SCUOLA DI DOTTORATO
UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

Dipartimento di / Department of

Medicina e Chirurgia

Dottorato di Ricerca in / PhD program Sanità Pubblica Ciclo / Cycle 38°

Curriculum in (se presente / if it is) Fisiopatologia clinica e prevenzione delle malattie

IL-1 blockade in the acute phase of ST-segment elevation myocardial infarction: pathophysiological insights from Phase II, investigator initiated randomized controlled trials

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ANNO ACCADEMICO / ACADEMIC YEAR 2024/2025

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Synopsys

Background – Innate immunity and inflammation are known to play a role in the development and progression of cardiovascular disease. A growing body of evidence, both pre-clinical and clinical, has implicated the intracellular sensing protein NLRP3 (for NACHT, LRR and PYD domains-containing protein 3) and its downstream product interleukin-1β as key pathogenic role in the formation and progression of atherosclerosis, as well as in its acute complications, i.e. acute myocardial infarction (AMI), in ischemic and non-ischemic myocardial damage and progression to heart failure. IL-1β selective inhibitors are currently clinically available, although not approved for use in cardiovascular disease. Canakinumab, a human monoclonal antibody targeting IL-1β, was shown to be effective in reducing recurrent ischemic events when used in secondary prevention in patients with prior AMI. Small pilot studies using Anakinra, recombinant IL-1 receptor antagonist, in patients with ST-elevation myocardial infarction (STEMI) showed promising result in terms of safety as well as effectiveness in reducing acute inflammation. The same studies seemed to suggest a reduction in mid-term mortality and new onset heart failure among patients treated with Anakinra.

Objectives – The objectives of this doctoral project are confirming the effectiveness of acute IL-1β blockade in reducing the incidence of mortality and new onset heart failure after STEMI. Secondarily, we sought to understand the pathophysiology of heart failure after STEMI, and how it is impacted IL-1β modulation.

Methods – The present work is articulated in two parts. First, we performed a pooled analysis of 3 pilot investigator initiated randomized controlled trials comparing treatment with Anakinra vs placebo for 14 days after STEMI successfully treated with primary percutaneous coronary intervention (pPCI). Primary endpoint was the composite of all-cause death, new hospitalization for heart failure (HF) and new episodes of HF, defined as new signs and symptoms of HF requiring unplanned outpatient visits or titration of HF therapy. Secondary outcomes included reduction of inflammatory burden, estimated by high-sensitivity C-reactive protein (hsCRP) area under curve (AUC) for repeated measures obtained at baseline, at 72h and at 14 days, and experimental drug safety. Non-prespecified subgroup analysis were subsequently performed to identify subjects more likely to benefit from experimental treatment. The exploratory analyses included stratifying patients according to inflammatory status (i.e. hsCRP) on presentation, left ventricular dysfunction, previous diagnosis of diabetes, sex and race.

Secondarily, we designed a new phase II, mechanistic investigator initiated randomized controlled trial to assess the effects of 14 days of treatment with Anakinra 100 mg daily or matching placebo

on cardio-respiratory fitness (CRF), cardiac function and cardiac remodeling after STEMI successfully treated with pPCI. Primary endpoint for the trial is cardiorespiratory fitness, measured as peak O_2 consumption (pVO_2) measured during cardiopulmonary exercise testing (CPET) 6 weeks after STEMI. Secondary endpoints of interest include comprehensive CPET evaluation, change in CPET parameters from 6 weeks to 1 year after the STEMI, cardiac systolic and diastolic reserve assessed by concomitant stress echocardiogram, left ventricular remodeling assessed by cardiac magnetic resonance (CMR), as well as clinical endpoints including all-cause mortality and new onset heart failure 1 year after the event.

Results – The pooled analysis included 139 patients enrolled in 3 previously conducted randomized controlled trials, the Virginia Commonwealth University Anakinra Remodeling Trial (VCUART – clinicaltrials.org identifier NCT00789724; n=10), the VCUART2 (NCT00175018; n=30) and VCUART3 (NCT01950299, n=99). Eighty-four subjects were assigned to experimental treatment with Anakinra for 14 days after STEMI, while 55 received matching placebo. Anakinra was associated with a lower incidence of the primary endpoint at 1 year follow up (7 [8.2%] vs 16 [29.1%], log-rank p=0.007).). As expected, hsCRP-AUC was lower in the Anakinra arm as compared to placebo (75.48 [41.7–147.47] vs. 222.82 [117.22–399.28] mg day/L, $P < 0.001$). Injection site reactions were more common in the active treatment arm (19 [22.6%] vs. 3 [5.5%], p=0.016). No difference in the incidence of serious infections was noted (11 [13.1%] vs 7 [12.7%], p= 1.0)

There was no difference in the response to treatment in subgroups based on race, sex, diabetes status, left ventricular ejection fraction or inflammatory burden on presentation.

Enrollment and baseline evaluation has been completed for 56/84 of the projected total enrollment of the new mechanistic phase II trial aimed at assessing the impact of Anakinra on CRF after STEMI (VirginiaART4, NCT05177822). Median time from STEMI to CPET was 41 (interquartile range [IQR] 39-43) days. The majority of subjects was treated with renin-angiotensin-aldosterone blockers (80%) and beta-blockers (86%). Baseline median left ventricular ejection fraction (LVEF) was 55% [IQR 49-58%]. Peak VO_2 was 19.8 [16.5-23.2] $mL \cdot kg^{-1} \cdot min^{-1}$. Fifty-two of the 56 (93%) had a peak VO_2 <100% of predicted, with 46 of the 56 (82%) <80% of predicted – indicating high prevalence of significantly impaired CRF at 6 weeks after STEMI. Follow up is projected to be completed by the end of 2026.

Conclusions – Preliminary data from small pilot randomized controlled trials suggest that IL-1 blockade using Anakinra for 14 days after a STEMI may reduce mortality and new onset HF at 1 year of follow up, and the effects are consistent across multiple subgroups. The ongoing VirginiaART4

will hopefully confirm these findings and help elucidating the pathophysiological impact of IL-1 blockade on cardiac remodeling and reserve after STEMI. In the preliminary analysis of baseline CPET and stress echocardiogram at 6 weeks we report a high prevalence of significantly impaired CRF in the subacute phase after STEMI.

Introduction

Acute myocardial infarction – epidemiology, health burden and unmet needs

Despite tremendous advances in prevention and treatment, cardiovascular diseases remain the leading cause of mortality and disability worldwide, accounting for approximately 20 million deaths worldwide.(Martin et al., 2025) Ischemic heart disease accounts for approximately half of those deaths.("Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021," 2024; Martin et al., 2025) The most severe acute manifestation of ischemic heart disease is ST-segment elevation myocardial infarction (STEMI), in which the sudden obstruction of a main coronary artery branch causes myocardial ischemia and tissue death.(Pfeffer, 2002) In the absence of prompt treatment, STEMI-related mortality exceeds 25% in the acute setting.(Pfeffer, 2002) In the current era, thanks to advancements in acute management , improved systems of care and access to treatment as well as awareness campaigns for the public, STEMI mortality at 30 days is <10%. However, STEMI survivors face a significant burden of delayed morbidity and mortality. STEMI patients are in fact at significant risk of heart failure (HF), with contemporary case series showing between 15% and 30% of subjects developing heart failure in the first 1 to 2 years after the event.(Ritsinger et al., 2020; Savarese et al., 2023) These outcomes are observed even with the implementation of contemporary optimal treatment, which include prompt reperfusion (i.e. rapid re-opening of the occluded vessel), aggressive secondary prevention strategies including anti-platelet treatment as well as risk factors optimization and neurohormonal treatment.(Byrne et al., 2023; Rao et al., 2025) Each of these treatments provide an incremental benefit towards myocardial preservation and reduction of infarct size as well as prevention of progressive left ventricular dilation and dysfunction – once considered the hallmarks of HF after STEMI. Notably, almost half of individuals that develop HF do not display the stigmata of remodeling and resting left ventricular dysfunction.(Lenselink et al., 2024) Understanding the mechanisms by which HF ensues despite optimal treatment is a critical step in the search for novel interventions to reduce the incidence, burden, and mortality of HF after STEMI. This changing paradigm calls for a search beyond traditional targets to measure progression toward HF beyond prevention of adverse remodeling or preservation of LV ejection fraction (LVEF).

Inflammation and cardiac healing after STEMI

The tissue damage induced by acute ischemia in the setting of STEMI, comprising cellular necrosis and extracellular matrix damage, is a potent trigger for inflammation.(Timmers et al., 2012) Local generation of pro-inflammatory signals attracts inflammatory cells into the injured tissue. Neutrophils are recruited early in the infarcted myocardium, where they phagocytize injured and dying cells and actively degrade extracellular matrix.(Westman et al., 2016) Neutrophils secrete chemotactic factors recruiting other immune-effector cells, including monocytes and lymphocytes.(Zouggari et al., 2013) All these cells act in concert to foster myocardial healing and scar formation (**Figure 1**).(Frangogiannis, 2014) Notably however, persistent or excessive inflammation after myocardial infarction has been shown to propagate damage and cause secondary injury, promoting infarct expansion and adverse remodeling. (Ørn et al., 2012; Westman et al., 2016) In particular, a paracrine effect of pro-inflammatory mediators appears to enhance the damage at the infarct border zone, the myocardium that was rescued by reperfusion but remained in jeopardy.(Dinarello, 2018) At the same time, a systemic response ensues.

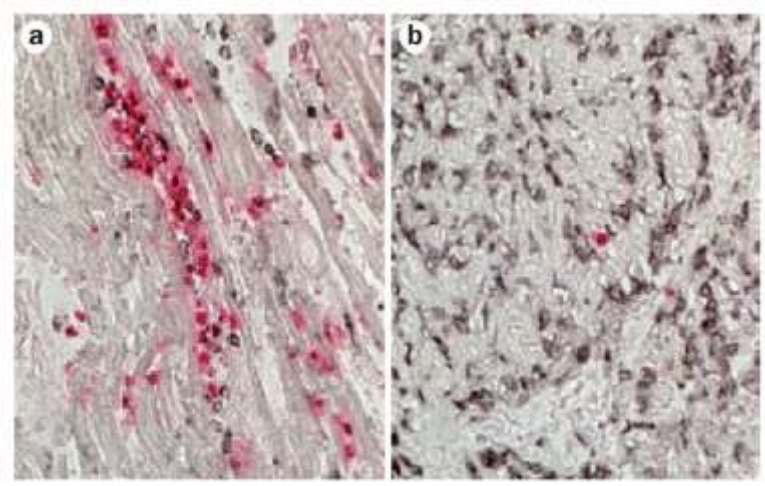

Figure 1 Inflammatory response and wound healing in acute myocardial infarction. Panel A shows necrotic cardiomyocytes and numerous myeloid cells in a canine model of myocardial infarction. Panel B shows appearance of fibrotic tissue with reduction of myeloid infiltrate. Adapted from Frangogiannis N, Nat Rev Cardiol 2014

