for oral daily administration.

With this in mind, this trial established the following eligibility criteria to ensure a well-defined and clinically relevant study population.

Inclusion criteria

- Age between 18 and 85 at screening.
- Patients with stable CAD OR stable post-ACS, including STEMI and/or NSTEMI.
- Stabilized myocardial necrosis markers, defined as Creatinine kinase-MB or troponins showing < 20% variation in two consecutive measurements taken ≥ 6h time distance before PCI, in accordance with the universal definition of peri-procedural MI.
- Randomization performed independent of the knowledge of coronary anatomy.
- Outcome evaluation to be primarily performed on patients who are randomized AND undergo PCI.

Exclusion criteria

- Any previously known increase in liver enzymes (AST, ALT) or liver dysfunction at baseline.
- History of liver toxicity or myopathy from previous treatment with statins.
- LVEF < 30%.
- Renal insufficiency with creatinine > 2 mg/dL at baseline.
- Pregnancy.

This trial is planned to last 12 months, including randomization and patients' follow-up, as it is essential to assess AEs, safety and efficacy. This trial also includes routine blood tests monitoring to determine if any biomarkers for myocardial injury are above UNL.

8.4. Summary of the Selected Clinical Trials in Secondary Prevention After ACS

To provide a comprehensive overview of the current research landscape, the following section summarizes the previous exposed clinical trials. *Table 1* below complies essential information from each study, including the trial name, biochemical target, intervention strategies, mechanisms of action and comparators. This structured summary is intended to provide a clear and concise reference point for understanding the scope and focus of current clinical investigations in this field.

Table 1. *Ongoing Phase III Clinical Trials evaluating Anti-inflammatory, Antithrombotic and Lipid-lowering Agents.*

Target	Intervention	Study name	Mechanism of action	Comparator
Anti-inflammatory Agents				
IL-6	Ziltivekimab (human IgG1 mAb)	**ARTEMIS**	Inhibits pro-inflammatory signaling by neutralizing IL-6 ligand.	Placebo
NLRP3 inflammasome	Colchicine	**CADENCE**	Tubulin disruption and inhibition of NLRP3 inflammasome and neutrophil chemotaxis.	Placebo
Antithrombotic and Antiplatelet Agents				
P2Y12	AAs and/or Clopidogrel	**ADEN**	Inhibition of COX-1 and P2Y12, preventing activation of GPIIb/IIIa receptor, reducing platelet aggregation.	High-potency SAPT
	Clopidogrel	**TADCLOT**	Inhibition of P2Y12, preventing GPIIb/IIIa receptor from activating, decreasing platelet aggregation and activation.	Ticagrelor
	Vicagrel	**VCP1-III-01**	Blockage of P2Y12, activated via esterase-mediated hydrolysis, preventing platelet activation and aggregation.	Clopidogrel
GPIIb/IIIa receptor	Zalunfiban	**CELEBRATE**	Displacement of Mg^{2+} from the GPIIIa domain, inactivating the receptor and reducing fibrinogen binding and therefore platelet aggregation.	Placebo

Factor Xa	Apixaban	**POTAMI**	Inhibition of FXa, reducing thrombin generation, consequent conversion of fibrinogen to fibrin, thus halting the development of blood clot.	Placebo
Factor XIa	Milvexian	**LIBREXIA-ACS**	Inhibition of FXIa, attenuating thrombin generation by inhibiting FXa-FVIIIa complex	Placebo
NOX2/ IL-6/STAT3	Xuesaitong	**2024XLA098-3**	Inhibition of NF-κB, JAK2/STAT3 pathways and down-regulation of NLRP3 inflammasome. ADP-receptor antagonist and inhibitor of COX, reducing thrombus formation	Placebo
Lipid-lowering Therapies				
HMG-CoA	Atorvastatin	**2009-012850-19**	Inhibition of HMG-CoA reductase, reducing cholesterol and APO-B100 molecule synthesis, such as VLDL and LDL.	Rosuvastatin
PCSK9	LIB003 (Lerodalcibep) Injectable	**LIBerate-HR**	Bindinf of circulating PCSK9 and prevention from binding to LDLRs, enhancing their recycling and clearance of LDL-C.	Placebo
	MK-0616 (Enlicitide Decanoate) Oral	**CORALreef**	Binding of circulating PCSK9 and prevents binding to LDLRs, preserving LDLR recycling and enhancing LDL-C clearance.	Placebo
CETP	Obicetrapib	**PREVAIL**	Inhibition of CETP, leading to cholesterol-poor LDL, resulting in elevated HDL-C and reduced LDL-C levels in the bloodstream.	Placebo
PCSK9 mRNA	Inclisiran sodium	**VICTORION-2 PREVENT**	siRNA targeting PCSK9 mRNA in hepatocytes, degrading it and preventing its translation, which leads to increased LDLR availability and reduced LDL-C.	Placebo
ATP-citrate liase	Bempedoic acid	**HACOL-ACS**	Inhibition of ATP-citrate liase, reducing acetyl-CoA levels and cholesterol synthesis, which activates SREBP-2 and enhances LDL-C clearance.	-
NPC1L1 and CETP	Ezetimibe and Obicetrapib	**REMBRANDT**	Inhibition of NPC1L1 protein, interfering with endocytosis of the NPC1L1-cholesterol complex increasing LDLR expression and LDL-C clearance. Inhibition of CETP resulting in reduced LDL-C and elevated HDL-C levels.	Placebo

