Evaluation of immunomodulatory mediators in chronic obstructive pulmonary disease and cardiovascular disease

Yuan Sun

Sidney Sussex College and Division of Experimental Medicine and Immunotherapeutics

This thesis is submitted for the degree of Doctor of Philosophy August 2022

Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text.

It is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text.

It does not exceed the prescribed word limit for the Clinical Medicine Degree Committee.

Abstract

Evaluation of immunomodulatory mediators in chronic obstructive pulmonary disease and cardiovascular disease

Author: Yuan Sun

Chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD) are among the leading causes of mortality and poor health burden in the world. Both COPD and CVD often co-exist, due to shared risk factors and common pathophysiological pathways. There is an unmet need for effective therapeutics in COPD, given that there has been a paucity of therapeutic progress over the last decades and currently very few therapeutics have been proven to improve survival in COPD. To this end, addressing cardiovascular risk and evaluating cardiovascular therapeutics in people with COPD is an attractive prospect to reduce mortality and morbidity in this patient group. Furthermore, there is an urgent need to improve understanding of immunomodulatory mediators that may have potential as therapeutics targets in both COPD and CVD, and thus health outcomes in COPD and CVD.

This thesis evaluated two distinct immunomodulatory pathways, which are of interest therapeutically in both COPD and CVD. Firstly, the Interleukin-33 (IL-33)/ST2 axis and secondly, specialised pro-resolving mediators (SPMs), specifically the specialised pro-resolving mediator Resolvin D1. Additionally, data pertaining to the anti-platelet drug aspirin in people with COPD was evaluated. The reason for this was to evaluate if aspirin may have therapeutic benefit in COPD. Given aspirin's mechanism of actions not only as an anti-inflammatory anti-platelet therapeutics but also possible enhancement of endogenous

production of specialised resolution mediators, it is helpful to evaluate patient level data relating to aspirin use and outcomes.

Firstly, a systematic review and meta-analysis of published human studies of the IL-33/ST2 axis across the spectrum of human cardiovascular disease was undertaken. The role of IL-33 in CVD is unclear with both ameliorative and harmful effects reported, and circulating ST2 is a biomarker in CVD. The review included 77 studies and 62075 participants. The main findings were that incremental increases in circulating ST2 levels were associated with increased risk of all-cause mortality and cardiovascular events in populations with cardiovascular disease. IL-33 levels were found to be lower in heart failure, coronary artery disease and acute coronary syndrome patient groups, compared to controls, with the opposite being observed in stroke patients. The results of this analysis have been published in PLOS One.

Secondly, levels of immunomodulatory mediators were measured in serum samples COPD patients and controls, specifically the specialised pro-resolving mediator Resolvin D1. Specialised pro-resolving mediators (SPMs) are metabolites of polyunsaturated fatty acids and are responsible for resolution after acute inflammatory events. There are several families of SPMs, including lipoxins, protectins, maresins and resolvins which are derived from different polyunsaturated fatty acids and are released at different times during the course of inflammation resolution. Of these SPM families, Resolvin D1 has been most extensively investigated in COPD pathogenesis and is thought to be dysregulated in COPD. Resolvin D1 levels were measured using enzyme linked immunosorbent assays in serum samples from 86 stable COPD patients, 140 COPD patients with recorded exacerbations at baseline and 146 healthy controls, to evaluate the differences in circulating levels between the groups. Resolvin D1 levels were significantly lower in stable COPD patients compared with COPD patients with exacerbations at baseline, which suggests potentially dysregulated resolution pathways in COPD. The finding that samples from patients who had reported acute exacerbations at

baseline had higher Resolvin D1 levels than in stable COPD patients suggests that it plays an essential role in the natural resolution of inflammation in COPD.

Lastly, aspirin use has in some observational studies showed encouraging data that it was associated with reduced exacerbation rates. Therefore, work was undertaken to evaluate aspirin use at baseline for its association with health outcomes in COPD populations, in a comprehensive statistical analysis of two large high quality clinical trial datasets (SUMMIT trial n=16485, IMPACT trial n=10355), which included patients with both moderate (SUMMIT) and severe COPD (IMPACT) respectively, and those with a history/risk of cardiovascular disease (SUMMIT). The patient level data from both trials were obtained and patient level analysis performed. The analysis showed that aspirin use was associated with increased risk of mortality [SUMMIT hazard ratio 1.15 (1.00-1.33), p=0.048] fully adjusted for known and possible confounders, exacerbations and cardiovascular events (including myocardial infarction and stroke) over follow up of 52 weeks (IMPACT) and median 1.8 years (SUMMIT) respectively.

In summary, the IL-33 has potential as a therapeutic target and biomarker across the range of human cardiovascular disease although further studies with larger sample sizes are needed. Whilst IL-33 levels were lower in patients than in controls for several cardiovascular diseases, further investigation is needed to explain the contradictory findings in stroke patients. Resolvin D1 is dysregulated in stable COPD patients and has potential in future treatment regimens. The results of the Resolvin D1 study led to an ongoing clinical study which measures changes in patient Resolvin D1 levels over time, following a COPD exacerbation. While previous observational studies had suggested aspirin use was associated with reduced mortality risk and exacerbations in COPD patients, the findings of this extensive analysis suggest that aspirin use is associated with increased mortality risk and exacerbations, and should not presently be indicated for treating COPD patients. Future work would include assessing IL-33 and ST2

receptor expression in tissue samples from CVD patients which could identify any disruptions to this pathway. Additionally, levels of SPMs including resolvins need to be measured in COPD patients at set time points after an exacerbation, to identify changes in the trajectory of SPM levels. Finally, a randomised control trial of aspirin in COPD patients would be useful to evaluate the effect of aspirin use on patient exacerbations and all-cause mortality.

Table of Contents

Declaration 2
Abstract 3
Acknowledgements 10
Abbreviations 11
List of Figures 14
List of Tables 15
Chapter One: Introduction 16
1.1 Thesis overview 16
1.2 Cardiovascular disease prevalence and impact 16
1.2.1 Cardiovascular system 17
1.2.2 Cardiovascular disease pathogenesis 19
1.3 Chronic obstructive pulmonary disease impact 22
1.3.1 Chronic obstructive pulmonary disease pathogenesis 22
1.3.2 Comorbidity of cardiovascular disease in chronic obstructive pulmonary disease patients 24
1.4 Interleukin-33 signalling axis 25
1.4.1 Interleukin-33 in chronic obstructive pulmonary disease and cardiovascular disease 27
1.4.2 Planned wire myography work 29
1.5 Specialised pro-resolving mediators 29
1.5.1 Specialised pro-resolving mediator biosynthesis 31
1.5.2 Specialised pro-resolving mediators in chronic obstructive pulmonary disease and cardiovascular disease 32
1.5.3 Resolvin D1 in chronic obstructive pulmonary disease 33
1.6 Current treatments for chronic obstructive pulmonary disease 34
1.7 Aspirin use and chronic obstructive pulmonary disease health outcomes 35
1.7.1 Platelets in chronic obstructive pulmonary disease and cardiovascular disease 38
1.7.2 Aspirin's potential in chronic obstructive pulmonary disease treatment 38
1.8 Summary 40
1.9 Hypotheses 40
1.10 Aims 41
Chapter Two: Methods 42
2.1 Overview 42
2.1.1 Datasets used 42
2.1.2 Data extraction 43
2.1.3 Serum analysis 43
2.1.4 Statistical analysis 44
2.1.5 Software 45

Chapter Three: IL-33/ST2 axis in cardiovascular disease 46
3.1 Background 46
3.1.1 Interleukin-33 structure 46
3.1.2 Interleukin-33 storage and release 46
3.1.3 Interleukin-33 in pathogenesis of chronic obstructive pulmonary disease and cardiovascular disease 47
3.1.4 Role of Interleukin-33 signalling in cardiovascular disease 49
3.2 Hypothesis 50
3.3 Aims 50
3.4 Methods 50
3.4.1 Study design 50
3.4.2 Search strategy 50
3.4.3 Data extraction and quality assessment 52
3.4.4 Statistical analysis 53
3.5 Results 53
3.5.1 Interleukin-33 and soluble ST2 levels in cardiovascular disease patients versus controls 54
3.5.2 Association of soluble ST2 levels and clinical outcomes in cardiovascular disease and community cohorts 58
3.5.3 Heterogeneity and meta-regression 59
3.6 Discussion 59
3.6.1 Limitations 62
3.6.2 Impact and future work 63
3.6.3 Summary 63
Chapter Four: Evaluation of Resolvin D1 levels in COPD patients and controls 65
4.1 Background 65
4.1.1 Biosynthesis of specialised pro-resolving mediators 65
4.1.2 Resolvin D1 in chronic obstructive pulmonary disease 67
4.1.3 Interleukin-17 and Del-1 in COPD 69
4.2 Hypothesis 70
4.3 Aims 70
4.4 Methods 71
4.4.1 Study design 71
4.4.2 Sourcing of samples 71
4.4.3 Measurement of serum Resolvin D1, Interleukin-17, Del-1 71
4.4.4 Statistical analysis 72
4.5 Results 72
4.5.1 Resolvin D1 74
4.5.2 Del-1 75
4.5.3 Interleukin-17 76

4.5.4 Regression analyses 76
4.6 Discussion 77
4.6.1 Limitations 80
4.6.2 Impact and future work 81
4.6.3 Summary 82
Chapter Five: Aspirin use and health outcomes in COPD populations 84
5.1 Background 84
5.1.1 Aspirin for chronic obstructive pulmonary disease treatment 84
5.1.2 Data analysis of aspirin and chronic obstructive pulmonary disease outcomes 85
5.2 Hypothesis 86
5.3 Aims 86
5.4 Methods 86
5.4.1 Study design 86
5.4.2 Datasets 87
5.4.3 Statistical analysis 88
5.5 Results 89
5.5.1 All-cause mortality 91
5.5.2 Exacerbations 92
5.5.3 Cardiovascular events 93
5.5.4 Sensitivity analysis 94
5.5.5 Bias analysis 94
5.5.6 Propensity score matched groups 95
5.5.7 Absolute Risk 95
5.6 Discussion 96
5.6.1 Limitations 98
5.6.2 Impact and future work 99
5.6.3 Summary 99
Chapter Six: Conclusions 101
References 104
Appendices 116
Appendix A 116
SUMMIT CODE 116
IMPACT CODE 199
Appendix B 226
Appendix C 227
Appendix D 228
Appendix E 230
Appendix F 231

Acknowledgements

I am extremely grateful to my supervisors Ian Wilkinson and Marie Fisk for their advice, knowledge, patience and support over the entire course of my PhD. Each stage in an academic career is more challenging than the last and I could not have asked for better supervisors to guide me through this journey. Although the COVID pandemic caused much disruption to my planned research, their unwavering support and guidance throughout helped me adapt to the situation and kept my work on track, for which I am very thankful. Despite their very busy work schedules, they have always been ready to offer their help and advice at any time. I feel very fortunate to have had the opportunity to learn from such pleasant, wise and patient supervisors.

Special mention goes to Holly Pavey and Duuamene Nyimanu, for their advice and help. Holly, thank you so much for your advice on statistical analysis and for being so generous with your time. Duuamene, I am very grateful for your help with troubleshooting in the lab and for being willing to give advice outside of work hours. Also, thank you very much to Janet Maguire and Kaisa Maki-Petaja for their help with my queries in the lab and to Beverley Reynolds for her organisation and prompt responses.

Finally, I would like to thank my parents for always being there for me and for always believing in me.

Abbreviations

ACM: All-cause mortality

ACS: Acute coronary syndrome

AF: Atrial fibrillation

ASPIRE: Effects of Aspirin on Specialised Pro-Resolving Mediators

BAL: Bronchoalveolar lavage fluid

BMI: Body mass index

CAD: Coronary artery disease

CHF: Chronic heart failure

COPD: Chronic obstructive pulmonary disease

COX: Cyclooxygenase

CRP: C-reactive protein

CVD: Cardiovascular disease

Del-1: Developmental endothelial locus-1

DHA: Docosahexaenoic acid

ELISA: Enzyme linked immunosorbent assays

EPA: Eicosapentaenoic acid

FDA: Food and Drug Administration

FF: Fluticasone furoate

GOLD: Global Initiative for Chronic Obstructive Lung Disease

HF: Heart failure

HR: Hazard ratio

IL-6: Interleukin-6

IL-8: Interleukin-8

IL-17: Interleukin-17

IL-33: Interleukin-33

LOX: Lipoxygenase

MI: Myocardial infarction

MACE: Composite endpoint of death or adverse cardiovascular event

MAPK: Mitogen-activated protein kinase

PAD: Peripheral artery disease

PT: Preferred term

PUFA: Polyunsaturated fatty acids

QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies 2

RESCUE: Resolution Mediators in Chronic Lung Disease

RvD1: Resolvin D1

SMD: Standardised mean difference

SMQ: Standardised MedDRA Queries

SNP: Single nucleotide polymorphism

SPM: Specialised pro-resolving mediators

VI: Vilanterol

VSMC: Vascular smooth muscle cell

List of Figures

Figure 1: Human cardiovascular system (made with BioRender) 18
Figure 2: Diagram showing atherosclerotic plaque in coronary arteries and process of atherosclerosis (made with BioRender). COPD=Chronic obstructive pulmonary disease 21
Figure 3: Diagram showing alveolar damage in human lungs with COPD (made with BioRender). COPD=Chronic obstructive pulmonary disease..... 23
Figure 4: Diagram showing IL-33/ST2 signalling axis. IL-33=Interleukin-33, sST2=Soluble ST2, ST2L=Transmembrane ST2, IL-1RAcP=ST2L accessory protein 26
Figure 5: Diagram showing the lipid mediator family. Red highlights indicate pro-inflammatory mediators, Green highlights indicate anti-inflammatory mediators. 31
Figure 6: Biosynthesis of aspirin triggered resolvins. AT-Resolvins= Aspirin triggered resolvins 32
Figure 7: Flow chart of search strategy 54
Figure 8: Summary forest plots showing the Meta-SMD and 95% CI of IL-33 levels in HF patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance. HF=Heart failure 55
Figure 9: Summary forest plots showing the Meta-SMD and 95% CI of IL-33 levels in stroke patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance. 56
Figure 10: Summary forest plots showing the Meta-SMD and 95% CI of sST2 levels in ACS patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance. ACS=Acute coronary syndrome 57
Figure 11: Summary forest plots showing the Meta-SMD and 95% CI of sST2 levels in AF patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance. AF=Atrial fibrillation..... 57
Figure 12: Summary forest plot showing the multivariate Meta-HR and 95% CI for risk of any adverse cardiovascular events in community populations and its relation to sST2 levels. The Meta-HR in the random effects model is shown by the black diamond at the bottom. The vertical line at 1 is the border for significance. AF=atrial fibrillation, ACM=all-cause mortality, CV=cardiovascular, HF=heart failure, CVD=cardiovascular disease, CHD=coronary heart disease, MI=myocardial infarction, MACE=major adverse cardiovascular events. 59
Figure 13: Biosynthesis of SPMs derived from arachidonic acid. COX-2= Cyclo-oxygenase 2, LOX=Lipoxygenase, HETE=Hydroxyeicosatetraenoic acid, AT-Lipoxins=Aspirin triggered lipoxins 66
Figure 14: Biosynthesis of SPMs derived from DHA. LOX=Lipoxygenase, HDHA=Hydroxydocosahexaenoic acid, HpDHA=Hydroperoxydocosahexaenoic acid 66
Figure 15: Effects of SPMs in resolving COPD inflammation (made with BioRender). COPD=Chronic obstructive pulmonary disease, CD8=Cluster of differentiation 8, TNF-alpha=Tumour necrosis factor alpha, IL-6=Interleukin-6, IL-17=Interleukin-17, SPMs=Specialised pro-resolving mediators 68
Figure 16: Box and Whisker Plot showing median RvD1 levels in COPD exacerbators and stable COPD. Outlier is any value more than 1.5 x IQR above quartile 3 or 1.5 x IQR less than quartile 1. Star=Extreme outlier, Circle=Outlier. 74
Figure 17: Box and Whisker Plot showing median Del-1 levels in COPD exacerbators and stable COPD. Outlier is any value more than 1.5 x IQR above quartile 3 or 1.5 x IQR less than quartile 1. Star=Extreme outlier, Circle=Outlier. 75
Figure 18: Forest plot showing multivariate adjusted HR and 95% CI for aspirin use and its relationship with ACM. ACM=All-cause mortality. SUMMIT multivariate HRs were adjusted for covariates of age, sex, BMI, smoking status, smoking pack years, FEV1% and history of stroke, HF, hypercholesterolemia, hypertension, heart disease, diabetes and PAD. IMPACT multivariate HRs were adjusted for covariates of age, sex, BMI, trial treatment arm, smoking status, smoking pack years, FEV1% and history of arrhythmia, stroke, HF, hypercholesterolemia, hypertension, heart disease, diabetes and PAD. 92
Figure 19: Forest plot showing multivariate adjusted HR and 95% CI for aspirin use and its relationship with moderate and severe exacerbations. Exac Sev= Severe exacerbation, Exac Mod= Moderate exacerbation. SUMMIT and IMPACT multivariate HRs were adjusted for covariates of age, sex, BMI, trial treatment arm, smoking status, smoking pack years, FEV1% and previous exacerbations. 93
Figure 20: Bland-Altman plot showing differences between measurements of RvD1 231
Figure 21: Bland-Altman plot showing differences between measurements of Del-1 231

List of Tables

TABLE 1: RANDOMISED CLINICAL TRIALS OF CVD DRUGS TO TREAT COPD PATIENTS 37

TABLE 2: DEMOGRAPHICS OF COPD PATIENTS AND CONTROLS IN ELISA STUDY 73

TABLE 3: MEDIAN (IQR) LEVELS OF RVD1, DEL-1 AND IL-17 IN STUDY GROUPS 73

TABLE 4: DEMOGRAPHICS AND CLINICAL HISTORY OF THE SUMMIT AND IMPACT POPULATIONS......90

TABLE 5: QUADAS-2 CRITERIA 226

TABLE 6: SUMMIT PROPENSITY MATCHED GROUPS 228

TABLE 7: IMPACT PROPENSITY MATCHED GROUPS.....229

TABLE 8: PRISMA 2009 CHECKLIST 230

Chapter One: Introduction

1.1 Thesis overview

In this thesis, several immunomodulatory mediators and the CVD indicated drug aspirin will be evaluated for their potential to improve treatment and understanding of CVD and COPD, which are two diseases that present a major burden to healthcare systems worldwide (1).

In Chapter 3, the immunomodulatory mediator IL-33 will be evaluated for its role in human CVD, using a systematic review and meta-analysis of published clinical studies measuring circulating levels of the mediator in CVD patients and healthy controls.

In Chapter 4, levels of the immunomodulatory mediators Resolvin D1, developmental endothelial locus-1 and Interleukin-17 will be measured using enzyme linked immunosorbent assays, in serum sourced from stable COPD patients, COPD patients with recorded exacerbations at baseline and healthy controls, to evaluate differences in circulating levels between the groups.

In Chapter 5, the association of aspirin use with health outcomes (risk of all-cause mortality, exacerbations and cardiovascular events) in COPD populations will be evaluated using statistical analysis of two large high quality datasets sourced from published clinical studies (SUMMIT and IMPACT), to identify potentially beneficial effects of aspirin use for COPD patients.

1.2 Cardiovascular disease prevalence and impact

Cardiovascular disease is an encompassing general term that means disease affecting the heart or blood vessels. It includes a spectrum of disorders with different clinical manifestations and sequelae, but many of which share the same pathological process of atherosclerosis (2).

Historically, CVD primarily afflicted countries with affluent societies (high income countries), where people live longer due to better access to healthcare but have a lifestyle that involves ready access to fat rich foods, tobacco and transportation that means risk factors of CVD are more common. More recently, low and middle income countries where historically the main cause of morbidity and mortality were infections, are now significantly more affected by non-communicable diseases such as CVD, with the prevalence and negative impacts of CVD steadily growing over the decades. The Global Burden of Disease study covering 204 countries and territories found a total global CVD caseload of 523 million in 2019, compared to 271 million in 1990 when the study began (3). In addition to the major increase in CVD caseload, there was an accompanying rise in CVD deaths (from 12.1 million in 1990 to 18.6 million in 2019) and years living with disabilities (17.7 million in 1990 and 34.4 million in 2019) (3). Closer to home, CVD caused 27% of UK deaths in 2020 (4). In 2022, there were 7.6 million people with CVD in the UK, with 64,000 deaths from coronary heart disease, 35,000 deaths from stroke and 100,000 hospitalisations due to MI (5, 6). CVD is also responsible for 1.18 million UK hospital admissions annually and costs the economy £19 billion (7). The human and economic costs shown by these statistics make CVD one of the greatest health challenges in the UK and across the world.

1.2.1 Cardiovascular system

Arteries are vessels of the cardiovascular system that carry oxygenated blood from the heart to the body (with pulmonary arteries being an exception). Arteries branch into smaller vessels called arterioles which provide blood to the organs (8, 9). The human arterial system is shown in Figure 1 below.

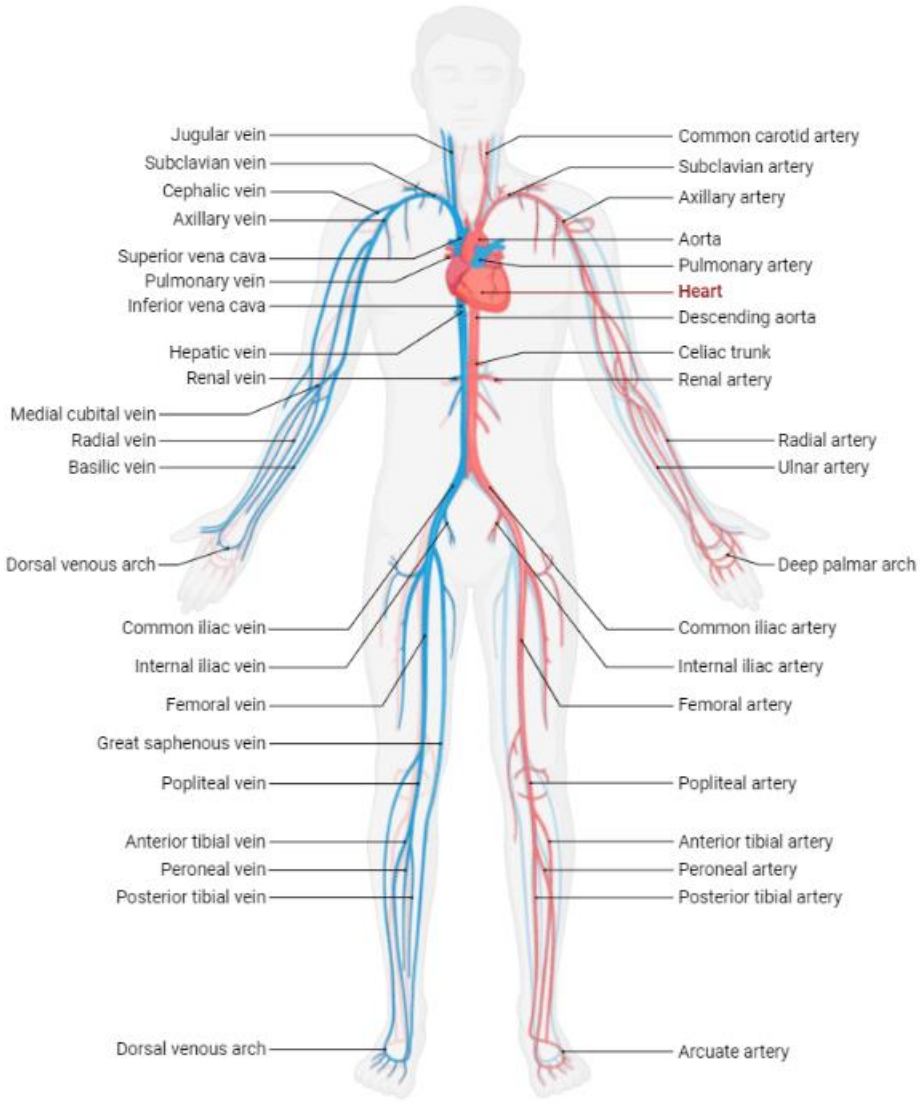

Figure 1: Human cardiovascular system (made with BioRender)

Due to the high pressure of blood leaving the heart, arteries have thick elastic walls (consisting of smooth muscle and elastin) that are capable of withstanding and regulating this pressure (8). Arteries have three layers, with the inner most layer (intima) being comprised of an endothelial monolayer (endothelium), smooth muscle and elastin. The middle layer (media) is comprised of smooth muscle that can regulate blood pressure and the outer layer (adventitia) interacts with vascular nerves which direct smooth muscle action (9). The endothelium regulates

vascular tone and extravasation of immune cells (movement from vessel into surrounding tissues) (10).

Coronary arteries are divided into the left and right coronary arteries that cover the heart and supply it with oxygenated blood (11). The carotid arteries (includes common, external and internal carotids) supply the brain with blood (12, 13). Peripheral arteries supply blood to the limbs (14).

1.2.2 Cardiovascular disease pathogenesis

Plaque build-up can occur inside the arteries which leads to arterial narrowing, in a process called atherosclerosis.

Atherosclerosis begins with endothelial dysfunction (which involves loss of endothelial integrity, increased smooth muscle cell proliferation and leukocyte adhesion) due to factors such as shear stress from blood flow, hypertension, and smoking, which is then followed by accumulation of low density lipoproteins in the intima and smooth muscle of the vessel (15, 16). These low density lipoproteins accumulate by binding to extracellular matrix proteins including proteoglycans such as heparin sulphate, eventually undergoing oxidation (15). These oxidized low density lipoproteins are involved in the activation of endothelial cells and increases monocyte recruitment which then infiltrate the intima (15). Monocyte recruitment is also enhanced by secretion of chemokines such as monocyte chemoattractant protein-1 (by cells such as endothelial and immune cells) (15, 17). After differentiating to macrophages (once monocytes enter the intima), these monocytes then ingest the oxidized lipoproteins and eventually become foam cells which accumulate to form lipid streaks (15). These monocytes also release signalling factors (such as tumour necrosis factor) and free radicals that can cause further endothelial dysfunction and lipoprotein oxidation (15). Smooth muscle cells then migrate to the lumen of the blood vessel wall (triggered by the release of factors including

Interleukin-1 and tumour necrosis factor by smooth muscle cells and endothelial cells), forming the fibrous cap of the plaque (15). This fibrous cap of smooth muscle cells, monocytes and T cells protrudes into the vessel lumen and disrupts blood flow (15). Over time, the plaque grows in size as more lipids accumulate and more monocytes are recruited, leading to arterial narrowing and impaired blood flow (15, 18). Atherosclerotic plaques present their greatest danger when they rupture, caused by destruction of the fibrous cap (due to lysis of the extracellular matrix by macrophage proteases) and subsequent exposure of the lipid core of the plaque, leading to blood clots forming around this lipid debris and a sudden blockage of blood flow in the major arteries (15, 19). These rupture events can also be caused by increased systemic inflammation such as during an acute COPD exacerbation, which increases thrombotic mediators including Interleukin-6 (IL-6), Interleukin-8 (IL-8) and tumour necrosis factor α (TNF-α) (20). Figure 2 below shows plaque build-up in the coronary arteries causing a blockage of blood flow.

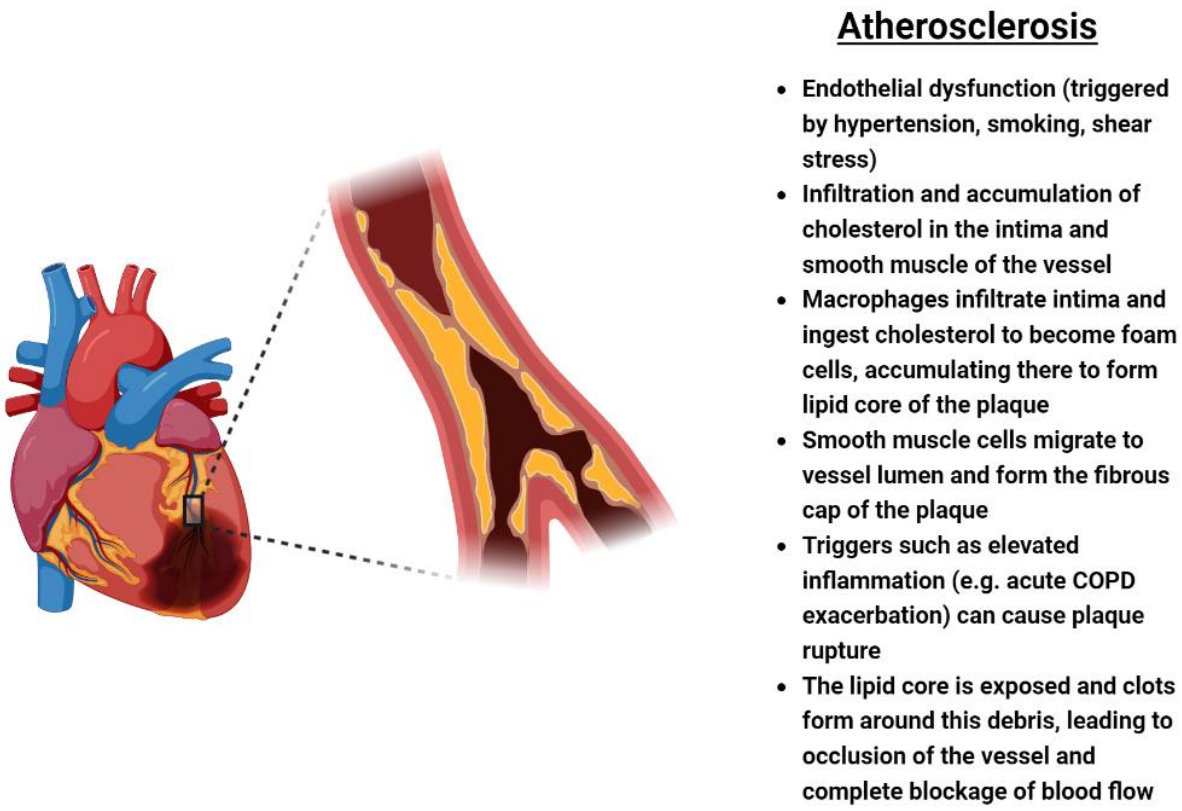

Figure 2: Diagram showing atherosclerotic plaque in coronary arteries and process of atherosclerosis (made with BioRender). COPD=Chronic obstructive pulmonary disease

Atherosclerosis in the coronary, carotid and peripheral arteries lead to different manifestations of CVD. Rupture of atherosclerotic plaque in the coronary arteries that causes a blockage of blood flow is an MI (or heart attack), which results in death of cardiac tissue and a weakened heart (21). Similarly, a blockage of the carotid arteries by plaque rupture causes an ischaemic stroke that leads to necrosis of brain tissue (22). Peripheral artery disease (PAD, caused by plaque formation in the peripheral arteries) typically affects the abdominal aorta, iliac and femoral arteries and can be marked by pain and muscle discomfort (14).

Atherosclerosis and subsequent narrowing of the coronary, carotid and peripheral arteries are primarily responsible for the manifestation of cardiovascular disease.

1.3 Chronic obstructive pulmonary disease impact

COPD describes a type of lung disease, primarily characterised by chronic bronchitis (inflammation of the airways) and emphysema (destruction of the alveoli). Globally, there were approximately 391.9 million cases of COPD in 2019 (in the 30-79 age group) (23). Additionally, COPD was the third most common global cause of death in 2019, with 3.23 million people dying from the disease (24). Whilst less than a quarter of global COPD cases are found in high income countries, COPD is still a heavy burden even in those countries, with the British Lung Foundation reporting an annual cost of COPD to the UK economy of £1.9 billion (23, 25).

1.3.1 Chronic obstructive pulmonary disease pathogenesis

In the respiratory system, the trachea (windpipe) divides into a left and right bronchus that each branch into bronchioles, which branch into alveoli. The alveoli are responsible for gaseous exchange in the lungs, delivering oxygen to the vascular system (26).

COPD disrupts the normal functioning of the respiratory system and is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2018 as a ‘common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities, usually caused by significant exposure to noxious particles or gases’ (27). Figure 3 below shows damaged alveoli in human lungs with COPD.

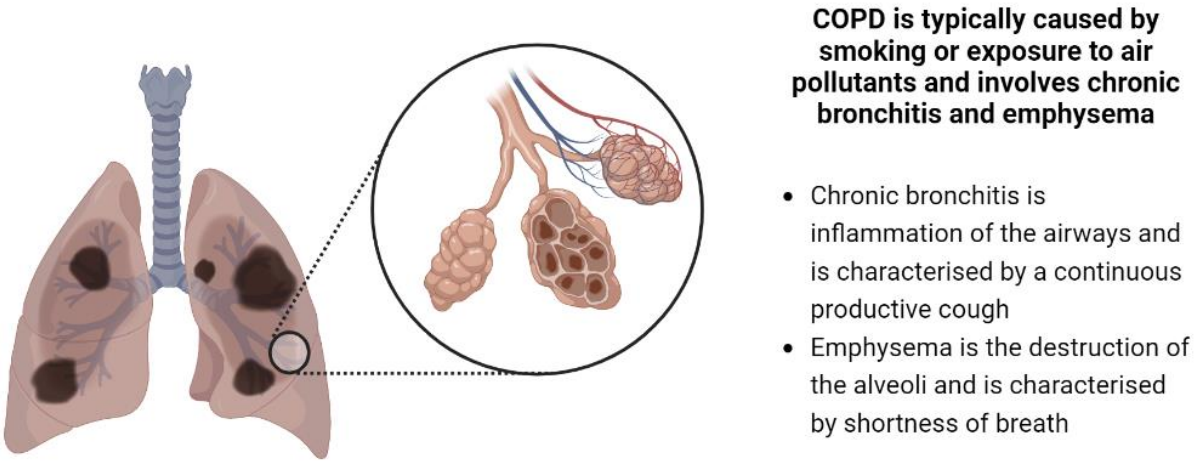

Figure 3: Diagram showing alveolar damage in human lungs with COPD (made with BioRender). COPD=Chronic obstructive pulmonary disease

COPD typically begins with an insult to the pulmonary system caused by exposure to cigarette smoke or air pollution. These trigger inflammatory responses that recruit pro-inflammatory neutrophils which can release reactive oxygen species as part of the immune response. The oxidative stress in this inflammatory environment promotes elevated protease levels (e.g. elastase) and inactivates various anti-proteases (e.g. α-1 antitrypsin) that results in the breakdown of alveolar walls and narrowing of the airways (28-30). The incomplete cessation of this pro-inflammatory response leads to chronic inflammation resulting in COPD. Bacterial or viral infection can trigger an exacerbation, which is an acute worsening of COPD symptoms that leads to lower quality of life and increased risk of mortality (31). Although the majority of COPD cases are caused by long term smoking or pollutants exposure, a minority of cases (approximately 5% of all COPD cases) are caused by a mutation in the SERPINA1 gene that leads to a deficiency in the protein α-1 antitrypsin, that is needed to inhibit neutrophil elastase (which contributes to the pathogenesis of COPD by breaking down elastin in alveolar walls) (32).

1.3.2 Comorbidity of cardiovascular disease in chronic obstructive pulmonary disease patients

In addition to being reported together as leading causes of death, CVD is a major comorbidity reported in COPD patients. In a meta-analysis of 29 datasets, Chen *et al* 2015 showed that COPD patients are almost two and a half times more likely to have CVD compared to a non-COPD population (33). Additionally, during up to 3 years follow up, CVD patients with COPD were observed to have a higher risk of mortality compared to those without COPD (34). Apart from lifestyle factors such as smoking, systemic inflammation (persistent elevated levels of inflammatory markers in the blood) is associated with the presence of COPD, particularly during exacerbations, and is thought to be a major cause of increased CVD risk. Evidence cited to support this theory include the association of COPD with increased carotid intima medial thickness, higher levels of circulating inflammatory markers in COPD patients with CVD compared to those without CVD and increased risk of MI following exacerbations (1, 35). This is potentially caused by increased expression of leukocyte adhesion molecules in endothelial cells (due to increased leukocyte extravasation in COPD) which increases risk of atherosclerosis, as well as acute inflammation (e.g. exacerbations) causing plaque rupture (1, 36). However, other factors such as older age causing elastin degradation (via enzymatic degradation, calcification and oxidative stress) in the vasculature leading to arterial stiffness have also been suggested (1, 37). In particular, age is a major independent risk factor for CVD (including hypertension, myocardial infarction and arrhythmia) due to increased oxidative stress during the aging process, and while older age is not as significant a risk factor in COPD (smoking, air pollution and alpha-1 antitrypsin deficiency are the main risk factors), most COPD patients are older, likely due to COPD pathogenesis being triggered by prolonged exposure to air pollutants/cigarette smoke over time (38).

Moderately effective treatment options for CVD (e.g. beta blockers, ACEIs) and COPD (e.g. bronchodilators) exist and are commonly used. However, it is important to identify novel treatments for COPD patients who are at risk of, or have been diagnosed with CVD, due to the increased risk of mortality in these patients. Furthermore, patients living with CVD or COPD are also more vulnerable to other diseases compared with healthy populations, which has been clearly highlighted by the COVID-19 pandemic.

Given the status of CVD as a commonly reported comorbidity of COPD, it is clear that there is an unmet need for expanded therapeutic options for COPD patients that are compatible and synergistic with underlying CVD and concomitant CVD medications. There are immunomodulatory mediators that have potential in the treatment of both COPD and CVD, particularly the Interleukin-33 (IL-33)/ST2 axis, the specialised pro-resolving mediator (SPM) Resolvin D1 (RvD1). Additionally, the anti-platelet drug aspirin, currently indicated for CVD patients, also has promise as a treatment for COPD patients.

Systemic inflammation underpins the pathogenesis of COPD and CVD and better understanding of immunomodulatory mediators that can reduce this inflammation can lead to improved treatment regimens.

1.4 Interleukin-33 signalling axis

The IL-33/ST2 axis has been implicated in the pathogenesis of both COPD and CVD.

Within this signalling axis, IL-33 is the ligand and ST2 is the receptor. First discovered in 1989, ST2 was designated an orphan receptor until its ligand, IL-33, was identified in 2005 (39). ST2 occurs in two forms, the transmembrane ST2L form (found in cells such as macrophages, cardiomyocytes, T regulatory cells, neutrophils, natural killer cells) which is responsible for initiating the cellular effects of IL-33 binding, and the circulating soluble ST2 (sST2) form which acts as a decoy receptor to dampen the effects of IL-33 signalling (40). The IL-33 protein

is organised into a tertiary structure of 12 β-strands in a β-trefoil fold with 270 residues in total (41). In humans, the cytokine IL-33 is constitutively expressed (accumulating in the cell nucleus in health and linked with chromatin) by endothelial, epithelial and smooth muscle cells of the vasculature, and released (still linked with chromatin) outside the cell after a trigger such as cell damage or infection, serving as an alarmin signal (39, 40, 42). Figure 4 below shows the process of IL-33 release and binding to its receptor on target cells.

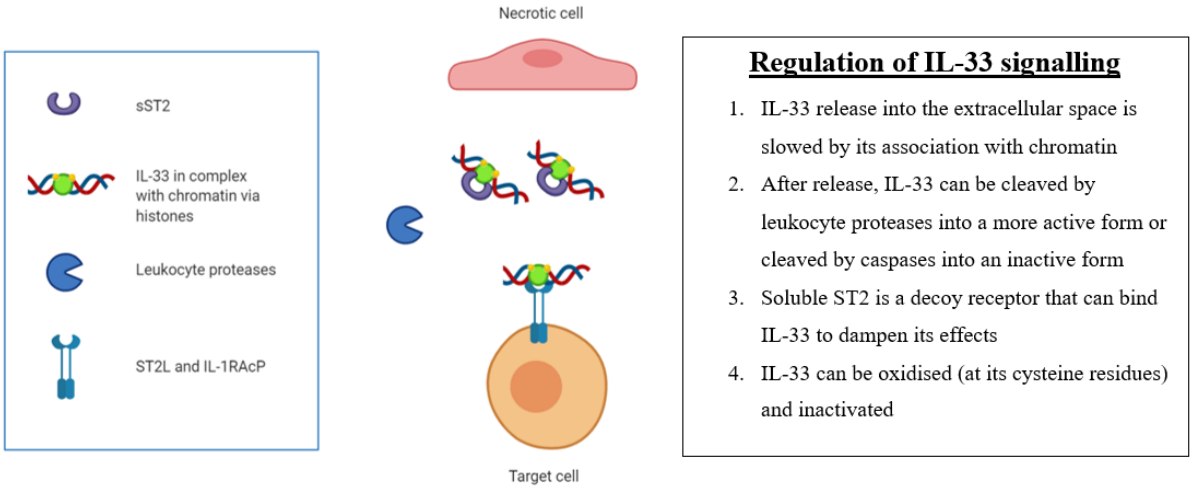

Figure 4: Diagram showing IL-33/ST2 signalling axis. IL-33=Interleukin-33, sST2=Soluble ST2, ST2L=Transmembrane ST2, IL-1RAcP=ST2L accessory protein

Due to its strong downstream signalling effects, IL-33 signalling is controlled through several mechanisms to dampen its effects. Firstly, being linked to chromatin in the nucleus slows down the release of IL-33 outside the cell. Secondly, if IL-33 has been released as part of apoptosis, it is cleaved into an inactive form by caspase 3 and caspase 7 (42). Thirdly, once released into the extracellular space, IL-33 is rapidly oxidised and inactivated (43). Finally, the decoy receptor sST2 can bind to IL-33, preventing it from exerting downstream effects through binding to its transmembrane receptor ST2L (44).

If IL-33 is not inactivated following release, it will bind to its transmembrane receptor ST2L and the accessory protein IL-1RAcP on target cells such as the previously listed immune cells, and activate the mitogen-activated protein kinase pathway, allowing it to exert its signalling effects (45).

IL-33 has many wide ranging and significant downstream effects including enhanced human mast cell activity (implicated in allergic diseases), longer human eosinophil survival and IL-8 production (recruits neutrophils) as well as IL-6 production (46-48).

The IL-33/ST2 axis has been implicated in many diseases, including in tumour development where there it appears to have both pro and anti-tumour effects (49).

1.4.1 Interleukin-33 in chronic obstructive pulmonary disease and cardiovascular disease

In COPD patients, serum IL-33, ST2 and IL-1RAcP levels have been found to be higher than in healthy controls and cigarette smoke extract increased expression of IL-33 by peripheral blood monocytes and bronchial epithelial cells (50). In a 2019 study, 210 COPD patients and 180 controls had serum levels of IL-33 measured, with the COPD group having higher levels of IL-33 (51). Additionally, IL-33 gene expression was linked to mucin gene expression in COPD patients, where mucus hypersecretion is a symptom of COPD (52, 53). Taken together, these findings suggest that IL-33 plays a significant harmful role in COPD pathogenesis and disease progression. There are several ongoing clinical studies using antibodies to target IL-33 and its receptor ST2 (54).

However, in CVD, the role of the IL-33/ST2 signalling axis has been less clear.

IL-33 prevents cardiomyocyte apoptosis in an *in vitro* setting and slowed the development of atherosclerosis in mice (55, 56). Whilst sST2 is a Food and Drug Administration (FDA)

approved prognostic biomarker of mortality for chronic heart failure, and has been studied in several CVD including CAD and HF which associated elevated levels with poor prognosis, there have been fewer studies focusing on circulating levels of IL-33. Although there have been some measurements of IL-33 in MI and HF patients which have inconclusive results or are limited by small sample size (57-61).

IL-33 causes human monocytes to release pro-coagulant microvesicles which suggests potential pro-atherosclerotic effects (62). Additionally, an *in vitro* study showed that IL-33 enhanced the adhesion of human leukocytes to endothelial cells, and increased expression (on a protein and mRNA level) of vascular cell adhesion molecule-1, intracellular adhesion molecule-1 and monocyte chemoattractant protein-1 in atherosclerotic plaques *ex vivo* (63). Furthermore, in a 2016 study, 41 atherosclerotic plaques taken from human carotid arteries were analysed for ST2L expression, and ST2L was highly expressed on macrophages and more so in plaques from symptomatic patients compared to asymptomatic. These findings suggest IL-33 signalling could be promoting plaque progression (64). Taken together with the fact that in humans, platelets are one of the cell types that constitutively express IL-33, the IL-33/ST2 axis appears to have a potentially harmful role in CVD, which is contrary to some previous findings and are not well supported by studies measuring circulating levels of IL-33 (65).

Given the importance of the IL-33/ST2 axis in COPD and CVD, the clinical studies targeting this axis in COPD, and COPD patients being at higher risk of CVD, it is essential to further develop understanding of IL-33/ST2 in CVD, to see if they have the same role as in COPD. This will help identify potential contraindications with future COPD medications that target the IL-33/ST2 axis.

Moreover, there have been several published studies measuring IL-33 and sST2 levels in CVD patients. However, contradictory findings, small individual study sizes and the heterogeneity

of the preceding studies indicate that a systematic review and meta-analysis of the published studies is needed, to elucidate differences in IL-33 and sST2 levels in CVD patients and controls and identify any associations of the biomarker levels with health outcomes over a follow up period.

1.4.2 Planned wire myography work

Before the outbreak of COVID-19, the plan was to assess the effects of IL-33 (speculated to regulate vascular constriction) on the human vascular system *in vitro*, using wire myography and human resistance and pulmonary vessels sourced from excess surgical tissue (abdominal and lung).

Briefly, we planned to obtain up to 100 resistance vessel samples from excess subcutaneous tissue removed during abdominoplasty or breast reconstruction surgery at Cambridge University Hospitals NHS Foundation Trust. 30 pulmonary vessel samples were to be sourced from lung tissue removed during thoracic surgery at Royal Papworth Hospital tissue bank. Samples were to be less than 500 μM in diameter for resistance vessels and 3-5 mm diameter for pulmonary vessels. The vessels were to be mounted onto a dual wire myograph system (Danish Myo Technology Myo-Interface, model 500A and 610M). Vascular constriction would then be assessed after IL-33 treatment.

The project was approved but not carried out due to the pandemic.

1.5 Specialised pro-resolving mediators

Eicosanoids are a large and diverse group of lipid mediators that includes pro-inflammatory mediators such as leukotrienes (recruits neutrophils) and thromboxane (promotes platelet aggregation) as well as anti-inflammatory mediators such as resolvins and lipoxins, which are pro-resolving (66). Eicosanoids primarily signal target cells such as macrophages and

neutrophils via G-protein coupled receptors, with effects limited to their area of production (66).

SPMs are a highly important member of the eicosanoid family that is responsible for the natural cessation of inflammation after infection or tissue damage. There are many types of SPMs such as resolvins, lipoxins, protectins and maresins, all of which are derived from different polyunsaturated fatty acids (PUFA) (67). These different SPMs have varying roles during sequential phases of inflammation resolution, but all are pro-resolving, with effects including inhibition of neutrophil recruitment, reduced production of pro-inflammatory cytokines such as IL-6 and IL-8 and decreased levels of reactive oxygen species (68, 69). They are critical to the complete cessation of inflammation, the lack of which underpins COPD and CVD pathogenesis. Their importance in maintaining a balanced immune response is highlighted by their near ubiquitous presence in the body, being identified in tissues ranging from serum to breast milk to cerebrospinal fluid (70). Figure 5 below shows the lipid mediator family and the PUFAs from which they are derived.