The inflammasome, interleukin-1 and acute myocardial infarction

Ischemic damage to the heart causes extracellular release of intracellular components – which are sensed by surrounding cells as a damage signal. These cellular debris are collectively known as damage-associated molecular patterns (DAMPs). (Toldo & Abbate, 2018) DAMPs are sensed by specialized receptors, the most studied of which are Toll-like receptors.(Moresco et al., 2011) The activation of Toll-like receptors triggers a downstream cascade of genes transcription an d protein synthesis. Amongst the end-products of the activation, the NACHT, LRR and PYD domain-containing protein 3 (NLRP3) inflammasome has drawn significant attention in the setting of cardiovascular disease in general and acute myocardial infarction in particular.(Toldo & Abbate, 2018; Toldo et al., 2015) NLRP3 is an intracellular damage sensor, which is triggered by lysosomal alterations or

imbalances in intracellular K^+ concentrations (**Figure 2**). Intracellular K^+ is depleted in ischemic cells, mostly due to the combination of Na^+/K^+ failure and the purinergic P2X7 receptor, which causes K^+ efflux in response to extracellular adenosine-triphosphate (ATP) secreted by leukocytes and dying cells.(Toldo & Abbate, 2018; Toldo et al., 2018) Upon activation, it activates several effector proteins, including interleukin-1 (IL-1). IL-1 is activated by cleavage in is a signaling molecule, implicated in the development and amplification of inflammation at both a local and systemic inflammatory responses.(Dinarello, 2018) Two isoforms of IL-1 have been described, IL-1β which is an inducible molecule mostly expressed by myeloid cells upon activation, and IL-1α, which is constitutively expressed by mesenchymal cells, such as cardiomyocytes.(Dinarello, 2018) Both IL-1 isoforms exert their action by binding to its transmembrane protein receptors. Interelukin-1 receptor 1 (IL-1R1) is the ligand binding chain of IL-1 receptor. Upon binding with IL-1, IL-1R1 undergoes a conformational change which allows it to form a heterodimer with interleukin-1 receptor 3 (IL-1R3).(Dinarello, 2018) The approximation of the intracellular domains of IL-1R1 and IL-1R3 triggers signal transduction.(Abbate, Toldo, et al., 2020) This process is tightly regulated and balanced by anti-inflammatory mediators. Among those counter regulators, interleukin-1 receptor antagonist (IL-1RA) is a soluble molecule that binds to IL-1R1 and prevents dimerization with IL-1R3.(Abbate, Toldo, et al., 2020)

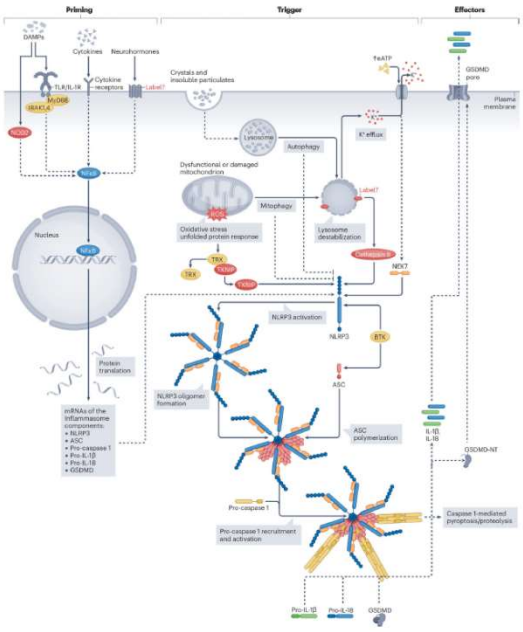

Figure 2 NLRP3 Inflammasome activation in cardiomyocytes. Damage associated molecular patterns liberated by ischemic damage causes synthesis of the inflammasome components. The inflammasome subsequently gets triggered by imbalances in intracellular K+ concentrations, mostly secondary to Na/K pump function impairment and P7X receptor activation by extracellular ATP liberated from dying cells. The activated inflammasome in turn activates effector cytokines of which IL-1 has shown to play a prominent role in cardiovascular disease. NLRP3 also activates gasdermins, membrane pore proteins that induce cellular death and may be responsible for initial infarct expansion. Adapted from Abbate A, Toldo S, Nat Rev Card 2024

The detrimental role of NLRP3 and IL-1β in acute myocardial infarction has been shown in several preclinical models. In particular, mice lacking the Nlrp3 gene were shown to have smaller infarct size compared to wild type mice after having been subject to cardiac ischemia-reperfusion, and similar results were obtained by selectively inhibiting the inflammasome and its components.(Liu et al., 2014; Mezzaroma et al., 2011) The mechanism of infarct expansion in the initial phase is mostly mediated by NLRP3-induced cell death, a mechanism called pyroptosis that involves activation and

membrane translocation of porin proteins called gasdermins.(Mezzaroma et al., 2011; Toldo & Abbate, 2024) The activation of NLRP3 however causes a surge of IL-1. Unopposed IL-1 activity during acute myocardial infarction mobilized myeloid cells from the bone marrow into the ischemic site and promotes pathological healing, favoring adverse remodeling and myocardial rupture in mice.(Abbate et al., 2011) This deleterious effects appear to be mostly mediated by IL-1α. Inhibition of IL-1 directly or at the receptor level consistently reduced infarct size, adverse cardiac remodeling and cardiac dysfunction after experimental myocardial infarction in pre-clinical models.(Abbate et al., 2011; Toldo et al., 2012)

Clinical evidence supporting inflammasome and IL-1 modulation in STEMI

Colchicine is a naturally occurring alkaloid that bind in a poorly reversible fashion tubulin, a cytoskeletal protein instrumental in the assembly of NLRP3 inflammasome.(Leung et al., 2015) In an early phase II randomized controlled study, 151 subjects presenting with STEMI within 12 hours from symptoms onset and successfully treated with primary percutaneous coronary intervention (pPCI) were randomized to receive a weight adjusted dose of colchicine vs matched placebo for 5 days.(Deftereos et al., 2015) Subjects randomized to the active treatment arm had smaller infarct size measured as area under the curve (AUC) of biomarkers of myocardial necrosis and as visualized on cardiac magnetic resonance (CMR).(Deftereos et al., 2015) A second trial using colchicine in the setting of acute myocardial infarction – the Low Dose Colchicine Myocardial Infarction (LoDoCo-MI) trial – randomized 237 subjects within 7 days from an acute myocardial infarction to receive colchicine 0.5 mg daily or matching placebo for 30 days.(Hennessy et al., 2019) Colchicine did not reduce 30 days inflammatory markers more than placebo.(Hennessy et al., 2019) Although not powered for clinical events, the trial did show a significant reduction in all-cause rehospitalization associated with active treatment.(Hennessy et al., 2019) Anakinra is a recombinant IL-RA, able therefore to directly inhibit signal transduction downstream of IL-1. In the MRC-ILA Heart trial, investigators randomized 182 subjects presenting with small, non-ST elevation myocardial infarction within 48h from symptoms onset to receive either anakinra 100 mg or matching placebo daily for 14 days.(Morton et al., 2015) Anakinra was successful in reducing C-reactive protein (CRP) – a biomarker of active inflammation – at day 7, but failed to improve clinical outcomes.(Morton et al., 2015) Interleukin 6 (IL-6) is synthesized by immune and non-immune cells mostly in response to IL-1β, although other activation pathways playing a lesser role have been described.(Grebenciucova & VanHaerents, 2023) Therefore, IL-6 is broadly considered a downstream effector of IL-1 in the inflammatory cascade. In the Assessing the effects of anti-IL-6 treatment in myocardial infarction

(ASSAIL-MI) 199 STEMI treated patients with pPCI were randomized to receive a single infusion of Tocilizumab – a recombinant antibody targeted to IL-6 – or placebo within 6h from presentation.(Broch et al., 2021) Myocardial salvage index, i.e. the ratio of the non-infarcted border zone over the total area at risk, and microvascular obstruction were significantly improved on CMR obtained 3-7 days after the intervention.(Broch et al., 2021) No difference in overall infarct size nor in clinical events at 6 months was observed.(Broch et al., 2021) Table 1 summarizes the above mentioned trials.

Non-IL-1 based anti-inflammatory and immunomodulatory strategies in STEMI

Other non-inflammasome, non-IL-1-based immune-modulatory strategies have been tested in the setting of STEMI.(Waksman et al., 2024) Cyclosporin A (CsA) is an immune modulator that predominantly inhibits T lymphocytes activation via preventing activation of transcription factor Nuclear Factor of Activated T-cells.(Patocka et al., 2021) Of note, CsA is also a potent inhibitor of the mitochondrial transition pore, a protein complex involved in cellular apoptosis, likely a molecular mediator of ischemia reperfusion damage. CsA therefore appears to be a particularly attractive molecule in the setting of acute myocardial infarction given the dual role – immunomodulatory and directly anti-apoptotic. While an early proof of principle RCT including 58 subjects with STEMI showed a significant reduction in infarct size amongst patients treated with CsA prior to revascularization,(Piot et al., 2008) the subsequent Cyclosporine before PCI in patients with Acute Myocardial Infarction (CIRCUS) failed to prove effectiveness of CsA in improving mortality, incident HF or adverse LV remodeling in patients with STEMI after successful reperfusion.(Cung et al., 2015) Methotrexate (MTX) competitively inhibits dihydrofolate reductase (DHFR), effectively interfering with thymidine synthesis hence with mitosis in rapidly dividing cells – such as activated immune cells.(Cronstein, 1997) MTX is highly effective in treating auto-immune and inflammatory conditions, including rheumatoid arthritis and psoriatic arthritis, and was shown in observational studies to reduce cardiovascular mortality among patients with autoimmune diseases.(Westlake et al., 2010) In the MethotrexaTE THerapy in ST-Segment Elevation MYocardial InfarctionS (THETYS) trial, 84 patients presenting with STEMI were randomized 1:1 to receive either MTX at the dose of 0.05 mg/kg prior to PCI followed by 0.05 mg/kg/h for 6 hours after reperfusion or matching placebo.(Moreira et al., 2017) At 3 months of follow up, no clinical difference was noted between the two groups, but the active treatment arm had a trend towards lower LVEF.(Moreira et al., 2017) Finally, glucocorticoids exert a pleiotropic anti-inflammatory and immunomodulatory effect by inhibiting the prostaglandin and leukotrienes pathways as well as by altering the gene expression in

a variety of immune and inflammatory cells.(Stortz et al., 2017; Wang et al., 2021) In the recent Prehospital Pulse-Dose Glucocorticoid in ST-Segment Elevation Myocardial Infarction (PULSE-MI), 530 subjects presenting with STEMI were randomized 1:1 to receive a single dose of 250 mg of methylprednisolone or matching placebo in the pre-hospital setting.(Madsen et al., 2024) While acutely patients in the active treatment arm had smaller infarct sizes, less microvascular obstruction and higher LVEF, these benefits were not sustained at 3 months follow up. No difference in clinical endpoints was noted.(Madsen et al., 2024)

The Virginia Commonwealth University – Anakinra Remodeling Trials (VCU-ARTs)

Our group has conducted three separate, sequential trials of IL-1 inhibition in the setting of STEMI, including a total of 139 patients.(Abbate et al., 2010; Abbate, Trankle, et al., 2020; Abbate et al., 2013) In each study, patients with successfully reperfused STEMI within 12h from presentation were randomized to receive Anakinra or matching placebo for 14 days. Treatment with Anakinra was well tolerated and associated with significant reduction of inflammatory markers.

Table 1 Summary of prior randomized controlled trials not part of the Anakinra Remodeling Trials initiative concerned with inflammasome-IL-1 pathway modulation in acute myocardial infarction. STEMI, ST-elevation myocardial infarction; pPCI, primary PCI; CRP, C-reactive protein; AUC, area under curve; CK-MB, creatin kinase-MB isoform.

Trial	n	Inclusion criteria	Intervention	Summary of results
Deftereos et al, 2015 (Deftereos et al., 2015)	151	STEMI within 12 hours from symptoms onset and successful pPCI	Colchicine 2 mg loading then dose weight adjusted Colchicine (0.5 mg qd if <60kg, bid if ≥60kg) for 5 days or matched placebo	Active treatment was associated to lower AUC for CK-MB. In the CMR substudy (n=60) colchicine associated with smaller infarct size.
LoDoCo-MI, 2019 (Hennessy et al., 2019)	237	Type 1 myocardial infarction within 7 days from presentation	Colchicine 0.5 mg daily for 30 days or matching placebo.	Colchicine was not effective in reducing CRP at follow up. 30-days rehospitalizations were lower in the active treatment arm.
MRC-ILA Heart, 2015 (Morton et al., 2015)	182	Non-ST elevation myocardial infarction<48h from symptoms onset	Anakinra 100 mg daily for 14 days or matching placebo.	Anakinra was effective in reducing CRP. No significant impact on clinical events at 1 year – reportedly possible numerical rebound (nonsignificant).
ASSAIL-MI, 2021 (Broch et al., 2021)	199	STEMI after successful pPCI, within 6 hours from presentation	Tocilizumab 280 mg single i.v. infusion or matching placebo	Active treatment arm associated with increased myocardial salvage index and reduced microvascular obstruction.