NPC1L1 and HMG-CoA reductase	Rosuvastatin	**REMEDY**	Inhibition of HMG-CoA reductase and reduction of hepatic cholesterol synthesis, triggering enhanced LDLR expression and LDL-C clearance. Blockage of NPC1L1 transporter reducing cholesterol delivery to the liver and stimulating LDLR expression.	Ezetimibe and Atorvastatin

9. Discussion Based on Relevant Findings from Clinical Trials

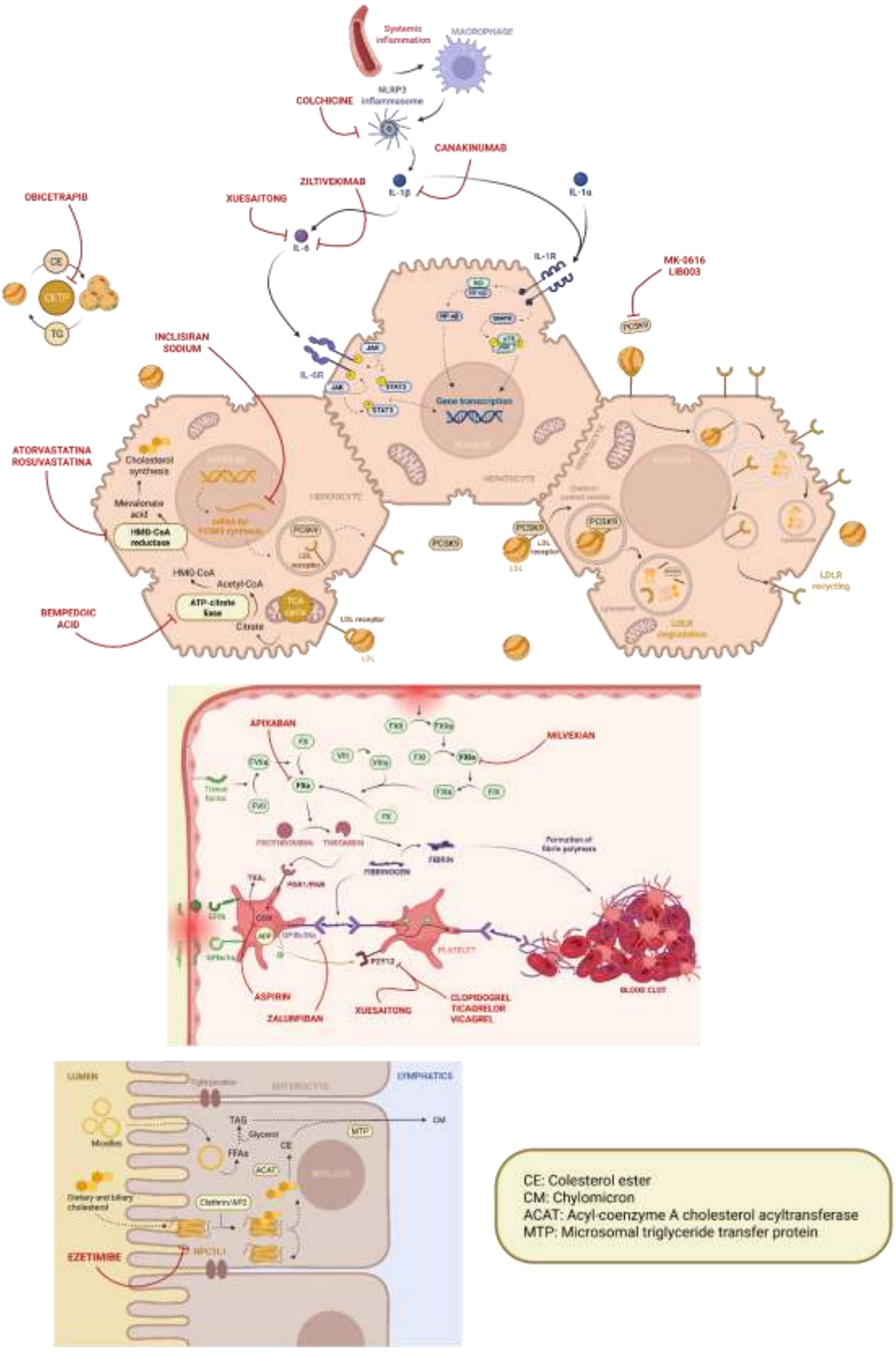

Figure 8. *Integrative visual summary of the mechanisms of action of each therapy of the ongoing phase III clinical trials previously reviewed in this work involving inflammation, thrombosis and lipids.*

To fully understand the rationale behind the current secondary prevention strategies in post-ACS patients prior observed, it is critical to examine how each therapeutic agent mechanistically targets key elements in signaling pathways that play an essential role in the pathogenesis of atherosclerosis. *Figure 8* provides a visual abstract of the mechanism of action of the agents included in the meta-analysis in each tissue, categorized into anti-inflammatory, antiplatelet/antithrombotic and lipid-lowering therapies.

Figure 8 is organized along the atherothrombotic cascade, the inflammation cascade and the absorption of dietary and biliary lipids, highlighting therapeutic targets at different stages – from inflammation inhibition and lipid degradation to thrombus formation and coagulation cascade repression:

- **Anti-inflammatory agents** such as Canakinumab, Colchicine, Ziltivekimab and, in some measure, Xuesaitong, target the innate immune activation that contributes to plaque destabilization. By dampening inflammatory cytokine production (IL-1, IL-6), their aim is to reduce residual inflammatory risk.
- **Antiplatelet and antithrombin agents** like Apixaban, Milvexian, Vicagrel and Clopidogrel among others, intervene at the terminal thrombotic stage, reducing the likelihood of clot propagation following plaque rupture. These agents often present a tarde-off between efficacy and bleeding risk.
- **Lipid-lowering agents** such as PCSK9 inhibitors (MK-0616, LIB003 and in a certain way, Inclisiran sodium), Obicetrapib, Bempedoic acid, statins and Ezetimibe act upstream by reducing circulating LDL-C and Lp(a), thereby mitigating foam cell formation and plaque progression.