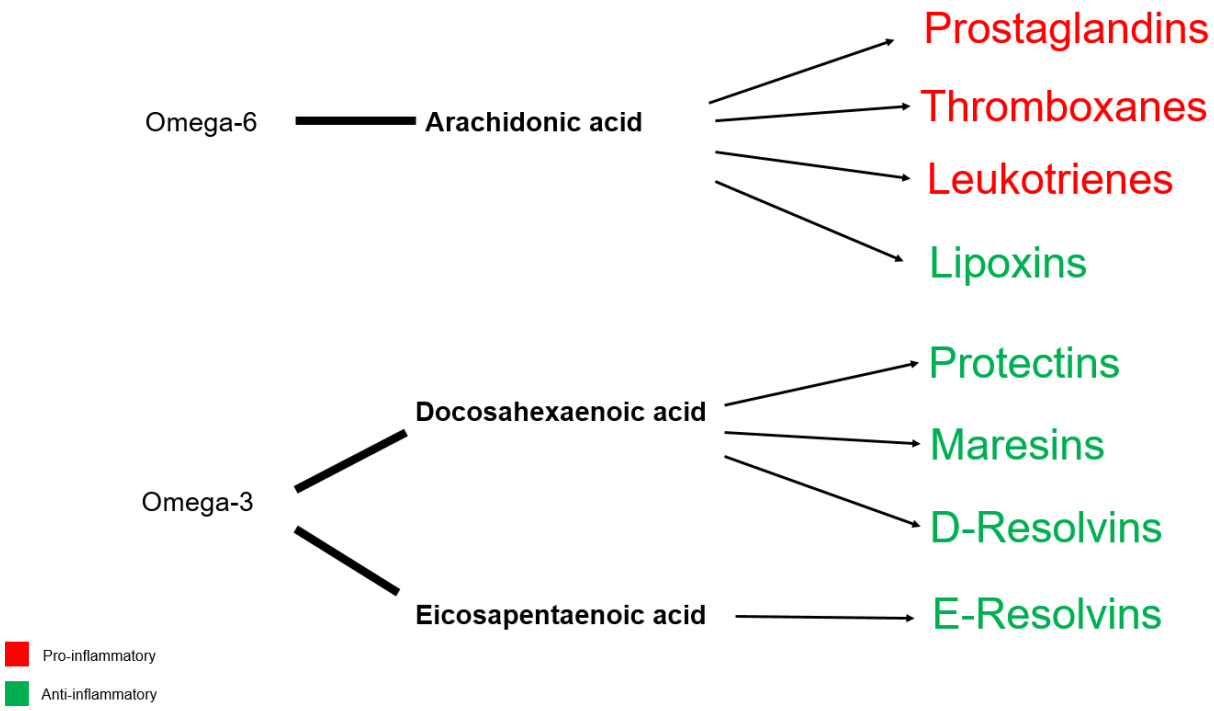

Figure 5: Diagram showing the lipid mediator family. Red highlights indicate pro-inflammatory mediators, Green highlights indicate anti-inflammatory mediators.

1.5.1 Specialised pro-resolving mediator biosynthesis

SPMs are synthesised via transcellular biosynthesis, which involves one cell producing an intermediate stage product before another cell finishes the synthesis. This transcellular biosynthesis involves three main pathways of SPM production, the lipoxygenase (LOX), cyclooxygenase (COX), cytochrome P450 pathways (66, 67). Depending on the SPM, synthesis can take place using only lipoxygenases such as 5-LOX and 12-LOX for lipoxins, or it can involve another enzyme such as cytochrome P450 producing the intermediate stage that is then finished by 5-LOX (67). Importantly, aspirin can also trigger the production of SPMs by acetylating the COX enzymes. Aspirin's well known anti-platelet effects are due to its inhibition of COX-1, which blocks the production of platelet activating thromboxanes. However, aspirin also acetylates COX-2, which modifies the enzyme to allow it to produce

intermediate stages of SPMs (such as lipoxins and resolvins), which are then completed by LOX enzymes. These aspirin triggered SPMs are epimers of natural SPMs and are as effective in their pro-resolving actions as well as being longer lasting (70, 71). Figure 6 shows the biosynthesis pathways for aspirin triggered resolvins.

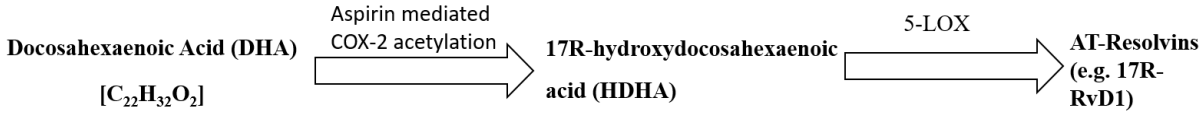

Figure 6: Biosynthesis of aspirin triggered resolvins. AT-Resolvins= Aspirin triggered resolvins

SPMs are essential for inflammatory resolution and their expression has been found to be disrupted in many diseases from chronic conditions such as multiple sclerosis (SPM levels decreased and correlated with disease progression) to acute conditions such as COVID-19 patients with severe acute respiratory syndrome (plasma levels of eicosanoids heavily biased towards pro-inflammatory mediators compared to SPMs) (72, 73).

1.5.2 Specialised pro-resolving mediators in chronic obstructive pulmonary disease and cardiovascular disease

The systemic inflammation present in CVD and COPD suggests the presence of dysregulated SPM production pathways in those diseases (74, 75). This is supported by the 2019 finding that plasma RvD1 levels were lower in 27 chronic heart failure patients compared to 23 healthy controls. This study also reported that T cells treated with exogenous RvD1 were not responsive, with production of inflammatory cytokines (TNF-α, Interleukin-17) being unchanged. This suggests that in CVD, both production and effectiveness of SPMs are reduced (76). Additionally, vulnerable regions of human atherosclerotic plaques sourced from carotid endarterectomy were found to have lower levels of RvD1, which suggests a potential

association between disrupted SPM expression and increased risk of plaque rupture (77). Similarly, COPD patients have lower serum RvD1 levels than controls, and resolvin treated macrophages produced lower levels of inflammatory cytokines (IL-6, IL-8) after exposure to cigarette smoke extract (78). Furthermore, levels of sputum PUFAs and PUFA metabolites were lower in stable COPD patients compared to controls. Interestingly, during acute exacerbations, levels of mediators produced by COX-2 were elevated (prostaglandins and thromboxanes), which draws further attention to the potential of aspirin in treating COPD via modulation of SPMs (79). The findings of these small studies suggest further investigation is needed to identify differences in SPM levels in COPD and CVD patients and expand understanding of how dysregulated SPM production is associated with disease progression and severity.

Whilst increased dietary intake of PUFAs has been recommended for decades to people at risk of CVD, interest in the role of PUFAs and their metabolites in treating COPD is a more recent phenomenon. In particular, there are few studies evaluating SPMs in COPD. In the literature, RvD1 has been the best represented compared to other SPMs in the study of COPD.

1.5.3 Resolvin D1 in chronic obstructive pulmonary disease

RvD1 is a D-series resolvin that is produced from docosahexaenoic acid, that has been shown to be expressed at lower levels in COPD patients compared to controls and had protective effects in lungs of cigarette smoke exposed mice emphysema models (78, 80). For homeostatic functions, RvD1 binds DRV1, and for resolution scenarios, RvD1 effects are exerted through G protein coupled receptors which are upregulated during neutrophil activation (81). RvD1 signalling causes activation of the mitogen-activated protein kinase pathway in target cells, leadings to effects such as reduced neutrophil recruitment to inflammatory sites, direction of macrophages to an anti-inflammatory phenotype and reducing differentiation of Th17 cells

(that produce inflammatory IL-17) (82-84). The reported effects of RvD1 signalling suggests it has an important resolution role in COPD and it is important to assess if this pathway is dysregulated in COPD and if it is associated with disease severity.

Current therapeutics for COPD, while effective, are inadequate for tackling the challenge of the global COPD burden, as highlighted by the rising caseload, mortality and poor quality of life caused by COPD over the decades. In particular, despite the increased risk of developing CVD in COPD populations, there are no specific treatment regimens that help these patients who are at higher risk of death than other COPD patients. Commonly indicated drugs such as bronchodilators may have harmful effects on the cardiovascular system and corticosteroids are a blunt tool that increases risk of infection from immunosuppression, which can trigger an acute exacerbation that is known to worsen prognosis (31, 85, 86).

RvD1, as an immunomodulatory mediator, has the potential to promote the natural resolution of the systemic inflammation found in COPD and also has an ameliorative role in CVD, making them an attractive target for further study. It is important to evaluate differences in the expression levels of RvD1 in COPD patients (both stable and unstable) and controls to further understanding of the role of RvD1 in COPD pathogenesis and disease progression.

1.6 Current treatments for chronic obstructive pulmonary disease

COPD patients are encouraged to manage their condition through exercise and therapeutics such as bronchodilators and corticosteroids. Bronchodilators are agents which dilate the bronchi by targeting smooth muscle, resulting in increased airflow. The three classes of bronchodilators are β-2 adrenergic receptor agonists, anti-muscarinic and methylxanthines, which are often used in combination treatment with corticosteroids. Corticosteroids have

powerful anti-inflammatory effects, which can counter the chronic inflammation associated with COPD progression. Bronchodilators and corticosteroids have been found to be effective in reducing the symptoms of COPD, with a systematic review of 33 bronchodilator studies finding that exercise capacity improved in half of the studies, and a meta-analysis of corticosteroid therapy (involving 1331 COPD patients) showing that systemic corticosteroid use was associated with successful treatment after exacerbations (87-93).

Although these therapeutics are effective in treating symptoms of COPD, they are also associated with harmful effects, including increased risk of arrhythmias and cardiomyopathy in bronchodilator users, while corticosteroid use is associated with increased risk of pneumonia (94, 95).

Although not currently indicated for COPD patients, aspirin has been of increasing interest for treating COPD, due to their anti-platelet effects (there is increased platelet activation in COPD which increases release of platelet factor 4 that stimulates elastase, causing the breakdown of lung elastin) and production of pro-resolving immunomodulatory molecules which can reduce lung inflammation (96-98). The status of aspirin as a widely prescribed CVD medication further increases interest in its potential for treating COPD, considering the increased cardiovascular risk of COPD populations.

1.7 Aspirin use and chronic obstructive pulmonary disease health outcomes

Current prevention and treatment regimens for COPD centre on lifestyle changes such as increased exercise and quitting smoking, as well as therapeutic options such as bronchodilators.

As previously described, there is a lack of effective COPD medications for patients with CVD, despite the elevated risk of CVD in COPD patients. While there remains a strong need for novel

therapies, repurposing existing CVD medications that are well studied has the potential to quickly improve patient treatment regimens.

Beta-blockers decrease heart rate and blood pressure by targeting β-adrenoceptors and are prescribed to treat conditions such as hypertension, HF and atrial fibrillation (99). There have been concerns of potential contraindications of beta-blocker use in COPD patients, but findings from a systematic review and meta-analysis of observational studies have suggested that beta-blockers are well tolerated in COPD patients and are associated with reduced exacerbation and mortality rates (100, 101). However, a recent trial of the beta-blocker metoprolol to treat COPD patients (532 patients randomised) found that metoprolol had little effect on time to first exacerbation compared to the placebo group and the metoprolol group was also more likely to be hospitalised after an exacerbation (102).

Other CVD drug classes have also been investigated for treating COPD, including a clinical trial of the statin (decreases cholesterol synthesis by inhibiting HMG-CoA reductase) simvastatin in 885 COPD patients, which did not find any effect on exacerbation numbers or time to first exacerbation compared to placebo (103, 104). Angiotensin converting enzyme inhibitors (inhibits production of the vasoconstrictor angiotensin II) and angiotensin receptor blockers (inhibits angiotensin II receptor) are prescribed to treat hypertension (105, 106). A small trial of the angiotensin converting enzyme inhibitor enalapril in 21 COPD patients found that treatment improved work rate compared to placebo, but a recent trial of the angiotensin receptor blocker losartan in 220 COPD patients found that treatment did not stop the progression of emphysema compared to placebo (107, 108). Table 1 lists the randomised clinical trials of CVD medications in COPD patients.

Table 1: Randomised Clinical Trials of CVD drugs to treat COPD patients

Year	Trial Drug	Sample Size	Population	Effects
1981	Propranolol (β-blocker)	13	Non-asthmatic COPD	Propranolol treatment caused worse pulmonary function compared to placebo (109).
1986	Atenolol, Bisoprolol (β-blocker)	12	Non-asthmatic COPD and co-existing angina	Atenolol treatment led to increased airway resistance compared to bisoprolol and placebo treatment (110).
2005	Propranolol, metoprolol, celiprolol (β-blocker)	15	Mild or moderate COPD and airway hyper-responsiveness	Forced expiratory volume 1 decreased only by propranolol treatment compared to placebo. Metoprolol and propranolol treatment increased airway hyper-responsiveness. Celiprolol had no effect on lung function (111).
2009	Bisoprolol (β-blocker)	27	HF and co-existing moderate/severe COPD	Treatment group had reduced forced expiratory volume 1s compared to placebo. Exacerbation number similar in both treatment and placebo groups (112).
2012	Bisoprolol (β-blocker)	27	Moderate or severe COPD	Treatment group associated with worse dynamic hyperinflation compared to placebo (113).
2019	Metoprolol (β-blocker)	532	Moderate and severe COPD	Time to first exacerbation similar in treatment and placebo groups. Treatment group more likely to be hospitalised for an exacerbation (102).
2014	Simvastatin (statin)	885	COPD with no diabetes or cardiovascular disease	Simvastatin treatment did not affect exacerbation numbers or time to first exacerbation compared to placebo (103).
2010	Enalapril (angiotensin converting enzyme inhibitor)	21	COPD without cardiovascular disease	Enalapril treatment improved work rate compared to placebo (107).
2022	Losartan (angiotensin receptor blocker)	220	Mild to moderate COPD	Losartan treatment did not stop emphysema progression compared to placebo (108).

1.7.1 Platelets in chronic obstructive pulmonary disease and cardiovascular disease

The anti-platelet class of CVD medications has also been of increasing interest for its potential in treating COPD.

Platelets play an essential role in blood clotting and are needed to stop bleeding. However, they are also implicated in CVD, where activated platelets increase the rate of atherosclerosis by promoting immune cell recruitment (114). Platelets also form clots around ruptured plaque, (which can be triggered by an acute COPD exacerbation) further increasing the risk of an ischaemic event (115). Platelet activity has been implicated in the pathogenesis of COPD, with higher platelet counts being reported in COPD patients compared to controls and also severe COPD patients compared to mild COPD patients (116, 117). Platelets are thought to drive COPD pathogenesis and the associated increased CVD risk through several mechanisms, including the release of platelet factor 4 which induces elastase breakdown of alveolar elastin (causing decreased alveolar wall elasticity), the formation of platelet-monocyte aggregates, increased release of thromboxane A2 (promotes platelet aggregation) and synthesis of plasminogen activator inhibitor-1 which is associated with thrombosis and reduced lung function in COPD patients (98). Considering the important role of platelets in COPD and CVD pathogenesis and the elevated risk of CVD in COPD populations, platelets are ideal targets for therapies aiming to improve health outcomes in COPD populations.

1.7.2 Aspirin's potential in chronic obstructive pulmonary disease treatment

There are many anti-platelet drugs which block platelet activity with different mechanisms of action, including clopidogrel which inhibits adenosine diphosphate receptors (needed for

platelet activation) and aspirin which irreversibly inhibits the COX-1 and COX-2 enzymes by acetylating the serine residue, inhibiting thromboxane A2 production which is needed for platelet aggregation (118, 119).

Aspirin, in particular, is of great interest for its potential as a CVD compatible COPD medication, as it is already indicated for CVD patients. Systematic reviews of 15 clinical trials found aspirin use reduced the risk of primary atherosclerotic CVD including MI and stroke (Risk Ratio: 0.90), although there was also an increased risk of bleeding (Risk Ratio: 1.54) (120). Other than its anti-platelet effects, aspirin is known to promote the production of immunomodulatory specialised pro-resolving molecules that are essential to the complete cessation of inflammation, which can be produced by aspirin modified COX-2 enzymes in addition to natural biosynthesis (121). These COX-2 derived SPMs are epimers of naturally produced SPMs (67). Given the major role of systemic inflammation in driving COPD and CVD pathogenesis, this immunomodulatory capability of aspirin is highly valued.

Their ready availability, well-tolerated status in CVD and ability to target pathways involved in the pathogenesis of CVD and COPD highlights their potential as a treatment to improve health outcomes in COPD patients, including those with a history/risk of CVD.

Whilst there have been no clinical trials of aspirin to treat COPD patients, several observational studies have reported a positive association between aspirin use and improved prognosis for COPD patients (reduced mortality and exacerbation rate) (122, 123). However, these studies made use of heterogeneous studies with varied population demographics, clinical backgrounds, disease definitions, follow up time, data collection methods and statistical analysis techniques. These limitations highlight the need for a comprehensive evaluation, in large high quality datasets, of aspirin use in COPD patients and its association with health outcomes over follow up.

1.8 Summary

CVD and COPD affect hundreds of millions of people across the world and are a major cause of mortality and decreased quality of life. Despite the availability of diverse therapeutics to treat these conditions, the number of cases and deaths from these diseases have only increased over the decades. Additionally, existing treatments for COPD and CVD have many drawbacks such as the increased risk of pneumonia in corticosteroid users. CVD is a commonly reported comorbidity in COPD patients, which complicates treatment options due to potential drug contraindications, further increasing the risk of mortality. Therefore, there is a need for novel treatments which can effectively treat COPD patients who have CVD or are at risk of CVD. Immunomodulatory mediators have great potential in improving the treatment of COPD and CVD, as systemic inflammation is the foundation of the pathogenesis of both diseases. Identifying mediators that promote resolution of inflammation and help regulate the immune response has the potential of providing a more effective and nuanced approach to tackling the challenge of CVD and COPD, while avoiding the drawbacks of existing therapies, such as the immunosuppressive effects of corticosteroids and the increased risk of arrhythmia for bronchodilator users. In this thesis, the following immunomodulatory mediators will be evaluated for their potential to improve the treatment and understanding of CVD and COPD: IL-33/ST2 and RvD1.

1.9 Hypotheses

1. The IL-33/ST2 axis is important in clinical presentations across the range of cardiovascular diseases. Soluble ST2 levels are higher in CVD patients than in controls and higher levels are associated with poor prognosis. Higher IL-33 levels are associated with disease.

2. Serum levels of RvD1 and developmental endothelial locus-1 (Del-1) are lower in COPD patients compared to healthy controls, while Interleukin-17 (IL-17) levels are higher in COPD patients.
3. Aspirin use is associated with decreased risk of mortality and exacerbations over follow up in moderate and severe COPD patients, including those with a risk/history of CVD.

1.10 Aims

To address the previously listed hypotheses, statistical and laboratory based studies were carried out. First a systematic review and meta-analysis of human IL-33/ST2 studies will be carried out to quantitatively assess circulating levels of IL-33 and ST2 in CVD patients and controls. Then enzyme linked immunosorbent assays will be used to measure and assess differences in the levels of RvD1, Del-1 and IL-17 in frozen serum samples sourced from COPD patients and controls. Finally, statistical analysis will be carried out on COPD datasets sourced from published clinical trials to assess the association between aspirin use and risk of all-cause mortality, exacerbations and cardiovascular composite events.

1. To evaluate the importance of the IL-33/ST2 axis in cardiovascular disease and to quantitatively analyse the differences in IL-33/ST2 levels in CVD patients and controls.
2. To evaluate differences in the serum levels of RvD1, Del-1 and IL-17 in COPD patients with exacerbations, stable COPD patients and controls.
3. To evaluate the association of aspirin use in COPD populations with risk of all-cause mortality, exacerbations and cardiovascular composite events over follow up.

Chapter Two: Methods

2.1 Overview

Here, an overview of the methods and techniques used in this research project are outlined. The methods are explained in more detail in the individual experimental chapters. I carried out all statistical analysis and laboratory work.

2.1.1 Datasets used

For the systematic review and meta-analysis of IL-33/ST2, the designated search terms (IL-33/Interleukin-33/ST2 combined with one of cardiovascular disease, stroke, myocardial infarction, heart failure, coronary disease, ischaemic heart disease and hypertension) were entered into each of the following databases: Pubmed, Web of Science, Cochrane Library, Prospero. Duplicates were then removed from the search results and primary and secondary screening of the abstract and main text (based on inclusion/exclusion criteria) was carried out. Only human studies that measured plasma or serum levels of IL-33 or sST2 were included. The number of eligible studies was 77.

For the evaluation of RvD1, Del-1 and IL-17 levels in COPD patients and controls, frozen serum samples were selected from manifests from the ERICA (assessed predictive value of cardiovascular abnormalities and fibrinogen for health outcomes in COPD patients) and ACCT (assessed effect of cardiovascular risk factors on central pulse pressure) studies.

For the evaluation of aspirin use and health outcomes in COPD populations, the full datasets of the SUMMIT (n=16485, moderate COPD with risk/history of CVD defined as CAD, PAD, stroke, MI and diabetes with target organ disease, median follow up 1.8 years, published 2016) and IMPACT (n=10355, severe COPD, 52 weeks follow up, published 2018) clinical trials were used.

2.1.2 Data extraction

For the systematic review and meta-analysis of IL-33/ST2, the eligible studies were scored on quality using the Quality Assessment of Diagnostic Accuracy Studies 2 criteria and data extraction was guided by the PRISMA statement. The main data extracted were the mean and standard deviation of circulating IL-33 and sST2 levels, as well as the hazard ratios and confidence intervals. Other data such as adjustment factors for multivariate hazard ratios are available in full in Appendix C.

For the evaluation of RvD1, Del-1 and IL-17 serum levels in COPD patients and controls, 140 COPD patients with exacerbations at baseline, 86 stable COPD patients and 146 controls were selected and matched based on factors including age, gender and body mass index (BMI) from the information provided in the manifests of the Evaluating the Role of Inflammation in Chronic Airways disease (ERICA) and Anglo-Cardiff Collaborative Trial II (ACCT) studies. Sample sizes of each group were limited by the number of available samples.

For the evaluation of aspirin use and health outcomes in COPD populations, data extracted from the SUMMIT and IMPACT studies included demographics, clinical background, concomitant medications, trial treatment arm, mortality status, exacerbation status, exacerbations number, cardiovascular composite event status.

2.1.3 Serum analysis

For the evaluation of RvD1, Del-1 and IL-17 levels in COPD patients and controls, selected and matched samples were identified from historic samples stored at -80 degrees Celsius. Levels of RvD1, Del-1 and IL-17 were measured using enzyme linked immunosorbent assays (ELISAs). An ELISA is a quantitative assay that uses specific antigen-antibody interactions linked to enzymes that react with substrates, to produce colour changes that are detected using a spectrometer at set wavelengths, from which the concentration of a target molecule in a

sample can be calculated (124). Serum samples were thawed and analysed using ELISA kits and according to the protocols supplied by the manufacturers. All samples were measured in duplicate and plates were read by FLUOstar Omega BMG LABTECH plate readers, at 450nm for RvD1, Del-1 and IL-17. The following assays were used: Human Resolvin D1 ELISA kit MBS053145 (from MyBioSource.com) for measuring RvD1, R&D Systems Quantikine HS ELISA Human IL-17 HS170 for measuring IL-17, R&D Systems DuoSet ELISA Human EDIL3 DY6046-05 for measuring Del-1.

2.1.4 Statistical analysis

For the systematic review and meta-analysis of IL-33/ST2, the outcomes were weighted pooled standardised mean difference of biomarker levels in patients vs controls and weighted pooled hazard ratios for risk of all-cause mortality, cardiovascular death and MACE (composite endpoint of death or adverse cardiovascular event) in random effects models. Results were displayed using forest plots. Heterogeneity and publication bias were assessed using I^2 statistics and funnel plots respectively.

For the evaluation of RvD1, Del-1 and IL-17 levels in COPD patients and controls, the median and interquartile range were calculated. The differences between the median levels detected in the COPD patients and controls were evaluated using Mann-Whitney U tests and associations between the groups were evaluated using linear regression.

For the evaluation of aspirin use and health outcomes in COPD populations, multivariate Cox Models were used to calculate hazard ratios for occurrence of all-cause mortality (ACM), moderate and severe exacerbations and cardiovascular events. Sensitivity analyses for the covariates of age (oldest strata), sex (M/F), race (White) and country (top five sources of participants) by carrying out analysis in separate subgroups. Bias analysis was carried out by calculating E-values, which identifies the associative strength of potential unmeasured

confounders (125). A low E-value would suggest that an unmeasured confounder could credibly explain away any observed associations. Propensity score matching was also used to create new matched groups of aspirin users and non-users, based on the following covariates that predict for being on/off aspirin: history of PAD, stroke, CAD, MI and percutaneous coronary intervention in the SUMMIT dataset and history of PAD, stroke, CAD, MI and angina in the IMPACT study. Propensity score matching is used to reduce the effect of confounding bias in observational studies (126). Hazard ratios for outcomes were then calculated using the newly matched groups.

2.1.5 Software

Figures 1, 2, 3, 4 and 15 were made using BioRender software.

Microsoft Excel was used throughout to view the datasets used in this thesis.

For the systematic review and meta-analysis of IL-33/ST2, the meta-analysis, forest plots, heterogeneity assessment and publication bias analysis were carried out using Comprehensive Meta-Analysis 3.0 software.

For the evaluation of RvD1, Del-1 and IL-17 serum levels in COPD patients and controls, calculation of median and interquartile range, Mann-Whitney U tests and linear regression analyses were carried out using SPSS version 28.

For the evaluation of aspirin use and health outcomes in COPD populations, data extraction, coding and hazard ratios were carried out using RStudio Desktop, R version 4.2.0. The full code is available in Appendix A. Hazard ratios were displayed in forest plots made with Microsoft Excel. E-values were calculated manually with the method detailed by VanderWeele *et al* 2017 (125).

Chapter Three: IL-33/ST2 axis in cardiovascular disease

3.1 Background

3.1.1 Interleukin-33 structure

IL-33 is a member of the Interleukin-1 family of cytokines that interacts with its receptor ST2 to play a pivotal role as an alarmin and mediator in the immune response to injury and infection.

The IL-33 protein is comprised of 270 residues and has a tertiary structure of 12 β-strands in a β-trefoil fold (127). IL-33 is constitutively expressed and stored in the nucleus of a range of cell types, including vascular endothelial, epithelial, smooth muscle cells as well as immune cells (128, 129). The receptor ST2 primarily exists in a transmembrane (ST2L) and soluble (sST2) form. IL-33 acts through ST2L binding and sST2 acts as a decoy receptor to dampen the effects of IL-33 signalling. ST2L is expressed on immune cells such as macrophages, mast cells and Th2 cells (130). Human aortic and coronary vascular endothelial cells are sources of sST2, whereas vascular smooth muscle cells do not secrete detectable levels of sST2 (131). The release of sST2 is enhanced in cardiac myocytes and lung alveolar epithelial cells after signalling by inflammatory cytokines such as TNFα and in mast cells after activation (132, 133).

3.1.2 Interleukin-33 storage and release

IL-33 remains stored in the nucleus in association with chromatin until cellular damage occurs, which triggers its release as a chromatin-cytokine complex into the extracellular space (42). Following its release, IL-33 can be cleaved by different proteases resulting in different effects. For example, caspases 3 and 7 inactivate IL-33 during apoptosis but neutrophil elastase cleaves

IL-33 to produce a highly active form during necrosis (42). IL-33 then binds to the transmembrane receptor ST2L and its associated protein IL-1RAcP to trigger an intracellular signalling cascade that activates the mitogen-activated protein kinase (MAPK) pathway (45). The presence of histones increases the strength of the resulting signal (42).

IL-33 mediates both the innate and adaptive immune response, through autocrine/paracrine effects including increased proliferation and cytokine secretion (e.g. IL-4, IL-6, IL-13) in target cells expressing ST2L such as macrophages, Th2 and mast cells (41, 134, 135).

Due to the significant downstream effects, IL-33 signalling is regulated by several mechanisms. Firstly, the association of IL-33 with chromatin in the nucleus slows its release into the extracellular space (42). Secondly, caspases can inactivate IL-33 after its release and sST2 acts as a decoy receptor. Finally, extracellular IL-33 can be inactivated via oxidation of its cysteine residues and formation of disulphide bonds (43).

3.1.3 Interleukin-33 in pathogenesis of chronic obstructive pulmonary disease and cardiovascular disease

IL-33 has been implicated in the pathogenesis of both COPD and CVD in mouse models and human clinical studies.

In mouse models, treatment with anti-IL-33 was protective against cigarette smoke induced changes in the lungs, although it should be noted that unlike in humans, mice endothelial cells do not constitutively express IL-33 (136, 137). Treatment with IL-33 also caused mice to develop pathophysiological changes associated with COPD and induced lung epithelial and endothelial cells to produce IL-6 and IL-8, which increased the migration of neutrophils to the lungs (54). In clinical studies of COPD patients and controls, there have been some contradictory observations, with serum IL-33 levels reported as lower in patients by Tang *et al*

2014 but higher in patients by Xia *et al* 2015 (50, 138). Additionally, Byer *et al* 2013 reported higher levels of IL-33 mRNA and protein in lung tissue from COPD patients (52). Moreover, most evidence points to a harmful role for IL-33 in COPD, and there are already ongoing clinical trials targeting the IL-33/ST2 axis in COPD patients (54).

The IL-33/ST2 axis has also been implicated in the development of CVD. In mouse models, endothelial IL-33 was responsible for the systemic inflammatory response after myocardial pressure overload (through transverse aortic restriction) but was also shown to reduce the rate of atherosclerosis (56, 139). In human genetic studies, single nucleotide polymorphisms (SNPs) in the IL-33 gene have been associated with both increased and decreased risk of coronary artery disease, suggesting a regulatory role in CVD (140, 141). Interestingly, IL-33 promotes leukocyte adhesion to human endothelial cells *in vitro* which suggests a role in atherosclerosis yet serum levels are lower in patients with coronary artery disease compared to controls (135). The importance of IL-33 in cardiovascular health remains poorly understood, with contrasting data from pre-clinical studies suggesting both cardio-protective and detrimental effects of IL-33. In contrast, sST2 has been evaluated as a prognostic factor in CVD such as HF and MI and is a FDA approved prognostic biomarker of mortality in chronic heart failure patients (57). In COPD patients, IL-33 has been targeted with anti-IL-33 and anti-ST2 receptor antibodies in ongoing clinical trials to reduce exacerbations (142, 143). In one of these trials involving 343 COPD patients, the IL-33 targeting monoclonal antibody Itepekimab (compared to placebo) improved lung function and decreased exacerbations within COPD patients who were also former smokers, although this was not observed for the main population (144). The COPD population was a mix of patients with moderate or severe disease and aged 40-75. Patients with alpha-1 antitrypsin deficiency and those who experienced an exacerbation 4 weeks before study start were excluded. Considering the fact that this drug is already being used to target IL-33 in patients and that COPD patients have increased risk of developing CVD, it is essential to

improve knowledge of the cardiovascular effects of the IL-33/ST2 signalling axis in clinical studies of CVD, to identify potential harmful effects of anti-IL-33 drugs.

3.1.4 Role of Interleukin-33 signalling in cardiovascular disease

There have been many clinical studies focused on the IL-33/ST2 axis (primarily studying sST2) in CVD. However, the conflicting evidence and heterogeneity of the studies show a need for a quantitative review of this axis to determine if IL-33 signalling is cardio-protective or not.

Previous meta-analyses showed that sST2 is prognostic for all-cause mortality in acute and chronic heart failure, CAD and following aortic valve replacement, as well as having reasonable diagnostic value for HF (145-150). As far as we are aware, no meta-analysis on sST2 covering CVD as a whole has yet been undertaken. There is a paucity of clinical studies on IL-33, which is likely due to difficulty measuring it in serum (151). However, *in vitro* effects of IL-33 signalling appears dependent on the cell type targeted, with pro-inflammatory effects produced by neutrophils and endothelial cells and anti-inflammatory effects produced by M2 macrophages (135).

To my knowledge, no previous meta-analysis has examined the role of IL-33 itself as a biomarker in specific CVD nor sST2 and/or IL-33 across CVD as a whole. A systematic review and meta-analysis is needed to synthesise and harmonise data from human clinical CVD studies to establish the clinical significance of the IL-33/ST2 axis in CVD as a whole and within subtypes of CVD. In particular, to determine the importance of IL-33 and/or sST2 levels in defining people with CVD versus those without. Also, to determine the association of biomarker levels with clinical outcomes of mortality and MACE in CVD or healthy cohort populations.

3.2 Hypothesis

Levels of sST2 are higher in CVD patients compared to controls and higher levels are associated with poor prognosis. Levels of IL-33 are higher in CVD patients compared to controls.

3.3 Aims

To quantitatively evaluate differences in IL-33 and sST2 levels in CVD patients and controls and identify any associations with prognosis over follow up, using published clinical studies.

3.4 Methods

3.4.1 Study design

As previously reported, the role of the IL-33/ST2 axis in the human cardiovascular system is unclear, with contrasting results from pre-clinical studies. This meta-analysis aimed to determine the importance of the IL-33/ST2 axis in the diagnosis and prognosis of human CVD. Clinical studies on circulating IL-33 and/or sST2 levels vary in cohort size and quality and there is a clear need for a quantitative overview to determine the effects of this axis in CVD and healthy populations. A systematic review and meta-analysis was carried out on the role of the IL-33/ST2 axis in human CVD, and has been published in PLOS One. I carried out all steps of this analysis, including search, data extraction, statistical analysis and data presentation.

3.4.2 Search strategy

Before beginning the literature search process, the meta-analysis was first registered on the Prospero website (https://www.crd.york.ac.uk/prospero/), which is an international database for prospectively registered systematic reviews. The meta-analysis ID is CRD42020168206.

Searches in the Pubmed (including MEDLINE), Web of Science, Cochrane Library and Prospero databases were carried out up to March 2020. The search terms were one of IL-33/Interleukin-33/ST2 combined with one of cardiovascular disease, stroke, myocardial infarction, heart failure, coronary disease, ischaemic heart disease and hypertension. The same search terms were entered into each database.

During primary screening of the articles, the title and abstract were assessed for relevance to the IL-33/ST2 in CVD, English language, full text availability and excluded studies about the following: vascular disease localised to renal or hepatic system, vasculitis, autoimmune disorders, transplant related diseases, parasite diseases, cancer, HIV, obesity, exercise, magnetic resonance imaging. All datasets included in this meta-analysis were *in vivo* human studies that involved measurement of plasma or serum levels of IL-33 or sST2 and a definition of CVD or a subtype of CVD (based on clinical diagnosis and/or supporting clinical test of this diagnosis). During secondary screening of the full text, the inclusion criteria were: clinical studies measuring protein levels of IL-33 and/or ST2 in plasma/serum/blood in CVD populations and/or controls, risk of event studies must report hazard ratios calculated using continuous and log transformed biomarker levels, study type must be one of patient/control comparison or event/no event over follow up comparison or time to first event over follow up with hazard ratios, reported biomarker levels at single time points. The exclusion criteria were: qualitative studies, studies published before 2000, genetic studies, review papers, animal studies, hazard ratios based on quadratic transformed data, time to event studies that reported only odds ratios or risk ratios, studies on cardiomyopathy/congenital disease/valve disease/pulmonary hypertension/paediatrics, non-extractable raw data such as graphical depiction of data only or biomarker levels below detection limits or unable to calculate biomarker mean and standard deviation.

Studies were undertaken in countries across the world, with China being the largest single source. Meta-analyses were performed for CVD versus controls based on CVD search terms and incorporated acute coronary syndrome (ACS, including unstable angina and myocardial infarction), coronary artery disease (CAD), atrial fibrillation (AF), systemic hypertension, acute heart failure (defined as hospital admission with decompensation or new hospital diagnosis) or chronic heart failure (CHF). Individual meta-analysis stratified by these distinct CVD subtypes were also performed if there were ≥2 eligible studies. The association of IL-33 and/or sST2 biomarker levels with clinical outcomes of all-cause mortality or MACE were evaluated in CVD (and separately within CVD subtypes) as well as community population cohorts. Stroke studies were omitted from combined CVD meta-analysis, since stroke represents cerebrovascular disease rather than CVD.

3.4.3 Data extraction and quality assessment

The PRISMA statement was used as the basis for extracting and recording data from eligible articles, the full checklist use is available in Appendix E. Data extracted includes circulating biomarker levels and associated standard deviation or confidence intervals, inclusion and exclusion criteria of the study, study type, sample size, hazard ratios, follow-up period, age and outcomes. Where median biomarker levels and quartile values were reported, they were converted to mean and standard deviations with the method reported by Wan *et al* 2014 to enable standardisation across studies (152). Studies reporting data that could not be converted to mean and standard deviation were not included in analyses where the outcome was standardised mean difference. Full data extracted, including adjustment factors of multivariate hazard ratio (HR) analysis are available in Appendix C. Study quality was assessed using a modified version of the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) criteria. Full criteria used are available in Appendix B.

3.4.4 Statistical analysis

The primary outcomes were weighted pooled standardised mean difference (SMD) of biomarker levels comparing cases vs controls (Meta-SMD) and weighted pooled hazard ratios (Meta-HR) for occurrence of all-cause mortality, cardiovascular death and MACE during follow up calculated using random effects models. Only studies presenting univariate or multivariate HRs (risk ratios and odds ratios not accepted) per (log) unit increase in biomarker levels were accepted for Meta-HR.

Data selected for meta-analysis were displayed in forest plots showing the SMDs or HRs of individual studies and the overall Meta-value. Heterogeneity was assessed using I^2 statistics and publication bias was tested for using funnel plots. Where possible, meta-regression was carried out to assess the effect of cohort age (mean or median), length of follow up (mean or median), publication year and biomarker assay type (Presage ST2 or not). Statistical analysis was carried out using Comprehensive Meta-Analysis 3.0 software.

3.5 Results

This systematic review and meta-analysis has been published in PLOS One (153).

The flow diagram summary of the search results is shown in Figure 7. In the literature search, 4745 articles were found. After duplicates were removed and screening was carried out, all studies were systematically reviewed, with 77 studies included (62075 participants) for meta-analysis and systematic review. The vast majority of included studies assessed subjects between 60-80 years old and heart failure was the most reported disease type (47% of studies).

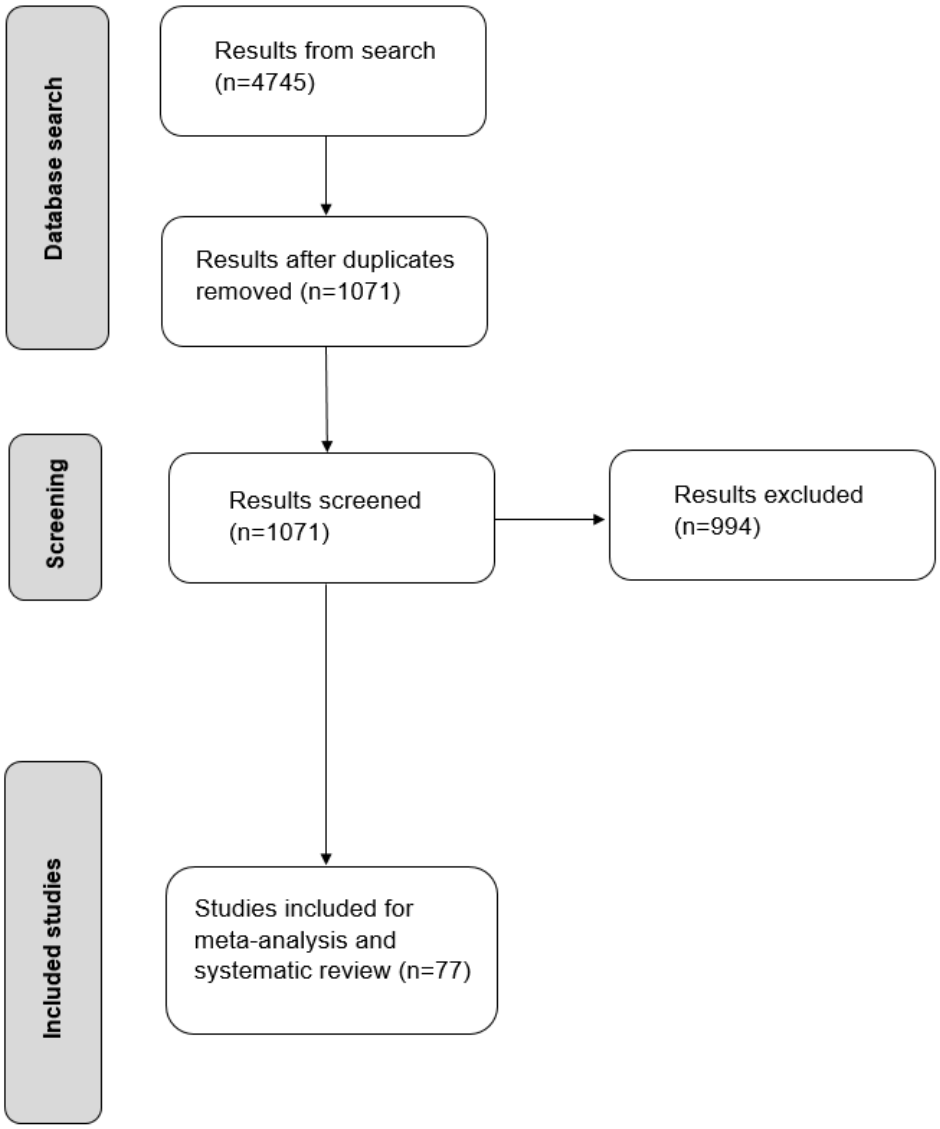

Figure 7: Flow chart of search strategy

3.5.1 Interleukin-33 and soluble ST2 levels in cardiovascular disease patients versus controls

3.5.1.1 Interleukin-33

In analysis of two CAD studies with a total sample size of 156 subjects, patients had lower IL-33 levels than controls, Meta-SMD of -0.972, 95% CI -1.307-(-0.638); $p<0.0001$, $I^2=0.0$. Additionally, analysis of two HF studies with 281 total subjects showed that patients had lower IL-33 levels than controls, Meta-SMD of -0.683, 95% CI -1.213-(-0.153); $p=0.012$, $I^2=68.467$

(Figure 8). ACS patients also had lower IL-33 levels compared to controls (four studies with total sample size of 331), although this did not reach statistical significance: Meta-SMD of -1.373, 95% CI -2.978-0.231; p=0.093, I^2=97.379.

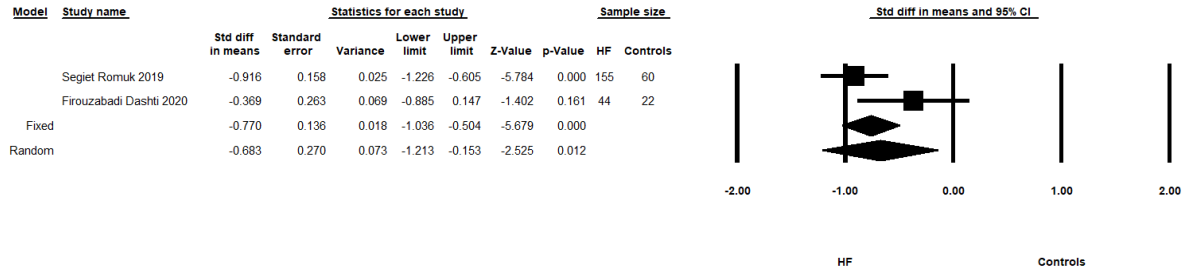

Figure 8: Summary forest plots showing the Meta-SMD and 95% CI of IL-33 levels in HF patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance. HF=Heart failure

Five studies reported (908 total subjects, sample size ranging from 90-287) IL-33 levels in acute ischaemic stroke patients vs controls. Stroke patients had consistently higher IL-33 levels than healthy controls [Meta-SMD 1.455, 95% CI 0.372-2.537; p=0.008, I^2=97.645] (Figure 9). Two studies (449 subjects) showed that stroke patients who had favourable outcomes (Barthel Index score above 85 or 90 respectively at 3 months and 1 year after admission respectively) had higher baseline IL-33 levels than those who did not: Meta-SMD 0.564, 95% CI 0.356-0.772; p<0.0001, I^2=0.0 (154, 155).

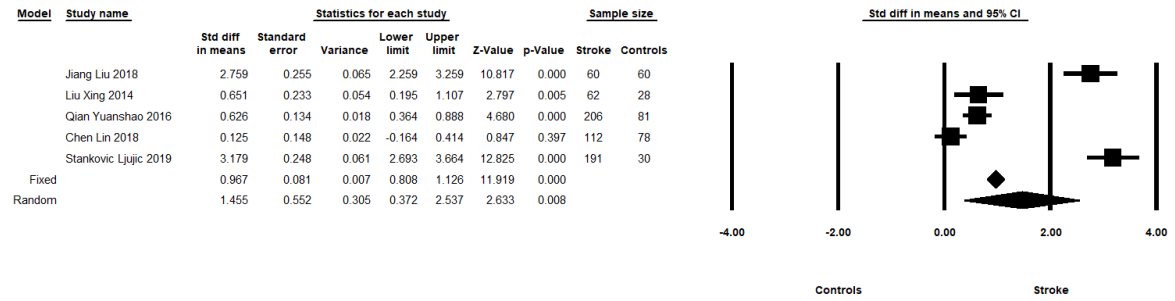

Figure 9: Summary forest plots showing the Meta-SMD and 95% CI of IL-33 levels in stroke patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance.

There was no difference in IL-33 levels between hypertensive subjects and controls (three studies with total sample size of 710): Meta-SMD of -0.024, 95% CI -0.443-0.395; p=0.912, I^2=81.874.

There were no clinical studies to evaluate the association of IL-33 with clinical outcomes based on the study's inclusion/exclusion criteria.

3.5.1.2 Soluble ST2

Analysis of two CAD studies (408 subjects) showed patients found no difference in levels of sST2 between patients and controls: Meta-SMD of 0.033 [95% CI -0.197-0.264; p=0.778, I^2=16.127]. Only one study across the meta-analysis of all CVD subtypes, Demyanets *et al* 2014 in CAD, reported patients having lower sST2 levels than controls (156). Four studies with a total of 470 subjects reported that HF patients had higher sST2 levels than controls: Meta-SMD of 2.178 [95% CI 0.653-3.704; p=0.005, I^2=96.939].

Meta-analyses of sST2 performed within ACS vs controls (total sample size of 1153) are shown below in Figure 10, where ACS patients had higher levels of sST2 compared with controls: Meta-SMD of 0.92 [95% CI 0.632-1.208; p<0.0001, I^2=77.797].

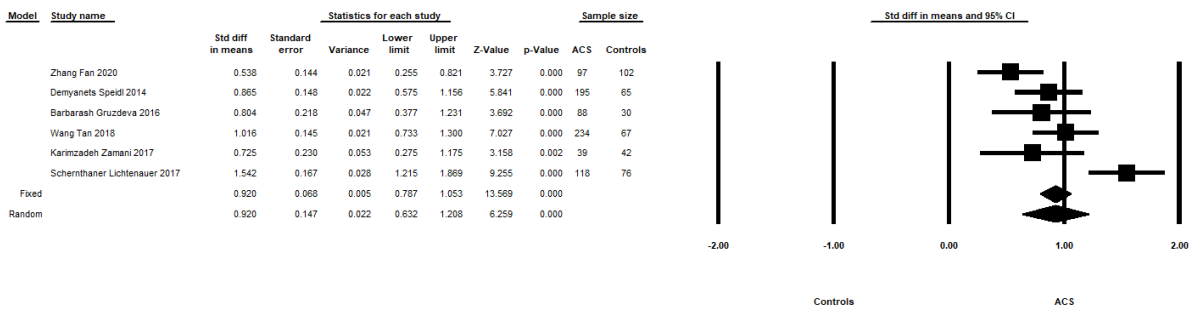

Figure 10: Summary forest plots showing the Meta-SMD and 95% CI of sST2 levels in ACS patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance. ACS=Acute coronary syndrome

Acute ischaemic stroke patients also had higher sST2 levels compared to controls, reported from two studies with a sample size of 190 and 221, although this did not reach statistical significance [Meta-SMD 3.96, 95% CI -0.839-8.760; p=0.106, I^2=99.334]. Additionally, AF patients also had higher sST2 levels than controls (Figure 11, 704 subjects from three studies): Meta-SMD of 2.825 [95% CI 0.607-5.043; p=0.013, I^2=99.132].

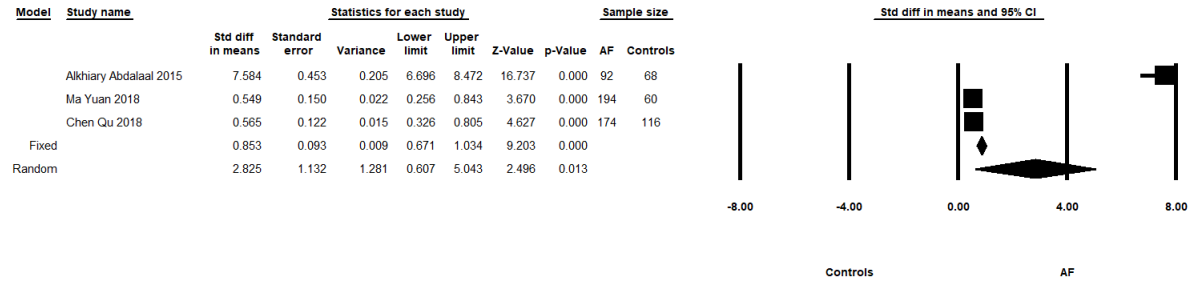

Figure 11: Summary forest plots showing the Meta-SMD and 95% CI of sST2 levels in AF patients and healthy controls. The Meta-SMD in the random effects model is shown by the black diamond at the bottom. The vertical line at 0.00 is the border for significance. AF=Atrial fibrillation

3.5.2 Association of soluble ST2 levels and clinical outcomes in cardiovascular disease and community cohorts

For CAD, three studies following 4371 patients for up to 12.3 years (median), showed that baseline sST2 levels of patients who died during follow up were higher than survivors, Meta-SMD of 0.502 [95% CI 0.273-0.730; p<0.0001, I^2=88.78].