The first of the Virginia Commonwealth University Anakinra Remodeling Trial (VCU-ART) enrolled 10 subjects, randomized 1:1 to either anakinra or placebo for 14 days, with follow up. The primary endpoint was difference in cardiac remodeling indices between the two treatment arms. Cardiac remodeling was assessed in terms of left ventricular volumes by CMR. Treatment with Anakinra was associated to lower increase in left ventricular end systolic volume index compared to placebo (+2 [interquartile range – IQR – +1; +11] ml/m^2 in the placebo group, -3.2 [IQR -4.5; -1.6] ml/m^2, p=0.033) between presentation and 3 months.(Abbate et al., 2010) The VCU-ART2 study attempted to reproduce the first trial results in a larger population including 30 subjects. Anakinra was effective in blunting the inflammatory response caused by STEMI, but no differences in terms of cardiac remodeling indices was detected between the two arms.(Abbate et al., 2013) Notably, a numerical difference in new onset HF was detected at 12 weeks after STEMI between the active treatment arm and the placebo arm (4 vs 1, p=0.13).(Abbate et al., 2013) Finally, the VCU-ART3 randomized 99 STEMI patients 1:1:1 to receive placebo, Anakinra 100 mg bid or 100 mg qd for 14 days.(Abbate, Trankle, et al., 2020) Inflammatory response, measured as high sensitivity CRP AUC, was significantly reduced by Anakinra compared to placebo (67 vs 214, p<0.0001), with no significant difference between the two Anakinra dosing regimens. No difference between the three groups was detected in terms of remodeling indices. Notably, patients treated with Anakinra had a significantly lower incidence of death or new HF at 12 months of follow up (9.4% vs 25.7%, p=0.046).(Abbate, Trankle, et al., 2020) None of the trial showed impact of treatment with Anakinra on recurrent ischemic events. **Table 2** summarizes the main findings of the VCU-ART trials.

Table 2 Summary of findings of the Virginia Commonwealth University Anakinra Remodeling Trials. VCU-ART, Virginia Commonwealth University Remodeling Trial; CRP, C-reactive protein; HF, heart failure; STEMI, ST-elevation myocardial infarction.

Trial	n	Inclusion	Intervention	Summary of results
VCU-ART, 2010 (Abbate et al., 2010)	10	STEMI after reperfusion <12 from symptoms	Anakinra 100 mg qd or matching placebo for 14 days	At 3 months of follow up treatment with Anakinra was associated with lower left ventricular systolic volumes
VCU-ART2, 2013 (Abbate et al., 2013)	30	STEMI after reperfusion <12 from symptoms	Anakinra 100 mg qd or matching placebo for 14 days	Anakinra associated with lower CRP but no effect on cardiac remodeling. Trend towards lower incidence of HF events at 3

				months follow up, no effect on recurrent ischemic events
VCU-ART3, 2020 (Abbate, Trankle, et al., 2020)	99	STEMI after reperfusion <12 from symptoms	Anakinra 100 mg qd or bid or matching placebo for 14 days	Anakinra was effective in reducing CRP. Active treatment was associated to lower incidence of HF events at 12 months, no difference in recurrent ischemic events

Notably, the VCU-ART trials did consistently suggest a reduction in new onset HF despite no change in resting left ventricular morphology and volumes, i.e. without the hypothesized prevention of adverse remodeling. Of note, a large number of individuals with HF have normal left ventricular function, measured as left ventricular ejection fraction (LVEF), and volumes.(Cannata & McDonagh, 2025) Therefore, additional cardiac abnormalities are responsible for the symptoms.(Cannata & McDonagh, 2025) Indeed, exercise testing has been included in HF diagnostic algorithms , to uncover limitations in cardiac reserve consistent with HF.(Pieske et al., 2019) Impaired cardiac reserve can include either reduced diastolic reserve, generally assessed by Doppler echocardiography, or reduced systolic reserve, i.e. inability to adequately augment cardiac output during exercise.(Del Buono et al., 2019) Very limited data exists on the impact of STEMI on cardiac reserve. In a study of 84 patients with STEMI, 54% had impaired exercise capacity (defined as peak oxygen consumption [pVO_2], an indirect measurement of cardiac output, <84% predicted) and impaired cardiac reserve correlated with serum levels natriuretic peptides.(Turski et al., 2019) In a study of 225 patients with recent myocardial infarction (38% STEMI), diastolic parameters were the strongest independent predictors of exercise capacity.(Fontes-Carvalho et al., 2015) The impact of inflammation and inflammatory modulation on cardiac reserve and exercise tolerance after STEMI have not been studied yet.

Hypotheses and objectives

Hypotheses and objectives of this doctoral work were:

1. *Primary analysis*: to confirm the beneficial effects of IL-1 blockade on post-STEMI HF prevention via a pooled analysis of extended follow up (up to 12 months) of the previously published VCU-ART trials. The pooled analysis will also include non-prespecified, hypothesis generating subgroup analyses aimed at identifying high risk subpopulation who may potentially derive a higher benefit from immune modulation after STEMI. A pooled analysis will possibly enhance the sensitivity to smaller effect sizes as compared to the primary trials, which individually include smaller treated population. The non-prespecified subgroup analyses will assist in identifying, if existing, subgroups more likely to benefit from IL-1 blockade. The latter point will aid in designing further investigator-initiated trial targeting the `super responders` subgroups.
2. *Mechanistic objective*: design and begin to implement a novel randomized controlled trial of IL-1 blockade in STEMI that incorporates assessment of non-remodeling mechanisms of HF development in order to elucidate the impact of inflammation modulation on post-STEMI HF pathophysiology. This builds on the observations made in the previous VCU-ART trials that patients in the active treatment arm did not differ in terms of resting cardiac volume or function as compared to patients randomized to the placebo arm.

Materials and methods

Trials' design and study population

The pooled analysis included individuals enrolled in the VCU-ART, VCU-ART2 and VCU-ART3 studies.(Abbate et al., 2010; Abbate, Trankle, et al., 2020; Abbate et al., 2013) The study designs are registered on clinicaltrials.org (NCT00789724, NCT01175018, and NCT01950299, respectively). As mentioned above, the three studies were intentionally designed to have overlapping inclusion and exclusion criteria. Patients were eligible to participate if presenting with STEMI, defined as acute onset chest pain and new or presumed new ST-segment elevation of at least 1 mm on two or more anatomically contiguous leads on an electrocardiogram, within 12h of symptoms onset, and enrolled within 12h of reperfusion. Exclusion criteria included resuscitated cardiac arrest, hemodynamic instability/cardiogenic shock, unsuccessful PCI, history of prior left ventricular dysfunction or congestive heart failure, severe mitral or aortic valvular heart disease, pregnancy, chronic infections, autoimmune or autoinflammatory conditions and active malignancy. Contraindications to the study drug, including allergy and advanced chronic kidney disease with estimated glomerular filtration rate (eGFR) ≤30 ml/min/m^2 were also exclusion criteria. VCU-ART and VCU-ART 2 included CMR, so individuals with contraindications to magnetic resonance imaging (including claustrophobia, ferromagnetic implants, allergy to contrast material) were excluded in those trials. **Table 3** reports the list of shared inclusion and exclusion criteria.

Table 3 List of inclusion and inclusion criteria for the Virginia Commonwealth University Anakinra Remodeling Trials. AHA, American Heart Association; ACC, American College of Cardiology; ECG, electrocardiogram; INR, international normalized ratio; NSAID, non-steroidal anti-inflammatory drug; PCI, percutaneous coronary intervention

Inclusion criteria	Exclusion criteria
Age >18 years	Inability to provide informed consent
New or presumably new ST elevation on ECG	Late presentation (>12h)
Successful primary PCI	Unsuccessful reperfusion
	Hemodynamic instability, shock
	Prior Q-waves myocardial infarction
	Known history of heart failure, AHA/ACC class C or D
	Severe left ventricular dysfunction
	Severe mitral or aortic valvular heart disease
	Chronic inflammatory or autoimmune disease

	Chronic infection
	Malignancy
	Any comorbidity precluding study completion
	Pregnancy
	Recent (<14 days) use of anti-inflammaory, non-NSAIDS drugs
	Coagulopathy (INR>1.5, platelets<50,000 mm^3)

A fourth trial – namely Virginia Anakinra Remodeling Trial 4 (VA-ART4) was designed and began enrollment in late 2022. Inclusion and exclusion criteria are consistent with the VCU-ART trials. VA-ART4 will include CMR among the study procedure, so contraindications to CMR are effectively contraindications to take part in the study. VA-ART4 is an investigator initiated, double blind, multicentric randomized controlled trial, with enrollment taking place both at Virginia Commonwealth University Medica Center in Richmond, Virginia and the University of Virginia Medical Center in Charlottesville, Virginia.

Investigational treatment

VCU-ART and VCU-ART2 randomly assigned participants to receive daily Anakinra 100 mg subcutaneously (Kineret, Swedish Orphan Biovitrum, Stockholm, Sweden) or matching normal saline for 14 days. VCU-ART3 randomized patients 1:1:1 to receive Anakinra 100 mg qd, Anakinra 100 mg bid or placebo for 14 days. Given that no difference in terms of inflammation reduction measured by high sensitivity CRP (hsCRP) reduction compared to placebo was detected between the two Anakinra arms in VCU-ART3,(Abbate, Trankle, et al., 2020) the two doses will be combined in a single active treatment arm.

Based on prior studies, VA-ART4 will randomize patients to receive either Anakinra 100 mg qd or matching placebo for 14 days.

Heart failure outcomes, safety endpoints and event adjudication

The clinical outcomes of interest for the pooled analysis include a composite of all-cause mortality and new-onset HF as well as a composite of all-cause mortality and HF-related hospitalization. HF specific endpoints are defined according to international consensus definitions:(Eapen et al., 2012)

- New onset HF (not hospitalized) – new or worsening shortness of breath and at least one between:

- Physical signs of HF, defined as two or more of:
  - Peripheral edema;
  - Crackles or rales on lung auscultation and tachypnea;
  - Jugular vein distension;
  - Hepatojugular reflex;
  - Rapid weight gain;
  - S3 gallop;
  - Abdominal distension or new ascites;
  - Radiographic evidence of pulmonary congestion;
  - Pulmonary artery wedge pressure >18 mmHg;
  - Cardiac index <2.2 l/min/m^2;
- Need for new or additional HF therapy including
  - New need or need to increase oral diuretic treatment, or need for intravenous diuretics;
  - Inotropes or vasodilators; ultrafiltration for diuretic resistant HF decompensation.

- Hospitalization for HF defined as a hospitalization with the primary diagnosis being HF as well as the above-mentioned criteria.

Safety events included injection site reactions and severe infections, the latter including infections requiring antibiotic treatment or hospitalization.

Patients had in person study visits at 2 and 12 weeks in all the VCU-ART studies. In addition, VCU-ART3 subjects had in-person study visits at 6 and 12 months. In VCU-ART and VCU-ART2 clinical events during the conduct of the studies (up until week 12) were assessed by a study investigator before unblinding. Subsequent events were adjudicated by two independent cardiologists blinded to patients' treatment allocation and inflammatory markers kinetics, based on documentation collected via chart review. For VCU-ART3 clinical events were adjudicated by an independent committee blinded to treatment allocation and not involved in the conduction of the study, using pre-specified criteria. Events for all the studies were censored at 12 months.

Inflammatory biomarkers after STEMI

hsCRP was measured at baseline, 72h and 14 days in all VCU-ART trials. hsCRP-AUC after 14 days was estimated using a linear trapezoidal method for each subject – as a measure of overall inflammatory burden.