By juxtaposing these agents in a mechanistic framework, the figure underscores both complementarity and potential synergy among therapies. For example, combining LDL-C reduction with inflammation control may more effectively stabilize plaques and suppress recurrent events than either strategy alone, a hypothesis being explored in combination clinical trials (57).

Notably, differences in trial populations, including factors such as age, sex, comorbidities and genetic background, combined with varying follow-up durations and endpoint definitions introduce significant heterogeneity, complicating direct comparisons across studies and potentially influencing the interpretation of efficacy and safety outcomes.

With each medication's mechanism of action comes a different target population, reflecting the distinct pathophysiological mechanisms these treatments address within cardiovascular disease.

Anti-inflammatory therapies, exemplified by the **ARTEMIS** and **CADENCE** trials (studying Ziltivekimab and Colchicine, respectively), target patients with a recent CV event who exhibit residual inflammatory risk, as identified by elevated hsCRP levels or coexisting comorbidities such as diabetes, chronic kidney disease, or prior vascular disease. These patients are typically clinically stable after the index event yet remain at increased risk of future CV events due to persistent inflammation. This target population is distinct from the boarder lipid-lowering

populations and from the patients in antithrombotic trials, who are generally in a more acute phase. ARTEMIS adopts a broader approach, targeting patients immediately post-AMI with known risk factors, and aims to reduce long-term CV events through systemic IL-6 inhibition. In contrast, CADENCE focuses specifically on patients with diabetes and a recent ischemic event, using advanced imaging (18F-FDG PET-CT) to quantify local vascular inflammation and correlate it with biomarker changes and therapeutic response. Additionally, Colchicine's oral administration and lower cost suggest greater accessibility and ease of integration into routine clinical practice, especially in resource-limited settings. This diverges with Ziltivekimab, which represents a novel, biologic-based precision therapy requiring subcutaneous administration and more intensive monitoring, potentially limiting its widespread use but offering a highly targeted approach to modulating inflammation in selected high-risk patients.