Soluble ST2 had a stronger association with risk of all-cause mortality in ACS (Four datasets, Meta-multivariate HR 2.207, 95% CI 1.160-4.198; p=0.016, I^2=95.661) than risk of all-cause mortality in HF (Fifteen datasets, Meta-multivariate HR 1.425, 95% CI 1.268-1.601; p<0.0001, I^2=92.276).

Two acute ischaemic stroke studies also evaluated baseline sST2 levels in patients stratified by survival status. During 90 days follow up, 132 patients who died had higher baseline sST2 levels than the 903 who survived [Meta-SMD 1.151, 95% CI 0.670-1.633, p<0.0001, I^2=83.474] (157, 158).

Five studies followed 18,264 individuals from community cohorts for up to 15 years (mean) to evaluate risk of adverse events (including all-cause mortality, MACE, and occurrence of specific CVD, such as development of AF) per log unit increase of sST2 levels (Figure 12). The Meta-multivariate HR was 1.035 [95% CI 1.005-1.065; p=0.021, I^2=2.114]. However, the significance of this finding should take into consideration the fact that several of the individual studies have the opposite effect direction or are not statistically significant and the overall effect is relatively small at 3.5%.

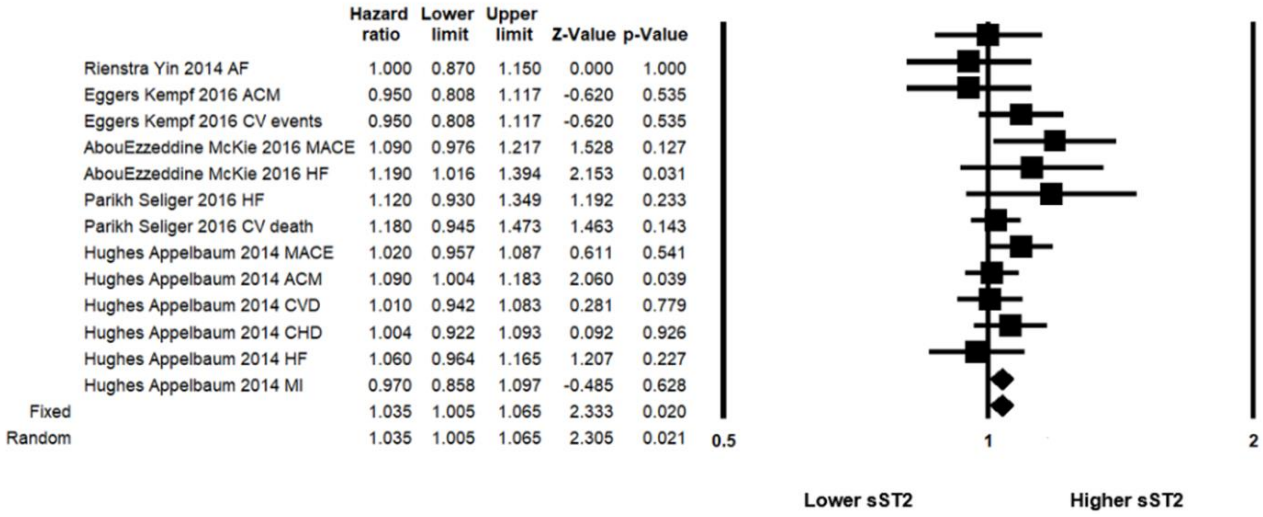

Figure 12: Summary forest plot showing the multivariate Meta-HR and 95% CI for risk of any adverse cardiovascular events in community populations and its relation to sST2 levels. The Meta-HR in the random effects model is shown by the black diamond at the bottom. The vertical line at 1 is the border for significance. AF=atrial fibrillation, ACM=all-cause mortality, CV=cardiovascular, HF=heart failure, CVD=cardiovascular disease, CHD=coronary heart disease, MI=myocardial infarction, MACE=major adverse cardiovascular events.

3.5.3 Heterogeneity and meta-regression

Most of the studies had high heterogeneity (I^2 value indicates heterogeneity levels, with greater than 50% considered high). For studies with high heterogeneity, meta-regression analyses (assessing the effect of age, follow up time, publication year and sST2 assay type) did not identify covariates that reduced I^2 value to below 50% in the vast majority of analyses.

3.6 Discussion

This analysis used systematic review methods to evaluate the associations between circulating levels of IL-33 and/or sST2 and CVD subtypes, and associations between these biomarkers and outcomes of mortality and MACE in CVD patients as well as community populations.

The main findings were that patients with various forms of CVD have higher sST2 levels compared with healthy controls, and that incremental increases in sST2 were associated with

poor clinical outcomes of mortality and MACE over several years follow up in both CVD and community based cohorts. IL-33 levels were lower in HF, CAD and ACS patients compared with controls, but higher in acute ischaemic stroke patients compared with controls. There were insufficient data to examine the association of IL-33 with clinical outcomes in CVD or community populations.

This is the first meta-analysis to systematically evaluate both IL-33 and sST2 levels across the spectrum of CVD. It suggests that peripheral circulating sST2 measurement may have clinical value in differentiating patients with CVD versus controls without CVD. The greatest difference in sST2 levels compared with controls was observed in AF and CHF.

Soluble ST2 is an FDA approved biomarker to evaluate prognosis of mortality in chronic heart failure. Previous meta-analyses of sST2 have also been performed in CAD and post-aortic valve replacement patients and showed that higher sST2 levels were associated with poor clinical outcomes in these populations (145-148). The current meta-analysis found sST2 was associated with risk of poor outcomes of mortality, MACE and adverse cardiovascular events in a wide definition of CVD patients as well as community populations without identified cardiovascular disease. It suggests sST2 may also have value as a risk biomarker in CVD patients generally (besides chronic heart failure) and in community cohorts, although notably to a lesser extent than in CVD. Despite methodological differences between a meta-analysis by Liu *et al* of ST2 (in ACS and CAD patients only) and this current study, meta-multivariate HRs of all-cause mortality in ACS patients are similar, with a respective meta-multivariate HR of 2.48, (95% CI 1.99-2.97) for Liu *et al* and HR 2.207, (95% CI 1.160-4.198) in this current study (148).

This is the first meta-analysis of human CVD studies of IL-33 and included 1638 participants. It suggests potential clinical value in measurement of IL-33, in that IL-33 levels were lower in

CAD, HF and ACS patients versus controls. However, it must be noted other individual studies (not included in this meta-analysis after considering inclusion/exclusion criteria) have shown different results, with IL-33 levels being higher in some heart failure patients compared with controls (159, 160). Interestingly, IL-33 levels were higher in stroke patients compared with controls; in contrast to findings from other CVD subtypes, based on five studies with a sample size of 908. The reasons for these contrasting results in stroke are unclear and require further investigation. Potentially, the unique setting and anatomy of stroke compared to other cardiovascular diseases could play a role. The blood brain barrier regulates the movement of blood solutes into the brain and is damaged during ischaemic stroke, resulting in increased permeability (161). This damage, and the increased movement of solutes, including inflammatory molecules, across the barrier could be the cause of the contrasting results in stroke compared to other non-cerebral CVD subtypes. Moreover, a major drawback to advancing understanding of IL-33 in human cardiovascular health and disease is that often levels are below the detection limit of an assay and there is wide divergence in the methodology of how studies processed and analysed IL-33 samples in the current published literature (151). In this meta-analysis, several IL-33 studies were not included due to levels of IL-33 being below the detection limit of the assay (156, 162, 163). A further point of consideration is that IL-33 exists extracellularly in both full length and cleaved forms and cleavage may either enhance its potency or inactivate it depending on whether serine proteases or apoptotic caspases facilitate cleavage. In this meta-analysis, the included studies did not identify whether full length or cleaved forms of IL-33 were measured. This is a drawback as neutrophil elastase (which cleaves IL-33 and increases its activity) levels are elevated in CVD such as MI (164).

This meta-analysis showed that circulating IL-33 levels were much lower than sST2 levels, which reflects sST2's role as a decoy receptor to limit IL-33 activity. Moreover, IL-33 exerts its effects in an autocrine/paracrine manner, and is quickly oxidised after its release from cells

(particularly important considering CVD are marked by elevated oxidative stress), making it more difficult to detect in circulation (43, 165). Due to these factors, sST2 is likely a much more clinically useful and attainable biomarker to measure in clinical studies as shown by the results of this meta-analysis.

Overall, it remains to be elucidated whether IL-33 itself is cardio-protective or deleterious as suggested by contrasting pre-clinical animal studies. There is a need to advance understanding of the IL-33/ST2 axis in CVD , and studying local tissue expression of IL-33 and the effects of blocking IL-33 or ST2 may be helpful (166, 167). Identifying the role of the IL-33/ST2 axis in CVD and COPD has the potential to improve the treatment of both, given its involvement in the pathogenesis of both diseases. Additionally, the multitude of downstream effects from IL-33 activity such as leukocyte activation and recruitment demonstrates the key immunomodulatory capabilities of IL-33 and highlights the potential health benefits of controlling this signalling axis. Of interest, a loss of function in the IL-33 gene has been shown to be protective against asthma in humans, with no harmful effects (i.e. cardiovascular abnormalities) reported (168).

3.6.1 Limitations

This study has several limitations. The first is the low number of IL-33 clinical studies available which limits the conclusions that can be drawn regarding IL-33's role in cardiovascular health. Additionally, differences in the sensitivity of IL-33 assays are a potential hindrance for repeatability and interpretation of clinical studies. The second is that Meta-multivariate HRs reported are adjusted for different factors at the individual study level that could impact IL-33/sST2 expression (e.g. age, gender, comorbidities, body mass index, and levels of other inflammatory biomarkers). The majority of studies adjusted for age and gender; and many included extensive adjustment for other cardiovascular risk factors. Furthermore, the length of

storage time for biomarkers before analysis varied, which may impact biomarker stability. Finally, causality between biomarker levels and outcomes could not be measured.

3.6.2 Impact and future work

The findings of this study are particularly impactful considering the recent clinical trial of the monoclonal antibody Itepekimab (targets IL-33) that included 343 COPD patients and showed that for COPD patients who are also former smokers, treatment lowered the exacerbation rate and improved lung function compared to placebo (144). The results of this meta-analysis showing that IL-33 levels are lower in HF, CAD and ACS but higher in stroke patients compared to controls are highly important given the existing Itepekimab COPD drug that targets IL-33, due to its potential effects on cardiovascular health. This is particularly significant considering the status of CVD as a commonly reported comorbidity in COPD patients. Any future therapies targeting the IL-33/ST2 axis will have to take into consideration the potential downstream effects on the cardiovascular and pulmonary systems, given the high position occupied by IL-33 in the immunomodulatory signalling network.

Future work should include more clinical studies measuring circulating levels of IL-33 in CVD patients and controls, to definitively establish any differences in levels. This would inform the safety considerations of clinical trials of drugs that target the IL-33 axis. Additionally, any future trials of anti-IL-33 drugs should particularly focus on observing cardiovascular health throughout the course of the trial.

3.6.3 Summary

In summary, this meta-analysis is the first to analyse the role of both IL-33 and sST2 across the spectrum of human CVD. The results showed that sST2 has diagnostic and prognostic value over several years of follow up across the spectrum of CVD. Similarly, IL-33 shows some promise as a biomarker to differentiate between CVD patients and controls. Meta-SMDs

showed that IL-33 levels are lower in HF and CAD patients compared to controls, while the reverse was observed in stroke patients. This observation and the small sample size means that further clinical studies are needed to determine if measurement of circulating IL-33 has diagnostic or prognostic value (similar to that of sST2) across the spectrum of CVD subtypes.

Chapter Four: Evaluation of Resolvin D1 levels in COPD patients and controls

4.1 Background

4.1.1 Biosynthesis of specialised pro-resolving mediators

SPMs are metabolites of PUFA such as arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and are found in many human tissues, including plasma, serum, saliva, sputum, breast milk, urine, lymph nodes and spleen (70). SPMs fall into the broad category of lipid mediators known as eicosanoids that also includes pro-inflammatory mediators such as leukotrienes. There are several known categories of SPMs, including lipoxins, protectins, maresins and resolvins that are essential to the resolution of inflammation after injury or infection. They are structurally unique (being derived from different PUFAs), released at different stages of resolution, and have a range of mainly local downstream pro-resolution effects, such as decreasing neutrophil migration and activity. SPMs are produced by transcellular biosynthesis, via a series of lipoxygenases, where a donor such as an endothelial cell synthesises and releases the intermediate component, which is then converted by an accessory cell (e.g. adhering leukocytes) into SPMs (67, 169). Figure 13 and Figure 14 show the biosynthesis pathways of resolvins, protectins and maresins.

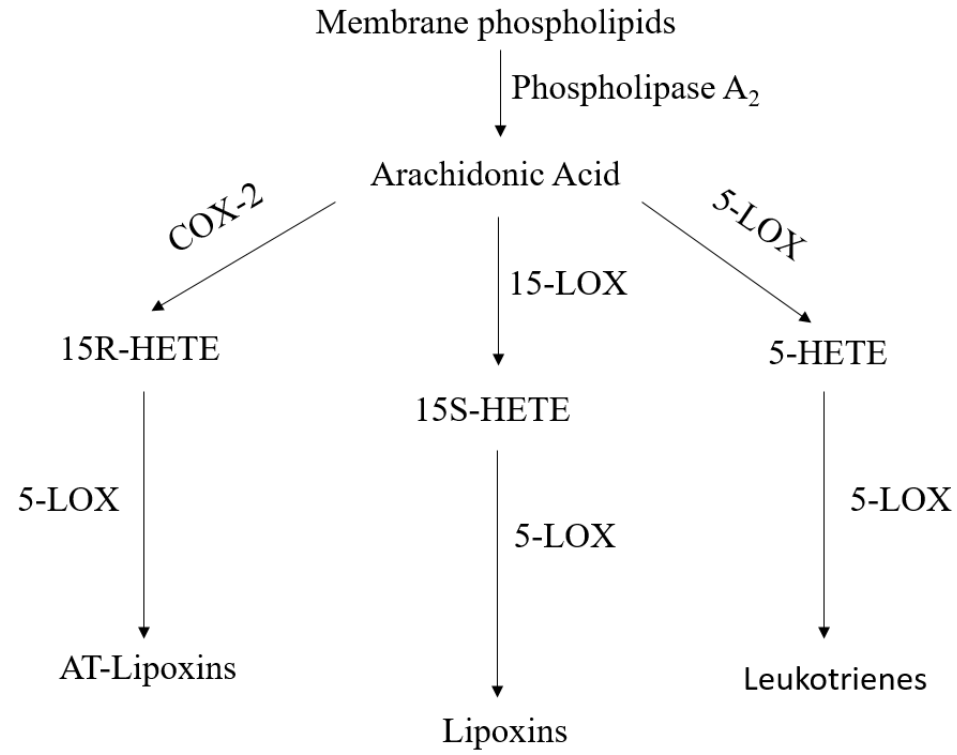

Figure 13: Biosynthesis of SPMs derived from arachidonic acid. COX-2= Cyclo-oxygenase 2, LOX=Lipoxygenase, HETE=Hydroxyeicosatetraenoic acid, AT-Lipoxins=Aspirin triggered lipoxins

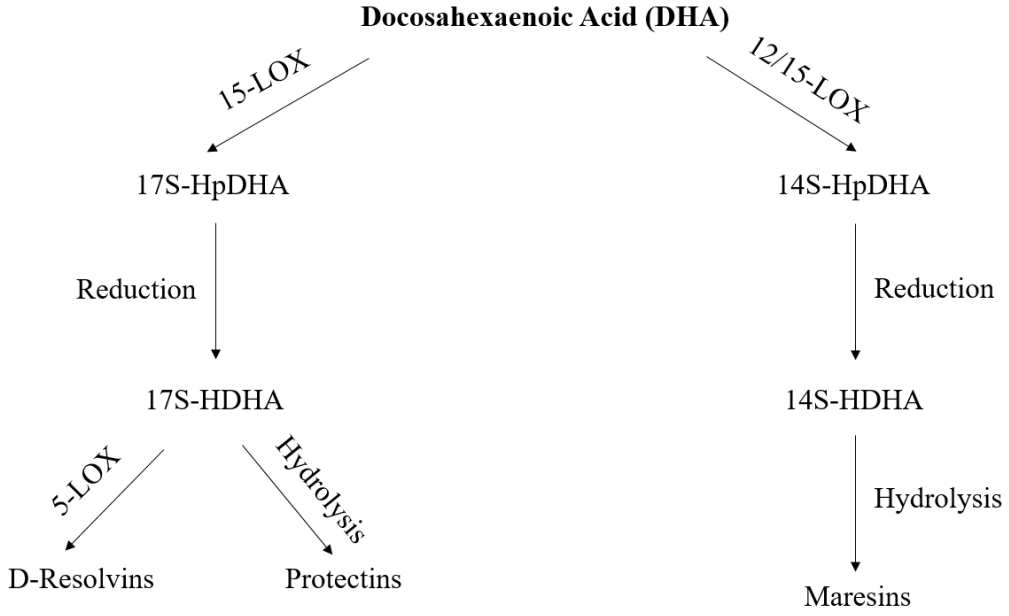

Figure 14: Biosynthesis of SPMs derived from DHA. LOX=Lipoxygenase, HDHA=Hydroxydocosahexaenoic acid, HpDHA=Hydroperoxydocosahexaenoic acid

Microbial infection or injury triggers the release of PUFAs from local cell membranes (by phospholipase A_2) or are delivered by tissue oedema to the inflammation site (67). Arachidonic acid (derived from omega-6) is rapidly metabolised to eicosanoids such as prostaglandins (regulates blood flow) and leukotrienes (attracts blood neutrophils), forming the inflammatory phase (67).

The beginning of the resolution phase is marked by the synthesis of lipoxins (also derived from arachidonic acid) which act as stop signals for neutrophils, followed by the production of other SPMs (67, 70).

4.1.2 Resolvin D1 in chronic obstructive pulmonary disease

Of the SPM family, resolvins have been the most extensively investigated for their role in COPD. A member of the resolvin family, Resolvin D1 (RvD1), derived from the omega-3 DHA, has shown promising results in pre-clinical studies that suggest it has an important ameliorative role in COPD. In health, RvD1 interacts with the receptor RvD1 receptor (DRV1). However, during inflammation, RvD1 signals via the G protein-coupled receptors formyl peptide receptor 2 (ALX/FPR2) and G protein-coupled receptor 32 (GPR32) which are expressed on the membranes of target cells such as polymorphonuclear leukocytes (e.g. neutrophils and eosinophils) and small airway epithelial cells (70, 170-172). This triggers an intracellular signalling cascade targeting the MAPK pathway (173). The effects of RvD1 include blocking neutrophil migration, decreasing neutrophil oxidative bursts and promoting neutrophil apoptosis, effects which could be protective against COPD (70, 174). Figure 15 shows the effects of SPMs in COPD.

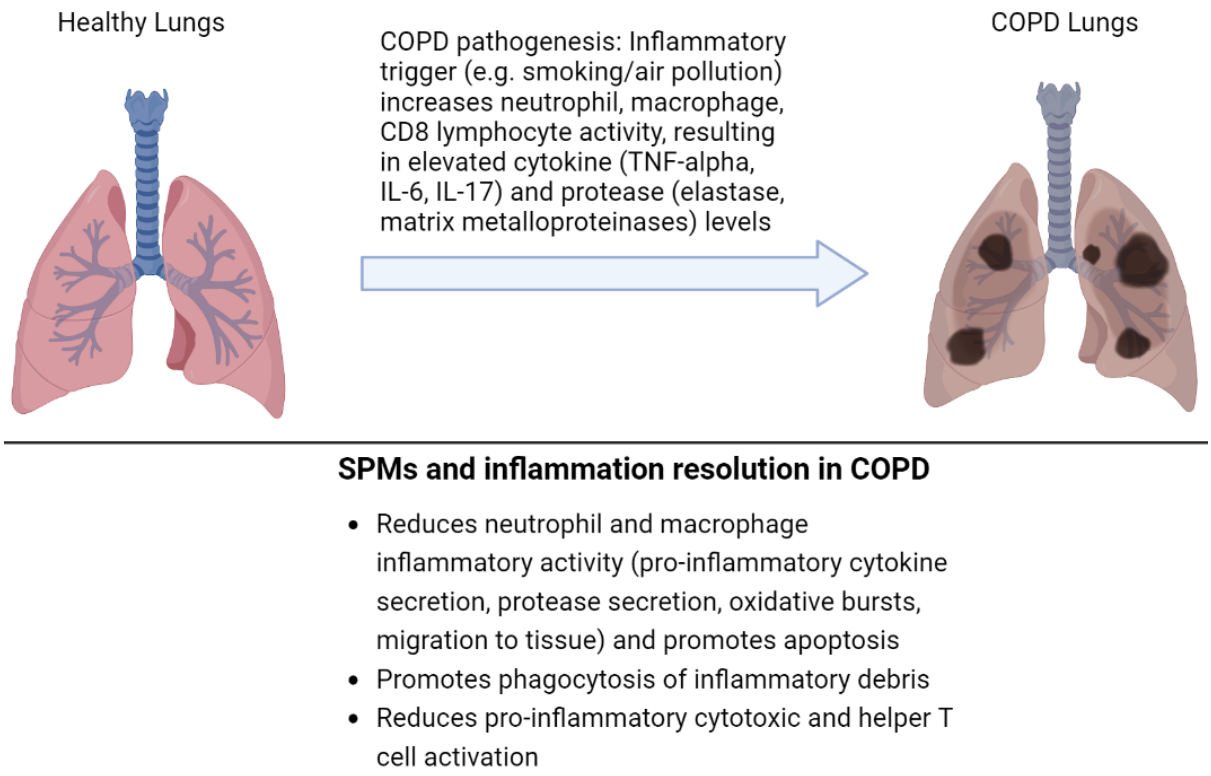

Figure 15: Effects of SPMs in resolving COPD inflammation (made with BioRender). COPD=Chronic obstructive pulmonary disease, CD8=Cluster of differentiation 8, TNF-alpha=Tumour necrosis factor alpha, IL-6=Interleukin-6, IL-17=Interleukin-17, SPMs=Specialised pro-resolving mediators

In murine models with cigarette smoke induced emphysema, RvD1 reduces inflammation by promoting the production of anti-inflammatory mediators, M2 macrophage differentiation, tissue regeneration and decreased emphysema (80, 175, 176). RvD1 also decreased pro-inflammatory signalling by human fibroblasts, small airway epithelial cells, blood monocytes and alveolar macrophage cells that were exposed to cigarette smoke during *in vitro* studies (78, 175). RvD1 also has an inhibitory effect on leukotrienes, which promote neutrophil recruitment to the inflammation site, an important mechanism during COPD pathogenesis (177, 178). In small clinical studies, COPD patients had lower levels of RvD1 in serum and bronchoalveolar lavage fluid (BAL) and have higher receptor expression in lung tissue compared to controls (78, 176). These results suggest that RvD1 has a potent anti-inflammatory role in COPD, but

further studies measuring levels of RvD1 in COPD patients and controls are needed to determine its clinical significance in COPD.

As previously discussed, therapeutics currently used to treat COPD such as bronchodilators and corticosteroids are insufficient in reducing mortality and improving long term quality of life. Furthermore, bronchodilators may cause a worsening of CVD (which COPD patients are at increased risk of) and corticosteroids have immunosuppressive effects that can lead to viral infections, causing an exacerbation (1). There is a particular lack of effective therapeutics in preventing or treating exacerbations (usually due to viral/bacterial infection), which are the major cause of mortality and reduced quality of life in COPD patients.

Pre-clinical studies in animals and human tissue have also revealed that treatment with resolvins has ameliorative effects for cardiovascular health, including decreased inflammatory markers (IL-6, TNF-α, myeloperoxidase) in mouse ischaemia models and reduced human vascular smooth muscle cell (VSMC) responses *in vitro* (implicated in atherosclerosis) (179). To be a potential future treatment for COPD patients, it is important that resolvins are compatible with cardiovascular health.

There have not been comprehensive studies comparing levels of SPMs in COPD patients and controls. A study measuring serum levels of RvD1 in COPD patients (including stable and those with exacerbations) and controls is needed to evaluate the role of SPMs in the pathogenesis and severity of COPD, and serve as an exploratory study for future clinical work.

4.1.3 Interleukin-17 and Del-1 in chronic obstructive pulmonary disease

In addition to RvD1, two further immunomodulatory molecules were of interest: IL-17 and Del-1. IL-17 is a pro-inflammatory cytokine primarily produced by T-helper 17 cells and has

been associated with the development of COPD through neutrophil recruitment (180). Del-1 (also known as EDIL3) is released by endothelial cells and blocks the adhesion of leukocytes to the vascular endothelium by inhibiting the interaction of leukocyte LFA-1 integrin with endothelial ICAM-1, resulting in reduced neutrophil migration (181). The measurement of Del-1 in COPD patients is very exploratory work.

Serum levels of IL-17 and Del-1 in COPD patients and controls are of interest, due to their relationship to each other and RvD1. In humans and mice, IL-17 and Del-1 expression is inversely related, with IL-17 inhibiting Del-1 expression. However, RvD1 can reverse this inhibitory effect of IL-17, while Del-1 is known to promote the production of resolvins, forming a positive feedback loop (182, 183). Previous studies have separately measured serum levels of IL-17 in COPD patients and controls, but not Del-1, and not all three molecules from the same population (184, 185).

A study measuring serum levels of RvD1, IL-17 and Del-1 in stable COPD patients and those with exacerbations is needed, to evaluate the role of these immunomodulatory molecules in COPD pathogenesis and during acute exacerbations. Levels of the immunomodulatory mediators will also be measured in controls to identify the baseline expression levels.

4.2 Hypothesis

Levels of serum RvD1 and Del-1 are lower in COPD patients than in controls and IL-17 levels are higher in COPD patients.

4.3 Aims

Measure levels of RvD1, Del-1 and IL-17 in serum sourced from COPD patients with exacerbations, stable COPD patients and controls.

4.4 Methods

4.4.1 Study design

Results from pre-clinical studies suggest RvD1 may have an immunomodulatory role in COPD pathogenesis. However, there is a paucity of studies measuring the levels of RvD1 in human COPD patients and controls. Additionally, there have been no studies comparing levels of RvD1 in stable COPD patients vs those with exacerbations. Identifying differences in the levels of RvD1 in COPD patients and controls would shed light on the potential ameliorative role of RvD1 in lung disease. Measurement of RvD1, IL-17 and Del-1 levels in frozen serum sourced from COPD patients and controls were carried out using ELISAs. I carried out all laboratory work, statistical analysis and data presentation.

4.4.2 Sourcing of samples

Frozen serum samples from COPD patients and healthy controls were sourced from the ERICA and ACCT studies respectively, with ethically approved storage (186, 187).

Briefly, the ERICA study published in 2014 investigated the predictive value of plasma fibrinogen and cardiovascular abnormalities for mortality or hospitalisation in 800 COPD patients (186). The ACCT study published in 2008 (study extended beyond publication) investigated the influence of cardiovascular risk factors on central pulse pressure in 10613 diabetics, CVD patients, subjects with one of hypertension/hypercholesterolemia/smoking and healthy individuals (187).

4.4.3 Measurement of serum Resolvin D1, Interleukin-17, Del-1

From the manifests of the ERICA and ACCT studies, 140 COPD patients with exacerbations at baseline, 86 stable COPD patients (no exacerbations at baseline) and 146 controls were selected and matched based on age, gender and BMI. Additionally, all controls selected had

FEV1% above 70 which is the minimum boundary of the normal range. All serum samples were located in -80 degrees Celsius freezers before measurement.

Serum levels of RvD1 (Human Resolvin D1 ELISA kit, MBS053145, MyBioSource.com), IL-17 (R&D Systems Quantikine HS ELISA Human IL-17, HS170) and Del-1 (R&D Systems DuoSet ELISA Human EDIL3, DY6046-05) were measured in duplicate, using ELISA kits according to the manufacturer's protocols. Readings of plates were carried out using the FLUOstar Omega BMG LABTECH plate reader.

4.4.4 Statistical analysis

Median biomarker levels in COPD patients with baseline exacerbations, stable COPD patients and controls were compared between groups with non-parametric Mann-Whitney U tests to assess differences in expression levels. The data were Log_{10} transformed due to non-normal distribution, then linear regression was used to assess for any associations between biomarker levels in the study groups. Bland-Altman analysis was carried out to assess the repeatability of the ELISA results. All statistical analyses were carried out using SPSS version 28.

4.5 Results

I used ELISAs to measure RvD1, Del-1 and IL-17 levels in 372 unique serum samples from unstable COPD patients with baseline exacerbations, stable COPD and controls, with different sample sizes across the groups due to differences in available samples and some measurements being unreadable (Table 2). Samples in stable and unstable groups were matched based on age, BMI and sex. Independent t-tests showed that the groups were well matched on age and BMI, with no significant difference in the means between the groups (for mean age: p=0.446, for mean BMI: p=0.343).

Table 2: Demographics of COPD patients and controls in ELISA study

	Unstable COPD	Stable COPD	Controls
Sample Size	140	86	146
Mean Age (years) ±SD	67±7	68±7	67±8
Sex (% male)	68	74	51
Mean BMI (kg/m^2) ±SD	26±6	27±5	27±4
Current smoker (%)	32	44	3
Mean FEV1 (%) ±SD	44±16	56±14	105±13

The median and interquartile range (IQR) of serum levels of RvD1, Del-1 and IL-17 in COPD patients and controls are shown below in Table 3. To assess statistically significant differences in biomarker levels across groups, the non-parametric Mann-Whitney U test was used to compare median biomarker values between groups, due to non-normal distribution of the data.

Table 3: Median (IQR) levels of RvD1, Del-1 and IL-17 in study groups

	Unstable COPD	Stable COPD	Controls
RvD1 (pg/ml)	143.25 (Q1: 88.99, Q3: 222.55)	109.04 (Q1: 76.41, Q3: 195.72)	139.96 (Q1: 84.05, Q3: 205.29)
Del-1 (pg/ml)	11.94 (Q1: 3.28, Q3: 28.94)	7.24 (Q1: 0.00, Q3: 20.71)	15.03 (Q1: 7.11, Q3: 149.21)
IL-17 (pg/ml)	0.15 (Q1: 0.091, Q3: 0.206)	0.13 (Q1: 0.082, Q3: 0.18)	0.08 (Q1: 0.032, Q3: 0.14)

4.5.1 Resolvin D1

Median RvD1 levels in 140 COPD patients with exacerbations were highest at 143.25 pg/ml, compared to 139.96 pg/ml for 146 controls and 109.04 pg/ml for 86 stable COPD patients (Figure 16). The difference in RvD1 levels between COPD patients with exacerbations and stable COPD patients was statistically significant (p=0.042). There was no significant difference (p>0.05) between exacerbators vs controls, stable COPD vs controls or combined COPD (exacerbators and stable) vs controls.

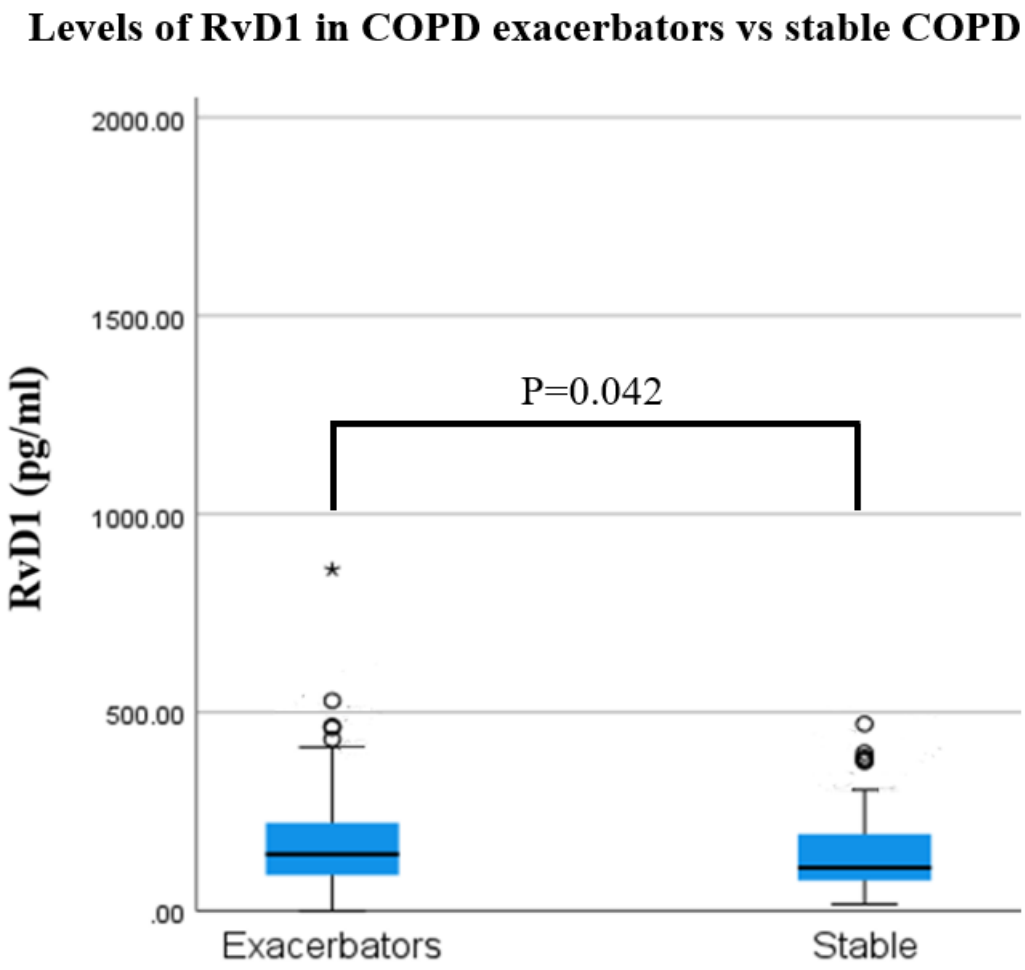

Figure 16: Box and Whisker Plot showing median RvD1 levels in COPD exacerbators and stable COPD. Outlier is any value more than 1.5 x IQR above quartile 3 or 1.5 x IQR less than quartile 1. Star=Extreme outlier, Circle=Outlier.

4.5.2 Del-1

Median Del-1 levels in healthy controls were highest at 15.03 pg/ml (n=101), followed by 11.94 pg/ml for 123 exacerbators and 7.24 pg/ml for 69 stable COPD patients. The difference between stable COPD and healthy controls was significant ($p<0.001$). There was no significant difference ($p>0.05$) between exacerbators vs controls, exacerbators vs stable or combined COPD (exacerbators and stable) vs controls. There was a large spread in recorded Del-1 values, shown in Figure 17.

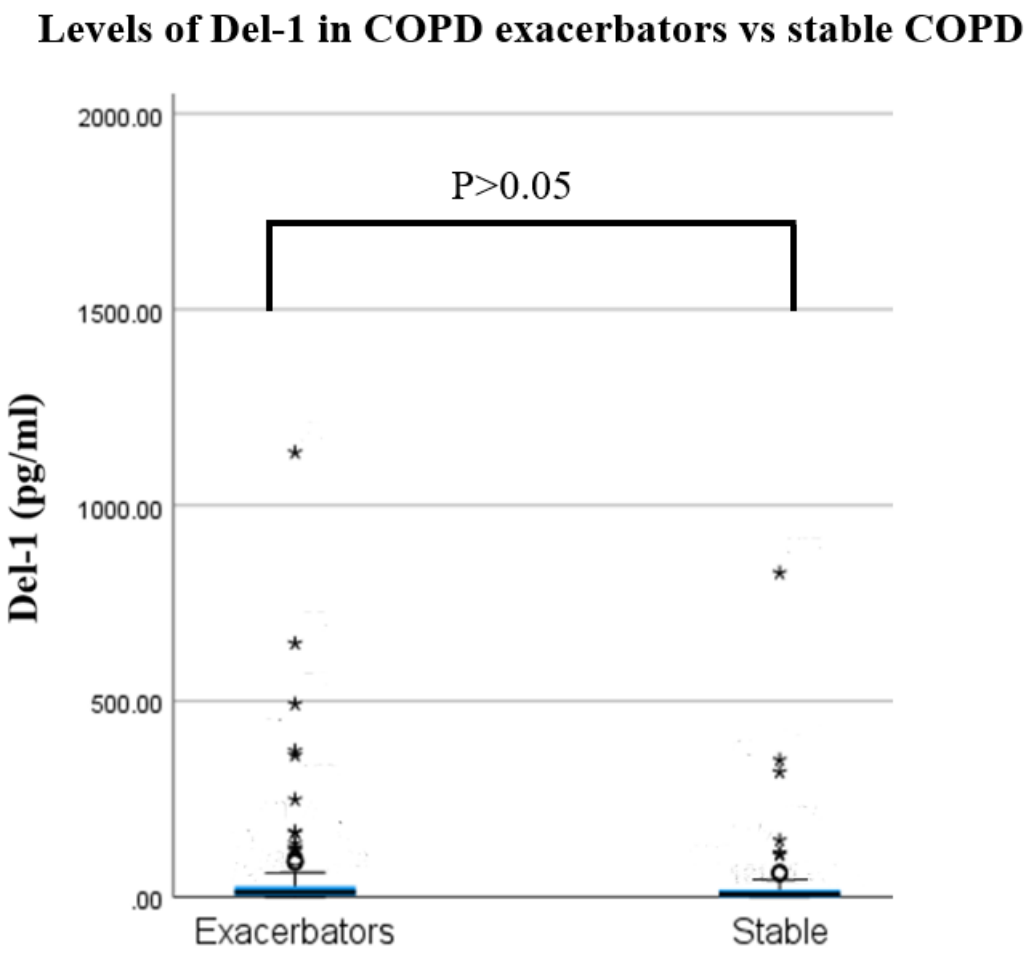

Figure 17: Box and Whisker Plot showing median Del-1 levels in COPD exacerbators and stable COPD. Outlier is any value more than 1.5 x IQR above quartile 3 or 1.5 x IQR less than quartile 1. Star=Extreme outlier, Circle=Outlier.

4.5.3 Interleukin-17

Median IL-17 levels were 0.15 pg/ml in 45 exacerbators, 0.13 pg/ml in 44 stable COPD patients and 0.08 pg/ml in 90 healthy controls. However, these measurements were clearly too low to be of statistical value, as the high sensitivity assay used (R&D Systems Quantikine HS ELISA Human IL-17, HS170) had a detection range of 0.2 – 15 pg/ml. The assays were carried out according to the manufacturer's instructions and the reasons for the abnormally low levels of IL-17 across the groups are currently unknown.

4.5.4 Regression analyses

Regression analyses were undertaken to evaluate the relationship between RvD1/Del-1 levels and RvD1/CRP (C-reactive protein, biomarker for cardiovascular injury) levels. Due to non-normal distributions, the data was first Log_{10} transformed before linear regression was carried out.

The relationship between RvD1 and Del-1 levels was expected to be positive as RvD1 can reverse the inhibitory effect of IL-17 on Del-1 expression and Del-1 is known to promote the production of resolvins (182, 188). However, regression analyses found no statistically significant relationship between RvD1 and Del-1 across any of the groups (exacerbators, stable, controls, combined COPD).

Regression analysis was also carried out to examine the relationship between RvD1 and CRP (biomarker for inflammation and cardiovascular injury) levels. There were no statistically significant relationships between RvD1 and CRP levels in exacerbators, stable or controls. However, in the combined COPD group (exacerbators and stable), there was a significant positive relationship between RvD1 and CRP: [Log_{10} RvD1= 2.055 + 0.111 (Log_{10} CRP), p=0.007]. This means that for every unit increase in Log_{10} CRP, Log_{10} RvD1 increases by 0.111.

Bland-Altman analysis (plots shown in Appendix F) was carried out for measurements of RvD1 and Del-1. For RvD1 measurements, the null hypothesis that the mean difference (-0.274) between measurements of the same sample was 0 remained intact ($p=0.111$), suggesting there was no proportional bias and high level of agreement between the measurements. For Del-1, the null hypothesis was rejected (mean difference 0.573, $p<0.001$), indicating the presence of proportional bias and different results between measurements of the same samples.

4.6 Discussion

ELISAs were used to measure levels of RvD1, Del-1 and IL-17 in 372 serum samples from COPD patients (exacerbators and stable) and healthy controls, to evaluate the role of SPMs and other immunomodulatory mediators in COPD pathogenesis and severity. This is the first study to measure RvD1, Del-1 and IL-17 in the same human COPD serum samples.

The main findings were that median RvD1 levels were highest in exacerbators, followed by healthy controls, with stable COPD having the lowest levels. A previous small study (n=11) found lower serum RvD1 levels in stable COPD patients compared to controls, which supports the findings in this study (78). However, only the difference in RvD1 levels between exacerbators and stable COPD was statistically significant. For Del-1, controls had the highest levels, followed by exacerbators and stable COPD had the lowest levels. Only the difference in Del-1 levels between stable COPD and controls was statistically significant. Median IL-17 levels were below the assay range in all groups so could not be included for meaningful analysis.

As previously explained, exacerbations are the major cause of mortality and reduced quality of life in COPD patients, often by aggravating underlying CVD conditions, which increases stress on the cardiovascular system and causes events such as plaque rupture. Currently, there is a lack of effective therapeutics for treating or preventing exacerbations, with steroidal options having major disadvantages, as previously noted. The results of this study showed that RvD1

levels were highest in exacerbators, suggesting that SPM levels are upregulated after an acute inflammatory event. This suggests that RvD1 upregulation is part of a natural resolution response to exacerbations and highlights its potential as a well-tolerated treatment for these acute inflammatory events. SPMs are an anti-inflammatory member of the broader eicosanoid mediator family and pro-inflammatory eicosanoids have already been explored as drug targets for lung disease, with anti-leukotrienes such as Montelukast being shown to successfully decrease exacerbations in asthma patients (189).

While RvD1 and Del-1 were speculated to have a positive relationship, regression analysis did not find a significant relationship between RvD1 and Del-1 levels. However, regression analysis revealed a positive relationship between RvD1 and CRP (a biomarker for cardiovascular injury) in the combined COPD (exacerbators and stable) group. This further suggests that RvD1 is upregulated in response to acute inflammation.

Interestingly, other studies assessing SPM levels in lung disease found that lower SPM levels were associated with more severe disease. A 2021 study in Covid-19 patients (n=38) showed that plasma levels of anti-inflammatory SPMs (including RvD1) were decreased in more severely ill patients, and higher SPM levels were associated with survival (190). Similarly, a study of 32 asthma patients revealed lipoxin levels in whole blood were lowest in severe asthma patients compared to moderate asthma, suggesting a defective lipoxin biosynthesis pathway in more severe disease (191). This contradicts the findings of this study that COPD patients with more severe disease (exacerbations) had the highest RvD1 levels, and needs to be further explored.

Due to time and monetary constraints, in this ELISA study, RvD1 was the only SPM measured. Further measurements of the full range of SPMs (including lipoxins, protectins, maresins) in COPD patients and analysis of their trajectory at different time points after an exacerbation are

needed to fully elucidate the clinical significance of SPMs in COPD and their potential as a novel treatment for preventing and treating exacerbations. To meet these objectives and to build on the findings of this study, the ongoing Resolution Mediators in Chronic Lung Disease (RESCUE) study is using mass spectrometry for lipid mediator profiling in fresh plasma samples taken from 24 COPD patients (12 exacerbators and 12 stable) and 12 controls at set time points, to track changes in SPM levels over time and after exacerbations.

While acute inflammation could lead to an increase in RvD1 levels, the finding that median RvD1 levels were lowest in stable COPD patients suggests that chronic inflammation could be the cause of disrupted SPM production. Unresolved inflammation leading to dysregulation of SPM production pathways is supported by results from a study showing that high doses of omega-3 supplements for patients with atherosclerosis and Type 2 diabetes increased serum DHA/EPA but did not influence levels of RvD1 or inflammatory markers (192). This suggests that omega-3 supplementation alone is not sufficient for treating disease, as patients with inflammatory diseases may have altered SPM production pathways, with reduced levels of potent SPM end products. It was found that sputum levels of arachidonic acid (derived from omega-6 and precursor for pro-inflammatory leukotrienes and anti-inflammatory lipoxins) were higher in COPD exacerbators compared to stable patients (79). Additionally, sputum levels of EPA (derived from omega-3) were lower in stable COPD patients compared to controls. These findings further suggest that SPM production pathways and PUFA metabolism in general are altered in COPD pathogenesis. To further explore this, the Effects of Aspirin on Specialised Pro-Resolving Mediators (ASPIRE) study will be launched to examine the effects of omega-3 and aspirin supplementation on plasma SPM levels and lung/cardiovascular function, in randomised groups of 24 COPD patients and 24 controls.

The finding that Del-1 levels were highest in controls suggests that Del-1 is highly expressed in health and plays an important role in regulating inflammation. These results showing that

Del-1 levels were lowest in stable COPD patients further suggests that chronic inflammation has a negative effect on the production of anti-inflammatory mediators like Del-1. The recorded biomarker levels recorded were highly variable, with a very large spread in exacerbators, stable COPD and healthy controls. The large range of values in all groups suggests that Del-1 levels are highly variable across individuals and could be influenced by factors that were unaccounted for in this study.

The findings of this study are supported by a recent ELISA study (with similar age and gender ratios to the samples used in this study) measuring plasma levels of Del-1 in 438 COPD patients and 99 controls (46 smokers, 53 never smoked). The study by Joo *et al* showed that COPD patients had significantly lower mean plasma levels of Del-1 than controls (385.9±523 pg/ml vs 535.4±903 pg/ml). The study also found that lower plasma levels of Del-1 was associated with increased risk of exacerbations (193). These results are in line with the findings of this study that controls have higher Del-1 levels than COPD patients and their reported standard errors for the mean values also suggest high variability in Del-1 expression.

While IL-17 is known to have an inverse relationship with Del-1 in humans and mice, it was not possible to examine this relationship due to measured IL-17 levels in all samples being below the assay range. While serum IL-17 levels were not measured successfully in these samples, IL-17 serum levels in COPD patients have been evaluated in prior studies, with exacerbators reporting the highest levels, followed by stable COPD patients, and controls had the lowest levels (184, 185).

4.6.1 Limitations

Much higher levels of Del-1 (more than 20 fold higher) were reported in COPD patients and controls by Joo *et al* compared to my results, suggesting that assay type and sample quality

may have influenced my findings, and further investigation is needed to identify the causes of these differences.

Additionally, this study is limited by the fact that RvD1, Del-1 and IL-17 levels were measured in COPD exacerbators at a single time point. In the ERICA study, all COPD exacerbators were stable at least 4 weeks before study start, so the levels of RvD1, Del-1 and IL-17 measured in the serum samples were reflective of patients already in recovery, potentially minimising observed differences with stable COPD samples.

While the demographics of stable and unstable COPD patients were matched well, stable COPD patients had a significantly higher proportion of current smokers, which could explain the lower levels of RvD1 reported in that group.

4.6.2 Impact and future work

There are many further areas to be explored in evaluating the potential of SPMs as a novel treatment for COPD exacerbations and improving understanding of their role during inflammation resolution.

The finding that RvD1 levels were significantly lower in stable COPD patients compared to those with exacerbations suggests an important role for RvD1 in COPD pathogenesis and resolution of inflammation after an exacerbation. Considering that COPD exacerbator samples were taken from patients who were stable for at least 4 weeks, it is important to carry out a clinical study measuring SPM levels in COPD patients at different time points after an exacerbation, to track their trajectory during the recovery stage. I am currently investigating this in the ongoing RESCUE study which is measuring SPM levels in stable COPD, COPD exacerbators and controls at set time points.

Importantly, SPM production may be a key mechanism for commonly used cardiovascular drugs to exert their effects, with aspirin and statins both producing epimers of SPMs that are

as potent as and more stable than naturally produced SPMs (70). The ASPIRE study will investigate this area further by measuring changes in SPM levels in COPD patients/smokers and controls who have been given omega-3 supplements and aspirin vs placebo.