Virginia Anakinra Remodeling Trial 4 procedures and endpoint

In order to assess for differences in cardiac reserve between STEMI patients treated with Anakinra vs placebo, the primary endpoint for the study will be peak O_2 consumption (pVO_2) during cardiopulmonary exercise testing (CPET) expressed as a percentage of maximum predicted per age and body size according to the Wasserman's equation.(Arena et al., 2009) Secondary endpoints will include other CPET parameters, stress echocardiogram assessment of cardiac reserve, CMR markers of remodeling, inflammatory biomarkers and neurohormonal HF biomarkers, quality of life scores and clinical outcomes, as specified below. **Figure 3** summarizes study procedures and timeline.

Combined cardiopulmonary exercise testing and stress echocardiogram

In order to assess exercise tolerance and cardiac reserve, each study subject will undergo a comprehensive evaluation with combined cardiopulmonary exercise testing (CPET) and stress echocardiogram. CPET combines exercise with indirect calorimetry and is currently considered the gold standard for cardiorespiratory fitness; on the other hand, cardiac reserve is broadly defined as the ability to increase heart rate, stroke volume and ultimately cardiac output with exercise. The combination of CPET and echocardiography allows to optimally assess cardiorespiratory fitness and cardiac reserve and possibly parse out which component of either (or both) is affected by STEMI and enhanced by the experimental treatment. The baseline exam will be scheduled 42±5 days after STEMI, and follow up exam will be performed at 365±30 days. The treadmill CPET will be performed with a metabolic cart (Viasys Vmax Encore, Yorba Linda, CA) adapted to a treadmill using a conservative ramping treadmill protocol with increases of approximately 0.3 metabolic equivalents every 30 seconds (BSU/Bruce protocol).(Kaminsky & Whaley, 1998) The highest 10-second average value of the volume of O_2 uptake during the final 30 seconds of exercise defined peak VO_2 in $ml \cdot kg^{-1} \cdot min^{-1}$. Only patients who achieve a peak respiratory exchange ratio (RER) ≥1.00 will be considered in the analysis. Percent (%) of predicted peak heart rate is reported using estimated peak heart rate = 220 – age. Percent (%) of predicted normal values for peak VO_2 are reported using the reference predicted values obtained with both the Wasserman and the FRIEND formula.(Arena et al., 2009; Myers et al., 2017)

Ventilatory efficiency will be assessed as the slope of the relation between total ventilation (VE) and carbon dioxide production (VCO_2).(Phillips et al., 2020) Anaerobic threshold will be identified using the ventilatory equivalent method; anaerobic threshold will be reported as VO_2 at anaerobic threshold (VAT).

A transthoracic echocardiogram will be performed before CPET and at peak exercise. Care will be taken in measuring left ventricular volumes (both systolic and diastolic) at rest and peak exercise. Cardiac output (CO) will be estimated using left ventricular outflow tract velocity-time integral both at baseline and at peak exercise. Diastolic function at baseline and peak exercise will be assessed with measurement of the mitral inflow peak early flow (E) to early mitral annulus early velocity (e').(Nagueh et al., 2025) Peripheral oxygen extraction will be estimated using Fick principle from pVO2 and echocardiographically derived CO as a-vO2 difference = pVO2/CO.

Cardiac Magnetic Resonance

CMR will be performed 4±1 days and 180±14 days after reperfusion, as recommended by experts in the field to optimally assess for cardiac remodeling.(Bulluck et al., 2018) Parameters of interest will be systolic and diastolic volumes, left ventricular mass, infarcts size and evidence and quantitation of microvascular obstruction. We will use a Siemens Vida 3.0T scanner. After locating the LV, a series of short axis cinematic views using a steady state free precession technique will be acquired perpendicular to the left ventricular long axis, covering from the base to the apex. These scans will incorporate a temporal resolution of ≤40msec in order to identify end-systole for the determinations of LV volumes, LVEF, and mass. Native T2 maps will be acquired to measure the extent of myocardial edema-based values exceeding normal ranges which have previously been established on the local scanner that will be used for the study. Subjects will be injected intravenously with a gadolinium contrast agent (Gadoteridol, Prohance, Bracco Diagnostic). Delayed acquisitions after contrast injection, short axis views (in the same slice positions as above) will be used for late-gadolinium enhancement (LGE) using a fast gradient echo preceded by a nonselective saturation pulse. The inversion time for the LGE acquisitions will be adjusted to null the normal remote myocardium. On the LGE images, the endocardial and epicardial surfaces will be manually contoured to identify the myocardium, with a region of interest (ROI) drawn within a non-infarcted region on 5 slices. The mean value of these voxels within normal myocardium will be determined, and those ≥6 SD in signal intensity above the mean will be defined as infarct. The total number of voxels ≥6 SD above the mean across all slices will be divided by the total number of voxels across all slices to determine the infarct volume %. Each assessment of LGE and LVEF will be analyzed separately by two individuals experienced in the use of imaging software Circle CVi (Canada) and blinded to patient identifiers. Measurements that differ <5% will be averaged, whereas those with >5% will be re-reviewed jointly and resolved by consensus or with the aid of a third reader.

Quality of life assessment

An assessment of patient's perception of symptoms and daily life limitations will be performed using the Duke Activity Status Index (DASI) questionnaire, administered on day 42±7 and 180±14 after reperfusion. The DASI is a twelve-item "yes/no" questionnaire that allows for the calculation of perceived functional capacity. Each question describes a different physical activity and asks the subjects if they feel they can perform the task. The questions are weighted according to their degree of physical exertion. The weighted values from the "yes" responses are summed to produce a score in metabolic equivalents. The DASI is a validated quality of life assessment tool for patients with coronary artery disease.(Parissis et al., 2009)

Biomarkers

Blood concentrations of hsCRP and N-terminal pro-B-type natriuretic peptide (NT-proBNP) will be obtained at baseline, at 72h±12 hours (or discharge, whichever comes first), 14±3 days, 42±7 days, 180±14 days and 365±30 days. HsCRP will aid in assessing the impact of Anakinra on acute inflammatory response, aiding in the interpretation of the results in the context of the anti-inflammatory effects of the intervention. NT-proBNP will aid in the diagnosis of HF.

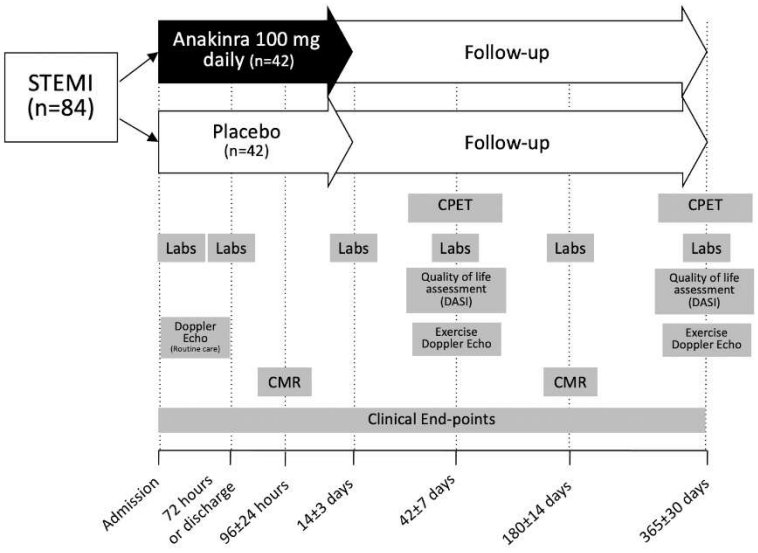

Figure 3 Timeline of study procedures for Virginia-ART4. CPET, cardiopulmonary exercise test; CMR, cardiac magnetic resonance; DASI, Duke Activity Status Index

Clinical outcomes

Clinical outcomes of interest will be all-cause mortality, cardiovascular mortality, HF hospitalization and new onset HF as defined above.

Sample size estimate for Virginia Anakinra Remodeling Trial 4

The sample size for this clinical trial is calculated for the primary endpoint of peak VO2 measured 42 days after STEMI and expressed as % of predicted (using the Wasserman-Hansen equation). Calculations are targeted for power >80% ($\alpha \leq 0.05$), with consideration of up to 20% patients with missing data. We reviewed data for patients with recent STEMI;(Giallauria et al., 2013; Turski et al., 2019) and preliminary data from a clinical cohort of 9 patients who underwent CPET 12 weeks after STEMI at Virginia Commonwealth University (VCU): mean peak VO2 was 79.7% and 75.6% predicted,

with a standard deviation of 23% and 20%, respectively. In the VCU data, those who went on to develop HF within 1 year had lower peak VO2 than those who did not (59.5% vs 80.1%). We therefore propose that patients treated with anakinra would have a higher peak VO2 than those treated with placebo with an expected effect size between 17-22% and a standard deviation of 21-25%.

A sample size of 84 patients (42 per group) would provide a power of >90% to detect a difference of 20±23% between 2 groups and maintain a power >90% for up to 20% missing data, and maintain a statistical power of at least 85% and no lower than 77.7% even for an effect size of 17±25% with a 20% data loss.

Statistical considerations

Pooled analysis

Continuous variables were tested for normality distribution using the Shapiro-Wilk test, and are reported as mean±standard deviation or median [interquartile range] as appropriate. Between group difference were assessed using Student's t-test (normally distributed variables) or Mann-Whitney U-test (non-normally distributed variables). Categorical variables are expressed as absolute number (percentage). Categorical variable distribution was tested using the Chi-square test or Fisher's exact test as appropriate. Kaplan–Meier curves for event-free survival were constructed for the time-dependent composite endpoints and compared using the log-rank (Mantel–Cox) test. Given the homogeneity in patient population and treatment protocol, no adjustment for trial effect was deemed necessary for what concerns the pooled analysis. Our published data have shown that the biological and clinical effects of Anakinra 100 mg daily are equivalent to those of Anakinra 100 mg bid.(Abbate, Trankle, et al., 2020) However, to account for potential heterogeneity in treatment dose, we performed a sensitivity analysis for the primary outcome excluding the Anakinra 100 mg bid arm.

Secondarily, predictors for end point events were estimated using multivariate Cox regression analysis. Given the low number of primary endpoints and the consequent risk of overfitting of the model, we ran a sensitivity analysis and pre-specified covariates mentioned before (sex, LVEF, diabetes mellitus, race and CRP on presentation). Proportionality assumptions for the variables included in the Cox models was tested by evaluating each variable interaction with time.

Missing data for CRP were handled using the 'last observation carried forward' method. For other relevant variables we excluded patients listwise. Statistical analyses were performed using SPSS

version 24.0 (SPSS; Chicago, IL), GraphPad Prism 9 or R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). The pooled analysis of the VCU-ART trials was not pre-specified and is an exploratory secondary analysis intended to be hypothesis generating. Caution should be exerted when interpreting the data, and no definitive nor mechanistic conclusion should be drawn.

Virginia-ART4

Virginia-ART4 will be a randomized-controlled double blind trial. It is registered to clinicaltrials.org under the identifier NCT05177822. Primary and secondary objectives are detailed above. The detailed statistical analysis plan (SAP) is included at the end of the manuscript as Appendix I.

Results – VCU-ART pooled analysis

Baseline clinical characteristics

A total of 139 subjects presenting with STEMI meeting inclusion criteria was enrolled in the VCU-ART trials. One hundred and ten (79%) were males, median age was 56 [49 - 63] years. A total of 84 (60%) subjects was assigned to the active treatment arm while 55 (40%) received placebo. The median duration of follow up was 365 [240 - 365] days. All patients received at least one dose of the treatment they were assigned to. Forty subjects (73%) in the placebo arm and 69 (82%) patients included in the active treatment arm completed the full 14 days of treatment. **Table 4** shows baseline characteristics of the population.