As opposed, **antithrombotic therapies** primarily target patients in the acute or early post-ACS phase, undergoing PCI, who are at elevated thrombotic risk. A common feature among these trials is the recognition of genetic variability in drug metabolism, especially CYP2C19 polymorphisms, which influence individual responses to Clopidogrel. The **ADEN** and **TADCLOT** trials both address this genetic variability by exploring alternatives in Clopidogrel regimens. Meanwhile, the **VCP1-III-01** trial investigates Vicagrel, a novel agent designed to overcome Clopidogrel resistance by opting for a different drug metabolism which does not involve CYP2C19 enzymes, avoiding the step that causes said resistance, aiming to reduce MACE without increasing bleeding risk. Additional trials, such as **POTAMI** and **LIBREXIA-ACS**, explore the role of direct oral anticoagulants (DOACs) – specifically Apixaban and Milvexian, respectively – post-ACS, to prevent thrombus formation and improve long-term outcomes. These studies focus on interrupting the coagulation cascades upstream from thrombin and fibrin generation and clot formation, potentially offering more targeted anticoagulation strategies with an improved safety profile. Where these trials diverge most is in their clinical settings. The POTAMI study focuses on patients with severe LV dysfunction post-anterior STEMI, testing low-dose Apixaban, a FXa inhibitor, for thrombus prevention, while LIBREXIA-ACS investigates a novel anticoagulant pathway via FXIa inhibition. On the other hand, the **CELEBRATE** trial uniquely evaluates the pre-hospital administration of Zalunfiban by paramedics, targeting platelet aggregation in the earliest stages of STEMI to optimize outcomes before PCI, normally including patients before written consent can be obtained and, instead, oral ICF is given. In contrast, the **Xuesaitong** trial overviews the effects of a traditional Chinese medicine extract post-PCI, assessing its advantages in platelet inhibition within population-specific context. These differences underscore the diversity in trial design, spanning novel drug mechanisms, personalized medicine, and pre-hospital interventions with various approaches, in order to guide research to the point where every possible finding is explored to aid in the cure of ACS.

Lipid-lowering therapies target a broad population of patients with elevated LDL-C levels or established ACVD, regardless of recent events. This group encompasses both primary and secondary prevention populations, including individuals with familial hypercholesterolemia, diabetes, or persistently high LDL-C despite statin therapy. However, each trial within this

category addresses a specific clinical question. For example, the **2009-012850-19** trial compares Atorvastatin and Rosuvastatin in reducing CRP levels in ACS patients undergoing PCI, notably excluding diabetics and patients with dyslipidemia. The **LIBerate-HR** trial investigates the efficacy of a monthly injectable agent (LIB003) for statin intolerant individuals, whereas **CORALreef** evaluates the novel oral PCSK9 inhibitor Enlicitide Decanoate for long-term risk reduction, including said diabetic patients if opportunity arises, as well as patients with stable lipid-lowering therapy prescribed. Moreover, in need of new biochemical targets, **PREVAIL** focuses on the administration of a CETP inhibitor in patients with persistent LDL-C elevation despite already receiving optimal lipid-lowering therapy, testing Obicetrapib as a novel drug. The **VICTORION-2 PREVENT** trial studies twice-yearly Inclisiran sodium administration in stable post-ACS patients already on high-intensity statin therapy. In contrast, the **HACOL-ACS** study addresses the early post-MI and post-PCI phase with Bempedoic acid in lipid-lowering therapy-naïve patients. While **REMBRANDT** uses imaging to assess plaque regression with a combination of Obicetrapib and Ezetimibe, being one of those trials that combines two targets but, in this case, of the same therapy, in patients on medication to lower cholesterol and whom have evidence of plaque in their coronary arteries. Finally, the **REMEDY** trial is a comparative study evaluating the efficacy of statin versus non-statin therapies in preventing peri-procedural MI during PCI. Unlike the inflammatory trials, which focus on immediate post-ACS secondary prevention, REMEDY requires patients to have stable CAD or be in a stable phase following ACS, highlighting its different focus on a more chronic disease stage.