Considering the primarily local activity of SPMs, measuring receptor expression in lung tissues may be more representative of SPM activity than systemic circulating levels. This would build on findings from a small study showing increased ALX/FPR2 expression in COPD lungs compared to controls (176).

One of the major advantages of SPMs as a novel treatment for COPD is their immunomodulatory effects (including clearance of pathogens), which is in contrast to the immunosuppressive effects of the current steroidal options, that can lead to pulmonary infections in vulnerable COPD patients. However, considering that the inflammatory phase is critical for clearing pathogens, ending this inflammation prematurely via SPM treatment could have harmful effects on health. A previous study using mouse pneumosepsis models showed that early treatment with Lipoxin A_4 was associated with worse infections and later treatment was associated with better survival (194). Further understanding of potential immunosuppressive side effects of SPMs are particularly important for treating COPD patients, due to their vulnerability to lung infections.

4.6.3 Summary

In summary, this is the first ELISA study to measure RvD1, Del-1 and IL-17 in the same human COPD serum samples. RvD1 and Del-1 were shown to be significantly decreased in stable COPD patients, suggesting that production pathways for mediators important to inflammation resolution are dysregulated in chronic inflammatory disease.

The findings also showed that an acute exacerbation at baseline is associated with a spike in RvD1 levels, suggesting a pivotal role for these SPMs in the natural resolution of inflammation

in COPD. However, it was not possible to carry out meaningful analysis of IL-17 levels and their relationship to RvD1/Del-1 due to measurements being below the assay range. These findings laid the groundwork for the ongoing RESCUE study to assess the full range of SPMs in COPD patients and their trajectories in the days and weeks following an exacerbation.

Chapter Five: Aspirin use and health outcomes in COPD populations

5.1 Background

5.1.1 Aspirin for chronic obstructive pulmonary disease treatment

COPD patients are at disproportionate risk of CVD but often do not receive the same treatments as patients with CVD alone (1, 195). This is a major problem as an exacerbation in COPD patients can cause or worsen underlying CVD and increase risk of mortality. Existing treatments for COPD exacerbations such as corticosteroids and beta-agonists are inadequate for reducing mortality from CVD and improving quality of life. Therefore, it is essential that treatment regimens for COPD are developed that are compatible with underlying CVD.

One promising avenue of treatment is the repurposing of existing CVD drugs to treat COPD, which may have ameliorative effects on exacerbations and survival in COPD patients, in addition to their beneficial effects for cardiovascular health. Aspirin is one such CVD drug that is widely prescribed and cheaply available. It is an anti-platelet that irreversibly inhibits the COX enzymes by acetylating the serine residue, which prevents thromboxane A_2 (promotes platelet aggregation) production in platelets (119). Aspirin is also known to promote the production of epimers of SPMs that have been shown to have anti-inflammatory effects in COPD and CVD animal models, as well as human *in vitro* studies (176, 196, 197). Furthermore, stable COPD patients have been shown to have elevated platelet levels compared to controls, which increase further during an exacerbation, which could be the cause of increased risk of CVD in COPD populations (117). The anti-platelet properties of aspirin suggests a potential to target this pathway.

5.1.2 Data analysis of aspirin and chronic obstructive pulmonary disease outcomes

While there have been no randomised control trials of aspirin for COPD treatment, previous observational studies of COPD patients have found that aspirin use was associated with fewer exacerbations, slower disease progression and lower mortality after exacerbations (122, 123, 198, 199).

However, these observational studies vary greatly in data quality and collection methods, follow up time and cohort demographics. There are large, high quality datasets available from published clinical trials involving thousands of COPD patients, which provide a wealth of information such as concomitant medications and CVD history/risk. These datasets are highly valued for their clear and consistent data entry and detailed records on the large populations of COPD patients involved, which can provide insights into the relationship between aspirin and outcomes such as risk of mortality and exacerbations.

COPD and CVD are still major causes of mortality and reduced quality of life worldwide, and constitute heavy economic burdens to health systems. While there is a need for novel drugs, the long development process for new therapeutics suggests that it is prudent to identify commonly used drugs such as aspirin that can be repurposed for treating COPD and are compatible with underlying CVD.

Taking into account previous reports of the beneficial effects of aspirin in COPD, there is a need to comprehensively investigate, using large high quality datasets, the potential of aspirin for reducing the risk of exacerbations and all-cause mortality in COPD patients with varying severity of disease and with a history/risk of CVD.

5.2 Hypothesis

Aspirin use is associated with decreased risk of mortality and exacerbations over follow up in COPD patients from the SUMMIT and IMPACT trials.

5.3 Aims

Evaluating the association of aspirin use in COPD populations with risk of all-cause mortality, exacerbations and cardiovascular composite events during follow up.

5.4 Methods

5.4.1 Study design

Despite the increased risk of mortality for COPD patients with CVD, there is currently a lack of CVD compatible therapeutics that reduces the risk of exacerbations and mortality for these patients. Considering the complex discovery process associated with developing new therapeutics, a cost and time effective method would be to repurpose existing drugs, such as aspirin, which is widely used in CVD populations and has been reported in observational studies to be associated with reduced exacerbations and mortality. I carried out all statistical analysis, coding and data presentation.

To further explore the potential of aspirin for improved health outcomes in COPD patients, I analysed using statistical methods, large datasets consisting of COPD patients from the SUMMIT (n=16485, moderate COPD with risk/history of CVD defined as CAD, PAD, stroke, MI and diabetes with target organ disease, median follow up 1.8 years, published 2016) and IMPACT (n=10355, severe COPD, 52 weeks follow up, published 2018) studies, to evaluate the effects of aspirin on risk of mortality, exacerbations and cardiovascular composite events (200, 201).

5.4.2 Datasets

The SUMMIT and IMPACT datasets were provided by Clinical Study Data Request (https://www.clinicalstudydatarequest.com). Dr Marie Fisk wrote the proposal for using the patient level datasets for analysis described in this chapter.

The SUMMIT study was a clinical trial assessing the effectiveness of fluticasone furoate (FF), vilanterol (VI) and FF/VI combination treatment on the primary outcome of all-cause mortality. The inclusion criteria for the study population included current or former smokers with minimum of ten-pack-year history, 40-80 years old, moderate COPD (FEV1% 50-70% of predicted value), history or increased risk (60 years or older using medication for more than two of hypercholesterolemia, hypertension, diabetes, PAD) of CVD (CAD, PAD, stroke, MI, diabetes with target organ disease) (200). The exclusion criteria were non-COPD respiratory disease, lung reduction surgery, oral corticosteroid use, on long term oxygen, severe heart failure, life expectancy below three years, end stage chronic renal disease (200). The SUMMIT study researchers found that the treatments did not have a significant effect on all-cause mortality or cardiovascular events, but did reduce exacerbations (200).

The IMPACT study was a clinical trial assessing the effectiveness of FF/VI/umeclidinium triple therapy, FF/VI dual therapy and VI/umeclidinium dual therapy on the primary outcome of exacerbation rate. The inclusion criteria for participants included severe COPD (FEV1% below 50% of predicted value and history of at least one moderate/severe exacerbation one year prior to study entry) and to be 40 years or older (201). The IMPACT study researchers found that triple therapy was significantly more effective in reducing exacerbation rate compared to the dual therapies, and therapies including FF were associated with lower mortality than VI/umeclidinium (201).

The datasets included detailed records of the concomitant medications (including aspirin) that the study participants were using at baseline. It is unknown how long the participants had been using reported concomitant medications or if they stopped using them during the course of the clinical study and follow up period. Only the intention to treat population will be used in data analysis.

5.4.3 Statistical analysis

The primary outcomes of this analysis were hazard ratios for time to first event occurrence of ACM, moderate and severe exacerbations (moderate defined as requiring antibiotics/glucocorticoid treatment, severe defined as requiring hospitalisation) and cardiovascular events, calculated using multivariate Cox Proportional Hazards models. The hazard ratios are presented using forest plots. Sensitivity analyses were carried out for the covariates of age (oldest strata), sex (M/F), race (white) and country (top five sources of participants), and the results were compared to those from the main population. Additionally, for bias analysis, E-values were calculated, which represent the minimum strength of association an unmeasured confounder would need to have with the treatment (aspirin) and the outcome to explain away an observed association between aspirin and the outcome (125). Propensity score matching was carried out on the SUMMIT and IMPACT datasets using covariates that predict for being on/off aspirin. In the SUMMIT dataset, 5038 matched pairs (of aspirin users and non-users) were selected. In the IMPACT dataset, 2037 matched pairs of aspirin users and non-users were selected. The scores were calculated with logistic regression and the matching method used was ‘nearest neighbour matching’, with a caliper of 0.2. The adjustment factors of the logistic regression for SUMMIT were history of PAD, stroke, CAD, MI and percutaneous coronary intervention, and for IMPACT were history of PAD, stroke, CAD, MI and angina. Hazard ratios for health outcomes were then calculated using the propensity score matched groups.

All analysis (except for E-values which were determined manually) was carried out with RStudio Desktop, R version 4.2.0. The full code used for this analysis is available in Appendix A.

5.5 Results

Datasets consisting of COPD patients from the SUMMIT and IMPACT studies were analysed to assess the association between aspirin use and risk of mortality, exacerbations and cardiovascular events over the follow up period.

From the SUMMIT and IMPACT studies, 16,485 moderate COPD patients with a history/risk of CVD and 10355 severe COPD patients were included respectively.

The demographics and clinical history/events of the populations are shown in Table 4. All subjects were from the intention to treat population. The SUMMIT and IMPACT populations had similar age, sex, BMI and ethnic backgrounds. The main difference is that the IMPACT subjects had more severe COPD (% FEV1 less than 50% of predicted value) and were not specifically recruited from a CVD risk/history population. SUMMIT had a median follow up time of 1.8 years and IMPACT had a follow up time of 52 weeks. Reported events were those that took place on treatment (trial drugs or placebo), except for ACM in SUMMIT, which included all deaths before the common end date.

Cardiovascular events for SUMMIT was defined as CV death, MI, stroke, unstable angina, transient ischaemic attack, and for IMPACT as CV death, acute MI Preferred Term (PT), CNS haemorrhage, heart disease Standardised MedDRA Queries (SMQ), MI PT, MI SMQ. For heart disease, definition for SUMMIT is myocardial infarction and/or coronary artery disease and/or percutaneous coronary intervention, and definition for IMPACT is myocardial infarction and/or coronary artery disease and/or angina.

Table 4: Demographics and clinical history of the SUMMIT and IMPACT populations.

Variable	**SUMMIT (n=16485)**	**IMPACT (n=10355)**
Age (mean years)	65	65.3
Sex	12289 (75%) male	6870 (66%) male
BMI (mean)	28.0	26.6
Race	13357 (81%) white	8083 (78%) white
Smoking Status	7678 (47%) current smoker	3587 (35%) current smoker
Pack Years (smoking)	41	47
FEV1%	59.1	45.5
Previous exacerbations prior to study entry (0, 1, >=2)	0 (61%) 1 (24%) >=2 (15%)	0 (<1%) 1 (45%) >=2 (55%)
Aspirin use (monotherapy)	6844 (41.5%)	2318 (22.4%)
Reported events (first time occurrence)		
All-cause mortality	1037 (6%)	138 (1%)
Moderate exacerbations	2858 (17%)	4401 (43%)
Severe exacerbations	1293 (8%)	1180 (11%)
Cardiovascular events	688 (4%)	299 (3%)
Clinical History (Yes)		
Diabetes	4376 (27%)	1599 (16%)
Hypercholesterolemia	10190 (62%)	3367 (33%)
Hypertension	14265 (87%)	5446 (53%)
Heart Disease	8599 (52%)	1684 (16%)
Arrhythmia	NA	816 (8%)
Stroke	1595 (10%)	458 (4%)
HF	3456 (21%)	539 (5%)
Peripheral Artery Disease	3145 (19%)	342 (3%)

5.5.1 All-cause mortality

Aspirin use in the SUMMIT study was associated with an increased risk of ACM before the common end date (January 25th 2015, date by which there would be at least 1000 deaths), HR of [1.15 (1.00-1.33), p=0.048], with 1037 deaths and 15448 survivors before the common end date. In the IMPACT dataset, aspirin use was also associated with increased risk of ACM, although the effect was not significant, HR of [1.45 (0.95-2.19), p=0.082] (Figure 18), with 138 deaths and 10217 survivors. SUMMIT multivariate HRs were adjusted for covariates of age, sex, BMI, smoking status, smoking pack years, FEV1% and history of stroke, HF, hypercholesterolemia, hypertension, heart disease, diabetes and PAD. IMPACT multivariate HRs were adjusted for covariates of age, sex, BMI, trial treatment arm, smoking status, smoking pack years, FEV1% and history of arrhythmia, stroke, HF, hypercholesterolemia, hypertension, heart disease, diabetes and PAD.

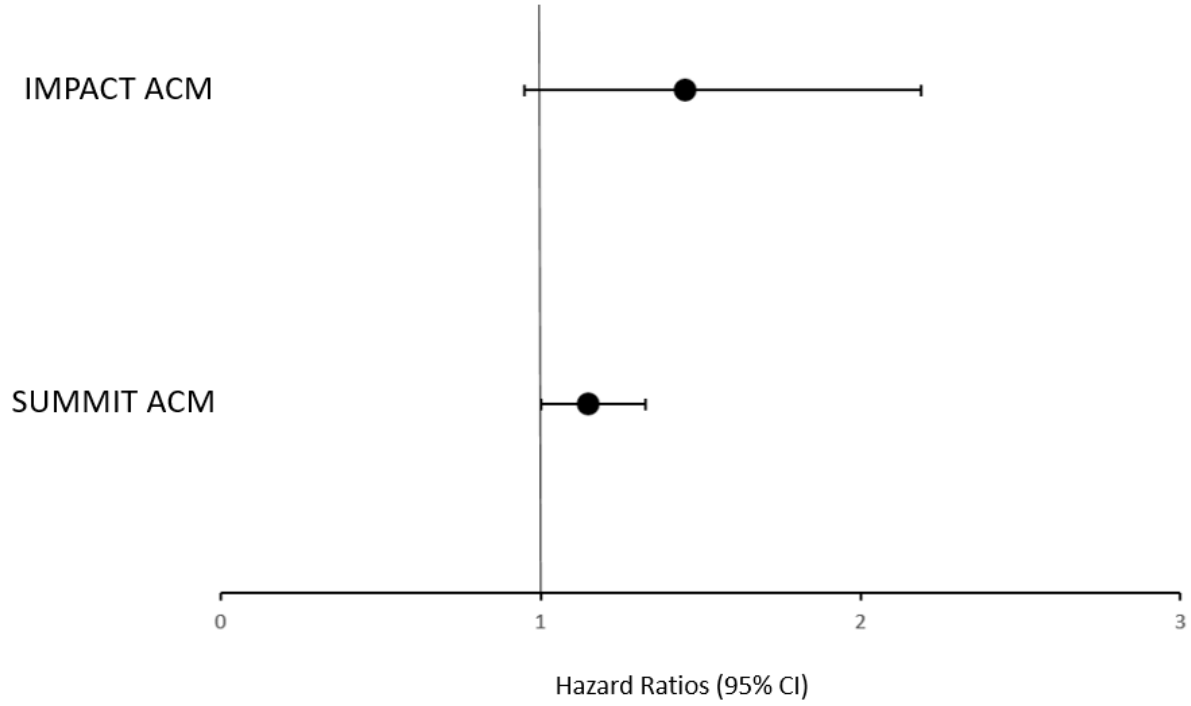

Figure 18: Forest plot showing multivariate adjusted HR and 95% CI for aspirin use and its relationship with ACM. ACM=All-cause mortality. SUMMIT multivariate HRs were adjusted for covariates of age, sex, BMI, smoking status, smoking pack years, FEV1% and history of stroke, HF, hypercholesterolemia, hypertension, heart disease, diabetes and PAD. IMPACT multivariate HRs were adjusted for covariates of age, sex, BMI, trial treatment arm, smoking status, smoking pack years, FEV1% and history of arrhythmia, stroke, HF, hypercholesterolemia, hypertension, heart disease, diabetes and PAD.

5.5.2 Exacerbations

Aspirin use was associated with increased risk of moderate exacerbations in the SUMMIT [HR=1.17 (1.08-1.27), p<0.001] and IMPACT [HR=1.10 (1.02-1.18), p=0.012] populations. There were 2858 patients who experienced a moderate exacerbation in the SUMMIT trial and 4401 patients who did so in the IMPACT trial. For severe exacerbations, aspirin use was also associated with an increased risk in the SUMMIT [HR=1.35 (1.19-1.52), p<0.001] trial with 1293 patients reporting a severe exacerbation, and in the IMPACT [HR=1.30 (1.14-1.49), p<0.001] trial with 1180 patients reporting a severe exacerbation (Figure 19). SUMMIT and

IMPACT multivariate HRs were adjusted for covariates of age, sex, BMI, trial treatment arm, smoking status, smoking pack years, FEV1% and previous exacerbations.

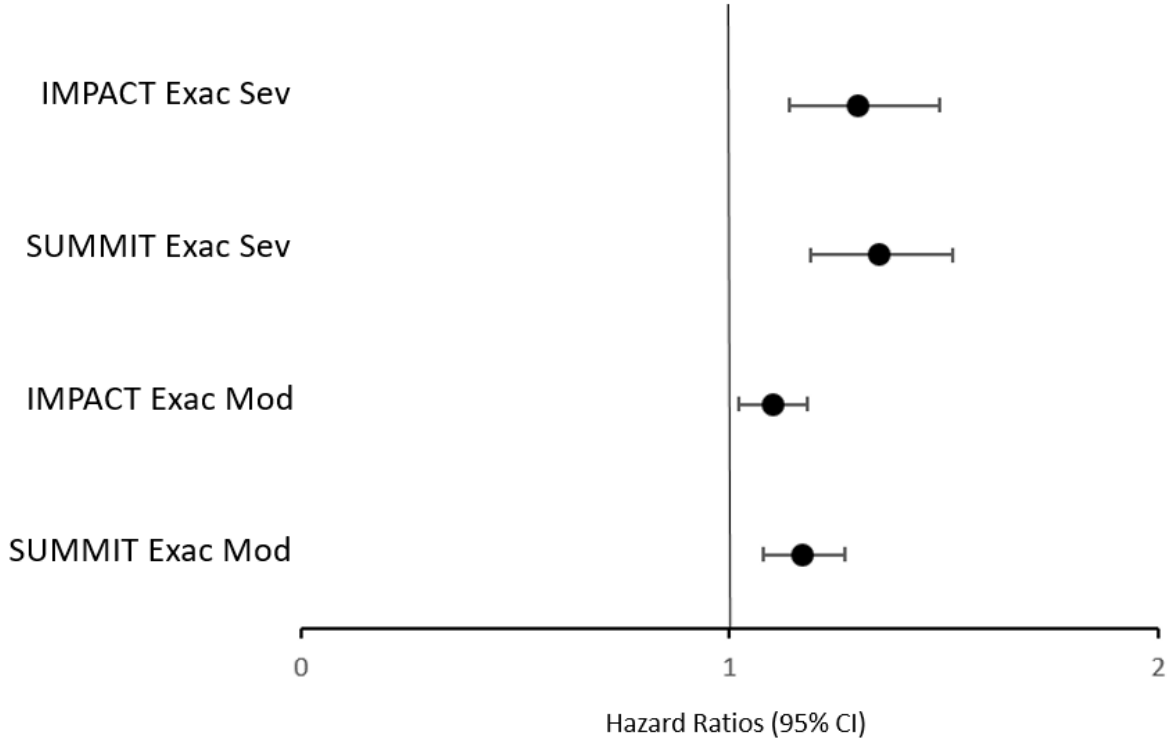

Figure 19: Forest plot showing multivariate adjusted HR and 95% CI for aspirin use and its relationship with moderate and severe exacerbations. Exac Sev= Severe exacerbation, Exac Mod= Moderate exacerbation. SUMMIT and IMPACT multivariate HRs were adjusted for covariates of age, sex, BMI, trial treatment arm, smoking status, smoking pack years, FEV1% and previous exacerbations.

5.5.3 Cardiovascular events

In the SUMMIT dataset, aspirin use was associated with an increased risk of cardiovascular events (688 patients experienced an event), with an adjusted HR of 1.65 (1.33-2.05), $p<0.001$. The adjustment factors were age, sex, BMI, trial treatment arm, smoking status, smoking pack years, history of stroke, HF, hypercholesterolemia, hypertension, heart disease, PAD, diabetes, previous exacerbations. For the IMPACT dataset, aspirin use was associated with an increased rate (adjusted) of cardiovascular events (rate ratio=2.22), with 299 patients experiencing an event. The adjustment factors were age, sex, BMI, trial treatment arm, smoking status, smoking

pack years, history of arrhythmia, stroke, HF, hypercholesterolemia, hypertension, heart disease, PAD, diabetes, previous exacerbations.

5.5.4 Sensitivity analysis

In sensitivity analysis, the subgroups of age (>=75), sex (M/F), race (white) and country (top five sources of patients) were analysed. Sensitivity analysis did not show any major differences from the main group, on aspirin use and association with increased risk of ACM, exacerbation and cardiovascular events. Subgroup analysis by race (white) found for moderate exacerbations a HR of [1.27 (1.15-1.40), p<0.001] in SUMMIT and a HR of [1.08 (1-1.17), p=0.049] in IMPACT. The same subgroup analysis for severe exacerbations found a HR of [1.33 (1.15-1.55), p<0.001] in SUMMIT and a HR of [1.31 (1.12-1.53), p<0.001] in IMPACT.

5.5.5 Bias analysis

Whilst many potential confounders were included as covariates in the Cox Models, E-values were also calculated (for the HR estimate and the lower estimate of the 95% CI) to identify the minimum association with aspirin and outcomes an unknown confounder would need to have to explain away the observed association of aspirin use and increased risk of ACM, exacerbations and cardiovascular events. For the outcome of ACM in the SUMMIT dataset, the E-value was 1 (for the lower estimate of CI), and for the outcome of moderate exacerbation, the E-values were 1.37 (SUMMIT) and 1.16 (IMPACT). For the outcome of severe exacerbation, the E-values for the lower estimate of CI were 1.67 (SUMMIT) and 1.54 (IMPACT). A low E-value (1-2) indicates an unknown confounding factor can credibly explain away observed associations of aspirin with COPD health outcomes.

5.5.6 Propensity score matched groups

After matching, there were 5038 pairs from the SUMMIT study and 2037 pairs from the IMPACT study. The analysis results of the propensity score matched groups were similar to the findings of the main group. For the matched pairs from the SUMMIT study, association of aspirin use with ACM risk was HR [1.22 (1.04-1.43), p=0.015], [1.19 (1.09-1.31), p<0.001] for moderate exacerbation risk and [1.32 (1.14-1.53), p<0.001] for severe exacerbation risk. For the matched pairs from the IMPACT study, association of aspirin use with risk of ACM, moderate exacerbation and severe exacerbation was [1.32 (0.79-2.19), p=0.3], [1.11 (1.01-1.23), p=0.031] and [1.16 (0.97-1.39), p=0.11] respectively. The demographics tables for the new matched SUMMIT and IMPACT groups are available in Appendix D. Risk of bleeding analysis (adjusted for age, sex, BMI and trial treatment arm) was also carried out, with aspirin use being associated (as expected) with increased risk of bleeding in SUMMIT [HR 1.25 (0.88-1.78), p=0.2] and IMPACT [HR 2.19 (1.09-4.41), p=0.028] populations.

5.5.7 Absolute Risk

In the SUMMIT population, the absolute risk of ACM in aspirin users was 6.9% compared to 5.3% in non-users. For risk of moderate exacerbations, aspirin users were also at increased risk compared to non-users, at 18.2% and 15.4% respectively. Aspirin users had an absolute risk of 8.4% for severe exacerbations, while non-users were at 6.2%.

In the IMPACT population, the absolute risk of ACM in aspirin users and non-users was 1.8% versus 1.2%. For risk of moderate exacerbations, aspirin users were at 44.2% risk compared to 41.5% for non-users. Additionally, aspirin users had 13.4% risk of severe exacerbations, while non-users were at 10.3% risk.

5.6 Discussion

Findings from previous observational studies had suggested a potential association of aspirin use with reduced risk of mortality and exacerbations in COPD patients. Those findings, in addition to aspirin's well known cardiovascular benefits, suggested a potential for aspirin as a well-tolerated and accessible treatment for COPD patients with a CVD background. In this project, two large high quality datasets from the SUMMIT (n=16485) and IMPACT (n=10355) trials were analysed, and the multivariate Cox models showed an association of aspirin use with increased risk of ACM, exacerbations (moderate and severe) and cardiovascular events. These associations were significant for outcomes in both datasets, with the exception of ACM in the IMPACT trial. These results are different to previous studies that suggested aspirin use was associated with ameliorative effects in COPD populations, and further investigation is needed to identify the causes of this (122).

Relative risk measures such as hazard ratios can overestimate an observed effect, so absolute risk of events was also calculated (202). When considering absolute risk of ACM, moderate and severe exacerbations, aspirin users were also at higher risk of an event compared to non-users, supporting the results of the hazard ratio analysis. Sensitivity analysis focusing on age (oldest group), sex, race (white) and country did not reveal major differences from the findings of the main group, nor did it find any association of aspirin use with reduced risk of ACM, exacerbations or cardiovascular events.

A previous observational study by Fawzy *et al* involving 503 propensity score matched participant pairs had suggested that aspirin use in COPD patients was associated with reduced exacerbation rate (122). The contrasting findings of this study could be due to the heterogeneity in methods and population clinical history of previous studies, as well as varied data quality. The study by Fawzy *et al* analysed a population which also included non-smokers and excluded

those with unstable CVD (122, 203). This analysis used high quality trial data from populations inclusive of moderate and severe COPD and those with a history/risk of CVD. The SUMMIT and IMPACT datasets had consistent data entry and well defined clinical conditions. Additionally, it has been noted by Bakshi *et al* 2021 that many previous observational studies investigating aspirin use in COPD were affected by biases such as collider-stratification bias (shows non-existent association between exposure and outcome) and exposure misclassification (204).

Additionally, considering aspirin's indication for CVD treatment regimens, patients who are prescribed aspirin have more CVD risk factors such as increased platelet count or have already been diagnosed with CVD. These risk factors such as increased platelet count have been implicated in COPD pathogenesis (including acute exacerbations) and are also associated with poor health outcomes. Potentially, the findings of this study that aspirin use is associated with worse outcomes in COPD patients could be due to confounding factors such as aspirin users having poorer health, more risk factors for mortality, and acute exacerbations at baseline, compared to non-users of aspirin.

However, even after creating new propensity score matched groups based on covariates that predict for aspirin use (e.g. CAD, MI and stroke), to reduce potential bias caused by confounding factors, aspirin was still associated with increased risk of ACM, moderate and severe exacerbations over follow up. Nevertheless, matching cannot fully exclude bias such as disease severity.

Bias analysis showed that E-values calculated using the lower estimate of the outcome HRs were relatively low, with values being between 1-2. This suggests that an unknown confounding factor could credibly explain away the observed association of aspirin use with negative health outcomes.

Dysregulated SPM pathways could also have contributed to the observed effects. It has been previously reported that T cells sourced from chronic heart failure patients were unresponsive to treatment with RvD1 and Resolvin D2 (76). Considering the systemic inflammation involved in COPD and the significant presence of CVD and CVD risk factors in the SUMMIT and IMPACT populations, it is possible that dysregulated SPM pathways in these patients prevented them from benefiting from the immunomodulatory effects of aspirin (production of aspirin triggered SPMs).

Additionally, the use of bronchodilators among significant portions of the study population could have increased the risk of mortality and development of CVD (which is a reported negative effect of bronchodilators), potentially masking the beneficial effects of aspirin (94).

Given the association of aspirin use with negative health outcomes shown in this study, and the high level of aspirin use in the COPD population (22.4% for IMPACT and 41.5% for SUMMIT), it is essential to investigate the causes of these findings. This is particularly important considering aspirin's status as a commonly prescribed CVD drug and the elevated risk of concurrent CVD in COPD patients. To identify potential molecular mechanisms that could explain the observed harmful effects of aspirin use in this study, levels of platelets and aspirin triggered SPMs should be measured in aspirin users and non-users, to assess if aspirin directed pathways are functioning as expected.

5.6.1 Limitations

There are several limitations to this analysis. For analysis of populations with the demographic characteristics seen in this study, it is difficult to find patients in statistically viable numbers who are only using the medication of interest, such as aspirin. While aspirin users were defined as patients who were only using one type of anti-platelet medication (aspirin), the other medications they were using such as statins, beta-blockers and ACEI/ARBs were not included

in the analysis. These medications could have an effect on health outcomes, including mortality and exacerbations. Particularly, statins have been shown to decrease mortality risk and beta-blockers are of increasing interest in treating COPD patients with concurrent CVD (101, 205).

Another limitation is the variation in aspirin dosages amongst the analysis population. The regularity of use, dosage and administrative route varied in the included population, which could have an influence on the health outcomes of interest. Additionally, while the trial drugs were included in the Cox Models if they were found to have had an effect on study outcomes (ACM, exacerbations and cardiovascular events), their potential interactions with aspirin and other CVD medications have not been assessed. To investigate this further, sensitivity analysis by trial drug subgroup could be carried out and compared with the designated placebo group from the SUMMIT study.

5.6.2 Impact and future work

The findings of this study that aspirin use is associated with increased risk of exacerbations and all-cause mortality are highly significant given the high proportion of COPD patients using aspirin and will be directly followed by the ASPIRE clinical study, which has already been approved. The ASPIRE study will involve randomised groups of 24 COPD patients/smokers and 24 controls given omega-3 and aspirin supplementation or placebo, over the course of 12 weeks. Plasma SPM levels and lung/cardiovascular function will be regularly measured during the study to assess the effects of aspirin use on production of SPMs as well as pulmonary and cardiovascular health.

5.6.3 Summary

This study comprehensively analysed large high quality datasets, comprised of moderate and severe COPD patients, including those with a history/risk of CVD. These demographic and clinical characteristics made the SUMMIT and IMPACT datasets ideal for assessing the

potential of aspirin use in reducing mortality and exacerbations in COPD patients, particularly those with CVD. Contrary to previous reports from observational studies, the findings of this study show that aspirin is associated with increased risk of ACM, exacerbations and cardiovascular events. In contrast to previous observational studies which observed beneficial effects of aspirin (smaller cohorts), at best, this work suggests no benefit and possible increased risk of harm. Although these data may simply reflect that patients prescribed aspirin have increased cardiovascular risk and poorer health than those that do not, and statistical adjustment for confounders (despite propensity score matching and other methodology) is not sufficient to mitigate this inherent association. Given these data, a randomised controlled trial would be the useful methodology to assess the effect of aspirin on exacerbations and mortality in patients with COPD.

Chapter Six: Conclusions

This project evaluated immunomodulatory mediators and the anti-platelet drug aspirin which have potential for improving health outcomes in COPD and CVD, with CVD being a frequently reported comorbidity of COPD patients.

A systematic review and meta-analysis investigated for the first time the potential ameliorative role of the IL-33/ST2 axis across the spectrum of human CVD, with contradictory findings from previous studies. IL-33 is known to have significant downstream effects as a mediator of innate and adaptive immunity, with ST2 as its receptor. Here, increased levels of circulating ST2 are associated with increased risk of mortality and MACE in CVD and community cohorts. This meta-analysis systematically evaluated IL-33 and sST2 levels and found that circulating sST2 measurement may have clinical value in differentiating patients with CVD versus controls without CVD, with the greatest difference in sST2 levels compared with controls being observed in AF and CHF. While IL-33 levels were lower in HF, CAD and ACS patients compared to controls, the opposite was observed in stroke patients which requires further investigation. These findings suggest a prognostic and diagnostic role for sST2 and IL-33 shows promise as a biomarker of CVD. Limitations of this study include the difficulty of measuring circulating IL-33 levels (circulating IL-33 levels were much lower than sST2 levels due to sST2's role as a decoy receptor to regulate IL-33 activity), the low number of IL-33 clinical studies and different adjustment factors being used for reported multivariate hazard ratios. Studies involving larger sample sizes would further improve understanding of this axis and its role in CVD. Further work to build on these findings should be carried out, including the evaluation of IL-33 levels and ST2L expression in brain and carotid artery tissue sections, to elucidate the causes of the higher levels of circulating IL-33 in stroke patients. I would also like to measure circulating IL-33 levels in COPD patients with and without CVD to further

increase understanding of the role of IL-33 in COPD and CVD pathogenesis, which would inform future COPD therapies targeting the IL-33/ST2 axis.

RvD1 and Del-1 were measured using ELISAs in the same human COPD serum samples, to evaluate differences in stable and unstable COPD patients and to analyse the relationship between the mediators. RvD1 and Del-1 levels were found to be significantly lower in stable COPD patients, suggesting dysregulated resolution pathways in chronic inflammatory disease. In COPD patients who had exacerbations, RvD1 levels were found to be increased, which suggests that these immunomodulatory SPMs are essential for the natural resolution of inflammation in COPD. These findings support a beneficial role for RvD1 in COPD, and provided the foundation for the ongoing RESCUE study which is assessing the full range of SPMs in COPD patients and their trajectories following an exacerbation. In addition to the ongoing RESCUE study, other future work I would like to carry out include the evaluation of SPM receptor expression in COPD lung tissue, which could provide a more accurate assessment of SPM activity in COPD patients, considering that SPMs are rapidly degraded.

A comprehensive analysis of two large trial datasets (SUMMIT n=16485, IMPACT n=10355) was carried out, involving moderate and severe COPD patients, and those with a history/risk of CVD, to evaluate the potential treatment benefits of aspirin, which is known to have anti-platelet properties as well as producing immunomodulatory aspirin triggered epimers of SPMs. The analysis showed that aspirin use is associated with an increased risk of ACM, exacerbations and cardiovascular events, which is contrary to the findings of previous observational studies. Analysis of propensity matched groups also found aspirin use was associated with increased ACM and exacerbation risk. However, E-values calculated for bias analysis were low (with values being between 1-2), which suggests that an unknown confounding factor could easily explain away the observed association of aspirin use with negative health outcomes. These findings suggests that aspirin should not be indicated for

COPD treatment regimens and further work is needed to identify the causes of this increased risk, as well as considering treatment recommendations for COPD patients who are often using aspirin. Limitations of this study include the potential of unknown confounding factors such as poorer baseline health for aspirin users influencing the findings, the varying dosages of aspirin reported across the populations and the use of other CVD medications.

In summary, this research project showed that sST2 has a prognostic and diagnostic role in CVD and IL-33 has potential as a biomarker of CVD and RvD1 levels are different in stable COPD patients, COPD patients with exacerbations at baseline and control groups. Additionally, aspirin use is associated with increased risk of all-cause mortality and exacerbations in COPD populations (moderate and severe) with and without CVD.

References

1. Morgan AD, Zakeri R, Quint JK. Defining the relationship between COPD and CVD: what are the implications for clinical practice? Ther Adv Respir Dis. 2018;12:1753465817750524.
2. NHS. Cardiovascular disease (CVD) 2022 [Available from: https://www.nhs.uk/conditions/cardiovascular-disease/.
3. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020;76(25):2982-3021.
4. Foundation BH. Heart Statistics British Heart Foundation2020 [cited 2020. Available from: https://www.bhf.org.uk/what-we-do/our-research/heart-statistics.
5. BHF. Facts and figures 2022 [Available from: https://www.bhf.org.uk/what-we-do/news-from-the-bhf/contact-the-press-office/facts-and-figures.
6. BHF. UK Factsheet 2022 [Available from: https://www.bhf.org.uk/-/media/files/research/heart-statistics/bhf-cvd-statistics---uk-factsheet.pdf.
7. Cheema KM, Dicks E, Pearson J, Samani NJ. Long-term trends in the epidemiology of cardiovascular diseases in the UK: Insights from the British Heart Foundation Statistical Compendium. Cardiovasc Res. 2022.
8. Tucker WD, Arora Y, Mahajan K. Anatomy, Blood Vessels. StatPearls. Treasure Island (FL)2022.
9. Mercadante AA, Raja A. Anatomy, Arteries. StatPearls. Treasure Island (FL)2022.
10. Kruger-Genge A, Blocki A, Franke RP, Jung F. Vascular Endothelial Cell Biology: An Update. Int J Mol Sci. 2019;20(18).
11. Ogobuiro I, Wehrle CJ, Tuma F. Anatomy, Thorax, Heart Coronary Arteries. StatPearls. Treasure Island (FL)2022.
12. Charlick M, J MD. Anatomy, Head and Neck, Internal Carotid Arteries. StatPearls. Treasure Island (FL)2022.
13. Flaherty ML, Kissela B, Khoury JC, Alwell K, Moomaw CJ, Woo D, et al. Carotid artery stenosis as a cause of stroke. Neuroepidemiology. 2013;40(1):36-41.
14. Zemaitis MR, Boll JM, Dreyer MA. Peripheral Arterial Disease. StatPearls. Treasure Island (FL)2022.
15. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med. 2014;5(8):927-46.
16. Park KH, Park WJ. Endothelial Dysfunction: Clinical Implications in Cardiovascular Disease and Therapeutic Approaches. J Korean Med Sci. 2015;30(9):1213-25.
17. Lin J, Kakkar V, Lu X. Impact of MCP-1 in atherosclerosis. Curr Pharm Des. 2014;20(28):4580-8.
18. Jebari-Benslaiman S, Galicia-Garcia U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, et al. Pathophysiology of Atherosclerosis. Int J Mol Sci. 2022;23(6).
19. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res. 2014;114(12):1852-66.
20. K. Milne DS. Acute Exacerbations of Chronic Lung Disease: Cardiac Considerations. Nature Public Health Emergency Collection 2020.
21. Frangogiannis NG. Pathophysiology of Myocardial Infarction. Compr Physiol. 2015;5(4):1841-75.

22. Kuriakose D, Xiao Z. Pathophysiology and Treatment of Stroke: Present Status and Future Perspectives. Int J Mol Sci. 2020;21(20).
23. Adeloye D, Song P, Zhu Y, Campbell H, Sheikh A, Rudan I, et al. Global, regional, and national prevalence of, and risk factors for, chronic obstructive pulmonary disease (COPD) in 2019: a systematic review and modelling analysis. Lancet Respir Med. 2022;10(5):447-58.
24. WHO. Chronic obstructive pulmonary disease (COPD) 2022 [Available from: https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd).
25. Foundation BL. The battle for breath - the economic burden of lung disease British Lung Foundation2020 [cited 2020. Available from: https://www.blf.org.uk/policy/economic-burden#:~:text=%C2%A31.2%20billion%20falls%20on,asthma%20(%C2%A33%20billion).
26. Chaudhry R, Bordoni B. Anatomy, Thorax, Lungs. StatPearls. Treasure Island (FL)2022.
27. Mirza S, Clay RD, Koslow MA, Scanlon PD. COPD Guidelines: A Review of the 2018 GOLD Report. Mayo Clin Proc. 2018;93(10):1488-502.
28. Tuder RM, Petrache I. Pathogenesis of chronic obstructive pulmonary disease. J Clin Invest. 2012;122(8):2749-55.
29. Pandey KC, De S, Mishra PK. Role of Proteases in Chronic Obstructive Pulmonary Disease. Front Pharmacol. 2017;8:512.
30. Manda-Handzlik A, Demkow U. Neutrophils: The Role of Oxidative and Nitrosative Stress in Health and Disease. Adv Exp Med Biol. 2015;857:51-60.
31. Qureshi H, Sharafkhaneh A, Hanania NA. Chronic obstructive pulmonary disease exacerbations: latest evidence and clinical implications. Ther Adv Chronic Dis. 2014;5(5):212-27.
32. Brode SK, Ling SC, Chapman KR. Alpha-1 antitrypsin deficiency: a commonly overlooked cause of lung disease. CMAJ. 2012;184(12):1365-71.
33. Chen W, Thomas J, Sadatsafavi M, FitzGerald JM. Risk of cardiovascular comorbidity in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. Lancet Respir Med. 2015;3(8):631-9.
34. Macnee W, Maclay J, McAllister D. Cardiovascular injury and repair in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2008;5(8):824-33.
35. Malo de Molina R, Aguado S, Arellano C, Valle M, Ussetti P. Ischemic Heart Disease during Acute Exacerbations of COPD. Med Sci (Basel). 2018;6(4).
36. Green CE, Turner AM. The role of the endothelium in asthma and chronic obstructive pulmonary disease (COPD). Respir Res. 2017;18(1):20.
37. Heinz A. Elastic fibers during aging and disease. Ageing Res Rev. 2021;66:101255.
38. Rodgers JL, Jones J, Bolleddu SI, Vanthenapalli S, Rodgers LE, Shah K, et al. Cardiovascular Risks Associated with Gender and Aging. J Cardiovasc Dev Dis. 2019;6(2).
39. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov. 2008;7(10):827-40.
40. Griesenauer B, Paczesny S. The ST2/IL-33 Axis in Immune Cells during Inflammatory Diseases. Front Immunol. 2017;8:475.
41. Cayrol C, Girard JP. Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family. Immunol Rev. 2018;281(1):154-68.
42. Travers J, Rochman M, Miracle CE, Habel JE, Brusilovsky M, Caldwell JM, et al. Chromatin regulates IL-33 release and extracellular cytokine activity. Nat Commun. 2018;9(1):3244.
43. Cohen ES, Scott IC, Majithiya JB, Rapley L, Kemp BP, England E, et al. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. Nat Commun. 2015;6:8327.

44. Homsak E, Gruson D. Soluble ST2: A complex and diverse role in several diseases. Clin Chim Acta. 2020;507:75-87.
45. Miller AM. Role of IL-33 in inflammation and disease. J Inflamm (Lond). 2011;8(1):22.
46. West PW, Bahri R, Garcia-Rodriguez KM, Sweetland G, Wileman G, Shah R, et al. Interleukin-33 Amplifies Human Mast Cell Activities Induced by Complement Anaphylatoxins. Front Immunol. 2020;11:615236.
47. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. J Allergy Clin Immunol. 2008;121(6):1484-90.
48. Chan BCL, Lam CWK, Tam LS, Wong CK. IL33: Roles in Allergic Inflammation and Therapeutic Perspectives. Front Immunol. 2019;10:364.
49. Larsen KM, Minaya MK, Vaish V, Pena MMO. The Role of IL-33/ST2 Pathway in Tumorigenesis. Int J Mol Sci. 2018;19(9).
50. Xia J, Zhao J, Shang J, Li M, Zeng Z, Zhao J, et al. Increased IL-33 expression in chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol. 2015;308(7):L619-27.
51. Sun BB, Ma LJ, Qi Y, Zhang GJ. Correlation of IL-33 gene polymorphism with chronic obstructive pulmonary disease. Eur Rev Med Pharmacol Sci. 2019;23(14):6277-82.
52. Byers DE, Alexander-Brett J, Patel AC, Agapov E, Dang-Vu G, Jin X, et al. Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. J Clin Invest. 2013;123(9):3967-82.
53. Ramos FL, Krahnke JS, Kim V. Clinical issues of mucus accumulation in COPD. Int J Chron Obstruct Pulmon Dis. 2014;9:139-50.
54. Gabryelska A, Kuna P, Antczak A, Bialasiewicz P, Panek M. IL-33 Mediated Inflammation in Chronic Respiratory Diseases-Understanding the Role of the Member of IL-1 Superfamily. Front Immunol. 2019;10:692.
55. Seki K, Sanada S, Kudinova AY, Steinhauser ML, Handa V, Gannon J, et al. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. Circ Heart Fail. 2009;2(6):684-91.
56. Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, et al. IL-33 reduces the development of atherosclerosis. J Exp Med. 2008;205(2):339-46.
57. FDA. SUBSTANTIAL EQUIVALENCE DETERMINATION

DECISION SUMMARY

ASSAY ONLY TEMPLATE 2020 [cited 2020 17/07/2020]. Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K111452.pdf.
58. Dieplinger B, Egger M, Haltmayer M, Kleber ME, Scharnagl H, Silbernagel G, et al. Increased soluble ST2 predicts long-term mortality in patients with stable coronary artery disease: results from the Ludwigshafen risk and cardiovascular health study. Clin Chem. 2014;60(3):530-40.
59. Pascual-Figal DA, Lax A, Perez-Martinez MT, del Carmen Asensio-Lopez M, Sanchez-Mas J, Network G. Clinical relevance of sST2 in cardiac diseases. Clin Chem Lab Med. 2016;54(1):29-35.
60. Zhang K, Zhang XC, Mi YH, Liu J. Predicting value of serum soluble ST2 and interleukin-33 for risk stratification and prognosis in patients with acute myocardial infarction. Chin Med J (Engl). 2013;126(19):3628-31.
61. Segiet OA, Romuk E, Nowalany-Kozielska E, Wojciechowska C, Piecuch A, Wojnicz R. The concentration of interleukin-33 in heart failure with reduced ejection fraction. Anatol J Cardiol. 2019;21(6):305-13.

62. Stojkovic S, Thulin A, Hell L, Thaler B, Rauscher S, Baumgartner J, et al. IL-33 stimulates the release of procoagulant microvesicles from human monocytes and differentially increases tissue factor in human monocyte subsets. Thromb Haemost. 2017;117(7):1379-90.
63. Demyanets S, Konya V, Kastl SP, Kaun C, Rauscher S, Niessner A, et al. Interleukin-33 induces expression of adhesion molecules and inflammatory activation in human endothelial cells and in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol. 2011;31(9):2080-9.
64. Marzullo A, Ambrosi F, Inchingolo M, Manca F, Devito F, Angiletta D, et al. ST2L Transmembrane Receptor Expression: An Immunochemical Study on Endarterectomy Samples. PLoS One. 2016;11(5):e0156315.
65. Takeda T, Unno H, Morita H, Futamura K, Emi-Sugie M, Arae K, et al. Platelets constitutively express IL-33 protein and modulate eosinophilic airway inflammation. J Allergy Clin Immunol. 2016;138(5):1395-403 e6.
66. Calder PC. Eicosanoids. Essays Biochem. 2020;64(3):423-41.
67. Basil MC, Levy BD. Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. Nat Rev Immunol. 2016;16(1):51-67.
68. Marginean A, Sharma-Walia N. Lipoxins exert antiangiogenic and anti-inflammatory effects on Kaposi's sarcoma cells. Transl Res. 2015;166(2):111-33.
69. Marcon R, Bento AF, Dutra RC, Bicca MA, Leite DF, Calixto JB. Maresin 1, a proresolving lipid mediator derived from omega-3 polyunsaturated fatty acids, exerts protective actions in murine models of colitis. J Immunol. 2013;191(8):4288-98.
70. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest. 2018;128(7):2657-69.
71. Fredman G, Serhan CN. Specialized proresolving mediator targets for RvE1 and RvD1 in peripheral blood and mechanisms of resolution. Biochem J. 2011;437(2):185-97.
72. Kooij G, Troletti CD, Leuti A, Norris PC, Riley I, Albanese M, et al. Specialized pro-resolving lipid mediators are differentially altered in peripheral blood of patients with multiple sclerosis and attenuate monocyte and blood-brain barrier dysfunction. Haematologica. 2020;105(8):2056-70.
73. Regidor PA, De La Rosa X, Santos FG, Rizo JM, Gracia Banzo R, Silva RS. Acute severe SARS COVID-19 patients produce pro-resolving lipids mediators and eicosanoids. Eur Rev Med Pharmacol Sci. 2021;25(21):6782-96.
74. Golia E, Limongelli G, Natale F, Fimiani F, Maddaloni V, Pariggiano I, et al. Inflammation and cardiovascular disease: from pathogenesis to therapeutic target. Curr Atheroscler Rep. 2014;16(9):435.
75. Oudijk EJ, Lammers JW, Koenderman L. Systemic inflammation in chronic obstructive pulmonary disease. Eur Respir J Suppl. 2003;46:5s-13s.
76. Chiurchiu V, Leuti A, Saracini S, Fontana D, Finamore P, Giua R, et al. Resolution of inflammation is altered in chronic heart failure and entails a dysfunctional responsiveness of T lymphocytes. FASEB J. 2019;33(1):909-16.
77. Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B, et al. An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun. 2016;7:12859.
78. Croasdell A, Thatcher TH, Kottmann RM, Colas RA, Dalli J, Serhan CN, et al. Resolvins attenuate inflammation and promote resolution in cigarette smoke-exposed human macrophages. Am J Physiol Lung Cell Mol Physiol. 2015;309(8):L888-901.
79. van der Does AM, Heijink M, Mayboroda OA, Persson LJ, Aanerud M, Bakke P, et al. Dynamic differences in dietary polyunsaturated fatty acid metabolism in sputum of COPD patients and controls. Biochim Biophys Acta Mol Cell Biol Lipids. 2019;1864(3):224-33.