Table 4 Baseline characteristics for the pooled population of participants to the Virginia Commonwealth University Anakinra Remodeling Trials. Data are expressed as median [interquartile range] or n (percentage) as appropriate. BMI, body mass index; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; RAASi, renin angiotensin aldosterone system inhibitor; NSAIDs, non-steroidal anti-inflammatory drugs; LAD, left anterior descending; LCx, left circumflex; RCA, right coronary arter; SVG, saphenous vein graft; WBC, white blood cells; hsCRP, high sensitivity C-reactive protein; CK-MB, creatin kinase – MB isoform; LVEF, left ventricular ejection fraction.

	Placebo (n=55)	**Anakinra (n=84)**	**p**
Clinical characteristics			
Age, years	57 [51-65]	55 [48-61]	0.801
Male	48 (87)	62 (74)	0.056
Black	20 (36)	32 (38)	0.837
BMI, kg/m^2	29 [27-35]	21 [25-34]	0.390
Diabetes mellitus	19 (34)	20 (24)	0.168
Hypertension	37 (67)	45 (54)	0.108
Tobacco use	29 (53)	51 (61)	0.352
Dyslipidemia	25 (45)	46 (55)	0.283
Peripheral arterial disease	3 (6)	7 (8)	0.740
COPD	5 (9)	3 (4)	0.264
Previous CABG	2 (4)	4 (5)	1
Medications on admission			
Beta blocker	9 (16)	21 (25)	0.232
Aspirin	14 (26)	25 (30)	0.595
RAASi	14 (26)	21 (25)	0.935
Statin	16 (29)	27 (32)	0.721

Spironolactone	0 (0)	2 (2)	0.519
Metformin	11 (20)	8 (10)	0.076
Clopidogrel	4 (7)	3 (3)	0.436
Insulin	7 (13)	6 (7)	0.279
NSAIDs	5 (9)	7(8)	0.893
Clinical presentation			
Killip class			0.176
I	43 (79)	75 (89)	
II	3 (6)	1 (1)	
III	5 (9)	5 (6)	
IV	4 (7)	3 (3)	
Symptoms to balloon time (min)	180 [112-360]	173 [106-334]	0.856
Culprit vessel			0.569
LAD	17 (31)	30 (36)	
LCx	10 (18)	16 (19)	
RCA	28 (51)	36 (43)	
SVG	0 (0)	2 (2)	
Labs at presentation			
WBC (x10^3/L)	11.2 [8.3-14.2]	11.1 [8.6-13.3]	0.918
Creatinine (mg/L)	0.98 [0.83-1.09]	0.96 [0.75-1.41]	0.902
hsCRP (mg/L)	4.95 [2.22-8.54]	4.70 [3.00-8.96]	0.500
Peak CK-MB (ng/mL)	101.70 [49.37-231.52]	134.10 [47.60-239.30]	0.595
Echocardiographic data			
LVEF (%)	54 [41-64]	52 [40-59]	0.871

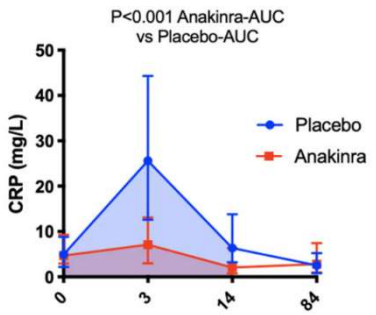

Figure 4 Effects of anakinra on high-sensitivity C-reactive protein (CRP). *Data are presented as median and interquartile range.*

Inflammatory response after STEMI

hsCRP-AUC for the first 14 days after STEMI was available in 115 (83%) subjects – namely 48 (87%) subjects in the placebo group and 67 (80%) in the active treatment group. Missing data were equally distributed in the two groups (p=0.252). As expected, treatment with Anakinra significantly reduce hsCRP-AUC (75.48

[41.7–147.47] vs. 222.82 [117.22–399.28] mg day/L, p<0.001). **Figure 3** graphically displays the hsCRP trajectory and AUC in the treatment groups.

Anakinra and clinical outcomes

Treatment with Anakinra was associated with a significant reduction of the combined endpoint of all-cause mortality and new onset HF (7 [8.2%] vs. 16 [29.1%], log-rank p=0.002) and the composite of HF hospitalization or death (0 [0] vs. 5 [9.1%], log-rank p=0.007). Only two deaths occurred during follow-up, both in the placebo group. **Figure 5** shows Kaplan-Meier curves for the outcomes of interest. The sensitivity analysis excluding patients treated with Anakinra 100 mg bid confirmed the results of the main analysis – with the primary outcome occurring in 16 (29.1%) of patients treated with placebo and 4 (7.5%) of patients receiving active treatment, log-rank p 0.008. On Cox proportional hazard analysis, treatment with Anakinra was confirmed to abate the risk of the composite outcome (hazard ratio – HR – 0.28, 95% confidence interval 0.11-0.63, p=0.005). Of note, the proportional hazard time-invariance assumption was found to be violated in case of race (Schoenfield residuals to time regression p=0.0065), hence race was not included in the model. In the multivariate model, treatment with Anakinra remained significantly associated with a lower risk of the composite outcome (HR 0.22, 95% confidence interval 0.08-0.60, p=0.003). LVEF on presentation was associated to the risk of the primary endpoint (HR for 1% increase in LVEF 0.94, 95% confidence interval 0.90-0.97, p=0.001), as was CRP on presentation (HR for 1 mg/dl increase in CRP 1.02, 95% confidence interval 1.00-1.03).

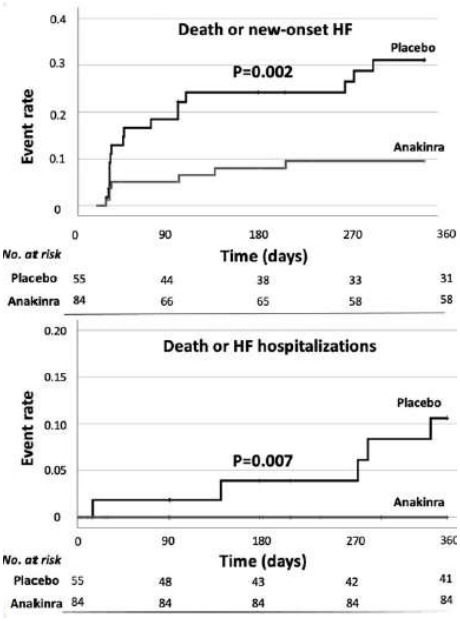

Figure 5 Kaplan–Meier curves for the incidence of the composite outcomes of all-cause mortality and new onset heart failure (upper panel) and combined mortality and heart failure hospitalization (lower panel). Reported p-value is derived from log-rank test. HF, heart failure.

Safety outcomes

Patients receiving anakinra had a significantly higher rate of injection site reactions (19 [22.6%] vs. 3 [5.5%], p=0.016), which nonetheless did not result in a higher rate of drug discontinuation due to these reactions (6 [7.1%] vs. 1 [1.8%] for anakinra and placebo, respectively, p=0.160). Serious infections occurred in 11 [13.1%] patients in the anakinra group and 7 [12.7%] in placebo (p=1.0).

Sex and treatment effect

Among the entire population enrolled, 110 (79%) were males and 29 (21%) females. Women were more likely to self-identify as Black or African American (59% vs 32%, p=0.008), a higher body mass index (BMI – 34 [31-43] vs 29 [26-34] kg/m^2) and a higher hsCRP (8.9 [5.2-13.4] vs 4.3 [2.0-7.6] mg/L, p<0.001). On the other hand, men had higher peak CK-MB (80.2 [22.8-164.0] vs 138.6 [52.5-160.5], p=0.021). Twenty-two (75%) females and 62 (56%) males were treated with Anakinra. hsCRP was lower in the active treatment arm both in females and males (73 [46-313] *vs.* 242 [102-988] mg day/L in females, 86 [37-130] *vs.* 223 [119-374] mg day/L in males, p<0.001 for multiple groups, p for interaction=0.22). **Figure 6** shows hsCRP in females and males, respectively.

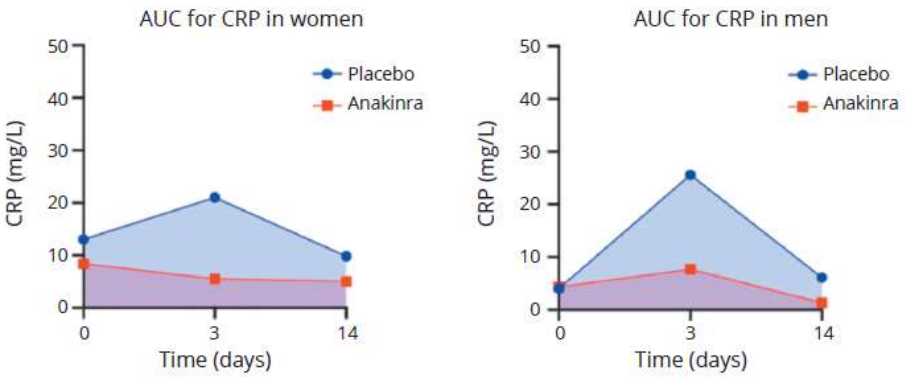

Figure 6 Effects of anakinra on high-sensitivity C-reactive protein, according to sex. Anakinra reduced the area under the curve for high-sensitivity C-reactive protein at 14 days (shaded areas) compared to placebo, both in men and wom

Overall, the composite outcome of death or new onset HF occurred in 5 (17%) of women and 18 (16%) of men. The incidence of the composite outcome was lower in the active treatment arm both in women and men (3 [13.6%] vs. 2 [28.5%] in women and 4 [6.4%] *vs.* 14 [29.1%], log-rang p=0.019). No interaction between treatment effect and sex was observed (p for interaction=0.22). **Figure 7** shows the Kaplan-Meier curve for the outcome of death and new onset HF stratified by sex.

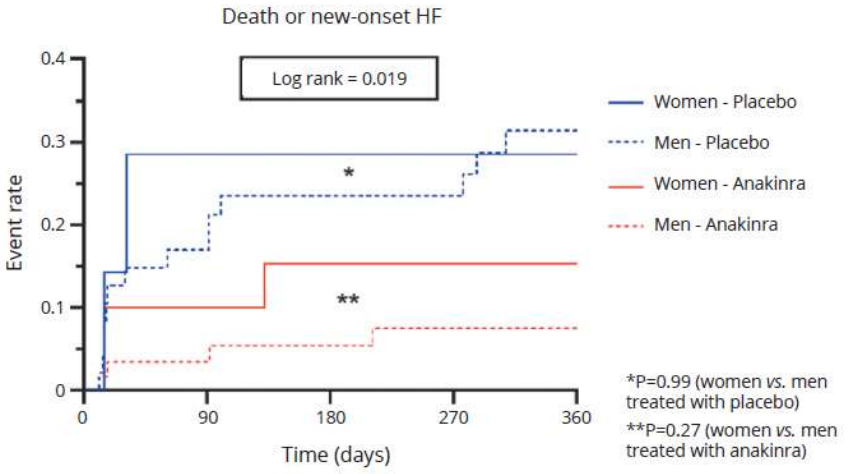

Figure 7. Kaplan-Meier curves for the incidence of the composite of death or new-onset HF according to treatment (placebo or anakinra) stratified by sex.

Impact of race on response to Anakinra after STEMI

Of the 139 patients enrolled in the trials, 52 (37%) self-identified as Black Americans, while 87 (63%) self-identified as White Americans. Black Americans had a numerically higher hsCRP on presentation, which did not reach statistical significance (6.4 [3.3-10.8] vs 4.2 [2.0-8.2] mg/L, p=0.056). Black Americans were more likely to be women (17 [32%] vs 12 [13%], p=0.008) and less likely to have a diagnosis of hyperlipidemia (20 [38%] vs 51 [58%], p=0.021). Thirty-two (62%) Black Americans and 52 (60%) White Americans were assigned to the active treatment group. Anakinra was effective in lowering inflammation in both subgroups (66 [46-128] vs 285 [109-490] mg day/L, p=0.007 in Black Americans and 80 [27-159] vs 207 [117-349] mg day/L, p<0.001 in White Americans). No difference in the effectiveness of Anakinra between the two subgroups was observed (p>0.05). The composite endpoint of all-cause mortality or new onset HF occurred in 10 (19%) Black Americans and in 13 (15%) White Americans. Anakinra was effective in preventing the occurrence of the composite outcome when compared to placebo in both Black American patients (3 [9.3%] vs 7 [35%], Log-rank p=0.033) and White American patients (4 [7.6%] vs 9 [25.7%], Log-rank p=0.027). No difference in the effectiveness of Anakinra between the two subgroups was observed (p>0.05). **Figure 8** shows the Kaplan-Meier curve for the outcome of death and new onset HF stratified by race.