Although none of these trials share exactly the same target population, a key resemblance across all studies is the exclusion of individuals who are currently enrolled in other interventional trials. This precaution ensures that experimental medications are not combined or crossed, which could lead to potential safety concerns, unanticipated adverse events, or confounding results that might compromise the validity of the findings, which would mean the study could have been conducted in vain.

Based on the findings of this work, it is expected that future research will increasingly integrate therapeutic strategies targeting multiple interconnected pathways such as the ones discussed previously (inflammation, thrombosis and lipid dysregulation), in order to optimize regimens for patients recovering from ACS. By combining therapies that modulate these distinct but overlapping pathways, future treatment regimens could be tailored more precisely to individual patient risk profiles. This multi-targeted strategy holds promise for improving not only CV outcomes but also overall quality of life for patients who have experienced an ACS event.

10.Conclusions and future directions

This work set out an integrative analysis of the complex pathophysiological mechanisms of atherosclerosis in the context of CAS, with a particular focus on secondary prevention strategies. By systematically exploring the interplay among inflammation, thrombosis, and

lipid metabolism, this thesis has underscored how these interrelated processes not only initiate but also perpetuate plaque formation, destabilization, and eventual rupture, which are hallmarks of the clinical manifestations of ACS. Chronic vascular inflammation, characterized by the activation of innate and adaptive immune pathways, sets the stage for endothelial dysfunction and the expression of pro-thrombotic mediators, which fosters platelet aggregation and ultimately occlusive thrombus formation. Simultaneously, dysregulated lipid metabolism fuels further inflammation and contributes to the necrotic core of plaques, exacerbating their vulnerability to rupture. By synthesizing current evidence on the molecular and cellular processes that drive plaque formation, destabilization, and rupture, this work highlights how chronic inflammation, pro-thrombotic states, and lipid dysregulation converge to influence the clinical manifestations of ACS and contribute to ischemic complications.

Building on this mechanistic foundation, this study then undertook a systematic search and meta-analysis of ongoing Phase III interventional clinical trials targeting these critical pathways in the setting of secondary prevention following ACS. This analysis revealed a dynamic landscape of therapeutic strategies, including anti-inflammatory agents, such as Ziltivekimab and Colchicine which target specific molecules like IL-6 and the NLRP3 inflammasome respectively, which were used for the first time as secondary prevention in ACS patients in the environment of inflammation. Novel antithrombotic approaches were found that aim to complement established regimens like DAPT or best actual treatments in order to reduce possible bleeding risk and overcome difficulties related to the metabolism of specific medication, as well as new agents like FX and FXI inhibitors aiming to halt coagulation cascade effects. In addition, lipid-lowering therapies that offer new administration routes to perfect the effect required and offer improved results are in the process of investigation. These trials underscore the growing emphasis on personalized and mechanism-based treatments that address the residual CV risk that persists despite standard care on patients with a previous ACS event. Trials vary widely in terms of eligibility criteria, primary and secondary endpoints, duration of follow-up, and therapeutic objectives. This diversity reflects the need to address the heterogeneity of the disease itself, encompassing different stages of the atherosclerotic process such as acute and/or stable phases, as well as patients-specific risk factors and comorbidities. Such variation emphasizes the importance of individualized approaches to both research design and clinical practice to optimize outcomes for patients with distinct risk profiles and disease presentations.

Wile many ongoing trials adopt a classical interventional approach, focusing on standard protocols, others, such as the CELEBRATE and Xuesaitong trials, are exploring innovative or population-specific strategies. These include pre-hospital interventions and the incorporation of traditional medicines, reflecting a broader effort to personalize treatment regimens by integrating cultural practices and early-phase management into secondary prevention of ACS.

Overall, this work highlights that an integrated approach which addresses inflammation, thrombosis, and lipid metabolism simultaneously – holds the potential to significantly reduce

recurrent CV events and improve long-term outcomes in ACS patients. Continued research is needed to refine therapeutic strategies, optimize risk stratification, and translate these insights into personalized clinical practice. Future directions should focus on leveraging advances in molecular biology and precision medicine to develop more effective, targeted secondary prevention therapies that can better address the multifactorial nature of atherosclerosis in ACS. Much has been learned about the characteristics of applying such medications for secondary prevention analyzed in this work in patients at high risk of recurrent CV event, but further investigation is essential to enhance survival, improve quality of life, and optimize patient adherence and satisfaction with these therapies.

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