80. Kim KH, Park TS, Kim YS, Lee JS, Oh YM, Lee SD, et al. Resolvin D1 prevents smoking-induced emphysema and promotes lung tissue regeneration. Int J Chron Obstruct Pulmon Dis. 2016;11:1119-28.
81. Norling LV, Perretti M. Control of myeloid cell trafficking in resolution. J Innate Immun. 2013;5(4):367-76.
82. Norling LV, Dalli J, Flower RJ, Serhan CN, Perretti M. Resolvin D1 limits polymorphonuclear leukocyte recruitment to inflammatory loci: receptor-dependent actions. Arterioscler Thromb Vasc Biol. 2012;32(8):1970-8.
83. Schmid M, Gemperle C, Rimann N, Hersberger M. Resolvin D1 Polarizes Primary Human Macrophages toward a Proresolution Phenotype through GPR32. J Immunol. 2016;196(8):3429-37.
84. Cheng T, Ding S, Liu S, Li X, Tang X, Sun L. Resolvin D1 Improves the Treg/Th17 Imbalance in Systemic Lupus Erythematosus Through miR-30e-5p. Front Immunol. 2021;12:668760.
85. Wang MT, Liou JT, Lin CW, Tsai CL, Wang YH, Hsu YJ, et al. Association of Cardiovascular Risk With Inhaled Long-Acting Bronchodilators in Patients With Chronic Obstructive Pulmonary Disease: A Nested Case-Control Study. JAMA Intern Med. 2018;178(2):229-38.
86. Yang M, Chen H, Zhang Y, Du Y, Xu Y, Jiang P, et al. Long-term use of inhaled corticosteroids and risk of upper respiratory tract infection in chronic obstructive pulmonary disease: a meta-analysis. Inhal Toxicol. 2017;29(5):219-26.
87. Liesker JJ, Wijkstra PJ, Ten Hacken NH, Koeter GH, Postma DS, Kerstjens HA. A systematic review of the effects of bronchodilators on exercise capacity in patients with COPD. Chest. 2002;121(2):597-608.
88. Kortianou EA, Nasis IG, Spetsioti ST, Daskalakis AM, Vogiatzis I. Effectiveness of Interval Exercise Training in Patients with COPD. Cardiopulm Phys Ther J. 2010;21(3):12-9.
89. Abroug F, Ouanes I, Abroug S, Dachraoui F, Abdallah SB, Hammouda Z, et al. Systemic corticosteroids in acute exacerbation of COPD: a meta-analysis of controlled studies with emphasis on ICU patients. Ann Intensive Care. 2014;4:32.
90. Kew KM, Seniukovich A. Inhaled steroids and risk of pneumonia for chronic obstructive pulmonary disease. Cochrane Database Syst Rev. 2014(3):CD010115.
91. Almadhoun K, Sharma S. Bronchodilators. StatPearls. Treasure Island (FL)2022.
92. Cazzola M, Matera MG. Bronchodilators: current and future. Clin Chest Med. 2014;35(1):191-201.
93. Falk JA, Minai OA, Mosenifar Z. Inhaled and systemic corticosteroids in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2008;5(4):506-12.
94. Gupta P, O'Mahony MS. Potential adverse effects of bronchodilators in the treatment of airways obstruction in older people: recommendations for prescribing. Drugs Aging. 2008;25(5):415-43.
95. Lee JH, Park YH, Kang DR, Lee SJ, Lee MK, Kim SH, et al. Risk of Pneumonia Associated with Inhaled Corticosteroid in Patients with Chronic Obstructive Pulmonary Disease: A Korean Population-Based Study. Int J Chron Obstruct Pulmon Dis. 2020;15:3397-406.
96. Sin DD. The devastating power of platelets in COPD exacerbations: can aspirin save lives in COPD? Thorax. 2014;69(7):603-4.
97. Duvall MG, Bruggemann TR, Levy BD. Bronchoprotective mechanisms for specialized pro-resolving mediators in the resolution of lung inflammation. Mol Aspects Med. 2017;58:44-56.

98. Mallah H, Ball S, Sekhon J, Parmar K, Nugent K. Platelets in chronic obstructive pulmonary disease: An update on pathophysiology and implications for antiplatelet therapy. Respir Med. 2020;171:106098.
99. Dezsi CA, Szentes V. The Real Role of beta-Blockers in Daily Cardiovascular Therapy. Am J Cardiovasc Drugs. 2017;17(5):361-73.
100. Salpeter SS, Ormiston T, Salpeter E, Poole P, Cates C. Cardioselective beta-blockers for chronic obstructive pulmonary disease. Cochrane Database Syst Rev. 2002(2):CD003566.
101. Gulea C, Zakeri R, Alderman V, Morgan A, Ross J, Quint JK. Beta-blocker therapy in patients with COPD: a systematic literature review and meta-analysis with multiple treatment comparison. Respir Res. 2021;22(1):64.
102. Dransfield MT, Voelker H, Bhatt SP, Brenner K, Casaburi R, Come CE, et al. Metoprolol for the Prevention of Acute Exacerbations of COPD. N Engl J Med. 2019;381(24):2304-14.
103. Criner GJ, Connett JE, Aaron SD, Albert RK, Bailey WC, Casaburi R, et al. Simvastatin for the prevention of exacerbations in moderate-to-severe COPD. N Engl J Med. 2014;370(23):2201-10.
104. Stancu C, Sima A. Statins: mechanism of action and effects. J Cell Mol Med. 2001;5(4):378-87.
105. Piepho RW. Overview of the angiotensin-converting-enzyme inhibitors. Am J Health Syst Pharm. 2000;57 Suppl 1:S3-7.
106. Hill RD, Vaidya PN. Angiotensin II Receptor Blockers (ARB). StatPearls. Treasure Island (FL)2022.
107. Di Marco F, Guazzi M, Vicenzi M, Santus P, Cazzola M, Pappalettera M, et al. Effect of enalapril on exercise cardiopulmonary performance in chronic obstructive pulmonary disease: A pilot study. Pulm Pharmacol Ther. 2010;23(3):159-64.
108. Wise RA, Holbrook JT, Brown RH, Criner GJ, Dransfield MT, He J, et al. Clinical Trial of Losartan for Pulmonary Emphysema: Pulmonary Trials Cooperative LEEP Trial. Am J Respir Crit Care Med. 2022.
109. Chester EH, Schwartz HJ, Fleming GM. Adverse effect of propranolol on airway function in nonasthmatic chronic obstructive lung disease. Chest. 1981;79(5):540-4.
110. Dorow P, Bethge H, Tonnesmann U. Effects of single oral doses of bisoprolol and atenolol on airway function in nonasthmatic chronic obstructive lung disease and angina pectoris. Eur J Clin Pharmacol. 1986;31(2):143-7.
111. van der Woude HJ, Zaagsma J, Postma DS, Winter TH, van Hulst M, Aalbers R. Detrimental effects of beta-blockers in COPD: a concern for nonselective beta-blockers. Chest. 2005;127(3):818-24.
112. Hawkins NM, MacDonald MR, Petrie MC, Chalmers GW, Carter R, Dunn FG, et al. Bisoprolol in patients with heart failure and moderate to severe chronic obstructive pulmonary disease: a randomized controlled trial. Eur J Heart Fail. 2009;11(7):684-90.
113. Mainguy V, Girard D, Maltais F, Saey D, Milot J, Senechal M, et al. Effect of bisoprolol on respiratory function and exercise capacity in chronic obstructive pulmonary disease. Am J Cardiol. 2012;110(2):258-63.
114. Badimon L, Padro T, Vilahur G. Atherosclerosis, platelets and thrombosis in acute ischaemic heart disease. Eur Heart J Acute Cardiovasc Care. 2012;1(1):60-74.
115. Wang L, Tang C. Targeting Platelet in Atherosclerosis Plaque Formation: Current Knowledge and Future Perspectives. Int J Mol Sci. 2020;21(24).
116. Moniruzzaman M, Karim MR, Ahamed F, Chowdhury M, Alam MS, Rouf MA, et al. Platelet Count as a Severity of Chronic Obstructive Pulmonary Disease. Mymensingh Med J. 2020;29(2):241-7.

117. Maclay JD, McAllister DA, Johnston S, Raftis J, McGuinnes C, Deans A, et al. Increased platelet activation in patients with stable and acute exacerbation of COPD. Thorax. 2011;66(9):769-74.
118. Schror K. Clinical pharmacology of the adenosine diphosphate (ADP) receptor antagonist, clopidogrel. Vasc Med. 1998;3(3):247-51.
119. Warner TD, Nylander S, Whatling C. Anti-platelet therapy: cyclo-oxygenase inhibition and the use of aspirin with particular regard to dual anti-platelet therapy. Br J Clin Pharmacol. 2011;72(4):619-33.
120. Karmali KN, Lloyd-Jones DM, Berendsen MA, Goff DC, Jr., Sanghavi DM, Brown NC, et al. Drugs for Primary Prevention of Atherosclerotic Cardiovascular Disease: An Overview of Systematic Reviews. JAMA Cardiol. 2016;1(3):341-9.
121. Lee CH. Role of specialized pro-resolving lipid mediators and their receptors in virus infection: a promising therapeutic strategy for SARS-CoV-2 cytokine storm. Arch Pharm Res. 2021;44(1):84-98.
122. Fawzy A, Putcha N, Aaron CP, Bowler RP, Comellas AP, Cooper CB, et al. Aspirin Use and Respiratory Morbidity in COPD: A Propensity Score-Matched Analysis in Subpopulations and Intermediate Outcome Measures in COPD Study. Chest. 2019;155(3):519-27.
123. Goto T, Faridi MK, Camargo CA, Hasegawa K. The association of aspirin use with severity of acute exacerbation of chronic obstructive pulmonary disease: a retrospective cohort study. NPJ Prim Care Respir Med. 2018;28(1):7.
124. Sakamoto S, Putalun W, Vimolmangkang S, Phoolcharoen W, Shoyama Y, Tanaka H, et al. Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. J Nat Med. 2018;72(1):32-42.
125. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. Ann Intern Med. 2017;167(4):268-74.
126. Kane LT, Fang T, Galetta MS, Goyal DKC, Nicholson KJ, Kepler CK, et al. Propensity Score Matching: A Statistical Method. Clin Spine Surg. 2020;33(3):120-2.
127. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. Nat Rev Immunol. 2016;16(11):676-89.
128. Gautier V, Cayrol C, Farache D, Roga S, Monsarrat B, Burlet-Schiltz O, et al. Extracellular IL-33 cytokine, but not endogenous nuclear IL-33, regulates protein expression in endothelial cells. Sci Rep. 2016;6:34255.
129. Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'? PLoS One. 2008;3(10):e3331.
130. Lu J, Kang J, Zhang C, Zhang X. The role of IL-33/ST2L signals in the immune cells. Immunol Lett. 2015;164(1):11-7.
131. Demyanets S, Kaun C, Pentz R, Krychtiuk KA, Rauscher S, Pfaffenberger S, et al. Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature. J Mol Cell Cardiol. 2013;60:16-26.
132. Mildner M, Storka A, Lichtenauer M, Mlitz V, Ghannadan M, Hoetzenecker K, et al. Primary sources and immunological prerequisites for sST2 secretion in humans. Cardiovasc Res. 2010;87(4):769-77.
133. Bandara G, Beaven MA, Olivera A, Gilfillan AM, Metcalfe DD. Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33. Eur J Immunol. 2015;45(11):3034-44.
134. Pinto SM, Subbannayya Y, Rex DAB, Raju R, Chatterjee O, Advani J, et al. A network map of IL-33 signaling pathway. J Cell Commun Signal. 2018;12(3):615-24.

135. Altara R, Ghali R, Mallat Z, Cataliotti A, Booz GW, Zouein FA. Conflicting vascular and metabolic impact of the IL-33/sST2 axis. Cardiovasc Res. 2018;114(12):1578-94.
136. Qiu C, Li Y, Li M, Li M, Liu X, McSharry C, et al. Anti-interleukin-33 inhibits cigarette smoke-induced lung inflammation in mice. Immunology. 2013;138(1):76-82.
137. Drake LY, Kita H. IL-33: biological properties, functions, and roles in airway disease. Immunol Rev. 2017;278(1):173-84.
138. Tang Y, Guan Y, Liu Y, Sun J, Xu L, Jiang Y. The role of the serum IL-33/sST2 axis and inflammatory cytokines in chronic obstructive pulmonary disease. J Interferon Cytokine Res. 2014;34(3):162-8.
139. Chen WY, Hong J, Gannon J, Kakkar R, Lee RT. Myocardial pressure overload induces systemic inflammation through endothelial cell IL-33. Proc Natl Acad Sci U S A. 2015;112(23):7249-54.
140. Tu X, Nie S, Liao Y, Zhang H, Fan Q, Xu C, et al. The IL-33-ST2L pathway is associated with coronary artery disease in a Chinese Han population. Am J Hum Genet. 2013;93(4):652-60.
141. Angeles-Martinez J, Posadas-Sanchez R, Llorente L, Alvarez-Leon E, Ramirez-Bello J, Villarreal-Molina T, et al. The rs7044343 Polymorphism of the Interleukin 33 Gene Is Associated with Decreased Risk of Developing Premature Coronary Artery Disease and Central Obesity, and Could Be Involved in Regulating the Production of IL-33. PLoS One. 2017;12(1):e0168828.
142. Sanofi. Proof-of-Concept Study to Assess the Efficacy, Safety and Tolerability of SAR440340 (Anti-IL-33 mAb) in Patients With Moderate-to-severe Chronic Obstructive Pulmonary Disease (COPD) NIH ClinicalTrials.gov2018 [cited 2020. Available from: https://clinicaltrials.gov/ct2/show/study/NCT03546907.
143. Leicester Uo. Anti-ST2 (MSTT1041A) in COPD (COPD-ST2OP) (COPD-ST2OP) NIH ClinicalTrials.gov2018 [cited 2020. Available from: https://clinicaltrials.gov/ct2/show/study/NCT03615040.
144. Rabe KF, Celli BR, Wechsler ME, Abdulai RM, Luo X, Boomsma MM, et al. Safety and efficacy of itepekimab in patients with moderate-to-severe COPD: a genetic association study and randomised, double-blind, phase 2a trial. Lancet Respir Med. 2021;9(11):1288-98.
145. Tse G, Ip C, Luk KS, Gong M, Ting YY, Lakhani I, et al. Prognostic value of soluble ST2 postaortic valve replacement: a meta-analysis. Heart Asia. 2018;10(1):e010980.
146. Aimo A, Vergaro G, Ripoli A, Bayes-Genis A, Pascual Figal DA, de Boer RA, et al. Meta-Analysis of Soluble Suppression of Tumorigenicity-2 and Prognosis in Acute Heart Failure. JACC Heart Fail. 2017;5(4):287-96.
147. Aimo A, Vergaro G, Passino C, Ripoli A, Ky B, Miller WL, et al. Prognostic Value of Soluble Suppression of Tumorigenicity-2 in Chronic Heart Failure: A Meta-Analysis. JACC Heart Fail. 2017;5(4):280-6.
148. Liu N, Hang T, Gao X, Yang W, Kong W, Lou Q, et al. The association between soluble suppression of tumorigenicity-2 and long-term prognosis in patients with coronary artery disease: A meta-analysis. PLoS One. 2020;15(9):e0238775.
149. Huang Z, Zhong J, Ling Y, Zhang Y, Lin W, Tang L, et al. Diagnostic value of novel biomarkers for heart failure : A meta-analysis. Herz. 2020;45(1):65-78.
150. Huang DH, Sun H, Shi JP. Diagnostic Value of Soluble Suppression of Tumorigenicity-2 for Heart Failure. Chin Med J (Engl). 2016;129(5):570-7.
151. Ketelaar ME, Nawijn MC, Shaw DE, Koppelman GH, Sayers I. The challenge of measuring IL-33 in serum using commercial ELISA: lessons from asthma. Clin Exp Allergy. 2016;46(6):884-7.

152. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol. 2014;14:135.
153. Sun Y, Pavey H, Wilkinson I, Fisk M. Role of the IL-33/ST2 axis in cardiovascular disease: A systematic review and meta-analysis. PLoS One. 2021;16(11):e0259026.
154. Qian L, Yuanshao L, Wensi H, Yulei Z, Xiaoli C, Brian W, et al. Serum IL-33 Is a Novel Diagnostic and Prognostic Biomarker in Acute Ischemic Stroke. Aging Dis. 2016;7(5):614-22.
155. Li XM, Wang XY, Feng XW, Shao MM, Liu WF, Ma QQ, et al. Serum interleukin-33 as a novel marker for long-term prognosis and recurrence in acute ischemic stroke patients. Brain Behav. 2019;9(9):e01369.
156. Demyanets S, Speidl WS, Tentzeris I, Jarai R, Katsaros KM, Farhan S, et al. Soluble ST2 and interleukin-33 levels in coronary artery disease: relation to disease activity and adverse outcome. PLoS One. 2014;9(4):e95055.
157. Dieplinger B, Bocksrucker C, Egger M, Eggers C, Haltmayer M, Mueller T. Prognostic Value of Inflammatory and Cardiovascular Biomarkers for Prediction of 90-Day All-Cause Mortality after Acute Ischemic Stroke-Results from the Linz Stroke Unit Study. Clin Chem. 2017;63(6):1101-9.
158. Wolcott Z, Batra A, Bevers MB, Sastre C, Khoury J, Sperling M, et al. Soluble ST2 predicts outcome and hemorrhagic transformation after acute stroke. Ann Clin Transl Neurol. 2017;4(8):553-63.
159. Zhang HF, Xie SL, Chen YX, Mai JT, Wang JF, Zhu WL, et al. Altered serum levels of IL-33 in patients with advanced systolic chronic heart failure: correlation with oxidative stress. J Transl Med. 2012;10:120.
160. Luo NS, Zhang HF, Liu PM, Lin YQ, Huang TC, Yang Y, et al. [Diagnostic value of combining serum soluble ST2 and interleukin-33 for heart failure patients with preserved left ventricular ejection fraction]. Zhonghua Xin Xue Guan Bing Za Zhi. 2017;45(3):198-203.
161. Bernardo-Castro S, Sousa JA, Bras A, Cecilia C, Rodrigues B, Almendra L, et al. Pathophysiology of Blood-Brain Barrier Permeability Throughout the Different Stages of Ischemic Stroke and Its Implication on Hemorrhagic Transformation and Recovery. Front Neurol. 2020;11:594672.
162. Dhillon OS, Narayan HK, Quinn PA, Squire IB, Davies JE, Ng LL. Interleukin 33 and ST2 in non-ST-elevation myocardial infarction: comparison with Global Registry of Acute Coronary Events Risk Scoring and NT-proBNP. Am Heart J. 2011;161(6):1163-70.
163. Dhillon OS, Narayan HK, Khan SQ, Kelly D, Quinn PA, Squire IB, et al. Pre-discharge risk stratification in unselected STEMI: is there a role for ST2 or its natural ligand IL-33 when compared with contemporary risk markers? Int J Cardiol. 2013;167(5):2182-8.
164. Ogura Y, Tajiri K, Murakoshi N, Xu D, Yonebayashi S, Li S, et al. Neutrophil Elastase Deficiency Ameliorates Myocardial Injury Post Myocardial Infarction in Mice. Int J Mol Sci. 2021;22(2).
165. Pignatelli P, Menichelli D, Pastori D, Violi F. Oxidative stress and cardiovascular disease: new insights. Kardiol Pol. 2018;76(4):713-22.
166. Tseng CCS, Huibers MMH, van Kuik J, de Weger RA, Vink A, de Jonge N. The Interleukin-33/ST2 Pathway Is Expressed in the Failing Human Heart and Associated with Pro-fibrotic Remodeling of the Myocardium. J Cardiovasc Transl Res. 2018;11(1):15-21.
167. Fairlie-Clarke K, Barbour M, Wilson C, Hridi SU, Allan D, Jiang HR. Expression and Function of IL-33/ST2 Axis in the Central Nervous System Under Normal and Diseased Conditions. Front Immunol. 2018;9:2596.

168. Smith D, Helgason H, Sulem P, Bjornsdottir US, Lim AC, Sveinbjornsson G, et al. A rare IL33 loss-of-function mutation reduces blood eosinophil counts and protects from asthma. PLoS Genet. 2017;13(3):e1006659.
169. Duvall MG, Levy BD. DHA- and EPA-derived resolvins, protectins, and maresins in airway inflammation. Eur J Pharmacol. 2016;785:144-55.
170. Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN. Resolvin D1 receptor stereoselectivity and regulation of inflammation and proresolving microRNAs. Am J Pathol. 2012;180(5):2018-27.
171. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, et al. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. Proc Natl Acad Sci U S A. 2010;107(4):1660-5.
172. Hsiao HM, Thatcher TH, Levy EP, Fulton RA, Owens KM, Phipps RP, et al. Resolvin D1 attenuates polyinosinic-polycytidylic acid-induced inflammatory signaling in human airway epithelial cells via TAK1. J Immunol. 2014;193(10):4980-7.
173. Lu Y, Xu Q, Yin G, Xu W, Jiang H. Resolvin D1 inhibits the proliferation of lipopolysaccharide-treated HepG2 hepatoblastoma and PLC/PRF/5 hepatocellular carcinoma cells by targeting the MAPK pathway. Exp Ther Med. 2018;16(4):3603-10.
174. Leuti A, Maccarrone M, Chiurchiu V. Proresolving Lipid Mediators: Endogenous Modulators of Oxidative Stress. Oxid Med Cell Longev. 2019;2019:8107265.
175. Hsiao HM, Sapinoro RE, Thatcher TH, Croasdell A, Levy EP, Fulton RA, et al. A novel anti-inflammatory and pro-resolving role for resolvin D1 in acute cigarette smoke-induced lung inflammation. PLoS One. 2013;8(3):e58258.
176. Hsiao HM, Thatcher TH, Colas RA, Serhan CN, Phipps RP, Sime PJ. Resolvin D1 Reduces Emphysema and Chronic Inflammation. Am J Pathol. 2015;185(12):3189-201.
177. Fredman G, Ozcan L, Spolitu S, Hellmann J, Spite M, Backs J, et al. Resolvin D1 limits 5-lipoxygenase nuclear localization and leukotriene B4 synthesis by inhibiting a calcium-activated kinase pathway. Proc Natl Acad Sci U S A. 2014;111(40):14530-5.
178. Beeh KM, Kornmann O, Buhl R, Culpitt SV, Giembycz MA, Barnes PJ. Neutrophil chemotactic activity of sputum from patients with COPD: role of interleukin 8 and leukotriene B4. Chest. 2003;123(4):1240-7.
179. Capo X, Martorell M, Busquets-Cortes C, Tejada S, Tur JA, Pons A, et al. Resolvins as proresolving inflammatory mediators in cardiovascular disease. Eur J Med Chem. 2018;153:123-30.
180. Rich HE, Alcorn JF. IL-17 Strikes a Chord in Chronic Obstructive Pulmonary Disease Exacerbation. Am J Respir Cell Mol Biol. 2018;58(6):669-70.
181. Shin J, Hosur KB, Pyaram K, Jotwani R, Liang S, Chavakis T, et al. Expression and function of the homeostatic molecule Del-1 in endothelial cells and the periodontal tissue. Clin Dev Immunol. 2013;2013:617809.
182. Maekawa T, Hosur K, Abe T, Kantarci A, Ziogas A, Wang B, et al. Antagonistic effects of IL-17 and D-resolvins on endothelial Del-1 expression through a GSK-3beta-C/EBPbeta pathway. Nat Commun. 2015;6:8272.
183. Hajishengallis G, Chavakis T. DEL-1-Regulated Immune Plasticity and Inflammatory Disorders. Trends Mol Med. 2019;25(5):444-59.
184. Zou Y, Chen X, Liu J, Zhou DB, Kuang X, Xiao J, et al. Serum IL-1beta and IL-17 levels in patients with COPD: associations with clinical parameters. Int J Chron Obstruct Pulmon Dis. 2017;12:1247-54.
185. Kubysheva N, Boldina M, Eliseeva T, Soodaeva S, Klimanov I, Khaletskaya A, et al. Relationship of Serum Levels of IL-17, IL-18, TNF-alpha, and Lung Function Parameters in Patients with COPD, Asthma-COPD Overlap, and Bronchial Asthma. Mediators Inflamm. 2020;2020:4652898.

186. Mohan D, Gale NS, McEniery CM, Bolton CE, Cockcroft JR, MacNee W, et al. Evaluating the role of inflammation in chronic airways disease: the ERICA study. COPD. 2014;11(5):552-9.
187. McEniery CM, Yasmin, McDonnell B, Munnery M, Wallace SM, Rowe CV, et al. Central pressure: variability and impact of cardiovascular risk factors: the Anglo-Cardiff Collaborative Trial II. Hypertension. 2008;51(6):1476-82.
188. Kourtzelis I, Li X, Mitroulis I, Grosser D, Kajikawa T, Wang B, et al. DEL-1 promotes macrophage efferocytosis and clearance of inflammation. Nat Immunol. 2019;20(1):40-9.
189. Zhang HP, Jia CE, Lv Y, Gibson PG, Wang G. Montelukast for prevention and treatment of asthma exacerbations in adults: Systematic review and meta-analysis. Allergy Asthma Proc. 2014;35(4):278-87.
190. Palmas F, Clarke J, Colas RA, Gomez EA, Keogh A, Boylan M, et al. Dysregulated plasma lipid mediator profiles in critically ill COVID-19 patients. PLoS One. 2021;16(8):e0256226.
191. Levy BD, Bonnans C, Silverman ES, Palmer LJ, Marigowda G, Israel E, et al. Diminished lipoxin biosynthesis in severe asthma. Am J Respir Crit Care Med. 2005;172(7):824-30.
192. Poreba M, Mostowik M, Siniarski A, Golebiowska-Wiatrak R, Malinowski KP, Haberka M, et al. Treatment with high-dose n-3 PUFAs has no effect on platelet function, coagulation, metabolic status or inflammation in patients with atherosclerosis and type 2 diabetes. Cardiovasc Diabetol. 2017;16(1):50.
193. Joo D-H, Lee K-H, Lee C-H, Woo J, Kim J, Park SJ, et al. Developmental endothelial locus-1 as a potential biomarker for the incidence of acute exacerbation in patients with chronic obstructive pulmonary disease. Respiratory Research. 2021;22(1):297.
194. Sandhaus S, Swick AG. Specialized proresolving mediators in infection and lung injury. Biofactors. 2021;47(1):6-18.
195. Andell P, Koul S, Martinsson A, Sundstrom J, Jernberg T, Smith JG, et al. Impact of chronic obstructive pulmonary disease on morbidity and mortality after myocardial infarction. Open Heart. 2014;1(1):e000002.
196. Liu G, Liu Q, Shen Y, Kong D, Gong Y, Tao B, et al. Early treatment with Resolvin E1 facilitates myocardial recovery from ischaemia in mice. Br J Pharmacol. 2018;175(8):1205-16.
197. Ho KJ, Spite M, Owens CD, Lancero H, Kroemer AH, Pande R, et al. Aspirin-triggered lipoxin and resolvin E1 modulate vascular smooth muscle phenotype and correlate with peripheral atherosclerosis. Am J Pathol. 2010;177(4):2116-23.
198. Aaron CP, Schwartz JE, Hoffman EA, Angelini E, Austin JHM, Cushman M, et al. A Longitudinal Cohort Study of Aspirin Use and Progression of Emphysema-like Lung Characteristics on CT Imaging: The MESA Lung Study. Chest. 2018;154(1):41-50.
199. Harrison MT, Short P, Williamson PA, Singanayagam A, Chalmers JD, Schembri S. Thrombocytosis is associated with increased short and long term mortality after exacerbation of chronic obstructive pulmonary disease: a role for antiplatelet therapy? Thorax. 2014;69(7):609-15.
200. Vestbo J, Anderson JA, Brook RD, Calverley PM, Celli BR, Crim C, et al. Fluticasone furoate and vilanterol and survival in chronic obstructive pulmonary disease with heightened cardiovascular risk (SUMMIT): a double-blind randomised controlled trial. Lancet. 2016;387(10030):1817-26.
201. Lipson DA, Barnhart F, Brealey N, Brooks J, Criner GJ, Day NC, et al. Once-Daily Single-Inhaler Triple versus Dual Therapy in Patients with COPD. N Engl J Med. 2018;378(18):1671-80.

202. Noordzij M, van Diepen M, Caskey FC, Jager KJ. Relative risk versus absolute risk: one cannot be interpreted without the other. Nephrol Dial Transplant. 2017;32(suppl_2):ii13-ii8.
203. Couper D, LaVange LM, Han M, Barr RG, Bleecker E, Hoffman EA, et al. Design of the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS). Thorax. 2014;69(5):491-4.
204. Bakshi A, Suissa S. Effectiveness of Aspirin in COPD: Biases in the Observational Studies. COPD. 2021;18(4):449-55.
205. Eilat-Tsanani S, Mor E, Schonmann Y. Statin Use Over 65 Years of Age and All-Cause Mortality: A 10-Year Follow-Up of 19 518 People. J Am Geriatr Soc. 2019;67(10):2038-44.

Appendices

Appendix A

SUMMIT CODE

```
install.packages(c("survival","survminer","ranger","ggplot2","ggfortify","tidyr","dplyr","janitor","lubridate","mass","magrittr","ggplot","tidyverse"))
install.packages(c("MASS","magrittr"))


concomitantmeds <- gsk_113782_adcm
cvcompevents <- gsk_113782_adcv
acm <- gsk_113782_addth
exac<-gsk_113782_adexac
sla<-gsk_113782_adsl
cvcrit<-gsk_113782_adcvcrit
cvmeds<-gsk_113782_adcmcv
adverse<-gsk_113782_adae
glucose<-gsk_113782_adlb
fev<-gsk_113782_adpft
cvhist<-gsk_113782_adcvhist
smoking<-gsk_113782_adsu
t2e<-gsk_113782_adtte


#Selecting data
library("survival","survminer")
library("tidyverse","dplyr")
library("tibble")
install.packages(c("reshape2"))
library(reshape2)
library("MatchIt")
library("Hmisc")
library("nnet")
```

```
library("tableone")
library("DMwR")
library("cobalt")
library("weights")
library("Zelig")
library("rbounds")
library("randomForest")
library(arsenal)
library("broom")
library("gtsummary")
library("MASS")
library("magrittr")
library("MatchThem")
library("mice")



cvcrit<-dplyr::select(gsk_113782_adcvcrit,USUBJID,AVALC,PARAM)
cvcrit[1,]
cvcrit$USUBJID<-as.factor(cvcrit$USUBJID)
cvcrit<-reshape2::dcast(cvcrit,USUBJID~PARAM,value.var="AVALC")



cvhist<-dplyr::select(gsk_113782_adcvhist,USUBJID,AVALC,PARAM)
cvhist[1,]
colnames(cvhist)[colnames(cvhist)=="PARAM"]<-"EVENT_HIST"
cvhist%<>%filter(EVENT_HIST=="Ever Diagnosed with Congestive Heart Failure")
cvhist$EVENT_HIST=droplevels(cvhist$EVENT_HIST)
cvhist$AVALC[cvhist$AVALC==""]=NA
cvhist$AVALC[cvhist$AVALC=="U"]=NA
cvhist$AVALC=droplevels(cvhist$AVALC)
colnames(cvhist)[colnames(cvhist)=="AVALC"]<-"HF"
cvhist$EVENT_HIST<-NULL
```

```
concomitantmeds<-
dplyr::select(gsk_113782_adcm,USUBJID,ONTRTFL,DCL2T,CMBASE)

concomitantmeds[1,]

colnames(concomitantmeds)[colnames(concomitantmeds)=="ONTRTFL"]<-
"USED_DURING_TRIAL"

table(c(concomitantmeds$DCL2T))

diabetesmeds<-concomitantmeds%>%filter(DCL2T=="DRUGS USED IN DIABETES")

diabetesmeds[1,]

library(magrittr)

diabetesmeds%<>%distinct(USUBJID,CMBASE,.keep_all=TRUE)


diabetesmeds%<>%mutate(CLASS=case_when(CMBASE=="ACARBOSE"~"GLUCOSID
ASE_INHIB",
                       CMBASE=="MIGLITOL"~"GLUCOSIDASE_INHIB",
                       CMBASE=="VOGLIBOSE"~"GLUCOSIDASE_INHIB",
                       CMBASE=="INSULIN ASPART"~"INSULINS",
                       CMBASE=="INSULIN ASPART PROTAMINE"~"INSULINS",
                       CMBASE=="INSULIN DETEMIR"~"INSULINS",
                       CMBASE=="INSULIN GLARGINE"~"INSULINS",
                       CMBASE=="INSULIN GLULISINE"~"INSULINS",
                       CMBASE=="INSULIN HUMAN"~"INSULINS",
                       CMBASE=="INSULIN HUMAN
SEMISYNTHETIC"~"INSULINS",
                       CMBASE=="INSULIN INJECTION, BIPHASIC
ISOPHANE"~"INSULINS",
                       CMBASE=="INSULIN ISOPHANE, HUMAN
BIOSYNTHETIC"~"INSULINS",
                       CMBASE=="INSULIN LISPRO"~"INSULINS",
                       CMBASE=="INSULIN LISPRO PROTAMINE"~"INSULINS",
                       CMBASE=="INSULIN NOS"~"INSULINS",
                       CMBASE=="INSULIN PORCINE"~"INSULINS",
                       CMBASE=="INSULIN, HUMAN BIOSYNTHETIC"~"INSULINS",
```

```
                    CMBASE=="ISOPHANE INSULIN"~"INSULINS",
                    CMBASE=="BUFORMIN"~"BIGUANIDES",
                    CMBASE=="METFORMIN"~"BIGUANIDES",
                    CMBASE=="PHENFORMIN"~"BIGUANIDES",
                    CMBASE=="GLUCOMET (NOS)"~"BIGUANIDES",
                    CMBASE=="GLUCONORM
(NOS)"~"SULFONYLUREAS_BIGUANIDES",
                    CMBASE=="GLUCORED
NOS"~"SULFONYLUREAS_BIGUANIDES",
                    CMBASE=="ZOMARIST (NOS)"~"DPP4_BIGUANIDES",

CMBASE=="GLUCONORM"~"SULFONYLUREAS_BIGUANIDES",
                    CMBASE=="ALOGLIPTIN"~"DPP4",
                    CMBASE=="GALVUS (NOS)"~"DPP4",
                    CMBASE=="GEMIGLIPTIN"~"DPP4",
                    CMBASE=="LINAGLIPTIN"~"DPP4",
                    CMBASE=="SAXAGLIPTIN"~"DPP4",
                    CMBASE=="SITAGLIPTIN"~"DPP4",
                    CMBASE=="VILDAGLIPTIN"~"DPP4",
                    CMBASE=="EXENATIDE"~"GLPR_AGONIST",
                    CMBASE=="LIRAGLUTIDE"~"GLPR_AGONIST",
                    CMBASE=="LIXISENATIDE"~"GLPR_AGONIST",
                    CMBASE=="CHLORPROPAMIDE"~"SULFONYLUREAS",
                    CMBASE=="DIABETA (NOS)"~"SULFONYLUREAS",
                    CMBASE=="GLIBENCLAMIDE"~"SULFONYLUREAS",
                    CMBASE=="GLIBETIC (NOS)"~"SULFONYLUREAS",
                    CMBASE=="GLICLAZIDE"~"SULFONYLUREAS",
                    CMBASE=="GLIM (NOS)"~"SULFONYLUREAS",
                    CMBASE=="GLIMEL (NOS)"~"SULFONYLUREAS",
                    CMBASE=="GLIMEPIRIDE"~"SULFONYLUREAS",
                    CMBASE=="GLIPID NOS"~"SULFONYLUREAS",
                    CMBASE=="GLIPIZIDE"~"SULFONYLUREAS",
                    CMBASE=="GLIQUIDONE"~"SULFONYLUREAS",
```

```
                        CMBASE=="GLYCRON (NOS)"~"SULFONYLUREAS",
                        CMBASE=="TOLBUTAMIDE"~"SULFONYLUREAS",

CMBASE=="CANAGLIFLOZIN"~"SODIUM_TRANSPORT_INHIB",

CMBASE=="DAPAGLIFLOZIN"~"SODIUM_TRANSPORT_INHIB",
                        CMBASE=="MITIGLINIDE"~"MEGLITINIDES",
                        CMBASE=="NATEGLINIDE"~"MEGLITINIDES",
                        CMBASE=="REPAGLINIDE"~"MEGLITINIDES",
                        CMBASE=="LOBEGLITAZONE"~"THIAZOLIDINEDIONE",
                        CMBASE=="PIOGLITAZONE"~"THIAZOLIDINEDIONE",
                        CMBASE=="ROSIGLITAZONE"~"THIAZOLIDINEDIONE",
                        CMBASE=="THIAZOLIDINEDIONE
(NOS)"~"THIAZOLIDINEDIONE",
                        CMBASE=="CINNAMOMUM VERUM  EXTRACT"~"OTHER",
                        CMBASE=="COLESEVELAM"~"OTHER",
                        CMBASE=="D.B.I. (NOS)"~"OTHER",
                        CMBASE=="DIABETOL (NOS)"~"OTHER",
                        CMBASE=="EPALRESTAT"~"OTHER",
                        CMBASE=="ORAL HYPOGLYCEMICS NOS"~"OTHER",
                        CMBASE=="PRAMLINTIDE"~"OTHER",
                        CMBASE=="THIOCTIC
ACID"~"OTHER",TRUE~NA_character_))


diabetesmeds[1,]
diabetesmeds$USED_DURING_TRIAL<-diabetesmeds$DCL2T<-
diabetesmeds$CMBASE<-NULL
diabetesmeds%<>%distinct(USUBJID,CLASS,.keep_all=TRUE)
diabetesmeds$VALUE<-1
diabetesmeds<-reshape2::dcast(diabetesmeds,USUBJID~CLASS,value.var="VALUE")
diabetesmeds[is.na(diabetesmeds)]<-0



cvmeds<-dplyr::select(gsk_113782_adcmcv,USUBJID,CVGROUP1)
```

```
cvmeds$CVMEDS<-1

cvmeds$USUBJID<-as.factor(cvmeds$USUBJID)
cvmeds[1,]
cvmeds%<>%distinct(USUBJID,CVGROUP1,.keep_all=TRUE)
cvmeds$CVGROUP1[cvmeds$CVGROUP1==""]=NA

cvmeds<-reshape2::dcast(cvmeds,USUBJID~CVGROUP1,value.var="CVMEDS")
cvmeds[is.na(cvmeds)]<-0

medstable<-merge(cvmeds,diabetesmeds,by="USUBJID",all.x=TRUE,all.y=TRUE)

cvcompevents<-dplyr::select(gsk_113782_adcv,USUBJID,ADY,AVALC,ADT,LSTCT)
cvcompevents[1,]
colnames(cvcompevents)[colnames(cvcompevents)=="AVALC"]<-"EVENT_TYPE"
cvcompevents%<>%filter(EVENT_TYPE=="Sudden Death"|EVENT_TYPE=="Myocardial Infarction"|EVENT_TYPE=="Stroke"|EVENT_TYPE=="Unstable Angina"|EVENT_TYPE=="Procedural Death (related to cardiac surgery)"|EVENT_TYPE=="Other CV Death"|
                       EVENT_TYPE=="Transient Ischemic Attack"|EVENT_TYPE=="Coronary Revascularization")

t2e<-dplyr::select(gsk_113782_adtte,USUBJID,AVAL,CNSR,PARAM)
t2e[1,]
t2e%<>%filter(PARAM=="Time to First On-treatment Moderate/Severe COPD Exacerbation")
colnames(t2e)[colnames(t2e)=="AVAL"]<-"TIME_EXAC_TOTAL"
colnames(t2e)[colnames(t2e)=="CNSR"]<-"CNSR_EXAC_TOTAL"
t2e$PARAM<-NULL
t2e%<>%distinct(USUBJID,TIME_EXAC_TOTAL,.keep_all=TRUE)


t2em<-dplyr::select(gsk_113782_adtte,USUBJID,AVAL,CNSR,PARAM)
t2em[1,]
```

```
t2em%<>%filter(PARAM=="Time to First On-treatment COPD Exacerbation Requiring Oral Corticosteroids")

colnames(t2em)[colnames(t2em)=="AVAL"]<-"TIME_EXAC_MOD"

colnames(t2em)[colnames(t2em)=="CNSR"]<-"CNSR_EXAC_MOD"

t2em$PARAM<-NULL

t2em%<>%distinct(USUBJID,TIME_EXAC_MOD,.keep_all=TRUE)



t2es<-dplyr::select(gsk_113782_adtte,USUBJID,AVAL,CNSR,PARAM)

t2es[1,]

t2es%<>%filter(PARAM=="Time to First On-treatment COPD Exacerbation Requiring Hospitalisation")

colnames(t2es)[colnames(t2es)=="AVAL"]<-"TIME_EXAC_SEV"

colnames(t2es)[colnames(t2es)=="CNSR"]<-"CNSR_EXAC_SEV"

t2es$PARAM<-NULL

t2es%<>%distinct(USUBJID,TIME_EXAC_SEV,.keep_all=TRUE)



test<-dplyr::select(gsk_113782_adtte,USUBJID,AVAL,CNSR,PARAM)

test[1,]

test%<>%filter(PARAM=="Time to First On-treatment Cardiovascular Composite Event")

colnames(test)[colnames(test)=="AVAL"]<-"TIME_CVCOMP"

colnames(test)[colnames(test)=="CNSR"]<-"CNSR_CVCOMP"

test$PARAM<-NULL

test%<>%distinct(USUBJID,TIME_CVCOMP,.keep_all=TRUE)



exac<-
dplyr::select(gsk_113782_adexac,USUBJID,ACESEV,ADURN,ASTDT,ASTDY,APHASE)

exac[1,]

colnames(exac)[colnames(exac)=="ACESEV"]<-"SEVERITY"

exac%<>%filter(APHASE=="On-treatment")

exac%<>%distinct(USUBJID,ASTDY,.keep_all=TRUE)
```

```
exac$VALUE<-1

exac$ADURN<-exac$ASTDT<-exac$ASTDY<-exac$APHASE<-NULL

exac<-reshape2::dcast(exac,USUBJID~SEVERITY,value.var="VALUE",fun.aggregate=sum)


exac%<>%mutate(TOTAL_EXAC=MODERATE+SEVERE)


smoking<-dplyr::select(gsk_113782_adsu,USUBJID,AVAL,PARAM)

smoking%<>%filter(PARAM=="Smoking Pack Years")

smoking%<>%distinct(USUBJID,PARAM,.keep_all=TRUE)

colnames(smoking)[colnames(smoking)=="AVAL"]<-"PACK_YRS"

smoking$PARAM<-NULL



cvmedsbreak<-dplyr::select(gsk_113782_adcmcv,USUBJID,ADECOD)

cvmedsbreak%<>%filter(ADECOD=="BISOPROLOL
FUMARATE"|ADECOD=="BISOPROLOL"|ADECOD=="AMLODIPINE BESILATE +
BISOPROLOL FUMARATE"|

        ADECOD=="BISOPROLOL FUMARATE +
HYDROCHLOROTHIAZIDE"|ADECOD=="BISOPROLOL +
HYDROCHLOROTHIAZIDE"|

        ADECOD=="NEBIVOLOL
HYDROCHLORIDE"|ADECOD=="NEBIVOLOL"|ADECOD=="HYDROCHLOROTHIA
ZIDE + NEBIVOLOL HYDROCHLORIDE"|

        ADECOD=="NEBIVILOL"|ADECOD=="AMLODIPINE +
NEBIVOLOL"|ADECOD=="HYDROCHLOROTHIAZIDE +
NEBIVOLOL"|ADECOD=="ATENOLOL"|

        ADECOD=="AMLODIPINE BESILATE +
ATENOLOL"|ADECOD=="AMLODIPINE + ATENOLOL"|ADECOD=="ATENOLOL +
HYDROCHLOROTHIAZIDE"|ADECOD=="ATENOLOL +
CHLORTALIDONE"|ADECOD=="ATENOLOL + NIFEDIPINE"|

        ADECOD=="ATENOLOL + CHLORTALIDONE +
NIFEDIPINE"|ADECOD=="ACETYLSALICYLIC ACID + ATORVASTATIN +
CLOPIDOGREL"|

         ADECOD=="ACETYLSALICYLIC ACID + ATORVASTATIN +
CLOPIDOGREL BISULFATE"|ADECOD=="ACETYLSALICYLIC ACID +
CLOPIDOGREL"|ADECOD=="ACETYLSALICYLIC ACID + CLOPIDOGREL
BISULFATE"|ADECOD=="ATORVASTATIN CALCIUM + CLOPIDOGREL
BISULFATE"|
```

```
            ADECOD=="CLOPIDOGREL"|ADECOD=="CLOPIDOGREL
BESYLATE"|ADECOD=="CLOPIDOGREL  BISULFATE"|ADECOD=="CLOPIDOGREL
RESINATE"|ADECOD=="CLOPIDOGREL NAPADISILATE")

cvmedsbreak%<>%distinct(USUBJID,ADECOD,.keep_all=TRUE)


cvmedsbreak%<>%mutate(CLASS=case_when(ADECOD=="BISOPROLOL
FUMARATE"~"BISOPROLOL",

                ADECOD=="BISOPROLOL"~"BISOPROLOL",

                ADECOD=="AMLODIPINE BESILATE + BISOPROLOL
FUMARATE"~"BISOPROLOL",

                ADECOD=="BISOPROLOL FUMARATE +
HYDROCHLOROTHIAZIDE"~"BISOPROLOL",

                ADECOD=="BISOPROLOL +
HYDROCHLOROTHIAZIDE"~"BISOPROLOL",

                ADECOD=="NEBIVOLOL HYDROCHLORIDE"~"NEBIVOLOL",

                ADECOD=="NEBIVOLOL"~"NEBIVOLOL",

                ADECOD=="HYDROCHLOROTHIAZIDE + NEBIVOLOL
HYDROCHLORIDE"~"NEBIVOLOL",

                ADECOD=="NEBIVILOL"~"NEBIVOLOL",

                ADECOD=="AMLODIPINE + NEBIVOLOL"~"NEBIVOLOL",

                ADECOD=="HYDROCHLOROTHIAZIDE +
NEBIVOLOL"~"NEBIVOLOL",

                ADECOD=="ATENOLOL"~"ATENOLOL",

                ADECOD=="AMLODIPINE BESILATE +
ATENOLOL"~"ATENOLOL",

                ADECOD=="AMLODIPINE + ATENOLOL"~"ATENOLOL",

                ADECOD=="ATENOLOL +
HYDROCHLOROTHIAZIDE"~"ATENOLOL",

                ADECOD=="ATENOLOL + CHLORTALIDONE"~"ATENOLOL",

                ADECOD=="ATENOLOL + NIFEDIPINE"~"ATENOLOL",

                ADECOD=="ATENOLOL + CHLORTALIDONE +
NIFEDIPINE"~"ATENOLOL",

                ADECOD=="ACETYLSALICYLIC ACID + ATORVASTATIN +
CLOPIDOGREL"~"ASP_STAT_CLOPIDOGREL",

                ADECOD=="ACETYLSALICYLIC ACID + ATORVASTATIN +
CLOPIDOGREL BISULFATE"~"ASP_STAT_CLOPIDOGREL",
```

```
                ADECOD=="ACETYLSALICYLIC ACID + CLOPIDOGREL"~"ASP_CLOPIDOGREL",
                ADECOD=="ACETYLSALICYLIC ACID + CLOPIDOGREL BISULFATE"~"ASP_CLOPIDOGREL",
                ADECOD=="ATORVASTATIN CALCIUM + CLOPIDOGREL BISULFATE"~"STAT_CLOPIDOGREL",
                ADECOD=="CLOPIDOGREL"~"CLOPIDOGREL",
                ADECOD=="CLOPIDOGREL BESYLATE"~"CLOPIDOGREL",
                ADECOD=="CLOPIDOGREL BISULFATE"~"CLOPIDOGREL",
                ADECOD=="CLOPIDOGREL RESINATE"~"CLOPIDOGREL",
                ADECOD=="CLOPIDOGREL NAPADISILATE"~"CLOPIDOGREL",TRUE~NA_character_))

cvmedsbreak$ADECOD<-NULL
cvmedsbreak$VALUE<-1
cvmedsbreak%<>%distinct(USUBJID,CLASS,.keep_all=TRUE)
cvmedsbreak<-reshape2::dcast(cvmedsbreak,USUBJID~CLASS,value.var="VALUE")

medstable<-merge(medstable,cvmedsbreak,by="USUBJID",all.x=TRUE,all.y=TRUE)



cvmedsbreak1<-dplyr::select(gsk_113782_adcmcv,USUBJID,ADECOD,CVGROUP1)
cvmedsbreak1%<>%filter(CVGROUP1=="Anti-platelet therapy")
cvmedsbreak1$CVGROUP1<-NULL
cvmedsbreak1%<>%distinct(USUBJID,ADECOD,.keep_all=TRUE)
cvmedsbreak1%<>%mutate(ANTIPLATE2=case_when(ADECOD=="ACETYLSALICYLATE LYSINE"~"ASPIRIN",
                        ADECOD=="ACETYLSALICYLIC ACID"~"ASPIRIN",
                        ADECOD=="ACETYLSALICYLIC ACID + ALUMINIUM GLYCINATE + MAGNESIUM CARBONATE"~"ASPIRIN",
                        ADECOD=="ACETYLSALICYLIC ACID + AMMONIUM CHLORIDE + ASCORBIC ACID + BAROSMA CRENATA + CAMPHOR + CINCHONA PUBESCENS + PERU BALSAM"~"ASPIRIN",
                        ADECOD=="ACETYLSALICYLIC ACID + AMMONIUM CHLORIDE + GLYCYRRHIZA (NOS) + LEVOMENTHOL"~"ASPIRIN",
```