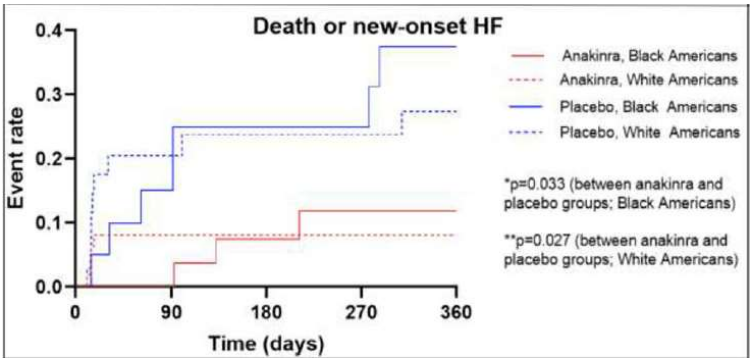

Figure 8 Kaplan–Meier curves for the incidence of the composite of death or new-onset HF according to treatment (placebo or anakinra) stratified by self-identified race (Black or White American.

Impact of diabetes on Anakinra treatment effect

Thirty-nine (28%) subjects were affected by diabetes mellitus at time of enrollment. Patients with diabetes were more likely to have hypertension than patients without diabetes (33 [87%] vs 49 [49%], p<0.001). Twenty (51%) subjects with diabetes and 64 (64%) subjects without diabetes were randomized to receive anakinra. Treatment with anakinra significantly dampened acute inflammation associated with STEMI in patients with diabetes (AUC-CRP 255.8 [119.7-478.4.3] vs 69.9 [39.9.7-137.4] mg day/L, p<0.001) and in those without diabetes (AUC-CRP 1922.8 [114.2-312.3] vs 86.5 [46.2-171.9] mg day/L, p<0.001). Among patients with

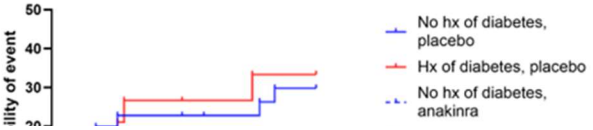

Figure 9 Kaplan–Meier curves for the incidence of the composite of death or new-onset heart failure according to treatment (placebo or anakinra) stratified by history of diabetes.

diabetes, the composite endpoint of all-cause mortality or new-onset HF occurred in 8 (21%) of subjects, while among patients without diabetes it occurred in 15 (15%) of patients (p=0.432). Treatment with Anakinra was effective in reducing the incidence of the endpoint in the group of patients without diabetes (5 [7.8%] vs 10 [28%], log-rank p=0.014) and in the group of patients with diabetes, although not quite reaching statistical significance (2 [10%] vs 6 [32%], log-rank p=0.083); no significant interaction between diabetes status and response to treatment was observed (p for interaction=0.921). Figure 9 shows the Kaplan-Meier curve for the outcome of all-cause mortality and new-onset HF stratified by history of diabetes.

Lef ventricular function and response to Anakinra

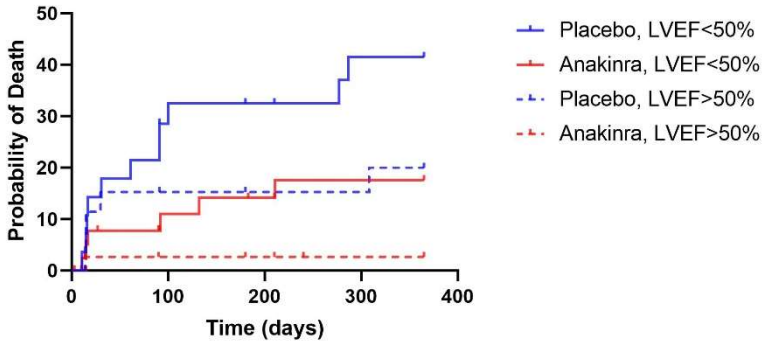

Figure 10 Kaplan–Meier curves for the incidence of the composite of death or new-onset heart failure according to treatment (placebo or anakinra) stratified by left ventricular ejection fraction

A transthoracic echocardiogram with evaluation of left ventricular ejection fraction (LVEF) obtained in the acute setting was available in 137 (98%) of subjects. Median LVEF for the study population was 50%. All-cause mortality or new onset HF occurred in 17 (25%) of subjects with baseline LVEF below the median, and in 6 (9%) of subjects with LVEF above it, p=0.013. Anakinra was effective in reducing the incidence of the composite outcome in both groups: 11 (39%) vs 6 (15%) in the anakinra group (log-rank p=0.039) among subjects with baseline LVEF above the median, and 5 (19%) in the placebo group as compared with 1 (2%) in the anakinra group (log-rank p=0.025) for subjects with LVEF above the median at presentation. No interaction between LVEF at presentation and treatment with Anakinra was detected (p for interaction=0.335). **Figure 10** shows the Kaplan-Meier curve for the effects of treatment on subgroup defined by LVEF. All patients with HF events had LVEF assessment at follow-up. In the placebo group, 8 (50%) patients with HF had LVEF < 40%, 4 (25%) had LVEF of 40–50%, and 4 (25%) had LVEF > 50%. In the anakinra group, of the patients with HF, 3 (43%) had an LVEF <40%, 1 (14%) had an LVEF of 40-50%, and 3 (43%) a preserved LVEF of >50%.

C-reactive protein on admission and treatment with Anakinra

Admission hsCRP was available in 129 (93%) subjects. Patient who experienced death or new onset HF at follow up had a numerically more elevated hsCRP at baseline, albeit the difference did not

reach statistical significance (6.45 [3.55-10.07] mg/L vs 4.56 [2.27-8.4] mg/L, p=0.093). Median hsCRP on presentation was 4.81 mg/L. The study population was stratified according to inflammatory burden at presentation as low burden if hsCRP was below the median and high burden if above the median. Anakinra was effective in reducing AUC-hsCRP both in subjects with low initial inflammatory burden (48.9 [26.4-89.9] in the Anakinra group vs 157.9 [95.9-312.4] in the placebo group, p<0.0001) and among subjects with high inflammatory burden (138.8 [69.1-297.1] in the Anakinra group vs 284.5 [134.5-607.2] in the placebo group, p=0.003). The outcome of interest occurred in 7 (11%) of subjects with low inflammatory burden on presentation and 15 (23%), p=0.056. Anakinra reduced the incidence of the combined outcome of death and new onset heart failure in the low inflammatory burden group (2 [5%] vs 5 [20%], log-rank p=0.081) and the high inflammatory burden group (4 [11%] vs 11 [41%], p=0.004). No interaction between inflammatory burden and treatment was identified (p for interaction=0.551), and in fact no difference in survival between initial inflammatory burden and incidence of the endpoint among individuals treated with Anakinra (log-rank p=0.521). **Figure 11** shows the Kaplan-Meier curves for the effects of treatment on subgroup defined by hsCRP on presentation.

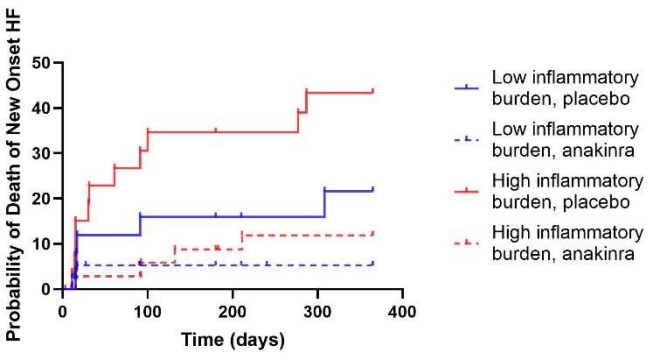

Figure 11 Kaplan–Meier curves for the incidence of the composite of death or new-onset heart failure according to treatment (placebo or anakinra) stratified by inflammatory burden on presentation. High inflammatory burden was defined as high sensitivity C-reactive protein above baseline on presentation.

Results – Virginia Anakinra Remodeling Trial 4

Patients' population

At the time of writing, 56 patients have been enrolled in the Virginia-ART4 study, have completed 14 days of blinded therapy as well as baseline evaluation with combined CPET and stress echocardiogram. Therefore, we will be presenting a purely baseline analysis of the partial enrollment in the trial available so far. We are on track to hopefully complete enrollment by the third quarter of 2026 and to complete the 12 months prespecified follow up by the end of 2027. Median age was 58 [51-66] years and 21 (38%) were females. Patients underwent testing after a median of 41 [39-43] days after the STEMI. **Table 5** shows baseline characteristics of the population enrolled so far.

Table 5. Baseline characteristics of patients currently enrolled in the Virginia Anakinra Remodeling Trial. BMI, body mass index; RAASi, renin angiotensin aldosterone inhibition; MRA, mineralcorticoid receptor antagonists; SGLT2i, sodium-glucose cotransporter 2 inhibitor.

Variable	N=56
Female sex – n (%)	21 (38)
Age (years)	58 [51-66]
BMI (kg/m^2)	30.2 [26.9-34.2]
RAASi – n (%)	45 (80%)
Betablocker – n (%)	48 (86%)
MRA – n (%)	11 (20%)
SGLT2i – n (%)	18 (32%)
Loop diuretic – n (%)	3 (5%)

Resting echocardiographic data

LVEF at rest was 55 [49-58] %, LVEDV 100 [79-127] mL, LVESV 49 [34-58] mL, LAV 49 [42-60] mL, e' 8.8 [7.4-10.5] cm/s, and E/e' 8.0 [6.5-9.9]. **Table 6** shows a summary of resting echocardiographic parameter.

Table 6 Resting echocardiographic characteristics of patients currently enrolled in the Virginia Anakinra Remodeling Trial. LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end systolic volume; LVEF, left ventricular ejection fraction; LAV, left atrial volume; TAPSE, tricuspid annular plane systolic excursion; E/e' early mitral inflow velocity/early diastolic mitral annual velocity ratio

Variable	N=56
LVEDV (mL)	100 [79-127]

LVESV (mL)	49 [34-58]
LVEF (%)	55 [49-58]
LAV (mL)	49 [42-60]
TAPSE (mm)	2.1 [1.9-2.5]
E/e'	8.0 [6.5-9.9]

Cardiopulmonary exercise test and stress echocardiography data

Peak VO_2 was 19.8 [16.5-23.2] $mL \cdot kg^{-1} \cdot min^{-1}$. Using the Wasserman formula, 52 of the 56 (93%) had a peak VO_2 <100% of predicted, with 46 of the 56 (82%) <80% of predicted. Using the FRIEND formula, 46 of the 56 (82%) had a peak VO_2 <100% of predicted, with 31 of the 56 (55%) <80% of predicted.

Heart rate increased from rest (65 [57-71] bpm) to peak exercise (137 [123-151] bpm), at 87 [76-93]% of age-predicted. Stroke volume increased from 75 [63-84] at rest to 79 [70-94] mL with exercise (p<0.0001) and stroke volume index increased from 35 [31-39] to 40 [34-44] mL/m^2 (p<0.0001). Cardiac output increased from 4.9 [4.3-5.6] L/min at rest to 11.3 [9.0-13.1] L/min with exercise (p<0.0001) and cardiac index increased from 2.3 [2.0-2.6] to 5.3 [4.6-6.2] $L/min/m^2$ (p<0.0001). Resting a-v O_2diff increased from 7.3 [6.0-8.6] to 17.1 [13.8-20.2] mL/dL (p<0.0001). Similarly, e' velocity increased from rest (8.8 [7.4-10.5] cm/s) to peak exercise (13.9 [11.5-16.1] cm/s; p<0.0001). **Table 7** summarizes exercise parameters in the Virginia-ART4 population.

Table 7 Exercise test parameter in currently enrolled Virginia Anakinra Remodeling Trial 4. BP, blood pressure; a-v O2 diff, arteriovenous O2 difference; VO2, oxygen consumption; FRIEND, Fitness Registry and the Importance of Exercise National Database; RER, respiratory equivalents ratio.

Stress echocardiography			
	Rest	*Stress*	*p*
e' (cm/s)	8.8 [7.4-10.5]	13.9 [11.5-16.1]	**<0.0001**
E velocity (cm/s)	72 [64-83]	106 [87-127]	**<0.0001**
E/e'	8.0 [6.5-9.9]	8.4 [6.4-9.7]	0.95
Cardiac Output (L/min)	4.9 [4.3-5.6]	11.3 [9.0-13.1]	**<0.0001**
Cardiac Index ($L/min/m^2$)	2.3 [2.0-2.6]	5.3 [4.6-6.2]	**<0.0001**
Heart Rate (bpm)	65 [57-71]	137 [123-151]	**<0.0001**
Stroke Volume (mL)	75 [63-84]	79 [70-94]	**<0.0001**
Stroke Volume Index (mL/m^2)	35 [31-39]	40 [34-44]	**<0.0001**

a-v O_2diff (mL/dL)	7.3 [6.0-8.6]	17.1 [13.8-20.2]	**<0.0001**
Systolic BP (mmHg)	120 [108-130]	162 [148-180]	**<0.0001**
Diastolic BP (mmHg)	70 [64-78]	73 [68-82]	**0.04**
Cardiopulmonary exercise test			
Peak VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)		19.8 [16.5-23.2]	
Peak VO_2 ($L \cdot min^{-1}$)		1.8 [1.4-2.3]	
Peak VO_2 (% Predicted Wasserman)		71 [63-78]	
Peak VO_2 (% Predicted FRIEND)		77 [67-93]	
Peak RER		1.14 [1.06-1.23]	
Peak O_2 Pulse		14 [11-17]	
Exercise Time (sec)		414 [289-489]	

Discussion

The main findings of this work are:

1. New onset heart failure is still fairly common in the first year after STEMI, affecting up to one patient in 3 despite gold standard interventional and medical treatment. Approximately half of subjects appear to have a depressed left ventricular function, while half has only mild or no systolic dysfunction.
2. In a pooled analysis of 3 Phase II, investigator-initiated trials of IL-1 blockade in the acute phase of successfully reperfused STEMI Anakinra was found to be successful in reducing the incidence of the composite of all-cause mortality and new onset heart failure. The effects were consistent across multiple subgroups defined by presence or absence of high-risk features.
3. A large proportion of subjects have significantly impaired cardiorespiratory fitness after approximately one month from successfully treated STEMI, despite mostly normal resting systolic and diastolic function.

Despite the reduction in mortality brought about by early reperfusion by means of primary PCI and early initiation of anti-remodeling treatment focused on neuro-hormonal modulation,(Thrane et al., 2024) the incidence of new HF remains significant. Among participants in our studies, which were conducted between 2008 and 2017, 1 subject in 4 experienced a new heart failure event at 1 year follow up while 1 in 11 required inpatient admission due to heart failure exacerbation. In addition, 50% of subjects that had experienced a new heart failure event did not have significant left ventricular systolic impairment at baseline. This contrasts, at least in part, with the classic notion that the pathway of heart failure development in the setting of ischemic heart disease has in cardiomyocyte loss and myocardial hibernation leading to cardiac adverse remodeling and drop in left ventricular ejection fraction its necessary antecedents.(Minicucci et al., 2011) Other factors, including latent or overt diastolic dysfunction and impaired cardiac reserve are likely implicated in new onset heart failure after STEMI, especially when resting function appears intact.(Del Buono et al., 2019)

Treatment with Anakinra for 14 days starting within 12 hours from successful reperfusion was effective in reducing new heart failure events from 29% in the placebo arm to 8% in the active treatment arm. This is an absolute risk reduction of roughly 20%, leading to a number needed to treat of 5. Treatment with Anakinra was not associated to major adverse events. The only drug related adverse event recorded in our trial being injection site reaction, which nonetheless did not

lead to increased rates of treatment discontinuation when compared to placebo. Patients with evidence of higher inflammatory burden on presentation and patients with impaired left ventricular systolic function on admission were at higher risk for the outcome of interest. Anakinra showed efficacy regardless of the presence of high-risk characteristics. In particular, no difference in response was seen with different left ventricular ejection fractions. In the original VCU-ART and VCU-ART2, no difference in infarct size and remodeling parameters measured by cardiac magnetic resonance.(Abbate et al., 2010; Abbate et al., 2013)

The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) showed that targeting inflammation by means of an IL-1 directed recombinant antibody, confers a survival advantage in patients with atherosclerotic heart disease who have survived a myocardial infarction and have residual inflammatory risk.(Ridker et al., 2017) In the CANTOS trial canakinumab was also shown to significantly reduce heart failure related hospitalizations and mortality.(Everett et al., 2019) In addition, canakinumab was shown to improve cardio-respiratory fitness and exercise capacity, assessed by means of pVO_2 among patients with reduced left ventricular ejection fraction after a myocardial infarction.(Trankle et al., 2018) This is in line with our prior data showing that treatment with Anakinra in patients with heart failure can indeed improve cardiac function and cardiorespiratory fitness.(Buckley et al., 2018; Van Tassell et al., 2017) Modulation of cardiac reserve and improvement of cardiorespiratory fitness by IL-1 inhibition therefore appears to be a likely mechanism for the observed clinical benefit in this study. We designed therefore a study, which is ongoing, aimed at assessing the impact of Anakinra on cardiac reserve and cardiorespiratory fitness. So far, we have enrolled and completed baseline analysis in approximately two thirds of the target enrollment and are on track to complete the study by the end of 2026. Our preliminary data show that indeed many patients with STEMI maintain relatively normal cardiac dimensions and resting left ventricular ejection fraction. These changes likely reflect a benefit of prompt reperfusion and with modern neurohormonal blockade on remodeling. However, despite these advances in treatment which enable preservation of resting cardiac function, cardiac reserve may still be impaired and contribute to dyspnea and exercise intolerance after STEMI – and ultimately development of new heart failure signs and symptoms. Along these lines, we show here that cardio-respiratory fitness after STEMI is reduced compared to age-predicted norms despite the relatively preserved left ventricular function and lack of widespread remodeling, further implicating cardiac reserve as a major determinant of exercise capacity and cardiorespiratory fitness. There was an increase in heart rate, stroke volume, cardiac output, and e' during exercise, each representing

exercise-induced cardiac augmentation. In addition, we noticed an increase in peripheral O_2 extraction induced by exercise. Exercise induced increase in heart rate, stroke volume and peripheral O_2 extraction all contribute to pVO_2, playing a crucial role in determining exercise capacity.(Higginbotham et al., 1986) While there are no clearly established normative values for the three component, some prior observations may come of help in parsing out which of them may be contributing to the observed exercise limitations. Shimiaie et al., reported that healthy controls had a peak VO_2 of 24.8 $mL \cdot kg^{-1} \cdot min^{-1}$ and peak cardiac output derived by echocardiography of 14.5 L/min with recumbent cycling, while patients with HF had a peak VO_2 of ~15 $mL \cdot kg^{-1} \cdot min^{-1}$ and peak cardiac output of ~9 L/min.(Shimiaie et al., 2015) Both impaired stroke volume augmentation with exercise and chronotropic incompetence were noticed among patients with HF, while peripheral O2 extraction was comparable between HF patients and healthy controls.(Shimiaie et al., 2015) In line with their observations, the cohort we describe appears to have an impaired peak exercise cardiac output of 11.3 [9.0-13.1], suggesting that this could be the main driver for the observed impaired cardiorespiratory fitness. We eagerly await the completion of the study to reproduce these observations on a larger population, and possibly to assess the longitudinal effects of Anakinra on modulating cardiac reserve and cardiorespiratory fitness after STEMI.

Study limitations

There are several limitations to this work. The pooled analysis of previously performed trials was non-prespecified, and different experimental drug dosing were combined into a single active treatment arm for the purpose of this analysis. The three trials individually had limited power to detect statistically significant differences in clinical outcomes. Therefore, the results should be interpreted as hypothesis generating only. In addition, a large proportion of patients were enrolled before widespread use of new highly effective HF treatment including sacubitril-valsartan and sodium-glucose cotransporter 2 inhibitors, which may impact the occurrence of HF-related events after STEMI.(Rao et al., 2025) The interim analysis of the Virginia-ART4 is also one of a small sample size. In addition, CPET data from the patients prior to the STEMI is unavailable for comparison to determine the extent of deconditioning present prior to the index event. Data on cardiac rehabilitation and physical activity were not taken into account. Furthermore, the lack of normative data on cardiac reserve makes the interpretation of differential contribution to impaired cardiorespiratory fitness less straightforward.

Conclusions

Preliminary data from small pilot randomized controlled trials including 139 patients suggest that IL-1 blockade using Anakinra for 14 days after a STEMI may reduce mortality and new onset HF at 1 year of follow up. Treatment effects consistent across multiple subgroups, including high risk patients with reduced left ventricular function or high inflammatory burden at presentation. The ongoing Virginia-ART4 will hopefully confirm these findings and help elucidating the pathophysiological impact of IL-1 blockade on cardiac remodeling and reserve after STEMI. In the preliminary analysis of baseline CPET and stress echocardiogram at 6 weeks of the first 56 patients enrolled in Virginia-ART4, we report for the first time a high prevalence of significantly impaired cardiorespiratory fitness in the subacute phase after STEMI. The observed reduction in cardiorespiratory fitness occurs in the context of preserved baseline cardiac function and is associated an impairment in cardiac output augmentation with exercise. Virginia-ART4 data will help further characterize the pathophysiology of heart failure after STEMI in the current era of early reperfusion and neuro-hormonal modulation, as well as the impact of IL-1 blockade on cardiac reserve, cardiorespiratory fitness and incidence of heart failure post STEMI.

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Appendix 1: Statistical Analysis Plan

1. Sample size:

The sample size for this clinical trial is calculated for the primary endpoint of peak VO_2 measured 42 days after STEMI and expressed as % of predicted (using the Wasserman-Hansen equation). Calculations are targeted for power >80% (≤0.05), with consideration of up to 20% patients with missing data. We reviewed data for patients with recent STEMI(Giallauria et al., 2013; Turski et al., 2019); and preliminary data from 9 patients who underwent CPET 12 weeks after STEMI at VCU: mean peak VO_2 was 79.7% and 75.6% predicted, with a standard deviation of 23% and 20%, respectively. In the VCU data, those who went on to develop HF within 1 year had lower peak VO_2 than those who did not (59.5% vs 80.1%). We therefore propose that patients treated with anakinra would have a higher peak VO_2 than those treated with placebo with an expected effect size between 17-22% and a standard deviation of 21-25%.

A sample size of 84 patients (42 per group) would provide a power of >90% to detect a difference of 20±23% between 2 groups and maintain a power >90% for up to 20% missing data, and maintain a statistical power of at least 85% and no lower than 77.7% even for an effect size of 17±25% with a 20% data loss.

The table below shows estimated power analysis across a variety of effect sizes and SD.