ADECOD=="ACETYLSALICYLIC ACID + ASCORBIC ACID"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ASCORBIC ACID + CALCIUM GLUCONATE + DIPHENHYDRAMINE + METAMIZOLE SODIUM + RUTOSIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ASCORBIC ACID + CALCIUM LACTOGLUCONATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ASCORBIC ACID + CHLORPHENAMINE MALEATE + MOROXYDINE HYDROCHLORIDE + PHENYLEPHRINE HYDROCHLORIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ASCORBIC ACID + PARACETAMOL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ASCORBIC ACID + RUTOSIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ATORVASTATIN"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ATORVASTATIN + RAMIPRIL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ATORVASTATIN CALCIUM"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + BISOPROLOL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + BISOPROLOL FUMARATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + BUTALBITAL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + BUTALBITAL + CAFFEINE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + CITRIC ACID + PARACETAMOL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + CODEINE + PARACETAMOL + PHENOBARBITAL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + CODEINE + PHENACETIN + PHENOBARBITAL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + CODEINE PHOSPHATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + ETHENZAMIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + ORPHENADRINE CITRATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + PARACETAMOL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + PHENACETIN"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + QUININE SULPHATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE + SALICYLAMIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CAFFEINE CITRATE + FILIPENDULA ULMARIA"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CALCIUM"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CHLORPHENAMINE + DEXTROMETHORPHAN"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CHLORPHENAMINE MALEATE + PHENYLPROPANOLAMINE BITARTRATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CHLORPHENAMINE + DEXTROMETHORPHAN + PHENYLEPHRINE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CHLORPHENAMINE MALEATE + PHENYLPROPANOLAMINE BITARTRATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CITRIC ACID + SODIUM BICARBONATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CODEINE PHOSPHATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CODEINE PHOSPHATE + PARACETAMOL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + CODEINE PHOSPHATE + DIPHENHYDRAMINE HYDROCHLORIDE + MEPROBAMATE + NICOTINIC ACID"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + DEXTROMETHORPHAN HYDROBROMIDE + PHENYLEPHRINE HYDROCHLORIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + DIPHENHYDRAMINE CITRATE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + MAGNESIUM HYDROXIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + GLYCINE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ESOMEPRAZOLE MAGNESIUM"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + METHOCARBAMOL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + PARACETAMOL"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + MAGNESIUM OXIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + ROSUVASTATIN"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + PRAVASTATIN"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + PSEUDOEPHEDRINE HYDROCHLORIDE"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + RAMIPRIL + SIMVASTATIN"~"ASPIRIN",