Table 1. Power analysis	N=84 (42 per group)			20% data loss → N=66 (33 per group)		
	Effect size 17%	Effect size 20%	Effect size 22%	Effect size 17%	Effect size 20%	Effect size 22%
SD 21%	95.6%	99.2%	99.8%	90.0%	96.8%	98.7%
SD 23%	91.7%	97.6%	99.2%	84.1%	93.6%	96.9%
SD 25%	86.8%	95.2%	97.8%	77.7%	89.3%	94.1%

In HF-ACTION study, for every 6% increase in peak VO_2 there was a significant 8% lower risk of cardiovascular mortality or HF hospitalization (hazard ratio=0.92; CI=0.88–0.96; *P*<0.001), therefore

the estimated effect size of 20% improvement in peak VO_2 would serve as a surrogate for an approximate 25% reduction in the composite of HF hospitalization and death(Swank et al., 2012), an effect that would be considered clinically significant and worth exploring in future studies.

Based on the results of the VCUART3 clinical trial(Abbate, Trankle, et al., 2020) showing a significant >50% reduction in CRP_{AUC} (P<0.001) with anakinra, we anticipate that the sample size of 84 will provide a >80% power to detect a significant reduction in CRP_{AUC}. CRP_{AUC} will serve as the preferred measure for systemic inflammation, and correlate with other parameters. We will also measure levels of IL-6 and IL-18 as they have shown to determine residual risk, especially after IL-1 blockade in the CANTOS trial, both IL-6 and IL-18 are targetable by drugs.

A sample size of 84 patients will provide a power of 81.7% to detect a difference in the DASI of 5 points, that is considered clinically significant(Parissis et al., 2009), between the two groups, with an expected standard deviation of 8 points. Based on the quality-of-life DASI data on HF patients paired with peak VO_2 from the REDHART study(Van Tassell et al., 2017) with a total size of 84 patients, a two-sided Fisher's z test will also have a 75% power to detect a statistically significant Pearson correlation coefficient different from zero.

Based on the results of prior VCUART studies, we do not anticipate a significant effect of anakinra on infarct size or cardiac remodeling. However, we will incorporate these variables in multiple regression models as covariates to determine their marginal contributions to clinical outcomes like HF, in addition to important predictors such as peak VO_2, and CRP_{AUC}.

Based on the VCUART2(Abbate et al., 2013) and VCUART3(Abbate, Trankle, et al., 2020), patient data on time to first HF onset show an estimated hazard ratio (HR) of 0.366 between anakinra and placebo group (see figures in the Research Plan), with the attainable power over 98% using Cox regression for comparing two Kaplan-Meier survival curves.

2. Analysis plan:

2.1 Intention-to-treat (ITT):

The ITT population will include all randomized subjects. Following the ITT principle, subjects will be analyzed according to the treatment they were assigned at randomization regardless of withdrawals or treatment duration. The ITT population will be the primary efficacy population. All efficacy analyses, demographic and baseline summaries will be performed using this population.

2.2 Per Protocol (PP)

The PP population will include all subjects in the ITT population who meet all the inclusion and exclusion criteria and received sufficient study treatment as defined in the protocol (i.e., ≥80% adherence, no treatment interruption >72 hours during the 14 days) (not excluding subjects who die prior to receiving the full dose of 10-14 days). This population will be used to perform confirmatory analyses of the primary efficacy evaluation.

2.3 Safety

The safety population will include all subjects who received any anakinra treatment. The safety population will be used for all summaries of safety data.

3. Statistical Methods:

All analyses and reporting of study results will be conducted using SAS Version 9.4 (SAS Institute Inc., Cary, North Carolina). All variables will be presented using appropriate descriptive statistics according to the variable nature: (1) continuous variables: number of non-missing observations, mean, SD, minimum, first quartile, median, third quartile, maximum – median and interquartile range will be preferred for external reporting, due to the potential deviation from Gaussian distribution (2) categorical variables: number of non-missing observations, absolute and relative frequency (n and percentages [%]), (3) time-to-event variables: number of non-missing observations, number and percentage of censored observations, first quartile, median (and 95% CI), third quartile, and Kaplan-Meier survival curves. (4) The behavior over time of continuous variables

will be analyzed by presenting descriptive statistics for each time point and the difference compared to baseline (actual values and change from baseline). (5) The behavior over time of categorical data will be analyzed by presenting the descriptive statistics for each time point and the shift compared to baseline.

3.1 Demographic and Baseline Characteristics

Demographic variables (age, sex, ethnicity and race), and baseline characteristics (hsCRP, culprit vessel, time from presentation, etc..) will be summarized for each treatment group and overall using appropriate descriptive statistics. They will also be examined for potential imbalances following randomization.

3.2 Efficacy Analysis

Primary Efficacy Analysis

The primary efficacy variable of the peak VO_2, expressed as % of predicted measured at 42 days after STEMI admission, will be compared between the treatment groups. The hypothesis that the observed median peak VO_2 values in each treatment group are the same will be tested using the nonparametric Mann-Whitney test.

$$H_0: m_1 = m_2, \text{ versus } H_1: m_1 \neq m_2$$

where m_1 is the median peak VO_2 value in the anakinra treated group and m_2 is the median peak VO_2 value in placebo group. A 2-sided, 5% significance level will be used to test the null hypothesis. If the null hypothesis is rejected ($p < 0.05$), it will be concluded that there is a statistically significant difference in the primary efficacy end point between the two treatment groups.

Secondary Variables (non-clinical)

A list of secondary efficacy variables are described in the proposal. For continuous variables, the analysis of the secondary endpoints will proceed as described for the primary endpoint. For categorical variables, Fisher's exact test will be conducted for difference between two groups.

Secondary Variables (clinical events)

For the clinical endpoints, these efficacy variables are obtained by collecting the survival status (event or death) from the Day 1 of the randomized treatment until Day 365 (1 year) or longest available follow up as lost to follow-up, consent withdrawn, readmission, alive, dead or other. Cases will be censored at time of last available follow up, which in some cases may coincide with hospital discharge. The hypothesis that the event (new onset heart failure or hospitalization) or mortality rate observed in each treatment group is the same will be tested using the Fisher's Exact test.

$$H_0: p_1 = p_2, \qquad H_1: p_1 \neq p_2$$

where p_1 = proportion of event or deaths in the anakinra treated group and p_2 = proportion of event or deaths in the placebo group. A 2-sided, 5% significance level will be used to test the null hypothesis. If rejected ($p < 0.05$), it will be concluded that there is a statistically significant difference in the proportion of readmissions or deaths between the two treatment groups. In addition, 2-sided 95% confidence intervals for the difference in the proportion of readmissions or deaths between the groups will also be calculated. Summaries of events and mortality will be presented for both the ITT and PP populations.

In addition, the actual time to event or death will be analyzed for the ITT population. If a subject experiences no events nor dies during the conduct of the study, they will be censored at the last point of contact. The LIFETEST procedure in SAS will be used to produce Kaplan-Meier plots and estimates of 25^{th}, 50^{th}, and 75^{th} quartiles and corresponding 95% confidence limits of the median time to readmission or death. The two treatment groups will be compared using a 2-sided log-rank test. If this test is statistically significant ($p < 0.05$), it will be concluded that the time to readmission or death is significantly different between the anakinra and placebo treatment groups at the 5% level. Examination of the point estimates will indicate the direction of the treatment difference. The cumulative percent mortality based on the Kaplan-Meier survival probability estimates will be plotted for each treatment group.

The time to readmission or death (in days) will be further analyzed using a Cox proportional hazard model. The comparison of the treatment effect on the time to readmission or death will be based on the hazard ratio (HR) from the Cox regression model using the PHREG procedure in SAS. Estimates of HR and 95% confidence limits will be presented. The null hypothesis that the hazard of readmission or death is the same in both treatment groups, i.e.

$$H_0: HR_{anakinra/placebo} = 1, \quad H_1: HR_{anakinra/placebo} \neq 1$$

will be tested at the 2-sided, 5% significance level. If the null hypothesis is rejected ($p < 0.05$), it will be concluded that there is a statistically significant difference between the treatment groups. With the placebo group being considered as the reference category, if the $HR_{anakinra/placebo} < 1$, the hazard of readmission or death will be considered to be less in the anakinra treatment group compared to placebo. The Cox model is a standard model used in failure time analysis, its assumptions are fairly unspecified and will yield robust estimates of the treatment effect. The model will be applied using the Efron approach for handling ties, since this approach is known to give a reasonable approximation of the exact likelihood in the presence of ties. The Cox model relies on the assumption of proportional hazards. This assumption will be examined using a plot of the log(-log S(t)) versus time, where S(t) is the estimated survival distribution. The graphs of these functions for each treatment group should be parallel under the proportional hazards assumption. A lack of parallelism suggests deviations from the proportional hazard assumption. In this situation, other appropriate analytic approach such as the piecewise constant hazards model will be considered.

Interim analysis

No interim efficacy analyses are planned. Interim safety analyses can be performed upon request of the Data and Safety Monitoring Board.

4. Missing data

When the primary endpoint peak VO_2 at 42 days is missing, we will first assess baseline covariates associated with missingness for peak VO_2. If missing at random (MAR) assumption is assumed, the conditional regression model will be used to perform multiple imputation on missing peak VO_2 data, with the utilization of associated baseline covariates, and covariates of clinical importance. Sensitivity analyses using all available data (ITT), or patients who meet inclusion/exclusion criteria (PP), with various imputation strategies such as baseline carried forward or last observation carried backward will be conducted. ITT analysis without imputation, taking advantage of mixed effect modeling treating anakinra versus placebo as fixed effect will be performed as well. The mixed effect modeling approach not only allows us to produce primary unadjusted estimates and their confidence intervals of peak VO_2 between anakinra and placebo, but also allows for adjusted estimates while controlling for important covariates, such as sex, age, cardiac reserve and cardiac remodeling.

For data pertaining to the secondary endpoints, the analyses will be performed on available data only, without missing data imputation.

5. Subgroup analysis

No subgroup analyses are planned, in consideration of the limited sample size affecting the statistical power of secondary analyses. All subgroup analyses will be performed post-hoc and considered hypothesis generating only.

6. Pre-specified exploratory analysis

We will assess several other measures of cardiorespiratory fitness and exercise capacity such as the VE/VCO_2 slope and OUES between treatment groups, following the same manner assessing %predicted peak VO_2.

Bivariate parametric and nonparametric correlation measures (Pearson/Spearman correlation coefficients) will be estimated between peak VO_2 at 42 days and AUC_{CRP} from admission to the 14

days follow up, for all STEMI patients. In addition, stratified correlation estimation will be provided for anakinra and placebo group, separately, to study if there exists statistically significant heterogeneity in correlations between CRF measures (peak VO_2) and IL-1 activity biomarkers (CRP_{AUC}). The mixed effect model would also quantify the association between peak VO_2 at 42 days and AUC_{CRP} from admission to 14 days follow up, while simultaneously controlling for other direct/indirect relationships, such as between peak VO_2 and cardiac reserve.

Longitudinally followed biomarkers will also be investigated using mixed effect analysis of repeated measures ANOVA to study the effects of time and treatment group allocation. Similarly, other important covariates besides time and treatment will be included in the mixed effect ANCOVA models, to produce adjusted effect estimates.

To determine if reduced CRF and/or impaired cardiac reserve predict HF in patients with STEMI, logistic regression models will be implemented to assess the impact of peak VO_2, diastolic and systolic reserve for the onset of HF events, while controlling for possibly confounding covariates. ROC analysis will also be considered to study best possible cutoffs for peak VO_2, diastolic and systolic reserve measures, based on the sensitivity and specificity tradeoff, for the dichotomous prediction of a future HF event. Such cutoffs may be used to define low versus high-risk group for the purpose of assessing differential survival prognostics, for instance, with respect to time to first HF onset, or time to death associated with HF. The log-rank test, the Cox proportional-hazards model, or the piecewise constant hazards model if PH assumption is violated, will be considered to compare Kaplan-Meier survival curves, and to estimate hazard ratios of various important prognostic factors.

We will exclude patients that have a respiratory exchange ratio (RER<1.0) at cardiopulmonary exercise test (CPET) as the test may not be reflective of maximal cardiac performance. As an additional exploratory analysis will repeat the analysis to include all individuals, to explore whether reduced RER, reflecting deconditioning may also be affected by IL-1 blockade with anakinra.