ADECOD=="ACETYLSALICYLIC ACID + PHENYLPROPANOLAMINE BITARTRATE"~"ASPIRIN",

ADECOD=="ABCIXIMAB"~"ABCIXIMAB",

ADECOD=="ACETYLSALICYLIC ACID + DIPYRIDAMOLE"~"ASPDIP",

ADECOD=="CARBASALATE CALCIUM"~"CARB",

ADECOD=="CAFFEINE + CARBASALATE CALCIUM + PARACETAMOL"~"CARB",

ADECOD=="DIPYRIDAMOLE"~"DIP",

ADECOD=="ETHYL ICOSAPENTATE"~"ETHICO",

ADECOD=="DIPYRIDAMOLE + GINKGO BILOBA EXTRACT"~"DIP",

ADECOD=="ILOPROST"~"ILO",

ADECOD=="ICOSAPENT"~"ICO",

ADECOD=="MESOGLYCAN SODIUM"~"MESOGLYCAN",

ADECOD=="OZAGREL SODIUM"~"OZAGREL",

ADECOD=="OZAGREL"~"OZAGREL",

ADECOD=="SARPOGRELATE HYDROCHLORIDE"~"SARPO",

ADECOD=="PRASUGREL"~"PRAS",

ADECOD=="SARPOGRELATE"~"SARPO",

```
                    ADECOD=="PLATELET AGGREGATION INHIBITOR (NOS)"~"PAI",
                    ADECOD=="TICAGRELOR"~"TICA",
                    ADECOD=="TICLOPIDINE HYDROCHLORIDE"~"TICLO",
                    ADECOD=="TICLOPIDINE"~"TICLO",
                    ADECOD=="TRIFLUSAL"~"TRIF",
                    ADECOD=="TIROFIBAN HYDROCHLORIDE"~"TIRO",
                    ADECOD=="TREPROSTINIL"~"TREPR",TRUE~NA_character_))

cvmedsbreak1$ADECOD<-NULL
cvmedsbreak1$VALUE<-1
cvmedsbreak1%<>%distinct(USUBJID,ANTIPLATE2,.keep_all=TRUE)
cvmedsbreak1<-
reshape2::dcast(cvmedsbreak1,USUBJID~ANTIPLATE2,value.var="VALUE")
medstable<-merge(medstable,cvmedsbreak1,by="USUBJID",all.x=TRUE,all.y=TRUE)

fev<-dplyr::select(gsk_113782_adpft,USUBJID,AVISIT,PARAM,ATPT,AVAL)
fev%<>%filter(PARAM=="% Predicted FEV1 (recalculated)")
fev%<>%filter(ATPT=="Post-Bronchodilator")
fev%<>%filter(AVISIT=="Baseline (Visit 2)")
fev$AVISIT<-fev$ATPT<-fev$PARAM<-NULL
colnames(fev)[colnames(fev)=="AVAL"]<-"FEV1"


acm<-dplyr::select(gsk_113782_addth,USUBJID,ADY)
acm[1,]
acm%<>%distinct(USUBJID,ADY,.keep_all=TRUE)


acm2<-dplyr::select(gsk_113782_addth,USUBJID,AVALCAT1,PARAM,ADY)
acm2[1,]
acm2%<>%distinct(USUBJID,AVALCAT1,.keep_all=TRUE)
```

```
colnames(acm2)[colnames(acm2)=="AVALCAT1"]<-"MORT_CAUSE"

acm2%<>%filter(MORT_CAUSE=="Pulmonary"|MORT_CAUSE=="Cardiovascular"|MORT_CAUSE=="Cancer"|MORT_CAUSE=="Other Cause of Death")

acm2%<>%filter(PARAM=="Primary Death Class Category")

acm2%<>%distinct(USUBJID,ADY,.keep_all = TRUE)

acm2$PARAM<-NULL


sla<-dplyr::select(gsk_113782_adsl,USUBJID,AGE,SEX,TRTPN,CVBL1,BMIBL,REGION,COUNTRY,AGEGR3,RACE,EXDUR,LSTCT,ITTFL,RANDDT,STUDEDT,PREVEXCT,SMKBLN,IHDIN)

sla[1,]

colnames(sla)[colnames(sla)=="STUDEDT"]<-"COMMON_END_DATE"

colnames(sla)[colnames(sla)=="RANDDT"]<-"RANDOMISATION_DATE"

colnames(sla)[colnames(sla)=="EXDUR"]<-"TREATMENT_YEARS"

colnames(sla)[colnames(sla)=="CVBL1"]<-"CV_CRITERIA"

colnames(sla)[colnames(sla)=="BMIBL"]<-"BMI"

colnames(sla)[colnames(sla)=="ITTFL"]<-"INTENTION_TO_TREAT"


library(lubridate)

sla%>%mutate(RANDOMISATION_DATE=ymd(RANDOMISATION_DATE),COMMON_END_DATE=ymd(COMMON_END_DATE))

sla$RANDOMISATION_DATE<-as.Date(sla$RANDOMISATION_DATE)

sla$COMMON_END_DATE<-as.Date(sla$COMMON_END_DATE)

sla%<>%mutate(os_yrs=as.duration(RANDOMISATION_DATE%--%COMMON_END_DATE)/dyears(1))



sla$PREVEXCT[sla$PREVEXCT==""]=NA

sla$PREVEXCT=droplevels(sla$PREVEXCT)



#All cause mortality analysis
```

```
library(survival)

library(survminer)

a<-merge(sla,medstable,by="USUBJID",all.x=TRUE,all.y=TRUE)

b<-merge(a,cvcrit,by="USUBJID",all.x=TRUE,all.y=TRUE)

c<-merge(b,acm,by="USUBJID",all.x=TRUE,all.y=TRUE)

c%<>%mutate(STATUS=case_when(LSTCT=="Alive"~0,LSTCT=="Dead"~1,TRUE~NA_
real_))

c$STATUS<-as.numeric(c$STATUS)

x<-merge(c,glucose,by="USUBJID",all.x=TRUE,all.y=TRUE)

x1<-merge(x,fev,by="USUBJID",all.x=TRUE,all.y=TRUE)

x2<-merge(x1,cvhist,by="USUBJID",all.x=TRUE,all.y=TRUE)

x3<-merge(x2,smoking,by="USUBJID",all.x=TRUE,all.y=TRUE)



ACM<-x3

colnames(ACM)[colnames(ACM)=="Alpha and beta blocking"]<-"ALPHABETABLOCK"

colnames(ACM)[colnames(ACM)=="Angiotensin-converting Enzyme Inhibitors"]<-"ACEI"

colnames(ACM)[colnames(ACM)=="Angiotensin receptor blockers"]<-"ARB"

colnames(ACM)[colnames(ACM)=="Anti-coagulant therapy"]<-"ANTI_COAG"

colnames(ACM)[colnames(ACM)=="Anti-platelet therapy"]<-"ANTI_PLATE"

colnames(ACM)[colnames(ACM)=="Cholesterol and bile acid absorption inhibitors"]<-
"CHOL_BILE_ABSORB_INHIB"

colnames(ACM)[colnames(ACM)=="Class III"]<-"CLASS3"

colnames(ACM)[colnames(ACM)=="Dihydropyridine"]<-"DIHYDROPYRIDINE"

colnames(ACM)[colnames(ACM)=="Direct Renin Inhibitors"]<-"DIR_RENIN_INHIB"

colnames(ACM)[colnames(ACM)=="Long-acting"]<-"LONG_NITRATES"

colnames(ACM)[colnames(ACM)=="Short-acting"]<-"SHORT_NITRATES"

colnames(ACM)[colnames(ACM)=="Mineralocorticoid Receptor Antagonists"]<-"MRA"

colnames(ACM)[colnames(ACM)=="Non-dihydropyridine"]<-
"NON_DIHYDROPYRIDINE"

colnames(ACM)[colnames(ACM)=="Non-selective beta-adrenergic receptor blocker"]<-
"NON_SELEC_B_BLOCK"

colnames(ACM)[colnames(ACM)=="Other lipid modifying"]<-"SUPPLEMENTS"
```

```
colnames(ACM)[colnames(ACM)=="Selective beta1-adrenergic receptor blocker"]<-"SELEC_B_BLOCK"

colnames(ACM)[colnames(ACM)=="Statins"]<-"STATINS"

colnames(ACM)[colnames(ACM)=="Thiazides and Thiazide like Diuretics"]<-"THIAZIDES_DIUR"

colnames(ACM)[colnames(ACM)=="Being treated for diabetes mellitus"]<-"DIABETES"

colnames(ACM)[colnames(ACM)=="Being treated for hypercholesterolemia"]<-"HYPERCHOLESTEROL"

colnames(ACM)[colnames(ACM)=="Being treated for hypertension"]<-"HYPERTENSION"

colnames(ACM)[colnames(ACM)=="Diabetes mellitus with target organ disease"]<-"DIABETES_ORGAN_DISEASE"

colnames(ACM)[colnames(ACM)=="Diabetes mellitus with target organ disease: eyes"]<-"DIABETES_EYES"

colnames(ACM)[colnames(ACM)=="Being treated for peripheral arterial disease"]<-"TREATED_FOR_PAD"

colnames(ACM)[colnames(ACM)=="Diabetes mellitus with target organ disease: limbs/extremities"]<-"DIABETES_LIMBS"

colnames(ACM)[colnames(ACM)=="Diabetes mellitus with target organ disease: kidneys"]<-"DIABETES_KIDNEYS"

colnames(ACM)[colnames(ACM)=="Established coronary artery disease (CAD)"]<-"CAD"

colnames(ACM)[colnames(ACM)=="Established peripheral arterial disease (PAD)"]<-"PAD"

colnames(ACM)[colnames(ACM)=="Previous MI"]<-"PREV_MI"

colnames(ACM)[colnames(ACM)=="Previous stroke"]<-"PREV_STROKE"

colnames(ACM)[colnames(ACM)=="CV risk criteria at study entry only"]<-"CV_RISK_CRIT_ONLY"

ACM[1,]


ACM$TRTPN<-as.factor(ACM$TRTPN)

ACM$PREV_MI<-as.factor(ACM$PREV_MI)

ACM$PREV_STROKE<-as.factor(ACM$PREV_STROKE)

ACM$CAD<-as.factor(ACM$CAD)

ACM$PAD<-as.factor(ACM$PAD)

ACM$HYPERTENSION<-as.factor(ACM$HYPERTENSION)

ACM$HYPERCHOLESTEROL<-as.factor(ACM$HYPERCHOLESTEROL)

ACM$TREATED_FOR_PAD<-as.factor(ACM$TREATED_FOR_PAD)
```

```
ACM$DIABETES_ORGAN_DISEASE<-as.factor(ACM$DIABETES_ORGAN_DISEASE)

ACM$DIABETES_EYES<-as.factor(ACM$DIABETES_EYES)

ACM$DIABETES_KIDNEYS<-as.factor(ACM$DIABETES_KIDNEYS)

ACM$DIABETES_LIMBS<-as.factor(ACM$DIABETES_LIMBS)

ACM$'Met protocol CV entry criteria'<-as.factor(ACM$'Met protocol CV entry criteria')

ACM$'History of CV disease at study entry'<-as.factor(ACM$'History of CV disease at study entry')

ACM$DIABETES<-as.factor(ACM$DIABETES)

ACM$PREVEXCT<-as.factor(ACM$PREVEXCT)

ACM$IHDIN<-as.factor(ACM$IHDIN)


ACM%<>%mutate(ALPHABETABLOCK_1=case_when(ALPHABETABLOCK=="0"~"N"
,ALPHABETABLOCK=="1"~"Y",

ALPHABETABLOCK=="2"~"Y",ALPHABETABLOCK=="3"~"Y",

ALPHABETABLOCK=="4"~"Y",ALPHABETABLOCK=="5"~"Y",

ALPHABETABLOCK=="6"~"Y",ALPHABETABLOCK=="7"~"Y",TRUE~NA_character_
))


ACM$ALPHABETABLOCK_1<-as.factor(ACM$ALPHABETABLOCK_1)


ACM%<>%mutate(ACEI_1=case_when(ACEI=="0"~"N",ACEI=="1"~"Y",
                    ACEI=="2"~"Y",ACEI=="3"~"Y",
                    ACEI=="4"~"Y",ACEI=="5"~"Y",
                    ACEI=="6"~"Y",ACEI=="7"~"Y",
                    ACEI=="8"~"Y",ACEI=="9"~"Y",
                    ACEI=="10"~"Y",ACEI=="11"~"Y",
                    ACEI=="18"~"Y",ACEI=="19"~"Y",
                    ACEI=="26"~"Y",TRUE~NA_character_))


ACM$ACEI_1<-as.factor(ACM$ACEI_1)
```

```
ACM%<>%mutate(ARB_1=case_when(ARB=="0"~"N",ARB=="1"~"Y",
                              ARB=="2"~"Y",ARB=="3"~"Y",
                              ARB=="4"~"Y",ARB=="5"~"Y",
                              ARB=="6"~"Y",ARB=="7"~"Y",
                              ARB=="8"~"Y",ARB=="9"~"Y",
                              ARB=="10"~"Y",ARB=="11"~"Y",
                              ARB=="12"~"Y",TRUE~NA_character_))

ACM$ARB_1<-as.factor(ACM$ARB_1)

ACM%<>%mutate(ANTI_COAG_1=case_when(ANTI_COAG=="0"~"N",ANTI_COAG==
"1"~"Y",
                                    ANTI_COAG=="2"~"Y",ANTI_COAG=="3"~"Y",
                                    ANTI_COAG=="4"~"Y",ANTI_COAG=="5"~"Y",
                                    ANTI_COAG=="6"~"Y",ANTI_COAG=="7"~"Y",
                                    ANTI_COAG=="8"~"Y",ANTI_COAG=="9"~"Y",
                                    ANTI_COAG=="10"~"Y",ANTI_COAG=="11"~"Y",
                                    ANTI_COAG=="12"~"Y",ANTI_COAG=="13"~"Y",

ANTI_COAG=="14"~"Y",ANTI_COAG=="19"~"Y",TRUE~NA_character_))

ACM$ANTI_COAG_1<-as.factor(ACM$ANTI_COAG_1)

ACM%<>%mutate(ANTI_PLATE_1=case_when(ANTI_PLATE=="0"~"N",ANTI_PLATE
=="1"~"Y",
                                     ANTI_PLATE=="2"~"Y",ANTI_PLATE=="3"~"Y",
                                     ANTI_PLATE=="4"~"Y",ANTI_PLATE=="5"~"Y",
                                     ANTI_PLATE=="6"~"Y",ANTI_PLATE=="7"~"Y",
                                     ANTI_PLATE=="8"~"Y",ANTI_PLATE=="9"~"Y",
                                     ANTI_PLATE=="10"~"Y",ANTI_PLATE=="11"~"Y",

ANTI_PLATE=="12"~"Y",ANTI_PLATE=="13"~"Y",TRUE~NA_character_))
```

```
ACM$ANTI_PLATE_1<-as.factor(ACM$ANTI_PLATE_1)

ACM%<>%mutate(CHOL_BILE_ABSORB_INHIB_1=case_when(CHOL_BILE_ABSORB
_INHIB=="0"~"N",CHOL_BILE_ABSORB_INHIB=="1"~"Y",

CHOL_BILE_ABSORB_INHIB=="2"~"Y",CHOL_BILE_ABSORB_INHIB=="3"~"Y",TR
UE~NA_character_))



ACM$CHOL_BILE_ABSORB_INHIB_1<-
as.factor(ACM$CHOL_BILE_ABSORB_INHIB_1)

ACM%<>%mutate(CLASS3_1=case_when(CLASS3=="0"~"N",CLASS3=="1"~"Y",
                    CLASS3=="2"~"Y",CLASS3=="3"~"Y",
                    CLASS3=="4"~"Y",CLASS3=="5"~"Y",
                    CLASS3=="6"~"Y",CLASS3=="10"~"Y",TRUE~NA_character_))

ACM$CLASS3_1<-as.factor(ACM$CLASS3_1)

ACM%<>%mutate(DIHYDROPYRIDINE_1=case_when(DIHYDROPYRIDINE=="0"~"N"
,DIHYDROPYRIDINE=="1"~"Y",

DIHYDROPYRIDINE=="2"~"Y",DIHYDROPYRIDINE=="3"~"Y",

DIHYDROPYRIDINE=="4"~"Y",DIHYDROPYRIDINE=="5"~"Y",

DIHYDROPYRIDINE=="6"~"Y",DIHYDROPYRIDINE=="7"~"Y",

DIHYDROPYRIDINE=="8"~"Y",DIHYDROPYRIDINE=="9"~"Y",
                        DIHYDROPYRIDINE=="11"~"Y",TRUE~NA_character_))

ACM$DIHYDROPYRIDINE_1<-as.factor(ACM$DIHYDROPYRIDINE_1)

ACM%<>%mutate(DIR_RENIN_INHIB_1=case_when(DIR_RENIN_INHIB=="0"~"N",DI
R_RENIN_INHIB=="1"~"Y",
                        DIR_RENIN_INHIB=="2"~"Y",TRUE~NA_character_))
```

```
ACM$DIR_RENIN_INHIB_1<-as.factor(ACM$DIR_RENIN_INHIB_1)

ACM%<>%mutate(Fibrates_1=case_when(Fibrates=="0"~"N",Fibrates=="1"~"Y",
                        Fibrates=="2"~"Y",Fibrates=="3"~"Y",
                        Fibrates=="4"~"Y",Fibrates=="5"~"Y",
                        Fibrates=="6"~"Y",TRUE~NA_character_))

ACM$Fibrates_1<-as.factor(ACM$Fibrates_1)

ACM%<>%mutate(LONG_NITRATES_1=case_when(LONG_NITRATES=="0"~"N",LON
G_NITRATES=="1"~"Y",
                            LONG_NITRATES=="2"~"Y",LONG_NITRATES=="3"~"Y",
                            LONG_NITRATES=="4"~"Y",LONG_NITRATES=="5"~"Y",
                            LONG_NITRATES=="6"~"Y",TRUE~NA_character_))

ACM$LONG_NITRATES_1<-as.factor(ACM$LONG_NITRATES_1)

ACM%<>%mutate(Loop_1=case_when(Loop=="0"~"N",Loop=="1"~"Y",
                        Loop=="2"~"Y",Loop=="3"~"Y",
                        Loop=="4"~"Y",Loop=="5"~"Y",
                        Loop=="6"~"Y",Loop=="7"~"Y",
                        Loop=="8"~"Y",Loop=="10"~"Y",
                        Loop=="11"~"Y",Loop=="12"~"Y",

Loop=="13"~"Y",Loop=="21"~"Y",Loop=="24"~"Y",TRUE~NA_character_))

ACM$Loop_1<-as.factor(ACM$Loop_1)

ACM%<>%mutate(MRA_1=case_when(MRA=="0"~"N",MRA=="1"~"Y",
                        MRA=="2"~"Y",MRA=="3"~"Y",
                        MRA=="4"~"Y",MRA=="5"~"Y",
                        MRA=="6"~"Y",TRUE~NA_character_))
```

```
ACM$MRA_1<-as.factor(ACM$MRA_1)

ACM%<>%mutate(NON_DIHYDROPYRIDINE_1=case_when(NON_DIHYDROPYRIDI
NE=="0"~"N",NON_DIHYDROPYRIDINE=="1"~"Y",

NON_DIHYDROPYRIDINE=="2"~"Y",NON_DIHYDROPYRIDINE=="3"~"Y",

NON_DIHYDROPYRIDINE=="4"~"Y",NON_DIHYDROPYRIDINE=="5"~"Y",

NON_DIHYDROPYRIDINE=="6"~"Y",NON_DIHYDROPYRIDINE=="10"~"Y",

NON_DIHYDROPYRIDINE=="12"~"Y",TRUE~NA_character_))

ACM$NON_DIHYDROPYRIDINE_1<-as.factor(ACM$NON_DIHYDROPYRIDINE_1)

ACM%<>%mutate(NON_SELEC_B_BLOCK_1=case_when(NON_SELEC_B_BLOCK=="
0"~"N",NON_SELEC_B_BLOCK=="1"~"Y",

NON_SELEC_B_BLOCK=="2"~"Y",NON_SELEC_B_BLOCK=="3"~"Y",

NON_SELEC_B_BLOCK=="4"~"Y",NON_SELEC_B_BLOCK=="7"~"Y",TRUE~NA_cha
racter_))

ACM$NON_SELEC_B_BLOCK_1<-as.factor(ACM$NON_SELEC_B_BLOCK_1)

ACM%<>%mutate(SELEC_B_BLOCK_1=case_when(SELEC_B_BLOCK=="0"~"N",SEL
EC_B_BLOCK=="1"~"Y",
                SELEC_B_BLOCK=="2"~"Y",SELEC_B_BLOCK=="3"~"Y",
                SELEC_B_BLOCK=="4"~"Y",SELEC_B_BLOCK=="5"~"Y",
                SELEC_B_BLOCK=="6"~"Y",SELEC_B_BLOCK=="7"~"Y",
                SELEC_B_BLOCK=="8"~"Y",SELEC_B_BLOCK=="9"~"Y",
                SELEC_B_BLOCK=="10"~"Y",TRUE~NA_character_))

ACM$SELEC_B_BLOCK_1<-as.factor(ACM$SELEC_B_BLOCK_1)
```

```
ACM%<>%mutate(SHORT_NITRATES_1=case_when(SHORT_NITRATES=="0"~"N",S
HORT_NITRATES=="1"~"Y",
                              SHORT_NITRATES=="2"~"Y",SHORT_NITRATES=="3"~"Y",
                              SHORT_NITRATES=="4"~"Y",SHORT_NITRATES=="5"~"Y",
                              SHORT_NITRATES=="6"~"Y",SHORT_NITRATES=="8"~"Y",
                              SHORT_NITRATES=="9"~"Y",TRUE~NA_character_))

ACM$SHORT_NITRATES_1<-as.factor(ACM$SHORT_NITRATES_1)

ACM%<>%mutate(STATINS_1=case_when(STATINS=="0"~"N",STATINS=="1"~"Y",
                            STATINS=="2"~"Y",STATINS=="3"~"Y",
                            STATINS=="4"~"Y",STATINS=="5"~"Y",
                            STATINS=="6"~"Y",STATINS=="7"~"Y",
                            STATINS=="8"~"Y",STATINS=="9"~"Y",
                            STATINS=="10"~"Y",STATINS=="12"~"Y",TRUE~NA_character_))

ACM$STATINS_1<-as.factor(ACM$STATINS_1)

ACM%<>%mutate(THIAZIDES_DIUR_1=case_when(THIAZIDES_DIUR=="0"~"N",THI
AZIDES_DIUR=="1"~"Y",
                              THIAZIDES_DIUR=="2"~"Y",THIAZIDES_DIUR=="3"~"Y",
                              THIAZIDES_DIUR=="4"~"Y",THIAZIDES_DIUR=="5"~"Y",
                              THIAZIDES_DIUR=="6"~"Y",THIAZIDES_DIUR=="10"~"Y",
                              THIAZIDES_DIUR=="11"~"Y",TRUE~NA_character_))

ACM$THIAZIDES_DIUR_1<-as.factor(ACM$THIAZIDES_DIUR_1)

ACM%<>%mutate(BIGUANIDES_1=case_when(BIGUANIDES=="0"~"N",BIGUANIDES
=="1"~"Y",TRUE~NA_character_))

ACM$BIGUANIDES_1<-as.factor(ACM$BIGUANIDES_1)
```

```
ACM%<>%mutate(DPP4_1=case_when(DPP4=="0"~"N",DPP4=="1"~"Y",DPP4=="2"~"Y
",DPP4=="3"~"Y",TRUE~NA_character_))


ACM$DPP4_1<-as.factor(ACM$DPP4_1)


ACM%<>%mutate(DPP4_BIGUANIDES_1=case_when(DPP4_BIGUANIDES=="0"~"N",
DPP4_BIGUANIDES=="1"~"Y",TRUE~NA_character_))


ACM$DPP4_BIGUANIDES_1<-as.factor(ACM$DPP4_BIGUANIDES_1)


ACM%<>%mutate(GLPR_AGONIST_1=case_when(GLPR_AGONIST=="0"~"N",GLPR_
AGONIST=="1"~"Y",GLPR_AGONIST=="2"~"Y",TRUE~NA_character_))


ACM$GLPR_AGONIST_1<-as.factor(ACM$GLPR_AGONIST_1)


ACM%<>%mutate(GLUCOSIDASE_INHIB_1=case_when(GLUCOSIDASE_INHIB=="0"
~"N",GLUCOSIDASE_INHIB=="1"~"Y",GLUCOSIDASE_INHIB=="2"~"Y",GLUCOSID
ASE_INHIB=="3"~"Y",TRUE~NA_character_))


ACM$GLUCOSIDASE_INHIB_1<-as.factor(ACM$GLUCOSIDASE_INHIB_1)


ACM%<>%mutate(INSULINS_1=case_when(INSULINS=="0"~"N",INSULINS=="1"~"Y",
                    INSULINS=="2"~"Y",INSULINS=="3"~"Y",
                    INSULINS=="4"~"Y",INSULINS=="5"~"Y",
                    INSULINS=="6"~"Y",TRUE~NA_character_))


ACM$INSULINS_1<-as.factor(ACM$INSULINS_1)


ACM%<>%mutate(MEGLITINIDES_1=case_when(MEGLITINIDES=="0"~"N",MEGLITI
NIDES=="1"~"Y",TRUE~NA_character_))


ACM$MEGLITINIDES_1<-as.factor(ACM$MEGLITINIDES_1)
```

```
ACM%<>%mutate(SODIUM_TRANSPORT_INHIB_1=case_when(SODIUM_TRANSPO
RT_INHIB=="0"~"N",SODIUM_TRANSPORT_INHIB=="1"~"Y",TRUE~NA_character_))


ACM$SODIUM_TRANSPORT_INHIB_1<-
as.factor(ACM$SODIUM_TRANSPORT_INHIB_1)


ACM%<>%mutate(SULFONYLUREAS_1=case_when(SULFONYLUREAS=="0"~"N",SU
LFONYLUREAS=="1"~"Y",SULFONYLUREAS=="2"~"Y",SULFONYLUREAS=="3"~"
Y",TRUE~NA_character_))


ACM$SULFONYLUREAS_1<-as.factor(ACM$SULFONYLUREAS_1)


ACM%<>%mutate(SULFONYLUREAS_BIGUANIDES_1=case_when(SULFONYLUREA
S_BIGUANIDES=="0"~"N",SULFONYLUREAS_BIGUANIDES=="1"~"Y",TRUE~NA_c
haracter_))


ACM$SULFONYLUREAS_BIGUANIDES_1<-
as.factor(ACM$SULFONYLUREAS_BIGUANIDES_1)


ACM%<>%mutate(THIAZOLIDINEDIONE_1=case_when(THIAZOLIDINEDIONE=="0"
~"N",THIAZOLIDINEDIONE=="1"~"Y",TRUE~NA_character_))


ACM$THIAZOLIDINEDIONE_1<-as.factor(ACM$THIAZOLIDINEDIONE_1)




ACM%<>%mutate(ATENOLOL_1=case_when(ATENOLOL=="1"~"Y",TRUE~NA_charac
ter_))


ACM$ATENOLOL_1<-as.factor(ACM$ATENOLOL_1)


fct_explicit_na(ACM$ATENOLOL_1,na_level="N")

ACM%<>%mutate(ATENOLOL_1=fct_explicit_na(ATENOLOL_1,na_level="N"))


ACM%<>%mutate(BISOPROLOL_1=case_when(BISOPROLOL=="1"~"Y",TRUE~NA_ch
aracter_))
```

```
ACM$BISOPROLOL_1<-as.factor(ACM$BISOPROLOL_1)

fct_explicit_na(ACM$BISOPROLOL_1,na_level="N")
ACM%<>%mutate(BISOPROLOL_1=fct_explicit_na(BISOPROLOL_1,na_level="N"))


ACM%<>%mutate(CLOPIDOGREL_1=case_when(CLOPIDOGREL=="1"~"Y",TRUE~NA_character_))

ACM$CLOPIDOGREL_1<-as.factor(ACM$CLOPIDOGREL_1)

fct_explicit_na(ACM$CLOPIDOGREL_1,na_level="N")
ACM%<>%mutate(CLOPIDOGREL_1=fct_explicit_na(CLOPIDOGREL_1,na_level="N"))


ACM%<>%mutate(NEBIVOLOL_1=case_when(NEBIVOLOL=="1"~"Y",TRUE~NA_character_))

ACM$NEBIVOLOL_1<-as.factor(ACM$NEBIVOLOL_1)

fct_explicit_na(ACM$NEBIVOLOL_1,na_level="N")
ACM%<>%mutate(NEBIVOLOL_1=fct_explicit_na(NEBIVOLOL_1,na_level="N"))


ACM%<>%mutate(ASP_CLOPIDOGREL_1=case_when(ASP_CLOPIDOGREL=="1"~"Y",TRUE~NA_character_))

ACM$ASP_CLOPIDOGREL_1<-as.factor(ACM$ASP_CLOPIDOGREL_1)

fct_explicit_na(ACM$ASP_CLOPIDOGREL_1,na_level="N")
```

```
ACM%<>%mutate(ASP_CLOPIDOGREL_1=fct_explicit_na(ASP_CLOPIDOGREL_1,na_level="N"))



ACM%<>%mutate(ASP_STAT_CLOPIDOGREL_1=case_when(ASP_STAT_CLOPIDOGREL=="1"~"Y",TRUE~NA_character_))


ACM$ASP_STAT_CLOPIDOGREL_1<-as.factor(ACM$ASP_STAT_CLOPIDOGREL_1)


fct_explicit_na(ACM$ASP_STAT_CLOPIDOGREL_1,na_level="N")

ACM%<>%mutate(ASP_STAT_CLOPIDOGREL_1=fct_explicit_na(ASP_STAT_CLOPIDOGREL_1,na_level="N"))



ACM%<>%mutate(STAT_CLOPIDOGREL_1=case_when(STAT_CLOPIDOGREL=="1"~"Y",TRUE~NA_character_))


ACM$STAT_CLOPIDOGREL_1<-as.factor(ACM$STAT_CLOPIDOGREL_1)


fct_explicit_na(ACM$STAT_CLOPIDOGREL_1,na_level="N")

ACM%<>%mutate(STAT_CLOPIDOGREL_1=fct_explicit_na(STAT_CLOPIDOGREL_1,na_level="N"))









ACM$ASPIRIN[is.na(ACM$ASPIRIN)]<-0

ACM$ABCIXIMAB[is.na(ACM$ABCIXIMAB)]<-0

ACM$ASPDIP[is.na(ACM$ASPDIP)]<-0

ACM$CARB[is.na(ACM$CARB)]<-0

ACM$DIP[is.na(ACM$DIP)]<-0

ACM$ETHICO[is.na(ACM$ETHICO)]<-0

ACM$ILO[is.na(ACM$ILO)]<-0
```

```
ACM$ICO[is.na(ACM$ICO)]<-0
ACM$MESOGLYCAN[is.na(ACM$MESOGLYCAN)]<-0
ACM$OZAGREL[is.na(ACM$OZAGREL)]<-0
ACM$SARPO[is.na(ACM$SARPO)]<-0
ACM$PRAS[is.na(ACM$PRAS)]<-0
ACM$PAI[is.na(ACM$PAI)]<-0
ACM$TICA[is.na(ACM$TICA)]<-0
ACM$TICLO[is.na(ACM$TICLO)]<-0
ACM$TRIF[is.na(ACM$TRIF)]<-0
ACM$TIRO[is.na(ACM$TIRO)]<-0
ACM$TREPR[is.na(ACM$TREPR)]<-0

ACM%<>%distinct(USUBJID,INTENTION_TO_TREAT,.keep_all=TRUE)

library(forcats)

ACM$DIABETES<-as.factor(ACM$DIABETES)
fct_explicit_na(ACM$DIABETES,na_level="N")
ACM%<>%mutate(DIABETES=fct_explicit_na(DIABETES,na_level="N"))

fct_explicit_na(ACM$HYPERTENSION,na_level="N")
ACM%<>%mutate(HYPERTENSION=fct_explicit_na(HYPERTENSION,na_level="N"))

fct_explicit_na(ACM$HYPERCHOLESTEROL,na_level="N")
ACM%<>%mutate(HYPERCHOLESTEROL=fct_explicit_na(HYPERCHOLESTEROL,na_
level="N"))

fct_explicit_na(ACM$PAD,na_level="N")
ACM%<>%mutate(PAD=fct_explicit_na(PAD,na_level="N"))

fct_explicit_na(ACM$CAD,na_level="N")
ACM%<>%mutate(CAD=fct_explicit_na(CAD,na_level="N"))
```

```
fct_explicit_na(ACM$PREV_MI,na_level="N")
ACM%<>%mutate(PREV_MI=fct_explicit_na(PREV_MI,na_level="N"))

fct_explicit_na(ACM$PREV_STROKE,na_level="N")
ACM%<>%mutate(PREV_STROKE=fct_explicit_na(PREV_STROKE,na_level="N"))



ACM$STATUS[ACM$USUBJID=="1566"]<-0
ACM$STATUS[ACM$USUBJID=="2286"]<-0
ACM$STATUS[ACM$USUBJID=="2818"]<-0
ACM$STATUS[ACM$USUBJID=="4268"]<-0
ACM$STATUS[ACM$USUBJID=="4270"]<-0
ACM$STATUS[ACM$USUBJID=="4756"]<-0
ACM$STATUS[ACM$USUBJID=="5510"]<-0
ACM$STATUS[ACM$USUBJID=="6357"]<-0
ACM$STATUS[ACM$USUBJID=="7158"]<-0
ACM$STATUS[ACM$USUBJID=="9228"]<-0
ACM$STATUS[ACM$USUBJID=="11341"]<-0
ACM$STATUS[ACM$USUBJID=="12034"]<-0
ACM$STATUS[ACM$USUBJID=="13051"]<-0
ACM$STATUS[ACM$USUBJID=="15420"]<-0
ACM$STATUS[ACM$USUBJID=="15534"]<-0
ACM$STATUS[ACM$USUBJID=="15864"]<-0
ACM$STATUS[ACM$USUBJID=="15890"]<-0
ACM$STATUS[ACM$USUBJID=="16259"]<-0
ACM$STATUS[ACM$USUBJID=="16684"]<-0
ACM$STATUS[ACM$USUBJID=="20248"]<-0
ACM$STATUS[ACM$USUBJID=="21795"]<-0
ACM$STATUS[ACM$USUBJID=="22794"]<-0
ACM$STATUS[ACM$USUBJID=="22891"]<-0
ACM$STATUS[ACM$USUBJID=="23321"]<-0
```

```
ACM%<>%distinct(USUBJID,BMI,.keep_all=TRUE)

ACM%<>%mutate(RACE_CODE=case_when(RACE=="AMERICAN INDIAN OR ALASKA NATIVE"~"OTHER",
                                  RACE=="ASIAN"~"ASIAN",
                                  RACE=="BLACK OR AFRICAN AMERICAN"~"OTHER",
                                  RACE=="MULTIPLE"~"OTHER",
                                  RACE=="NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER"~"OTHER",
                                  RACE=="WHITE"~"WHITE",TRUE~NA_character_))

ACM$RACE_CODE<-as.character(ACM$RACE_CODE)
ACM$PREVEXCT<-as.factor(ACM$PREVEXCT)
ACM$SMKBLN<-as.factor(ACM$SMKBLN)
ACM$HYPERTENSION<-relevel(ACM$HYPERTENSION,ref="N")

ACM$PREVEXCT<-relevel(ACM$PREVEXCT,ref="0")

ACM$SMKBLN<-relevel(ACM$SMKBLN,ref="2")

ACM$HYPERCHOLESTEROL<-relevel(ACM$HYPERCHOLESTEROL,ref="N")

ACM$PAD<-relevel(ACM$PAD,ref="N")

ACM$CAD<-relevel(ACM$CAD,ref="N")

ACM$PREV_MI<-relevel(ACM$PREV_MI,ref="N")

ACM$PREV_STROKE<-relevel(ACM$PREV_STROKE,ref="N")

ACM$DIABETES<-relevel(ACM$DIABETES,ref="N")
```

```
ACM$ATENOLOL_1<-relevel(ACM$ATENOLOL_1,ref="N")

ACM$BISOPROLOL_1<-relevel(ACM$BISOPROLOL_1,ref="N")

ACM$NEBIVOLOL_1<-relevel(ACM$NEBIVOLOL_1,ref="N")

ACM$CLOPIDOGREL_1<-relevel(ACM$CLOPIDOGREL_1,ref="N")

ACM$ASPIRIN<-as.factor(ACM$ASPIRIN)
ACM$ASPIRIN<-relevel(ACM$ASPIRIN,ref="0")

ACM%<>%mutate(os.days=ACM$os_yrs*365.25)
ACM$os.days<-as.integer(ACM$os.days)
ACM%<>%mutate(ADY_FULL=case_when(os.days>ADY~ADY,TRUE~os.days))



ACM$BB_1<-paste(ACM$SELEC_B_BLOCK_1,ACM$NON_SELEC_B_BLOCK_1)

ACM%<>%mutate(BB_2=case_when(BB_1=="N N"~"N",BB_1=="N Y"~"Y",
                  BB_1=="Y N"~"Y",BB_1=="Y Y"~"Y",TRUE~NA_character_))

ACM$BB_2<-as.factor(ACM$BB_2)

ACM$ACEIARB_1<-paste(ACM$ACEI_1,ACM$ARB_1)

ACM%<>%mutate(ACEIARB_2=case_when(ACEIARB_1=="N
N"~"N",ACEIARB_1=="N Y"~"Y",
                     ACEIARB_1=="Y                    N"~"Y",ACEIARB_1=="Y
Y"~"Y",TRUE~NA_character_))

ACM$ACEIARB_2<-as.factor(ACM$ACEIARB_2)
```

```
ACM$IHD_CAD<-paste(ACM$IHDIN,ACM$CAD)

ACM%<>%mutate(IHD_CAD_1=case_when(IHD_CAD=="0 N"~"N",IHD_CAD=="0 Y"~"Y",
                                  IHD_CAD=="1 N"~"Y",IHD_CAD=="1 Y"~"Y",TRUE~NA_character_))

ACM$IHD_CAD_1<-as.factor(ACM$IHD_CAD_1)


fct_explicit_na(ACM$ACEIARB_2,na_level="N")
ACM%<>%mutate(ACEIARB_2=fct_explicit_na(ACEIARB_2,na_level="N"))

fct_explicit_na(ACM$BB_2,na_level="N")
ACM%<>%mutate(BB_2=fct_explicit_na(BB_2,na_level="N"))

fct_explicit_na(ACM$ANTI_COAG_1,na_level="N")
ACM%<>%mutate(ANTI_COAG_1=fct_explicit_na(ANTI_COAG_1,na_level="N"))

fct_explicit_na(ACM$ANTI_PLATE_1,na_level="N")
ACM%<>%mutate(ANTI_PLATE_1=fct_explicit_na(ANTI_PLATE_1,na_level="N"))

fct_explicit_na(ACM$DIHYDROPYRIDINE_1,na_level="N")
ACM%<>%mutate(DIHYDROPYRIDINE_1=fct_explicit_na(DIHYDROPYRIDINE_1,na_level="N"))

fct_explicit_na(ACM$LONG_NITRATES_1,na_level="N")
ACM%<>%mutate(LONG_NITRATES_1=fct_explicit_na(LONG_NITRATES_1,na_level="N"))

fct_explicit_na(ACM$Loop_1,na_level="N")
ACM%<>%mutate(Loop_1=fct_explicit_na(Loop_1,na_level="N"))
```

```
fct_explicit_na(ACM$NON_DIHYDROPYRIDINE_1,na_level="N")

ACM%<>%mutate(NON_DIHYDROPYRIDINE_1=fct_explicit_na(NON_DIHYDROPYRIDINE_1,na_level="N"))


fct_explicit_na(ACM$STATINS_1,na_level="N")

ACM%<>%mutate(STATINS_1=fct_explicit_na(STATINS_1,na_level="N"))


fct_explicit_na(ACM$THIAZIDES_DIUR_1,na_level="N")

ACM%<>%mutate(THIAZIDES_DIUR_1=fct_explicit_na(THIAZIDES_DIUR_1,na_level="N"))


fct_explicit_na(ACM$BIGUANIDES_1,na_level="N")

ACM%<>%mutate(BIGUANIDES_1=fct_explicit_na(BIGUANIDES_1,na_level="N"))


fct_explicit_na(ACM$INSULINS_1,na_level="N")

ACM%<>%mutate(INSULINS_1=fct_explicit_na(INSULINS_1,na_level="N"))



fct_explicit_na(ACM$SULFONYLUREAS_1,na_level="N")

ACM%<>%mutate(SULFONYLUREAS_1=fct_explicit_na(SULFONYLUREAS_1,na_level="N"))



ACM$CV_RISK_CRIT_ONLY<-as.factor(ACM$CV_RISK_CRIT_ONLY)


ACM$ASPCLOP<-paste(ACM$ASPIRIN_1,ACM$CLOPIDOGREL_1)


ACM%<>%mutate(ASPCLOPCOMBO=case_when(ASPCLOP=="N
N"~"N",ASPCLOP=="N Y"~"N",
                                     ASPCLOP=="Y                                     N"~"N",ASPCLOP=="Y
Y"~"Y",TRUE~NA_character_))


ACM$ASPCLOPCOMBO<-as.factor(ACM$ASPCLOPCOMBO)
```

```
ACM$CV_CRITERIA<-ACM$RACE<-ACM$SITEID<-ACM$LSTCT<-
ACM$TREATMENT_YEARS<-ACM$os_yrs<-ACM$ALPHABETABLOCK<-NULL

ACM$ACEI<-ACM$ARB<-ACM$ANTI_COAG<-ACM$ANTI_PLATE<-
ACM$CHOL_BILE_ABSORB_INHIB<-ACM$CLASS3<-NULL

ACM$DIHYDROPYRIDINE<-ACM$DIR_RENIN_INHIB<-ACM$Fibrates<-
ACM$LONG_NITRATES<-ACM$Loop<-ACM$MRA<-NULL

ACM$Niacin<-ACM$NON_DIHYDROPYRIDINE<-ACM$NON_SELEC_B_BLOCK<-
ACM$Other<-ACM$SUPPLEMENTS<-ACM$SELEC_B_BLOCK<-NULL

ACM$SHORT_NITRATES<-ACM$STATINS<-ACM$THIAZIDES_DIUR<-
ACM$NA.x<-ACM$BIGUANIDES<-ACM$DPP4<-ACM$DPP4_BIGUANIDES<-NULL

ACM$GLPR_AGONIST<-ACM$GLUCOSIDASE_INHIB<-ACM$INSULINS<-
ACM$MEGLITINIDES<-ACM$OTHER<-ACM$SODIUM_TRANSPORT_INHIB<-
NULL

ACM$SULFONYLUREAS<-ACM$SULFONYLUREAS_BIGUANIDES<-
ACM$THIAZOLIDINEDIONE<-ACM$NA.y<-ACM$TREATED_FOR_PAD<-NULL

ACM$`CV risk criteria at study entry`<-ACM$DIABETES_ORGAN_DISEASE<-
ACM$DIABETES_EYES<-ACM$DIABETES_KIDNEYS<-NULL

ACM$DIABETES_LIMBS<-ACM$`History of CV disease at study entry`<-ACM$`Met
protocol CV entry criteria`<-NULL

ACM$GLUC_CAT<-ACM$BB_1<-ACM$ACEIARB_1<-NULL

ACM$ATENOLOL<-ACM$BISOPROLOL<-ACM$CLOPIDOGREL<-
ACM$NEBIVOLOL<-NULL

ACM$ASP_BISOPROLOL<-ACM$ASP_CLOPIDOGREL<-
ACM$STAT_CLOPIDOGREL<-ACM$ASP_STAT_CLOPIDOGREL<-NULL

ACM$ASPCLOP<-ACM$IHD_CAD<-NULL


ACM$STATUS<-as.factor(ACM$STATUS)


ACM_TABLE<-
tableby(~AGE+SEX+TRTPN+BMI+SMKBLN+PACK_YRS+RACE_CODE+DIABETES+


HYPERCHOLESTEROL+PAD+CAD+HYPERTENSION+PREV_MI+PREV_STROKE+


ALPHABETABLOCK_1+ACEI_1+ARB_1+ANTI_PLATE_1+ANTI_COAG_1+
```

```
CHOL_BILE_ABSORB_INHIB_1+CLASS3_1+DIHYDROPYRIDINE_1+DIR_RENIN_I
NHIB_1+

Fibrates_1+LONG_NITRATES_1+Loop_1+MRA_1+NON_DIHYDROPYRIDINE_1+

NON_SELEC_B_BLOCK_1+SELEC_B_BLOCK_1+ACEIARB_2+BB_2+SHORT_NITR
ATES_1+

STATINS_1+THIAZIDES_DIUR_1+BIGUANIDES_1+DPP4_1+GLPR_AGONIST_1+

GLUCOSIDASE_INHIB_1+INSULINS_1+MEGLITINIDES_1+SULFONYLUREAS_1+

THIAZOLIDINEDIONE_1+DPP4_BIGUANIDES_1+SODIUM_TRANSPORT_INHIB_1+

SULFONYLUREAS_BIGUANIDES_1+STATUS+GLUCOSE+PREVEXCT+ADY+FEV1+

CV_RISK_CRIT_ONLY+IHDIN+HF+IHD_CAD_1+ATENOLOL_1+BISOPROLOL_1+

NEBIVOLOL_1+CLOPIDOGREL_1+ASPIRIN,data=subset(ACM,INTENTION_TO_TRE
AT=="Y"))


summary(ACM_TABLE,title="ACM_TABLE")


ACM$STATUS<-as.numeric(ACM$STATUS)

ACM%<>%mutate(STATUS=case_when(STATUS=="1"~0,STATUS=="2"~1,TRUE~NA_
real_))


coxph(Surv(ADY_FULL,STATUS)~AGE+SEX+BMI+SMKBLN+FEV1+PACK_YRS+PA
D+PREV_STROKE+IHD_CAD_1+HF+HYPERTENSION+HYPERCHOLESTEROL+

    DIABETES+ASPIRIN,

data=subset(ACM,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPIDOGREL_1=="N
"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="
N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&
```

```
ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&
SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&
TRIF=="0"&TIRO=="0"&TREPR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)



#CV composite events

cvcompevents[1,]
colnames(cvcompevents)[colnames(cvcompevents)=="LSTCT"]<-"STATUS_CV"
colnames(cvcompevents)[colnames(cvcompevents)=="ADY"]<-"ADY_CV"
colnames(cvcompevents)[colnames(cvcompevents)=="ADT"]<-"ADT_CV"

a<-merge(sla,medstable,by="USUBJID",all.x=TRUE,all.y=TRUE)
b<-merge(a,cvcrit,by="USUBJID",all.x=TRUE,all.y=TRUE)
d<-merge(b,cvcompevents,by="USUBJID",all.x=TRUE,all.y=TRUE)

#KEEP FIRST CV EVENT ONLY

d=d[order(d[,'USUBJID'],d[,'ADY_CV']),]
d=d[!duplicated(d$USUBJID),]

d%<>%mutate(CVSTATUS=ADY_CV)
d$CVSTATUS[d$CVSTATUS>0]<-1
d$CVSTATUS[is.na(d$CVSTATUS)]<-0

t<-merge(d,glucose,by="USUBJID",all.x=TRUE,all.y=TRUE)
t1<-merge(t,fev,by="USUBJID",all.x=TRUE,all.y=TRUE)
t2<-merge(t1,cvhist,by="USUBJID",all.x=TRUE,all.y=TRUE)
```

```
t3<-merge(t2,smoking,by="USUBJID",all.x=TRUE,all.y=TRUE)

CVCOMP<-t3

colnames(CVCOMP)[colnames(CVCOMP)=="Alpha and beta blocking"]<-"ALPHABETABLOCK"
colnames(CVCOMP)[colnames(CVCOMP)=="Angiotensin-converting Enzyme Inhibitors"]<-"ACEI"
colnames(CVCOMP)[colnames(CVCOMP)=="Angiotensin receptor blockers"]<-"ARB"
colnames(CVCOMP)[colnames(CVCOMP)=="Anti-coagulant therapy"]<-"ANTI_COAG"
colnames(CVCOMP)[colnames(CVCOMP)=="Anti-platelet therapy"]<-"ANTI_PLATE"
colnames(CVCOMP)[colnames(CVCOMP)=="Cholesterol and bile acid absorption inhibitors"]<-"CHOL_BILE_ABSORB_INHIB"
colnames(CVCOMP)[colnames(CVCOMP)=="Class III"]<-"CLASS3"
colnames(CVCOMP)[colnames(CVCOMP)=="Dihydropyridine"]<-"DIHYDROPYRIDINE"
colnames(CVCOMP)[colnames(CVCOMP)=="Direct Renin Inhibitors"]<-"DIR_RENIN_INHIB"
colnames(CVCOMP)[colnames(CVCOMP)=="Long-acting"]<-"LONG_NITRATES"
colnames(CVCOMP)[colnames(CVCOMP)=="Short-acting"]<-"SHORT_NITRATES"
colnames(CVCOMP)[colnames(CVCOMP)=="Mineralocorticoid Receptor Antagonists"]<-"MRA"
colnames(CVCOMP)[colnames(CVCOMP)=="Non-dihydropyridine"]<-"NON_DIHYDROPYRIDINE"
colnames(CVCOMP)[colnames(CVCOMP)=="Non-selective beta-adrenergic receptor blocker"]<-"NON_SELEC_B_BLOCK"
colnames(CVCOMP)[colnames(CVCOMP)=="Other lipid modifying"]<-"SUPPLEMENTS"
colnames(CVCOMP)[colnames(CVCOMP)=="Selective beta1-adrenergic receptor blocker"]<-"SELEC_B_BLOCK"
colnames(CVCOMP)[colnames(CVCOMP)=="Statins"]<-"STATINS"
colnames(CVCOMP)[colnames(CVCOMP)=="Thiazides and Thiazide like Diuretics"]<-"THIAZIDES_DIUR"
colnames(CVCOMP)[colnames(CVCOMP)=="Being treated for diabetes mellitus"]<-"DIABETES"
colnames(CVCOMP)[colnames(CVCOMP)=="Being treated for hypercholesterolemia"]<-"HYPERCHOLESTEROL"
```

```
colnames(CVCOMP)[colnames(CVCOMP)=="Being treated for hypertension"]<-"HYPERTENSION"

colnames(CVCOMP)[colnames(CVCOMP)=="Diabetes mellitus with target organ disease"]<-"DIABETES_ORGAN_DISEASE"

colnames(CVCOMP)[colnames(CVCOMP)=="Diabetes mellitus with target organ disease: eyes"]<-"DIABETES_EYES"

colnames(CVCOMP)[colnames(CVCOMP)=="Being treated for peripheral arterial disease"]<-"TREATED_FOR_PAD"

colnames(CVCOMP)[colnames(CVCOMP)=="Diabetes mellitus with target organ disease: limbs/extremities"]<-"DIABETES_LIMBS"

colnames(CVCOMP)[colnames(CVCOMP)=="Diabetes mellitus with target organ disease: kidneys"]<-"DIABETES_KIDNEYS"

colnames(CVCOMP)[colnames(CVCOMP)=="Established coronary artery disease (CAD)"]<-"CAD"

colnames(CVCOMP)[colnames(CVCOMP)=="Established peripheral arterial disease (PAD)"]<-"PAD"

colnames(CVCOMP)[colnames(CVCOMP)=="Previous MI"]<-"PREV_MI"

colnames(CVCOMP)[colnames(CVCOMP)=="Previous stroke"]<-"PREV_STROKE"

colnames(CVCOMP)[colnames(CVCOMP)=="CV risk criteria at study entry only"]<-"CV_RISK_CRIT_ONLY"



CVCOMP$TRTPN<-as.factor(CVCOMP$TRTPN)

CVCOMP$PREV_MI<-as.factor(CVCOMP$PREV_MI)

CVCOMP$PREV_STROKE<-as.factor(CVCOMP$PREV_STROKE)

CVCOMP$CAD<-as.factor(CVCOMP$CAD)

CVCOMP$PAD<-as.factor(CVCOMP$PAD)

CVCOMP$HYPERTENSION<-as.factor(CVCOMP$HYPERTENSION)

CVCOMP$HYPERCHOLESTEROL<-as.factor(CVCOMP$HYPERCHOLESTEROL)

CVCOMP$TREATED_FOR_PAD<-as.factor(CVCOMP$TREATED_FOR_PAD)

CVCOMP$DIABETES_ORGAN_DISEASE<-
as.factor(CVCOMP$DIABETES_ORGAN_DISEASE)

CVCOMP$DIABETES_EYES<-as.factor(CVCOMP$DIABETES_EYES)

CVCOMP$DIABETES_KIDNEYS<-as.factor(CVCOMP$DIABETES_KIDNEYS)

CVCOMP$DIABETES_LIMBS<-as.factor(CVCOMP$DIABETES_LIMBS)
```

```
CVCOMP$'Met protocol CV entry criteria'<-as.factor(CVCOMP$'Met protocol CV entry criteria')

CVCOMP$'History of CV disease at study entry'<-as.factor(CVCOMP$'History of CV disease at study entry')

CVCOMP$DIABETES<-as.factor(CVCOMP$DIABETES)

CVCOMP$CV_RISK_CRIT_ONLY<-as.factor(CVCOMP$CV_RISK_CRIT_ONLY)

CVCOMP$IHDIN<-as.factor(CVCOMP$IHDIN)



CVCOMP%<>%mutate(ALPHABETABLOCK_1=case_when(ALPHABETABLOCK=="0"
~"N",ALPHABETABLOCK=="1"~"Y",

ALPHABETABLOCK=="2"~"Y",ALPHABETABLOCK=="3"~"Y",

ALPHABETABLOCK=="4"~"Y",ALPHABETABLOCK=="5"~"Y",

ALPHABETABLOCK=="6"~"Y",ALPHABETABLOCK=="7"~"Y",TRUE~NA_character_
))


CVCOMP$ALPHABETABLOCK_1<-as.factor(CVCOMP$ALPHABETABLOCK_1)


CVCOMP%<>%mutate(ACEI_1=case_when(ACEI=="0"~"N",ACEI=="1"~"Y",
                  ACEI=="2"~"Y",ACEI=="3"~"Y",
                  ACEI=="4"~"Y",ACEI=="5"~"Y",
                  ACEI=="6"~"Y",ACEI=="7"~"Y",
                  ACEI=="8"~"Y",ACEI=="9"~"Y",
                  ACEI=="10"~"Y",ACEI=="11"~"Y",
                  ACEI=="18"~"Y",ACEI=="19"~"Y",
                  ACEI=="26"~"Y",TRUE~NA_character_))


CVCOMP$ACEI_1<-as.factor(CVCOMP$ACEI_1)


CVCOMP%<>%mutate(ARB_1=case_when(ARB=="0"~"N",ARB=="1"~"Y",
                 ARB=="2"~"Y",ARB=="3"~"Y",
```

```
                ARB=="4"~"Y",ARB=="5"~"Y",
                ARB=="6"~"Y",ARB=="7"~"Y",
                ARB=="8"~"Y",ARB=="9"~"Y",
                ARB=="10"~"Y",ARB=="11"~"Y",
                ARB=="12"~"Y",TRUE~NA_character_))

CVCOMP$ARB_1<-as.factor(CVCOMP$ARB_1)

CVCOMP%<>%mutate(ANTI_COAG_1=case_when(ANTI_COAG=="0"~"N",ANTI_COA
G=="1"~"Y",
                        ANTI_COAG=="2"~"Y",ANTI_COAG=="3"~"Y",
                        ANTI_COAG=="4"~"Y",ANTI_COAG=="5"~"Y",
                        ANTI_COAG=="6"~"Y",ANTI_COAG=="7"~"Y",
                        ANTI_COAG=="8"~"Y",ANTI_COAG=="9"~"Y",
                        ANTI_COAG=="10"~"Y",ANTI_COAG=="11"~"Y",
                        ANTI_COAG=="12"~"Y",ANTI_COAG=="13"~"Y",

ANTI_COAG=="14"~"Y",ANTI_COAG=="19"~"Y",TRUE~NA_character_))

CVCOMP$ANTI_COAG_1<-as.factor(CVCOMP$ANTI_COAG_1)

CVCOMP%<>%mutate(ANTI_PLATE_1=case_when(ANTI_PLATE=="0"~"N",ANTI_PL
ATE=="1"~"Y",
                        ANTI_PLATE=="2"~"Y",ANTI_PLATE=="3"~"Y",
                        ANTI_PLATE=="4"~"Y",ANTI_PLATE=="5"~"Y",
                        ANTI_PLATE=="6"~"Y",ANTI_PLATE=="7"~"Y",
                        ANTI_PLATE=="8"~"Y",ANTI_PLATE=="9"~"Y",
                        ANTI_PLATE=="10"~"Y",ANTI_PLATE=="11"~"Y",

ANTI_PLATE=="12"~"Y",ANTI_PLATE=="13"~"Y",TRUE~NA_character_))

CVCOMP$ANTI_PLATE_1<-as.factor(CVCOMP$ANTI_PLATE_1)
```

```
CVCOMP%<>%mutate(CHOL_BILE_ABSORB_INHIB_1=case_when(CHOL_BILE_ABS
ORB_INHIB=="0"~"N",CHOL_BILE_ABSORB_INHIB=="1"~"Y",

CHOL_BILE_ABSORB_INHIB=="2"~"Y",CHOL_BILE_ABSORB_INHIB=="3"~"Y",TR
UE~NA_character_))



CVCOMP$CHOL_BILE_ABSORB_INHIB_1<-
as.factor(CVCOMP$CHOL_BILE_ABSORB_INHIB_1)


CVCOMP%<>%mutate(CLASS3_1=case_when(CLASS3=="0"~"N",CLASS3=="1"~"Y",
                    CLASS3=="2"~"Y",CLASS3=="3"~"Y",
                    CLASS3=="4"~"Y",CLASS3=="5"~"Y",
                    CLASS3=="6"~"Y",CLASS3=="10"~"Y",TRUE~NA_character_))


CVCOMP$CLASS3_1<-as.factor(CVCOMP$CLASS3_1)


CVCOMP%<>%mutate(DIHYDROPYRIDINE_1=case_when(DIHYDROPYRIDINE=="0"
~"N",DIHYDROPYRIDINE=="1"~"Y",

DIHYDROPYRIDINE=="2"~"Y",DIHYDROPYRIDINE=="3"~"Y",

DIHYDROPYRIDINE=="4"~"Y",DIHYDROPYRIDINE=="5"~"Y",

DIHYDROPYRIDINE=="6"~"Y",DIHYDROPYRIDINE=="7"~"Y",

DIHYDROPYRIDINE=="8"~"Y",DIHYDROPYRIDINE=="9"~"Y",
                        DIHYDROPYRIDINE=="11"~"Y",TRUE~NA_character_))


CVCOMP$DIHYDROPYRIDINE_1<-as.factor(CVCOMP$DIHYDROPYRIDINE_1)


CVCOMP%<>%mutate(DIR_RENIN_INHIB_1=case_when(DIR_RENIN_INHIB=="0"~"N
",DIR_RENIN_INHIB=="1"~"Y",
                        DIR_RENIN_INHIB=="2"~"Y",TRUE~NA_character_))


CVCOMP$DIR_RENIN_INHIB_1<-as.factor(CVCOMP$DIR_RENIN_INHIB_1)
```

```
CVCOMP%<>%mutate(Fibrates_1=case_when(Fibrates=="0"~"N",Fibrates=="1"~"Y",
                        Fibrates=="2"~"Y",Fibrates=="3"~"Y",
                        Fibrates=="4"~"Y",Fibrates=="5"~"Y",
                        Fibrates=="6"~"Y",TRUE~NA_character_))

CVCOMP$Fibrates_1<-as.factor(CVCOMP$Fibrates_1)

CVCOMP%<>%mutate(LONG_NITRATES_1=case_when(LONG_NITRATES=="0"~"N",
LONG_NITRATES=="1"~"Y",
                           LONG_NITRATES=="2"~"Y",LONG_NITRATES=="3"~"Y",
                           LONG_NITRATES=="4"~"Y",LONG_NITRATES=="5"~"Y",
                           LONG_NITRATES=="6"~"Y",TRUE~NA_character_))

CVCOMP$LONG_NITRATES_1<-as.factor(CVCOMP$LONG_NITRATES_1)

CVCOMP%<>%mutate(Loop_1=case_when(Loop=="0"~"N",Loop=="1"~"Y",
                        Loop=="2"~"Y",Loop=="3"~"Y",
                        Loop=="4"~"Y",Loop=="5"~"Y",
                        Loop=="6"~"Y",Loop=="7"~"Y",
                        Loop=="8"~"Y",Loop=="10"~"Y",
                        Loop=="11"~"Y",Loop=="12"~"Y",

Loop=="13"~"Y",Loop=="21"~"Y",Loop=="24"~"Y",TRUE~NA_character_))

CVCOMP$Loop_1<-as.factor(CVCOMP$Loop_1)

CVCOMP%<>%mutate(MRA_1=case_when(MRA=="0"~"N",MRA=="1"~"Y",
                        MRA=="2"~"Y",MRA=="3"~"Y",
                        MRA=="4"~"Y",MRA=="5"~"Y",
                        MRA=="6"~"Y",TRUE~NA_character_))

CVCOMP$MRA_1<-as.factor(CVCOMP$MRA_1)
```

```
CVCOMP%<>%mutate(NON_DIHYDROPYRIDINE_1=case_when(NON_DIHYDROPYR
IDINE=="0"~"N",NON_DIHYDROPYRIDINE=="1"~"Y",

NON_DIHYDROPYRIDINE=="2"~"Y",NON_DIHYDROPYRIDINE=="3"~"Y",

NON_DIHYDROPYRIDINE=="4"~"Y",NON_DIHYDROPYRIDINE=="5"~"Y",

NON_DIHYDROPYRIDINE=="6"~"Y",NON_DIHYDROPYRIDINE=="10"~"Y",

NON_DIHYDROPYRIDINE=="12"~"Y",TRUE~NA_character_))


CVCOMP$NON_DIHYDROPYRIDINE_1<-
as.factor(CVCOMP$NON_DIHYDROPYRIDINE_1)


CVCOMP%<>%mutate(NON_SELEC_B_BLOCK_1=case_when(NON_SELEC_B_BLOC
K=="0"~"N",NON_SELEC_B_BLOCK=="1"~"Y",

NON_SELEC_B_BLOCK=="2"~"Y",NON_SELEC_B_BLOCK=="3"~"Y",

NON_SELEC_B_BLOCK=="4"~"Y",NON_SELEC_B_BLOCK=="7"~"Y",TRUE~NA_cha
racter_))



CVCOMP$NON_SELEC_B_BLOCK_1<-
as.factor(CVCOMP$NON_SELEC_B_BLOCK_1)


CVCOMP%<>%mutate(SELEC_B_BLOCK_1=case_when(SELEC_B_BLOCK=="0"~"N",
SELEC_B_BLOCK=="1"~"Y",
                                SELEC_B_BLOCK=="2"~"Y",SELEC_B_BLOCK=="3"~"Y",
                                SELEC_B_BLOCK=="4"~"Y",SELEC_B_BLOCK=="5"~"Y",
                                SELEC_B_BLOCK=="6"~"Y",SELEC_B_BLOCK=="7"~"Y",
                                SELEC_B_BLOCK=="8"~"Y",SELEC_B_BLOCK=="9"~"Y",
                                SELEC_B_BLOCK=="10"~"Y",TRUE~NA_character_))


CVCOMP$SELEC_B_BLOCK_1<-as.factor(CVCOMP$SELEC_B_BLOCK_1)
```

```
CVCOMP%<>%mutate(SHORT_NITRATES_1=case_when(SHORT_NITRATES=="0"~"
N",SHORT_NITRATES=="1"~"Y",
                                SHORT_NITRATES=="2"~"Y",SHORT_NITRATES=="3"~"Y",
                                SHORT_NITRATES=="4"~"Y",SHORT_NITRATES=="5"~"Y",
                                SHORT_NITRATES=="6"~"Y",SHORT_NITRATES=="8"~"Y",
                                SHORT_NITRATES=="9"~"Y",TRUE~NA_character_))

CVCOMP$SHORT_NITRATES_1<-as.factor(CVCOMP$SHORT_NITRATES_1)

CVCOMP%<>%mutate(STATINS_1=case_when(STATINS=="0"~"N",STATINS=="1"~"Y
",
                                STATINS=="2"~"Y",STATINS=="3"~"Y",
                                STATINS=="4"~"Y",STATINS=="5"~"Y",
                                STATINS=="6"~"Y",STATINS=="7"~"Y",
                                STATINS=="8"~"Y",STATINS=="9"~"Y",
                                STATINS=="10"~"Y",STATINS=="12"~"Y",TRUE~NA_character_))

CVCOMP$STATINS_1<-as.factor(CVCOMP$STATINS_1)

CVCOMP%<>%mutate(THIAZIDES_DIUR_1=case_when(THIAZIDES_DIUR=="0"~"N",
THIAZIDES_DIUR=="1"~"Y",
                                THIAZIDES_DIUR=="2"~"Y",THIAZIDES_DIUR=="3"~"Y",
                                THIAZIDES_DIUR=="4"~"Y",THIAZIDES_DIUR=="5"~"Y",
                                THIAZIDES_DIUR=="6"~"Y",THIAZIDES_DIUR=="10"~"Y",
                                THIAZIDES_DIUR=="11"~"Y",TRUE~NA_character_))

CVCOMP$THIAZIDES_DIUR_1<-as.factor(CVCOMP$THIAZIDES_DIUR_1)

CVCOMP%<>%mutate(BIGUANIDES_1=case_when(BIGUANIDES=="0"~"N",BIGUANI
DES=="1"~"Y",TRUE~NA_character_))

CVCOMP$BIGUANIDES_1<-as.factor(CVCOMP$BIGUANIDES_1)
```

```
CVCOMP%<>%mutate(DPP4_1=case_when(DPP4=="0"~"N",DPP4=="1"~"Y",DPP4=="2
"~"Y",DPP4=="3"~"Y",TRUE~NA_character_))


CVCOMP$DPP4_1<-as.factor(CVCOMP$DPP4_1)


CVCOMP%<>%mutate(GLPR_AGONIST_1=case_when(GLPR_AGONIST=="0"~"N",GL
PR_AGONIST=="1"~"Y",GLPR_AGONIST=="2"~"Y",TRUE~NA_character_))


CVCOMP$GLPR_AGONIST_1<-as.factor(CVCOMP$GLPR_AGONIST_1)


CVCOMP%<>%mutate(GLUCOSIDASE_INHIB_1=case_when(GLUCOSIDASE_INHIB=
="0"~"N",GLUCOSIDASE_INHIB=="1"~"Y",GLUCOSIDASE_INHIB=="2"~"Y",GLUC
OSIDASE_INHIB=="3"~"Y",TRUE~NA_character_))


CVCOMP$GLUCOSIDASE_INHIB_1<-as.factor(CVCOMP$GLUCOSIDASE_INHIB_1)


CVCOMP%<>%mutate(INSULINS_1=case_when(INSULINS=="0"~"N",INSULINS=="1"
~"Y",
                        INSULINS=="2"~"Y",INSULINS=="3"~"Y",
                        INSULINS=="4"~"Y",INSULINS=="5"~"Y",
                        INSULINS=="6"~"Y",TRUE~NA_character_))


CVCOMP$INSULINS_1<-as.factor(CVCOMP$INSULINS_1)


CVCOMP%<>%mutate(MEGLITINIDES_1=case_when(MEGLITINIDES=="0"~"N",MEG
LITINIDES=="1"~"Y",TRUE~NA_character_))


CVCOMP$MEGLITINIDES_1<-as.factor(CVCOMP$MEGLITINIDES_1)


CVCOMP%<>%mutate(SULFONYLUREAS_1=case_when(SULFONYLUREAS=="0"~"N
",SULFONYLUREAS=="1"~"Y",SULFONYLUREAS=="2"~"Y",SULFONYLUREAS=="
3"~"Y",TRUE~NA_character_))


CVCOMP$SULFONYLUREAS_1<-as.factor(CVCOMP$SULFONYLUREAS_1)
```

```
CVCOMP%<>%mutate(THIAZOLIDINEDIONE_1=case_when(THIAZOLIDINEDIONE==
="0"~"N",THIAZOLIDINEDIONE=="1"~"Y",TRUE~NA_character_))

CVCOMP$THIAZOLIDINEDIONE_1<-as.factor(CVCOMP$THIAZOLIDINEDIONE_1)

CVCOMP%<>%mutate(DPP4_BIGUANIDES_1=case_when(DPP4_BIGUANIDES=="0"~
"N",DPP4_BIGUANIDES=="1"~"Y",TRUE~NA_character_))

CVCOMP$DPP4_BIGUANIDES_1<-as.factor(CVCOMP$DPP4_BIGUANIDES_1)

CVCOMP%<>%mutate(SODIUM_TRANSPORT_INHIB_1=case_when(SODIUM_TRAN
SPORT_INHIB=="0"~"N",SODIUM_TRANSPORT_INHIB=="1"~"Y",TRUE~NA_charact
er_))

CVCOMP$SODIUM_TRANSPORT_INHIB_1<-
as.factor(CVCOMP$SODIUM_TRANSPORT_INHIB_1)

CVCOMP%<>%mutate(SULFONYLUREAS_BIGUANIDES_1=case_when(SULFONYLU
REAS_BIGUANIDES=="0"~"N",SULFONYLUREAS_BIGUANIDES=="1"~"Y",TRUE~
NA_character_))

CVCOMP$SULFONYLUREAS_BIGUANIDES_1<-
as.factor(CVCOMP$SULFONYLUREAS_BIGUANIDES_1)



CVCOMP$DIABETES<-as.factor(CVCOMP$DIABETES)

fct_explicit_na(CVCOMP$DIABETES,na_level="N")

CVCOMP%<>%mutate(DIABETES=fct_explicit_na(DIABETES,na_level="N"))

fct_explicit_na(CVCOMP$HYPERTENSION,na_level="N")

CVCOMP%<>%mutate(HYPERTENSION=fct_explicit_na(HYPERTENSION,na_level="N
"))

fct_explicit_na(CVCOMP$HYPERCHOLESTEROL,na_level="N")
```

```
CVCOMP%<>%mutate(HYPERCHOLESTEROL=fct_explicit_na(HYPERCHOLESTEROL,na_level="N"))

fct_explicit_na(CVCOMP$PAD,na_level="N")
CVCOMP%<>%mutate(PAD=fct_explicit_na(PAD,na_level="N"))

fct_explicit_na(CVCOMP$CAD,na_level="N")
CVCOMP%<>%mutate(CAD=fct_explicit_na(CAD,na_level="N"))

fct_explicit_na(CVCOMP$PREV_MI,na_level="N")
CVCOMP%<>%mutate(PREV_MI=fct_explicit_na(PREV_MI,na_level="N"))

fct_explicit_na(CVCOMP$PREV_STROKE,na_level="N")
CVCOMP%<>%mutate(PREV_STROKE=fct_explicit_na(PREV_STROKE,na_level="N"))


CVCOMP%<>%mutate(ATENOLOL_1=case_when(ATENOLOL=="1"~"Y",TRUE~NA_character_))

CVCOMP$ATENOLOL_1<-as.factor(CVCOMP$ATENOLOL_1)

fct_explicit_na(CVCOMP$ATENOLOL_1,na_level="N")
CVCOMP%<>%mutate(ATENOLOL_1=fct_explicit_na(ATENOLOL_1,na_level="N"))

CVCOMP%<>%mutate(BISOPROLOL_1=case_when(BISOPROLOL=="1"~"Y",TRUE~NA_character_))

CVCOMP$BISOPROLOL_1<-as.factor(CVCOMP$BISOPROLOL_1)

fct_explicit_na(CVCOMP$BISOPROLOL_1,na_level="N")
CVCOMP%<>%mutate(BISOPROLOL_1=fct_explicit_na(BISOPROLOL_1,na_level="N"))
```

```
CVCOMP%<>%mutate(CLOPIDOGREL_1=case_when(CLOPIDOGREL=="1"~"Y",TRU
E~NA_character_))

CVCOMP$CLOPIDOGREL_1<-as.factor(CVCOMP$CLOPIDOGREL_1)

fct_explicit_na(CVCOMP$CLOPIDOGREL_1,na_level="N")

CVCOMP%<>%mutate(CLOPIDOGREL_1=fct_explicit_na(CLOPIDOGREL_1,na_level="
N"))

CVCOMP%<>%mutate(NEBIVOLOL_1=case_when(NEBIVOLOL=="1"~"Y",TRUE~NA
_character_))

CVCOMP$NEBIVOLOL_1<-as.factor(CVCOMP$NEBIVOLOL_1)

fct_explicit_na(CVCOMP$NEBIVOLOL_1,na_level="N")

CVCOMP%<>%mutate(NEBIVOLOL_1=fct_explicit_na(NEBIVOLOL_1,na_level="N"))

CVCOMP%<>%mutate(ASP_CLOPIDOGREL_1=case_when(ASP_CLOPIDOGREL=="1"
~"Y",TRUE~NA_character_))

CVCOMP$ASP_CLOPIDOGREL_1<-as.factor(CVCOMP$ASP_CLOPIDOGREL_1)

fct_explicit_na(CVCOMP$ASP_CLOPIDOGREL_1,na_level="N")

CVCOMP%<>%mutate(ASP_CLOPIDOGREL_1=fct_explicit_na(ASP_CLOPIDOGREL_1
,na_level="N"))

CVCOMP%<>%mutate(ASP_STAT_CLOPIDOGREL_1=case_when(ASP_STAT_CLOPID
OGREL=="1"~"Y",TRUE~NA_character_))
```

```
CVCOMP$ASP_STAT_CLOPIDOGREL_1<-
as.factor(CVCOMP$ASP_STAT_CLOPIDOGREL_1)


fct_explicit_na(CVCOMP$ASP_STAT_CLOPIDOGREL_1,na_level="N")

CVCOMP%<>%mutate(ASP_STAT_CLOPIDOGREL_1=fct_explicit_na(ASP_STAT_CLO
PIDOGREL_1,na_level="N"))



CVCOMP%<>%mutate(STAT_CLOPIDOGREL_1=case_when(STAT_CLOPIDOGREL==
"1"~"Y",TRUE~NA_character_))


CVCOMP$STAT_CLOPIDOGREL_1<-as.factor(CVCOMP$STAT_CLOPIDOGREL_1)


fct_explicit_na(CVCOMP$STAT_CLOPIDOGREL_1,na_level="N")

CVCOMP%<>%mutate(STAT_CLOPIDOGREL_1=fct_explicit_na(STAT_CLOPIDOGRE
L_1,na_level="N"))


CVCOMP%<>%mutate(RACE_CODE=case_when(RACE=="AMERICAN INDIAN OR
ALASKA NATIVE"~"OTHER",
                 RACE=="ASIAN"~"ASIAN",
                 RACE=="BLACK OR AFRICAN AMERICAN"~"OTHER",
                 RACE=="MULTIPLE"~"OTHER",
                 RACE=="NATIVE HAWAIIAN OR OTHER PACIFIC
ISLANDER"~"OTHER",
                 RACE=="WHITE"~"WHITE",TRUE~NA_character_))


CVCOMP$ADT_CV<-as.Date(CVCOMP$ADT_CV)

CVCOMP%<>%mutate(CVSTATUS=case_when(CVSTATUS==0~0,ADT_CV>COMMO
N_END_DATE~0,TRUE~1))


CVCOMP$RACE_CODE<-as.character(CVCOMP$RACE_CODE)


CVCOMP$PREVEXCT<-as.factor(CVCOMP$PREVEXCT)
```

```
CVCOMP$SMKBLN<-as.factor(CVCOMP$SMKBLN)

CVCOMP$PREVEXCT<-relevel(CVCOMP$PREVEXCT,ref="0")

CVCOMP$SMKBLN<-relevel(CVCOMP$SMKBLN,ref="2")

CVCOMP$HYPERTENSION<-relevel(CVCOMP$HYPERTENSION,ref="N")

CVCOMP$HYPERCHOLESTEROL<-
relevel(CVCOMP$HYPERCHOLESTEROL,ref="N")

CVCOMP$PAD<-relevel(CVCOMP$PAD,ref="N")

CVCOMP$CAD<-relevel(CVCOMP$CAD,ref="N")

CVCOMP$PREV_MI<-relevel(CVCOMP$PREV_MI,ref="N")

CVCOMP$PREV_STROKE<-relevel(CVCOMP$PREV_STROKE,ref="N")

CVCOMP$DIABETES<-relevel(CVCOMP$DIABETES,ref="N")

CVCOMP$ATENOLOL_1<-relevel(CVCOMP$ATENOLOL_1,ref="N")

CVCOMP$BISOPROLOL_1<-relevel(CVCOMP$BISOPROLOL_1,ref="N")

CVCOMP$NEBIVOLOL_1<-relevel(CVCOMP$NEBIVOLOL_1,ref="N")

CVCOMP$CLOPIDOGREL_1<-relevel(CVCOMP$CLOPIDOGREL_1,ref="N")

CVCOMP$ASPIRIN[is.na(CVCOMP$ASPIRIN)]<-0
CVCOMP$ABCIXIMAB[is.na(CVCOMP$ABCIXIMAB)]<-0
CVCOMP$ASPDIP[is.na(CVCOMP$ASPDIP)]<-0
```

```
CVCOMP$CARB[is.na(CVCOMP$CARB)]<-0
CVCOMP$DIP[is.na(CVCOMP$DIP)]<-0
CVCOMP$ETHICO[is.na(CVCOMP$ETHICO)]<-0
CVCOMP$ILO[is.na(CVCOMP$ILO)]<-0
CVCOMP$ICO[is.na(CVCOMP$ICO)]<-0
CVCOMP$MESOGLYCAN[is.na(CVCOMP$MESOGLYCAN)]<-0
CVCOMP$OZAGREL[is.na(CVCOMP$OZAGREL)]<-0
CVCOMP$SARPO[is.na(CVCOMP$SARPO)]<-0
CVCOMP$PRAS[is.na(CVCOMP$PRAS)]<-0
CVCOMP$PAI[is.na(CVCOMP$PAI)]<-0
CVCOMP$TICA[is.na(CVCOMP$TICA)]<-0
CVCOMP$TICLO[is.na(CVCOMP$TICLO)]<-0
CVCOMP$TRIF[is.na(CVCOMP$TRIF)]<-0
CVCOMP$TIRO[is.na(CVCOMP$TIRO)]<-0
CVCOMP$TREPR[is.na(CVCOMP$TREPR)]<-0

CVCOMP$ASPIRIN<-as.factor(CVCOMP$ASPIRIN)
CVCOMP$ASPIRIN<-relevel(CVCOMP$ASPIRIN,ref="0")

CVCOMP%<>%mutate(os.days=CVCOMP$os_yrs*365.25)
CVCOMP$os.days<-as.integer(CVCOMP$os.days)
CVCOMP%<>%mutate(ADY_CV_FULL=case_when(os.days>ADY_CV~ADY_CV,TRUE
~os.days))

CVCOMP$BB_1<-
paste(CVCOMP$SELEC_B_BLOCK_1,CVCOMP$NON_SELEC_B_BLOCK_1)

CVCOMP%<>%mutate(BB_2=case_when(BB_1=="N N"~"N",BB_1=="N Y"~"Y",
                    BB_1=="Y N"~"Y",BB_1=="Y Y"~"Y",TRUE~NA_character_))

CVCOMP$BB_2<-as.factor(CVCOMP$BB_2)
```

```
CVCOMP$ACEIARB_1<-paste(CVCOMP$ACEI_1,CVCOMP$ARB_1)


CVCOMP%<>%mutate(ACEIARB_2=case_when(ACEIARB_1=="N
N"~"N",ACEIARB_1=="N Y"~"Y",
                 ACEIARB_1=="Y                    N"~"Y",ACEIARB_1=="Y
Y"~"Y",TRUE~NA_character_))


CVCOMP$ACEIARB_2<-as.factor(CVCOMP$ACEIARB_2)



CVCOMP$IHD_CAD<-paste(CVCOMP$IHDIN,CVCOMP$CAD)


CVCOMP%<>%mutate(IHD_CAD_1=case_when(IHD_CAD=="0  N"~"N",IHD_CAD=="0
Y"~"Y",
                 IHD_CAD=="1                    N"~"Y",IHD_CAD=="1
Y"~"Y",TRUE~NA_character_))


CVCOMP$IHD_CAD_1<-as.factor(CVCOMP$IHD_CAD_1)




fct_explicit_na(CVCOMP$ACEIARB_2,na_level="N")
CVCOMP%<>%mutate(ACEIARB_2=fct_explicit_na(ACEIARB_2,na_level="N"))


fct_explicit_na(CVCOMP$BB_2,na_level="N")
CVCOMP%<>%mutate(BB_2=fct_explicit_na(BB_2,na_level="N"))


fct_explicit_na(CVCOMP$ANTI_COAG_1,na_level="N")
CVCOMP%<>%mutate(ANTI_COAG_1=fct_explicit_na(ANTI_COAG_1,na_level="N"))


fct_explicit_na(CVCOMP$ANTI_PLATE_1,na_level="N")
CVCOMP%<>%mutate(ANTI_PLATE_1=fct_explicit_na(ANTI_PLATE_1,na_level="N"))
```

```
fct_explicit_na(CVCOMP$DIHYDROPYRIDINE_1,na_level="N")

CVCOMP%<>%mutate(DIHYDROPYRIDINE_1=fct_explicit_na(DIHYDROPYRIDINE_1
,na_level="N"))


fct_explicit_na(ACM$LONG_NITRATES_1,na_level="N")

ACM%<>%mutate(LONG_NITRATES_1=fct_explicit_na(LONG_NITRATES_1,na_level=
"N"))


fct_explicit_na(CVCOMP$Loop_1,na_level="N")

CVCOMP%<>%mutate(Loop_1=fct_explicit_na(Loop_1,na_level="N"))


fct_explicit_na(CVCOMP$NON_DIHYDROPYRIDINE_1,na_level="N")

CVCOMP%<>%mutate(NON_DIHYDROPYRIDINE_1=fct_explicit_na(NON_DIHYDRO
PYRIDINE_1,na_level="N"))


fct_explicit_na(CVCOMP$STATINS_1,na_level="N")

CVCOMP%<>%mutate(STATINS_1=fct_explicit_na(STATINS_1,na_level="N"))


fct_explicit_na(CVCOMP$THIAZIDES_DIUR_1,na_level="N")

CVCOMP%<>%mutate(THIAZIDES_DIUR_1=fct_explicit_na(THIAZIDES_DIUR_1,na_l
evel="N"))


fct_explicit_na(CVCOMP$BIGUANIDES_1,na_level="N")

CVCOMP%<>%mutate(BIGUANIDES_1=fct_explicit_na(BIGUANIDES_1,na_level="N"))


fct_explicit_na(CVCOMP$INSULINS_1,na_level="N")

CVCOMP%<>%mutate(INSULINS_1=fct_explicit_na(INSULINS_1,na_level="N"))



fct_explicit_na(CVCOMP$SULFONYLUREAS_1,na_level="N")

CVCOMP%<>%mutate(SULFONYLUREAS_1=fct_explicit_na(SULFONYLUREAS_1,na
_level="N"))
```

```
CVCOMP$ASPCLOP<-paste(CVCOMP$ASPIRIN_1,CVCOMP$CLOPIDOGREL_1)


CVCOMP%<>%mutate(ASPCLOPCOMBO=case_when(ASPCLOP=="N
N"~"N",ASPCLOP=="N Y"~"N",
                 ASPCLOP=="Y                 N"~"N",ASPCLOP=="Y
Y"~"Y",TRUE~NA_character_))


CVCOMP$ASPCLOPCOMBO<-as.factor(CVCOMP$ASPCLOPCOMBO)


#Had -1 value

CVCOMP$CVSTATUS[CVCOMP$USUBJID=="9466"]<-NA

CVCOMP$ADY_CV[CVCOMP$USUBJID=="9466"]<-NA

CVCOMP$ADY_CV_FULL[CVCOMP$USUBJID=="9466"]<-NA


CVCOMP%<>%distinct(USUBJID,AGE,.keep_all=TRUE)



CVCOMP$CV_CRITERIA<-CVCOMP$RACE<-CVCOMP$SITEID<-
CVCOMP$LSTCT<-CVCOMP$TREATMENT_YEARS<-CVCOMP$os_yrs<-
CVCOMP$ALPHABETABLOCK<-NULL

CVCOMP$ACEI<-CVCOMP$CVCOMP<-CVCOMP$ANTI_COAG<-
CVCOMP$ANTI_PLATE<-CVCOMP$CHOL_BILE_ABSORB_INHIB<-
CVCOMP$CLASS3<-NULL

CVCOMP$DIHYDROPYRIDINE<-CVCOMP$DIR_RENIN_INHIB<-
CVCOMP$Fibrates<-CVCOMP$LONG_NITRATES<-CVCOMP$Loop<-
CVCOMP$MRA<-NULL

CVCOMP$Niacin<-CVCOMP$NON_DIHYDROPYRIDINE<-
CVCOMP$NON_SELEC_B_BLOCK<-CVCOMP$Other<-CVCOMP$SUPPLEMENTS<-
CVCOMP$SELEC_B_BLOCK<-NULL

CVCOMP$SHORT_NITRATES<-CVCOMP$STATINS<-
CVCOMP$THIAZIDES_DIUR<-CVCOMP$NA.x<-CVCOMP$BIGUANIDES<-
CVCOMP$DPP4<-CVCOMP$DPP4_BIGUANIDES<-NULL

CVCOMP$GLPR_AGONIST<-CVCOMP$GLUCOSIDASE_INHIB<-
CVCOMP$INSULINS<-CVCOMP$MEGLITINIDES<-CVCOMP$OTHER<-
CVCOMP$SODIUM_TRANSPORT_INHIB<-NULL
```

```
CVCOMP$SULFONYLUREAS<-CVCOMP$SULFONYLUREAS_BIGUANIDES<-
CVCOMP$THIAZOLIDINEDIONE<-CVCOMP$NA.y<-
CVCOMP$TREATED_FOR_PAD<-NULL

CVCOMP$`CV risk criteria at study entry`<-CVCOMP$DIABETES_ORGAN_DISEASE<-
CVCOMP$DIABETES_EYES<-CVCOMP$DIABETES_KIDNEYS<-NULL

CVCOMP$DIABETES_LIMBS<-CVCOMP$`History of CV disease at study entry`<-
CVCOMP$`Met protocol CV entry criteria`<-NULL

CVCOMP$GLUC_CAT<-CVCOMP$BB_1<-CVCOMP$ACEIARB_1<-NULL

CVCOMP$ATENOLOL<-CVCOMP$BISOPROLOL<-CVCOMP$CLOPIDOGREL<-
CVCOMP$NEBIVOLOL<-NULL

CVCOMP$ASP_BISOPROLOL<-CVCOMP$ASP_CLOPIDOGREL<-
CVCOMP$ASP_STAT_CLOPIDOGREL<-CVCOMP$STAT_CLOPIDOGREL<-NULL

CVCOMP$ASPCLOP<-CVCOMP$IHD_CAD<-NULL




CVCOMP$CVSTATUS<-as.factor(CVCOMP$CVSTATUS)


CVCOMP_TABLE<-
tableby(~AGE+SEX+TRTPN+BMI+SMKBLN+PACK_YRS+RACE_CODE+DIABETES+

HYPERCHOLESTEROL+PAD+CAD+HYPERTENSION+PREV_MI+PREV_STROKE+

ALPHABETABLOCK_1+ACEI_1+ARB_1+ANTI_PLATE_1+ANTI_COAG_1+

CHOL_BILE_ABSORB_INHIB_1+CLASS3_1+DIHYDROPYRIDINE_1+DIR_RENIN_I
NHIB_1+

Fibrates_1+LONG_NITRATES_1+Loop_1+MRA_1+ACEIARB_2+BB_2+NON_DIHYDR
OPYRIDINE_1+

NON_SELEC_B_BLOCK_1+SELEC_B_BLOCK_1+SHORT_NITRATES_1+

STATINS_1+THIAZIDES_DIUR_1+BIGUANIDES_1+DPP4_1+GLPR_AGONIST_1+

GLUCOSIDASE_INHIB_1+INSULINS_1+MEGLITINIDES_1+SULFONYLUREAS_1+

THIAZOLIDINEDIONE_1+DPP4_BIGUANIDES_1+SODIUM_TRANSPORT_INHIB_1+
```

SULFONYLUREAS_BIGUANIDES_1+CVSTATUS+ADY_CV+FEV1+

CV_RISK_CRIT_ONLY+IHDIN+HF+IHD_CAD_1+ATENOLOL_1+BISOPROLOL_1+

NEBIVOLOL_1+CLOPIDOGREL_1+ASPIRIN+ASP_STAT_CLOPIDOGREL_1+

ASP_CLOPIDOGREL_1+STAT_CLOPIDOGREL_1,data=subset(CVCOMP,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPIDOGREL_1=="N"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&

ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&

SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&

TRIF=="0"&TIRO=="0"&TREPR=="0"))

summary(CVCOMP_TABLE,title="CVCOMP_TABLE")

CVCOMP$CVSTATUS<-as.numeric(CVCOMP$CVSTATUS)

CVCOMP%<>%mutate(CVSTATUS=case_when(CVSTATUS=="1"~0,CVSTATUS=="2"~1,TRUE~NA_real_))

coxph(Surv(ADY_CV_FULL,CVSTATUS)~AGE+SEX+BMI+SMKBLN+PACK_YRS+PAD+PREV_STROKE+IHD_CAD_1+HF+

HYPERTENSION+HYPERCHOLESTEROL+DIABETES+ASPIRIN,

data=subset(CVCOMP,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPIDOGREL_1=="N"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&

ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&

SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&

```
TRIF=="0"&TIRO=="0"&TREPR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)
#Exacerbation Rate

v<-merge(exac,sla,by="USUBJID",all.x=TRUE,all.y=TRUE)

w<-merge(v,medstable,by="USUBJID",all.x=TRUE,all.y=TRUE)

y<-merge(w,cvcrit,by="USUBJID",all.x=TRUE,all.y=TRUE)

z<-merge(y,glucose,by="USUBJID",all.x=TRUE,all.y=TRUE)

z1<-merge(z,fev,by="USUBJID",all.x=TRUE,all.y=TRUE)

z2<-merge(z1,cvhist,by="USUBJID",all.x=TRUE,all.y=TRUE)

z3<-merge(z2,t2e,by="USUBJID",all.x=TRUE,all.y=TRUE)

z4<-merge(z3,t2em,by="USUBJID",all.x=TRUE,all.y=TRUE)

z5<-merge(z4,t2es,by="USUBJID",all.x=TRUE,all.y=TRUE)

z6<-merge(z5,smoking,by="USUBJID",all.x=TRUE,all.y=TRUE)

z7<-merge(z6,test,by="USUBJID",all.x=TRUE,all.y=TRUE)

EXAC<-z7


colnames(EXAC)[colnames(EXAC)=="Alpha and beta blocking"]<-"ALPHABETABLOCK"
colnames(EXAC)[colnames(EXAC)=="Angiotensin-converting Enzyme Inhibitors"]<-
"ACEI"
colnames(EXAC)[colnames(EXAC)=="Angiotensin receptor blockers"]<-"ARB"
```

```
colnames(EXAC)[colnames(EXAC)=="Anti-coagulant therapy"]<-"ANTI_COAG"

colnames(EXAC)[colnames(EXAC)=="Anti-platelet therapy"]<-"ANTI_PLATE"

colnames(EXAC)[colnames(EXAC)=="Cholesterol and bile acid absorption inhibitors"]<-
"CHOL_BILE_ABSORB_INHIB"

colnames(EXAC)[colnames(EXAC)=="Class III"]<-"CLASS3"

colnames(EXAC)[colnames(EXAC)=="Dihydropyridine"]<-"DIHYDROPYRIDINE"

colnames(EXAC)[colnames(EXAC)=="Direct Renin Inhibitors"]<-"DIR_RENIN_INHIB"

colnames(EXAC)[colnames(EXAC)=="Long-acting"]<-"LONG_NITRATES"

colnames(EXAC)[colnames(EXAC)=="Short-acting"]<-"SHORT_NITRATES"

colnames(EXAC)[colnames(EXAC)=="Mineralocorticoid Receptor Antagonists"]<-"MRA"

colnames(EXAC)[colnames(EXAC)=="Non-dihydropyridine"]<-
"NON_DIHYDROPYRIDINE"

colnames(EXAC)[colnames(EXAC)=="Non-selective beta-adrenergic receptor blocker"]<-
"NON_SELEC_B_BLOCK"

colnames(EXAC)[colnames(EXAC)=="Other lipid modifying"]<-"SUPPLEMENTS"

colnames(EXAC)[colnames(EXAC)=="Selective beta1-adrenergic receptor blocker"]<-
"SELEC_B_BLOCK"

colnames(EXAC)[colnames(EXAC)=="Statins"]<-"STATINS"

colnames(EXAC)[colnames(EXAC)=="Thiazides and Thiazide like Diuretics"]<-
"THIAZIDES_DIUR"

colnames(EXAC)[colnames(EXAC)=="Being treated for diabetes mellitus"]<-"DIABETES"

colnames(EXAC)[colnames(EXAC)=="Being treated for hypercholesterolemia"]<-
"HYPERCHOLESTEROL"

colnames(EXAC)[colnames(EXAC)=="Being treated for hypertension"]<-
"HYPERTENSION"

colnames(EXAC)[colnames(EXAC)=="Diabetes mellitus with target organ disease"]<-
"DIABETES_ORGAN_DISEASE"

colnames(EXAC)[colnames(EXAC)=="Diabetes mellitus with target organ disease: eyes"]<-
"DIABETES_EYES"

colnames(EXAC)[colnames(EXAC)=="Being treated for peripheral arterial disease"]<-
"TREATED_FOR_PAD"

colnames(EXAC)[colnames(EXAC)=="Diabetes mellitus with target organ disease:
limbs/extremities"]<-"DIABETES_LIMBS"

colnames(EXAC)[colnames(EXAC)=="Diabetes mellitus with target organ disease:
kidneys"]<-"DIABETES_KIDNEYS"

colnames(EXAC)[colnames(EXAC)=="Established coronary artery disease (CAD)"]<-
"CAD"
```

```
colnames(EXAC)[colnames(EXAC)=="Established peripheral arterial disease (PAD)"]<-"PAD"

colnames(EXAC)[colnames(EXAC)=="Previous MI"]<-"PREV_MI"

colnames(EXAC)[colnames(EXAC)=="Previous stroke"]<-"PREV_STROKE"



EXAC$TRTPN<-as.factor(EXAC$TRTPN)

EXAC$PREV_MI<-as.factor(EXAC$PREV_MI)

EXAC$PREV_STROKE<-as.factor(EXAC$PREV_STROKE)

EXAC$CAD<-as.factor(EXAC$CAD)

EXAC$PAD<-as.factor(EXAC$PAD)

EXAC$HYPERTENSION<-as.factor(EXAC$HYPERTENSION)

EXAC$HYPERCHOLESTEROL<-as.factor(EXAC$HYPERCHOLESTEROL)

EXAC$TREATED_FOR_PAD<-as.factor(EXAC$TREATED_FOR_PAD)

EXAC$DIABETES_ORGAN_DISEASE<-
as.factor(EXAC$DIABETES_ORGAN_DISEASE)

EXAC$DIABETES_EYES<-as.factor(EXAC$DIABETES_EYES)

EXAC$DIABETES_KIDNEYS<-as.factor(EXAC$DIABETES_KIDNEYS)

EXAC$DIABETES_LIMBS<-as.factor(EXAC$DIABETES_LIMBS)

EXAC$'Met protocol CV entry criteria'<-as.factor(EXAC$'Met protocol CV entry criteria')

EXAC$'History of CV disease at study entry'<-as.factor(EXAC$'History of CV disease at study entry')

EXAC$DIABETES<-as.factor(EXAC$DIABETES)

EXAC$PREVEXCT<-as.factor(EXAC$PREVEXCT)


EXAC%<>%mutate(ALPHABETABLOCK_1=case_when(ALPHABETABLOCK=="0"~"
N",ALPHABETABLOCK=="1"~"Y",


ALPHABETABLOCK=="2"~"Y",ALPHABETABLOCK=="3"~"Y",


ALPHABETABLOCK=="4"~"Y",ALPHABETABLOCK=="5"~"Y",


ALPHABETABLOCK=="6"~"Y",ALPHABETABLOCK=="7"~"Y",TRUE~NA_character_
))
```

```
EXAC$ALPHABETABLOCK_1<-as.factor(EXAC$ALPHABETABLOCK_1)

EXAC%<>%mutate(ACEI_1=case_when(ACEI=="0"~"N",ACEI=="1"~"Y",
                    ACEI=="2"~"Y",ACEI=="3"~"Y",
                    ACEI=="4"~"Y",ACEI=="5"~"Y",
                    ACEI=="6"~"Y",ACEI=="7"~"Y",
                    ACEI=="8"~"Y",ACEI=="9"~"Y",
                    ACEI=="10"~"Y",ACEI=="11"~"Y",
                    ACEI=="18"~"Y",ACEI=="19"~"Y",
                    ACEI=="26"~"Y",TRUE~NA_character_))

EXAC$ACEI_1<-as.factor(EXAC$ACEI_1)

EXAC%<>%mutate(ARB_1=case_when(ARB=="0"~"N",ARB=="1"~"Y",
                   ARB=="2"~"Y",ARB=="3"~"Y",
                   ARB=="4"~"Y",ARB=="5"~"Y",
                   ARB=="6"~"Y",ARB=="7"~"Y",
                   ARB=="8"~"Y",ARB=="9"~"Y",
                   ARB=="10"~"Y",ARB=="11"~"Y",
                   ARB=="12"~"Y",TRUE~NA_character_))

EXAC$ARB_1<-as.factor(EXAC$ARB_1)

EXAC%<>%mutate(ANTI_COAG_1=case_when(ANTI_COAG=="0"~"N",ANTI_COAG=
="1"~"Y",
                       ANTI_COAG=="2"~"Y",ANTI_COAG=="3"~"Y",
                       ANTI_COAG=="4"~"Y",ANTI_COAG=="5"~"Y",
                       ANTI_COAG=="6"~"Y",ANTI_COAG=="7"~"Y",
                       ANTI_COAG=="8"~"Y",ANTI_COAG=="9"~"Y",
                       ANTI_COAG=="10"~"Y",ANTI_COAG=="11"~"Y",
                       ANTI_COAG=="12"~"Y",ANTI_COAG=="13"~"Y",

ANTI_COAG=="14"~"Y",ANTI_COAG=="19"~"Y",TRUE~NA_character_))
```

```
EXAC$ANTI_COAG_1<-as.factor(EXAC$ANTI_COAG_1)


EXAC%<>%mutate(ANTI_PLATE_1=case_when(ANTI_PLATE=="0"~"N",ANTI_PLATE=="1"~"Y",
                                      ANTI_PLATE=="2"~"Y",ANTI_PLATE=="3"~"Y",
                                      ANTI_PLATE=="4"~"Y",ANTI_PLATE=="5"~"Y",
                                      ANTI_PLATE=="6"~"Y",ANTI_PLATE=="7"~"Y",
                                      ANTI_PLATE=="8"~"Y",ANTI_PLATE=="9"~"Y",
                                      ANTI_PLATE=="10"~"Y",ANTI_PLATE=="11"~"Y",
ANTI_PLATE=="12"~"Y",ANTI_PLATE=="13"~"Y",TRUE~NA_character_))


EXAC$ANTI_PLATE_1<-as.factor(EXAC$ANTI_PLATE_1)


EXAC%<>%mutate(CHOL_BILE_ABSORB_INHIB_1=case_when(CHOL_BILE_ABSORB_INHIB=="0"~"N",CHOL_BILE_ABSORB_INHIB=="1"~"Y",
CHOL_BILE_ABSORB_INHIB=="2"~"Y",CHOL_BILE_ABSORB_INHIB=="3"~"Y",TRUE~NA_character_))



EXAC$CHOL_BILE_ABSORB_INHIB_1<-
as.factor(EXAC$CHOL_BILE_ABSORB_INHIB_1)


EXAC%<>%mutate(CLASS3_1=case_when(CLASS3=="0"~"N",CLASS3=="1"~"Y",
                                  CLASS3=="2"~"Y",CLASS3=="3"~"Y",
                                  CLASS3=="4"~"Y",CLASS3=="5"~"Y",
                                  CLASS3=="6"~"Y",CLASS3=="10"~"Y",TRUE~NA_character_))


EXAC$CLASS3_1<-as.factor(EXAC$CLASS3_1)


EXAC%<>%mutate(DIHYDROPYRIDINE_1=case_when(DIHYDROPYRIDINE=="0"~"N",DIHYDROPYRIDINE=="1"~"Y",
```

```
DIHYDROPYRIDINE=="2"~"Y",DIHYDROPYRIDINE=="3"~"Y",

DIHYDROPYRIDINE=="4"~"Y",DIHYDROPYRIDINE=="5"~"Y",

DIHYDROPYRIDINE=="6"~"Y",DIHYDROPYRIDINE=="7"~"Y",

DIHYDROPYRIDINE=="8"~"Y",DIHYDROPYRIDINE=="9"~"Y",
                        DIHYDROPYRIDINE=="11"~"Y",TRUE~NA_character_))

EXAC$DIHYDROPYRIDINE_1<-as.factor(EXAC$DIHYDROPYRIDINE_1)

EXAC%<>%mutate(DIR_RENIN_INHIB_1=case_when(DIR_RENIN_INHIB=="0"~"N",D
IR_RENIN_INHIB=="1"~"Y",
                        DIR_RENIN_INHIB=="2"~"Y",TRUE~NA_character_))

EXAC$DIR_RENIN_INHIB_1<-as.factor(EXAC$DIR_RENIN_INHIB_1)

EXAC%<>%mutate(Fibrates_1=case_when(Fibrates=="0"~"N",Fibrates=="1"~"Y",
                        Fibrates=="2"~"Y",Fibrates=="3"~"Y",
                        Fibrates=="4"~"Y",Fibrates=="5"~"Y",
                        Fibrates=="6"~"Y",TRUE~NA_character_))

EXAC$Fibrates_1<-as.factor(EXAC$Fibrates_1)

EXAC%<>%mutate(LONG_NITRATES_1=case_when(LONG_NITRATES=="0"~"N",LO
NG_NITRATES=="1"~"Y",
                        LONG_NITRATES=="2"~"Y",LONG_NITRATES=="3"~"Y",
                        LONG_NITRATES=="4"~"Y",LONG_NITRATES=="5"~"Y",
                        LONG_NITRATES=="6"~"Y",TRUE~NA_character_))

EXAC$LONG_NITRATES_1<-as.factor(EXAC$LONG_NITRATES_1)

EXAC%<>%mutate(Loop_1=case_when(Loop=="0"~"N",Loop=="1"~"Y",
```

```
                    Loop=="2"~"Y",Loop=="3"~"Y",
                    Loop=="4"~"Y",Loop=="5"~"Y",
                    Loop=="6"~"Y",Loop=="7"~"Y",
                    Loop=="8"~"Y",Loop=="10"~"Y",
                    Loop=="11"~"Y",Loop=="12"~"Y",

Loop=="13"~"Y",Loop=="21"~"Y",Loop=="24"~"Y",TRUE~NA_character_))

EXAC$Loop_1<-as.factor(EXAC$Loop_1)

EXAC%<>%mutate(MRA_1=case_when(MRA=="0"~"N",MRA=="1"~"Y",
                    MRA=="2"~"Y",MRA=="3"~"Y",
                    MRA=="4"~"Y",MRA=="5"~"Y",
                    MRA=="6"~"Y",TRUE~NA_character_))

EXAC$MRA_1<-as.factor(EXAC$MRA_1)

EXAC%<>%mutate(NON_DIHYDROPYRIDINE_1=case_when(NON_DIHYDROPYRIDI
NE=="0"~"N",NON_DIHYDROPYRIDINE=="1"~"Y",

NON_DIHYDROPYRIDINE=="2"~"Y",NON_DIHYDROPYRIDINE=="3"~"Y",

NON_DIHYDROPYRIDINE=="4"~"Y",NON_DIHYDROPYRIDINE=="5"~"Y",

NON_DIHYDROPYRIDINE=="6"~"Y",NON_DIHYDROPYRIDINE=="10"~"Y",

NON_DIHYDROPYRIDINE=="12"~"Y",TRUE~NA_character_))

EXAC$NON_DIHYDROPYRIDINE_1<-as.factor(EXAC$NON_DIHYDROPYRIDINE_1)

EXAC%<>%mutate(NON_SELEC_B_BLOCK_1=case_when(NON_SELEC_B_BLOCK==
"0"~"N",NON_SELEC_B_BLOCK=="1"~"Y",

NON_SELEC_B_BLOCK=="2"~"Y",NON_SELEC_B_BLOCK=="3"~"Y",
```

```
NON_SELEC_B_BLOCK=="4"~"Y",NON_SELEC_B_BLOCK=="7"~"Y",TRUE~NA_cha
racter_))



EXAC$NON_SELEC_B_BLOCK_1<-as.factor(EXAC$NON_SELEC_B_BLOCK_1)


EXAC%<>%mutate(SELEC_B_BLOCK_1=case_when(SELEC_B_BLOCK=="0"~"N",SE
LEC_B_BLOCK=="1"~"Y",
                              SELEC_B_BLOCK=="2"~"Y",SELEC_B_BLOCK=="3"~"Y",
                              SELEC_B_BLOCK=="4"~"Y",SELEC_B_BLOCK=="5"~"Y",
                              SELEC_B_BLOCK=="6"~"Y",SELEC_B_BLOCK=="7"~"Y",
                              SELEC_B_BLOCK=="8"~"Y",SELEC_B_BLOCK=="9"~"Y",
                              SELEC_B_BLOCK=="10"~"Y",TRUE~NA_character_))


EXAC$SELEC_B_BLOCK_1<-as.factor(EXAC$SELEC_B_BLOCK_1)


EXAC%<>%mutate(SHORT_NITRATES_1=case_when(SHORT_NITRATES=="0"~"N",S
HORT_NITRATES=="1"~"Y",
                               SHORT_NITRATES=="2"~"Y",SHORT_NITRATES=="3"~"Y",
                               SHORT_NITRATES=="4"~"Y",SHORT_NITRATES=="5"~"Y",
                               SHORT_NITRATES=="6"~"Y",SHORT_NITRATES=="8"~"Y",
                               SHORT_NITRATES=="9"~"Y",TRUE~NA_character_))


EXAC$SHORT_NITRATES_1<-as.factor(EXAC$SHORT_NITRATES_1)


EXAC%<>%mutate(STATINS_1=case_when(STATINS=="0"~"N",STATINS=="1"~"Y",
                            STATINS=="2"~"Y",STATINS=="3"~"Y",
                            STATINS=="4"~"Y",STATINS=="5"~"Y",
                            STATINS=="6"~"Y",STATINS=="7"~"Y",
                            STATINS=="8"~"Y",STATINS=="9"~"Y",
                            STATINS=="10"~"Y",STATINS=="12"~"Y",TRUE~NA_character_))
```

```
EXAC$STATINS_1<-as.factor(EXAC$STATINS_1)


EXAC%<>%mutate(THIAZIDES_DIUR_1=case_when(THIAZIDES_DIUR=="0"~"N",TH
IAZIDES_DIUR=="1"~"Y",
                                          THIAZIDES_DIUR=="2"~"Y",THIAZIDES_DIUR=="3"~"Y",
                                          THIAZIDES_DIUR=="4"~"Y",THIAZIDES_DIUR=="5"~"Y",
                                          THIAZIDES_DIUR=="6"~"Y",THIAZIDES_DIUR=="10"~"Y",
                                          THIAZIDES_DIUR=="11"~"Y",TRUE~NA_character_))


EXAC$THIAZIDES_DIUR_1<-as.factor(EXAC$THIAZIDES_DIUR_1)


EXAC%<>%mutate(BIGUANIDES_1=case_when(BIGUANIDES=="0"~"N",BIGUANIDE
S=="1"~"Y",TRUE~NA_character_))


EXAC$BIGUANIDES_1<-as.factor(EXAC$BIGUANIDES_1)


EXAC%<>%mutate(DPP4_1=case_when(DPP4=="0"~"N",DPP4=="1"~"Y",DPP4=="2"~"
Y",DPP4=="3"~"Y",TRUE~NA_character_))


EXAC$DPP4_1<-as.factor(EXAC$DPP4_1)


EXAC%<>%mutate(DPP4_BIGUANIDES_1=case_when(DPP4_BIGUANIDES=="0"~"N",
DPP4_BIGUANIDES=="1"~"Y",TRUE~NA_character_))


EXAC$DPP4_BIGUANIDES_1<-as.factor(EXAC$DPP4_BIGUANIDES_1)


EXAC%<>%mutate(GLPR_AGONIST_1=case_when(GLPR_AGONIST=="0"~"N",GLPR_
AGONIST=="1"~"Y",GLPR_AGONIST=="2"~"Y",TRUE~NA_character_))


EXAC$GLPR_AGONIST_1<-as.factor(EXAC$GLPR_AGONIST_1)


EXAC%<>%mutate(GLUCOSIDASE_INHIB_1=case_when(GLUCOSIDASE_INHIB=="0
"~"N",GLUCOSIDASE_INHIB=="1"~"Y",GLUCOSIDASE_INHIB=="2"~"Y",GLUCOSI
DASE_INHIB=="3"~"Y",TRUE~NA_character_))
```

```
EXAC$GLUCOSIDASE_INHIB_1<-as.factor(EXAC$GLUCOSIDASE_INHIB_1)


EXAC%<>%mutate(INSULINS_1=case_when(INSULINS=="0"~"N",INSULINS=="1"~"Y
",
                                    INSULINS=="2"~"Y",INSULINS=="3"~"Y",
                                    INSULINS=="4"~"Y",INSULINS=="5"~"Y",
                                    INSULINS=="6"~"Y",TRUE~NA_character_))


EXAC$INSULINS_1<-as.factor(EXAC$INSULINS_1)


EXAC%<>%mutate(MEGLITINIDES_1=case_when(MEGLITINIDES=="0"~"N",MEGLIT
INIDES=="1"~"Y",TRUE~NA_character_))


EXAC$MEGLITINIDES_1<-as.factor(EXAC$MEGLITINIDES_1)


EXAC%<>%mutate(SODIUM_TRANSPORT_INHIB_1=case_when(SODIUM_TRANSPO
RT_INHIB=="0"~"N",SODIUM_TRANSPORT_INHIB=="1"~"Y",TRUE~NA_character_))


EXAC$SODIUM_TRANSPORT_INHIB_1<-
as.factor(EXAC$SODIUM_TRANSPORT_INHIB_1)


EXAC%<>%mutate(SULFONYLUREAS_1=case_when(SULFONYLUREAS=="0"~"N",S
ULFONYLUREAS=="1"~"Y",SULFONYLUREAS=="2"~"Y",SULFONYLUREAS=="3"~
"Y",TRUE~NA_character_))


EXAC$SULFONYLUREAS_1<-as.factor(EXAC$SULFONYLUREAS_1)


EXAC%<>%mutate(SULFONYLUREAS_BIGUANIDES_1=case_when(SULFONYLURE
AS_BIGUANIDES=="0"~"N",SULFONYLUREAS_BIGUANIDES=="1"~"Y",TRUE~NA
_character_))


EXAC$SULFONYLUREAS_BIGUANIDES_1<-
as.factor(EXAC$SULFONYLUREAS_BIGUANIDES_1)
```

```
EXAC%<>%mutate(THIAZOLIDINEDIONE_1=case_when(THIAZOLIDINEDIONE=="0
"~"N",THIAZOLIDINEDIONE=="1"~"Y",TRUE~NA_character_))

EXAC$THIAZOLIDINEDIONE_1<-as.factor(EXAC$THIAZOLIDINEDIONE_1)

EXAC%<>%mutate(ATENOLOL_1=case_when(ATENOLOL=="1"~"Y",TRUE~NA_chara
cter_))

EXAC$ATENOLOL_1<-as.factor(EXAC$ATENOLOL_1)

fct_explicit_na(EXAC$ATENOLOL_1,na_level="N")
EXAC%<>%mutate(ATENOLOL_1=fct_explicit_na(ATENOLOL_1,na_level="N"))

EXAC%<>%mutate(BISOPROLOL_1=case_when(BISOPROLOL=="1"~"Y",TRUE~NA_c
haracter_))

EXAC$BISOPROLOL_1<-as.factor(EXAC$BISOPROLOL_1)

fct_explicit_na(EXAC$BISOPROLOL_1,na_level="N")
EXAC%<>%mutate(BISOPROLOL_1=fct_explicit_na(BISOPROLOL_1,na_level="N"))

EXAC%<>%mutate(CLOPIDOGREL_1=case_when(CLOPIDOGREL=="1"~"Y",TRUE~N
A_character_))

EXAC$CLOPIDOGREL_1<-as.factor(EXAC$CLOPIDOGREL_1)

fct_explicit_na(EXAC$CLOPIDOGREL_1,na_level="N")
EXAC%<>%mutate(CLOPIDOGREL_1=fct_explicit_na(CLOPIDOGREL_1,na_level="N"))

EXAC%<>%mutate(NEBIVOLOL_1=case_when(NEBIVOLOL=="1"~"Y",TRUE~NA_cha
racter_))
```

```
EXAC$NEBIVOLOL_1<-as.factor(EXAC$NEBIVOLOL_1)

fct_explicit_na(EXAC$NEBIVOLOL_1,na_level="N")
EXAC%<>%mutate(NEBIVOLOL_1=fct_explicit_na(NEBIVOLOL_1,na_level="N"))









EXAC$ASPIRIN[is.na(EXAC$ASPIRIN)]<-0
EXAC$ABCIXIMAB[is.na(EXAC$ABCIXIMAB)]<-0
EXAC$ASPDIP[is.na(EXAC$ASPDIP)]<-0
EXAC$CARB[is.na(EXAC$CARB)]<-0
EXAC$DIP[is.na(EXAC$DIP)]<-0
EXAC$ETHICO[is.na(EXAC$ETHICO)]<-0
EXAC$ILO[is.na(EXAC$ILO)]<-0
EXAC$ICO[is.na(EXAC$ICO)]<-0
EXAC$MESOGLYCAN[is.na(EXAC$MESOGLYCAN)]<-0
EXAC$OZAGREL[is.na(EXAC$OZAGREL)]<-0
EXAC$SARPO[is.na(EXAC$SARPO)]<-0
EXAC$PRAS[is.na(EXAC$PRAS)]<-0
EXAC$PAI[is.na(EXAC$PAI)]<-0
EXAC$TICA[is.na(EXAC$TICA)]<-0
EXAC$TICLO[is.na(EXAC$TICLO)]<-0
EXAC$TRIF[is.na(EXAC$TRIF)]<-0
EXAC$TIRO[is.na(EXAC$TIRO)]<-0
EXAC$TREPR[is.na(EXAC$TREPR)]<-0

EXAC$ASPIRIN<-as.factor(EXAC$ASPIRIN)
```

```
EXAC$ASPIRIN<-relevel(EXAC$ASPIRIN,ref="0")



EXAC$DIABETES<-as.factor(EXAC$DIABETES)

fct_explicit_na(EXAC$DIABETES,na_level="N")

EXAC%<>%mutate(DIABETES=fct_explicit_na(DIABETES,na_level="N"))


fct_explicit_na(EXAC$HYPERTENSION,na_level="N")

EXAC%<>%mutate(HYPERTENSION=fct_explicit_na(HYPERTENSION,na_level="N"))


fct_explicit_na(EXAC$HYPERCHOLESTEROL,na_level="N")

EXAC%<>%mutate(HYPERCHOLESTEROL=fct_explicit_na(HYPERCHOLESTEROL,na
_level="N"))


fct_explicit_na(EXAC$PAD,na_level="N")

EXAC%<>%mutate(PAD=fct_explicit_na(PAD,na_level="N"))


fct_explicit_na(EXAC$CAD,na_level="N")

EXAC%<>%mutate(CAD=fct_explicit_na(CAD,na_level="N"))


fct_explicit_na(EXAC$PREV_MI,na_level="N")

EXAC%<>%mutate(PREV_MI=fct_explicit_na(PREV_MI,na_level="N"))


fct_explicit_na(EXAC$PREV_STROKE,na_level="N")

EXAC%<>%mutate(PREV_STROKE=fct_explicit_na(PREV_STROKE,na_level="N"))


EXAC$TOTAL_EXAC[is.na(EXAC$TOTAL_EXAC)]<-0

EXAC$MODERATE[is.na(EXAC$MODERATE)]<-0

EXAC$SEVERE[is.na(EXAC$SEVERE)]<-0



EXAC%<>%mutate(RACE_CODE=case_when(RACE=="AMERICAN INDIAN OR
ALASKA NATIVE"~"OTHER",
```

```
                    RACE=="ASIAN"~"ASIAN",
                    RACE=="BLACK OR AFRICAN AMERICAN"~"OTHER",
                    RACE=="MULTIPLE"~"OTHER",
                    RACE=="NATIVE HAWAIIAN OR OTHER PACIFIC
ISLANDER"~"OTHER",
                    RACE=="WHITE"~"WHITE",TRUE~NA_character_))

EXAC$RACE_CODE<-as.character(EXAC$RACE_CODE)

EXAC$PREVEXCT<-as.factor(EXAC$PREVEXCT)

EXAC$SMKBLN<-as.factor(EXAC$SMKBLN)

EXAC$BB_1<-paste(EXAC$SELEC_B_BLOCK_1,EXAC$NON_SELEC_B_BLOCK_1)

EXAC%<>%mutate(BB_2=case_when(BB_1=="N N"~"N",BB_1=="N Y"~"Y",
                BB_1=="Y N"~"Y",BB_1=="Y Y"~"Y",TRUE~NA_character_))

EXAC$BB_2<-as.factor(EXAC$BB_2)

EXAC$ACEIARB_1<-paste(EXAC$ACEI_1,EXAC$ARB_1)

EXAC%<>%mutate(ACEIARB_2=case_when(ACEIARB_1=="N
N"~"N",ACEIARB_1=="N Y"~"Y",
                ACEIARB_1=="Y                N"~"Y",ACEIARB_1=="Y
Y"~"Y",TRUE~NA_character_))

EXAC$ACEIARB_2<-as.factor(EXAC$ACEIARB_2)



EXAC$IHD_CAD<-paste(EXAC$IHDIN,EXAC$CAD)
```

```
EXAC%<>%mutate(IHD_CAD_1=case_when(IHD_CAD=="0 N"~"N",IHD_CAD=="0 Y"~"Y",
                                   IHD_CAD=="1 N"~"Y",IHD_CAD=="1 Y"~"Y",TRUE~NA_character_))

EXAC$IHD_CAD_1<-as.factor(EXAC$IHD_CAD_1)

EXAC%<>%mutate(ASP_CLOPIDOGREL_1=case_when(ASP_CLOPIDOGREL=="1"~"Y",TRUE~NA_character_))

EXAC$ASP_CLOPIDOGREL_1<-as.factor(EXAC$ASP_CLOPIDOGREL_1)

fct_explicit_na(EXAC$ASP_CLOPIDOGREL_1,na_level="N")

EXAC%<>%mutate(ASP_CLOPIDOGREL_1=fct_explicit_na(ASP_CLOPIDOGREL_1,na_level="N"))

EXAC%<>%mutate(ASP_STAT_CLOPIDOGREL_1=case_when(ASP_STAT_CLOPIDOGREL=="1"~"Y",TRUE~NA_character_))

EXAC$ASP_STAT_CLOPIDOGREL_1<-
as.factor(EXAC$ASP_STAT_CLOPIDOGREL_1)

fct_explicit_na(EXAC$ASP_STAT_CLOPIDOGREL_1,na_level="N")

EXAC%<>%mutate(ASP_STAT_CLOPIDOGREL_1=fct_explicit_na(ASP_STAT_CLOPIDOGREL_1,na_level="N"))

EXAC%<>%mutate(STAT_CLOPIDOGREL_1=case_when(STAT_CLOPIDOGREL=="1"~"Y",TRUE~NA_character_))

EXAC$STAT_CLOPIDOGREL_1<-as.factor(EXAC$STAT_CLOPIDOGREL_1)

fct_explicit_na(EXAC$STAT_CLOPIDOGREL_1,na_level="N")
```

```
EXAC%<>%mutate(STAT_CLOPIDOGREL_1=fct_explicit_na(STAT_CLOPIDOGREL_1,
na_level="N"))

fct_explicit_na(EXAC$ACEIARB_2,na_level="N")
EXAC%<>%mutate(ACEIARB_2=fct_explicit_na(ACEIARB_2,na_level="N"))

fct_explicit_na(EXAC$BB_2,na_level="N")
EXAC%<>%mutate(BB_2=fct_explicit_na(BB_2,na_level="N"))

fct_explicit_na(EXAC$ANTI_COAG_1,na_level="N")
EXAC%<>%mutate(ANTI_COAG_1=fct_explicit_na(ANTI_COAG_1,na_level="N"))

fct_explicit_na(EXAC$ANTI_PLATE_1,na_level="N")
EXAC%<>%mutate(ANTI_PLATE_1=fct_explicit_na(ANTI_PLATE_1,na_level="N"))

fct_explicit_na(EXAC$DIHYDROPYRIDINE_1,na_level="N")
EXAC%<>%mutate(DIHYDROPYRIDINE_1=fct_explicit_na(DIHYDROPYRIDINE_1,na
_level="N"))

fct_explicit_na(EXAC$LONG_NITRATES_1,na_level="N")
EXAC%<>%mutate(LONG_NITRATES_1=fct_explicit_na(LONG_NITRATES_1,na_level
="N"))

fct_explicit_na(EXAC$Loop_1,na_level="N")
EXAC%<>%mutate(Loop_1=fct_explicit_na(Loop_1,na_level="N"))

fct_explicit_na(EXAC$NON_DIHYDROPYRIDINE_1,na_level="N")
EXAC%<>%mutate(NON_DIHYDROPYRIDINE_1=fct_explicit_na(NON_DIHYDROPY
RIDINE_1,na_level="N"))

fct_explicit_na(EXAC$STATINS_1,na_level="N")
EXAC%<>%mutate(STATINS_1=fct_explicit_na(STATINS_1,na_level="N"))
```

```
fct_explicit_na(EXAC$THIAZIDES_DIUR_1,na_level="N")

EXAC%<>%mutate(THIAZIDES_DIUR_1=fct_explicit_na(THIAZIDES_DIUR_1,na_level
="N"))


fct_explicit_na(EXAC$BIGUANIDES_1,na_level="N")

EXAC%<>%mutate(BIGUANIDES_1=fct_explicit_na(BIGUANIDES_1,na_level="N"))


fct_explicit_na(EXAC$INSULINS_1,na_level="N")

EXAC%<>%mutate(INSULINS_1=fct_explicit_na(INSULINS_1,na_level="N"))



fct_explicit_na(EXAC$SULFONYLUREAS_1,na_level="N")

EXAC%<>%mutate(SULFONYLUREAS_1=fct_explicit_na(SULFONYLUREAS_1,na_lev
el="N"))


EXAC$HYPERTENSION<-relevel(EXAC$HYPERTENSION,ref="N")


EXAC$HYPERCHOLESTEROL<-relevel(EXAC$HYPERCHOLESTEROL,ref="N")


EXAC$PAD<-relevel(EXAC$PAD,ref="N")


EXAC$CAD<-relevel(EXAC$CAD,ref="N")


EXAC$PREV_MI<-relevel(EXAC$PREV_MI,ref="N")


EXAC$PREV_STROKE<-relevel(EXAC$PREV_STROKE,ref="N")


EXAC$DIABETES<-relevel(EXAC$DIABETES,ref="N")


EXAC$ATENOLOL_1<-relevel(EXAC$ATENOLOL_1,ref="N")


EXAC$BISOPROLOL_1<-relevel(EXAC$BISOPROLOL_1,ref="N")
```

```
EXAC$NEBIVOLOL_1<-relevel(EXAC$NEBIVOLOL_1,ref="N")


EXAC$CLOPIDOGREL_1<-relevel(EXAC$CLOPIDOGREL_1,ref="N")


EXAC$SMKBLN<-relevel(EXAC$SMKBLN,ref="2")


EXAC$PREVEXCT<-relevel(EXAC$PREVEXCT,ref="0")


EXAC%<>%mutate(TREATMENT_YEARS_1=(EXAC$TREATMENT_YEARS)+1)

EXAC%<>%mutate(TREATMENT_YEARS_LOG=log(EXAC$TREATMENT_YEARS_1))

EXAC%<>%distinct(USUBJID,AGE,.keep_all=TRUE)

EXAC$CV_CRITERIA<-EXAC$RACE<-EXAC$SITEID<-EXAC$LSTCT<-
EXAC$os_yrs<-EXAC$ALPHABETABLOCK<-NULL

EXAC$ACEI<-EXAC$ARB<-EXAC$ANTI_COAG<-EXAC$ANTI_PLATE<-
EXAC$CHOL_BILE_ABSORB_INHIB<-EXAC$CLASS3<-NULL

EXAC$DIHYDROPYRIDINE<-EXAC$DIR_RENIN_INHIB<-EXAC$Fibrates<-
EXAC$LONG_NITRATES<-EXAC$Loop<-EXAC$MRA<-NULL

EXAC$Niacin<-EXAC$NON_DIHYDROPYRIDINE<-
EXAC$NON_SELEC_B_BLOCK<-EXAC$Other<-EXAC$SUPPLEMENTS<-
EXAC$SELEC_B_BLOCK<-NULL

EXAC$SHORT_NITRATES<-EXAC$STATINS<-EXAC$THIAZIDES_DIUR<-
EXAC$NA.x<-EXAC$BIGUANIDES<-EXAC$DPP4<-EXAC$DPP4_BIGUANIDES<-
NULL

EXAC$GLPR_AGONIST<-EXAC$GLUCOSIDASE_INHIB<-EXAC$INSULINS<-
EXAC$MEGLITINIDES<-EXAC$OTHER<-EXAC$SODIUM_TRANSPORT_INHIB<-
NULL

EXAC$SULFONYLUREAS<-EXAC$SULFONYLUREAS_BIGUANIDES<-
EXAC$THIAZOLIDINEDIONE<-EXAC$NA.y<-EXAC$TREATED_FOR_PAD<-NULL

EXAC$`CV risk criteria at study entry`<-EXAC$DIABETES_ORGAN_DISEASE<-
EXAC$DIABETES_EYES<-EXAC$DIABETES_KIDNEYS<-NULL

EXAC$DIABETES_LIMBS<-EXAC$`History of CV disease at study entry`<-EXAC$`Met
protocol CV entry criteria`<-NULL

EXAC$GLUC_CAT<-EXAC$BB_1<-EXAC$ACEIARB_1<-NULL

EXAC$ATENOLOL<-EXAC$BISOPROLOL<-EXAC$CLOPIDOGREL<-
EXAC$NEBIVOLOL<-NULL
```

```
EXAC%<>%mutate(CNSR_EXAC_TOTAL=case_when(CNSR_EXAC_TOTAL=="0"~1,CNSR_EXAC_TOTAL=="1"~0,TRUE~NA_real_))

EXAC%<>%mutate(CNSR_EXAC_MOD=case_when(CNSR_EXAC_MOD=="0"~1,CNSR_EXAC_MOD=="1"~0,TRUE~NA_real_))

EXAC%<>%mutate(CNSR_EXAC_SEV=case_when(CNSR_EXAC_SEV=="0"~1,CNSR_EXAC_SEV=="1"~0,TRUE~NA_real_))

EXAC%<>%mutate(CNSR_CVCOMP=case_when(CNSR_CVCOMP=="0"~1,CNSR_CVCOMP=="1"~0,TRUE~NA_real_))


EXAC$CNSR_EXAC_TOTAL<-as.factor(EXAC$CNSR_EXAC_TOTAL)

EXAC$CNSR_EXAC_MOD<-as.factor(EXAC$CNSR_EXAC_MOD)

EXAC$CNSR_EXAC_SEV<-as.factor(EXAC$CNSR_EXAC_SEV)

EXAC$CNSR_CVCOMP<-as.factor(EXAC$CNSR_CVCOMP)


EXAC_TABLE<-
tableby(~AGE+SEX+TOTAL_EXAC+TRTPN+BMI+SMKBLN+PACK_YRS+RACE_CODE+DIABETES+

HYPERCHOLESTEROL+PAD+CAD+HYPERTENSION+PREV_MI+PREV_STROKE+

ALPHABETABLOCK_1+ACEI_1+ARB_1+ANTI_PLATE_1+ANTI_COAG_1+

CHOL_BILE_ABSORB_INHIB_1+CLASS3_1+DIHYDROPYRIDINE_1+DIR_RENIN_INHIB_1+

Fibrates_1+LONG_NITRATES_1+Loop_1+MRA_1+NON_DIHYDROPYRIDINE_1+

NON_SELEC_B_BLOCK_1+SELEC_B_BLOCK_1+ACEIARB_2+BB_2+SHORT_NITRATES_1+

STATINS_1+THIAZIDES_DIUR_1+BIGUANIDES_1+DPP4_1+GLPR_AGONIST_1+

GLUCOSIDASE_INHIB_1+INSULINS_1+MEGLITINIDES_1+SULFONYLUREAS_1+

THIAZOLIDINEDIONE_1+DPP4_BIGUANIDES_1+SODIUM_TRANSPORT_INHIB_1+

        SULFONYLUREAS_BIGUANIDES_1+GLUCOSE+PREVEXCT+FEV1+
```

```
IHD_CAD_1+HF+CNSR_EXAC_TOTAL+CNSR_EXAC_MOD+CNSR_EXAC_SEV+CN
SR_CVCOMP+ATENOLOL_1+BISOPROLOL_1+

NEBIVOLOL_1+CLOPIDOGREL_1+ASPIRIN,data=subset(EXAC,INTENTION_TO_TRE
AT=="Y"))



summary(EXAC_TABLE,title="EXAC_TABLE")


EXAC$CNSR_EXAC_TOTAL<-as.numeric(EXAC$CNSR_EXAC_TOTAL)

EXAC%<>%mutate(CNSR_EXAC_TOTAL=case_when(CNSR_EXAC_TOTAL=="1"~0,C
NSR_EXAC_TOTAL=="2"~1,TRUE~NA_real_))

EXAC$CNSR_EXAC_MOD<-as.numeric(EXAC$CNSR_EXAC_MOD)

EXAC%<>%mutate(CNSR_EXAC_MOD=case_when(CNSR_EXAC_MOD=="1"~0,CNSR
_EXAC_MOD=="2"~1,TRUE~NA_real_))

EXAC$CNSR_EXAC_SEV<-as.numeric(EXAC$CNSR_EXAC_SEV)

EXAC%<>%mutate(CNSR_EXAC_SEV=case_when(CNSR_EXAC_SEV=="1"~0,CNSR_
EXAC_SEV=="2"~1,TRUE~NA_real_))

EXAC$CNSR_CVCOMP<-as.numeric(EXAC$CNSR_CVCOMP)

EXAC%<>%mutate(CNSR_CVCOMP=case_when(CNSR_CVCOMP=="1"~0,CNSR_CVC
OMP=="2"~1,TRUE~NA_real_))


coxph(Surv(TIME_EXAC_SEV,CNSR_EXAC_SEV)~AGE+SEX+BMI+SMKBLN+PACK
_YRS+IHD_CAD_1+PAD+HF+PREV_STROKE+HYPERTENSION+
      HYPERCHOLESTEROL+DIABETES+PREVEXCT+ASPIRIN,

data=subset(EXAC,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPIDOGREL_1=="
N"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="
N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&
            ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&
            SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&
```

```
TRIF=="0"&TIRO=="0"&TREPR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)

EXAC_RATE<-glm.nb(TOTAL_EXAC~AGE+SEX+FEV1+SMKBLN+
                  PREVEXCT+TRTPN+IHD_CAD_1+PAD+PREV_STROKE+HF+
HYPERTENSION+HYPERCHOLESTEROL+STATINS_1,data=subset(EXAC,INTENTIO
N_TO_TREAT=="Y"),offset(TREATMENT_YEARS_1))

summary(EXAC_RATE)
exp(coef(EXAC_RATE))

install.packages(c("broom"))
library("broom")
install.packages(c("gtsummary"))
library("gtsummary")

library("MASS")
library("magrittr")

#PROPENSITY SCORE MATCHING

describe(ACM)
nrow(ACM)

ACM$CV_CRITERIA<-ACM$RACE<-ACM$SITEID<-ACM$LSTCT<-
ACM$TREATMENT_YEARS<-ACM$os_yrs<-ACM$ALPHABETABLOCK<-NULL
ACM$ACEI<-ACM$ARB<-ACM$ANTI_COAG<-ACM$ANTI_PLATE<-
ACM$CHOL_BILE_ABSORB_INHIB<-ACM$CLASS3<-NULL
ACM$DIHYDROPYRIDINE<-ACM$DIR_RENIN_INHIB<-ACM$Fibrates<-
ACM$LONG_NITRATES<-ACM$Loop<-ACM$MRA<-NULL
ACM$Niacin<-ACM$NON_DIHYDROPYRIDINE<-ACM$NON_SELEC_B_BLOCK<-
ACM$Other<-ACM$SUPPLEMENTS<-ACM$SELEC_B_BLOCK<-NULL
ACM$SHORT_NITRATES<-ACM$STATINS<-ACM$THIAZIDES_DIUR<-
ACM$NA.x<-ACM$BIGUANIDES<-ACM$DPP4<-ACM$DPP4_BIGUANIDES<-NULL
```

```
ACM$GLPR_AGONIST<-ACM$GLUCOSIDASE_INHIB<-ACM$INSULINS<-
ACM$MEGLITINIDES<-ACM$OTHER<-ACM$SODIUM_TRANSPORT_INHIB<-
NULL

ACM$SULFONYLUREAS<-ACM$SULFONYLUREAS_BIGUANIDES<-
ACM$THIAZOLIDINEDIONE<-ACM$NA.y<-ACM$TREATED_FOR_PAD<-NULL

ACM$`CV risk criteria at study entry`<-ACM$DIABETES_ORGAN_DISEASE<-
ACM$DIABETES_EYES<-ACM$DIABETES_KIDNEYS<-NULL

ACM$DIABETES_LIMBS<-ACM$`History of CV disease at study entry`<-ACM$`Met
protocol CV entry criteria`<-NULL

ACM$GLUC_CAT<-ACM$BB_1<-ACM$ACEIARB_1<-NULL

ACM$ATENOLOL<-ACM$BISOPROLOL<-ACM$CLOPIDOGREL<-
ACM$NEBIVOLOL<-ACM$ASPIRIN<-NULL




ACM_PS<-matchit(ASPIRIN~PAD+PREV_STROKE+

IHD_CAD_1,data=subset(ACM,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPIDO
GREL_1=="N"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="
N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&
                ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&
                SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&

TRIF=="0"&TIRO=="0"&TREPR=="0"),method="nearest",caliper=0.2,distance="logit")

summary(ACM_PS,standardize=TRUE)

ACM_PS$match.matrix

ACM_PS$discarded
```

```
bal.tab(ACM_PS,m.threshold=0.1,un=TRUE)

bal.tab(ACM_PS,v.threshold=2)


ACM_PS1<-match.data(ACM_PS)

head(ACM_PS1)


ACM_PS1$STATUS<-as.factor(ACM_PS1$STATUS)


test_TABLE<-
tableby(~AGE+SEX+TRTPN+BMI+SMKBLN+RACE_CODE+DIABETES+

HYPERCHOLESTEROL+PAD+CAD+HYPERTENSION+PREV_MI+PREV_STROKE+

ALPHABETABLOCK_1+ACEI_1+ARB_1+ANTI_PLATE_1+ANTI_COAG_1+

CHOL_BILE_ABSORB_INHIB_1+CLASS3_1+DIHYDROPYRIDINE_1+DIR_RENIN_I
NHIB_1+

Fibrates_1+LONG_NITRATES_1+Loop_1+MRA_1+NON_DIHYDROPYRIDINE_1+

NON_SELEC_B_BLOCK_1+SELEC_B_BLOCK_1+ACEIARB_2+BB_2+SHORT_NITR
ATES_1+

STATINS_1+THIAZIDES_DIUR_1+BIGUANIDES_1+DPP4_1+GLPR_AGONIST_1+

GLUCOSIDASE_INHIB_1+INSULINS_1+MEGLITINIDES_1+SULFONYLUREAS_1+

THIAZOLIDINEDIONE_1+DPP4_BIGUANIDES_1+SODIUM_TRANSPORT_INHIB_1+

SULFONYLUREAS_BIGUANIDES_1+STATUS+GLUCOSE+PREVEXCT+ADY+FEV1+
                CV_RISK_CRIT_ONLY+IHDIN+HF+ATENOLOL_1+BISOPROLOL_1+

NEBIVOLOL_1+CLOPIDOGREL_1+ASPIRIN,data=subset(ACM_PS1,INTENTION_TO_
TREAT=="Y"))


summary(test_TABLE,title="test_TABLE")
```

```
test_TABLE<-
tableby(~AGE+SEX+TRTPN+BMI+SMKBLN+RACE_CODE+DIABETES+

HYPERCHOLESTEROL+PAD+CAD+HYPERTENSION+PREV_MI+PREV_STROKE+
                ALPHABETABLOCK_1+ACEI_1+ARB_1+ANTI_COAG_1+

CHOL_BILE_ABSORB_INHIB_1+CLASS3_1+DIHYDROPYRIDINE_1+DIR_RENIN_I
NHIB_1+

Fibrates_1+LONG_NITRATES_1+Loop_1+MRA_1+NON_DIHYDROPYRIDINE_1+

NON_SELEC_B_BLOCK_1+SELEC_B_BLOCK_1+ACEIARB_2+BB_2+SHORT_NITR
ATES_1+

STATINS_1+THIAZIDES_DIUR_1+BIGUANIDES_1+DPP4_1+GLPR_AGONIST_1+

GLUCOSIDASE_INHIB_1+INSULINS_1+MEGLITINIDES_1+SULFONYLUREAS_1+

THIAZOLIDINEDIONE_1+DPP4_BIGUANIDES_1+SODIUM_TRANSPORT_INHIB_1+

SULFONYLUREAS_BIGUANIDES_1+STATUS+GLUCOSE+PREVEXCT+ADY+FEV1+
                CV_RISK_CRIT_ONLY+IHDIN+HF+ATENOLOL_1+BISOPROLOL_1+

NEBIVOLOL_1+CLOPIDOGREL_1+ASPIRIN,data=subset(ACM_PS1,INTENTION_TO_
TREAT=="Y"&ASPIRIN=="1"))


ACM_PS1$STATUS<-as.numeric(ACM_PS1$STATUS)
ACM_PS1%<>%mutate(STATUS=case_when(STATUS=="1"~0,STATUS=="2"~1,TRUE~
NA_real_))



coxph(Surv(ADY_FULL,STATUS)~AGE+SEX+BMI+RACE_CODE+
    ASPIRIN+
    DIABETES+HYPERTENSION+
    HYPERCHOLESTEROL+SMKBLN+FEV1+HF,
```

```
data=subset(ACM_PS1,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPIDOGREL_1
=="N"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="
N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&
        ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&
        SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&

TRIF=="0"&TIRO=="0"&TREPR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)



str(EXAC)


EXAC$CV_CRITERIA<-EXAC$RACE<-EXAC$SITEID<-EXAC$LSTCT<-
EXAC$TREATMENT_YEARS<-EXAC$os_yrs<-EXAC$ALPHABETABLOCK<-NULL

EXAC$ACEI<-EXAC$ARB<-EXAC$ANTI_COAG<-EXAC$ANTI_PLATE<-
EXAC$CHOL_BILE_ABSORB_INHIB<-EXAC$CLASS3<-NULL

EXAC$DIHYDROPYRIDINE<-EXAC$DIR_RENIN_INHIB<-EXAC$Fibrates<-
EXAC$LONG_NITRATES<-EXAC$Loop<-EXAC$MRA<-NULL

EXAC$Niacin<-EXAC$NON_DIHYDROPYRIDINE<-
EXAC$NON_SELEC_B_BLOCK<-EXAC$Other<-EXAC$SUPPLEMENTS<-
EXAC$SELEC_B_BLOCK<-NULL

EXAC$SHORT_NITRATES<-EXAC$STATINS<-EXAC$THIAZIDES_DIUR<-
EXAC$NA.x<-EXAC$BIGUANIDES<-EXAC$DPP4<-EXAC$DPP4_BIGUANIDES<-
NULL

EXAC$GLPR_AGONIST<-EXAC$GLUCOSIDASE_INHIB<-EXAC$INSULINS<-
EXAC$MEGLITINIDES<-EXAC$OTHER<-EXAC$SODIUM_TRANSPORT_INHIB<-
NULL

EXAC$SULFONYLUREAS<-EXAC$SULFONYLUREAS_BIGUANIDES<-
EXAC$THIAZOLIDINEDIONE<-EXAC$NA.y<-EXAC$TREATED_FOR_PAD<-NULL

EXAC$`CV risk criteria at study entry`<-EXAC$DIABETES_ORGAN_DISEASE<-
EXAC$DIABETES_EYES<-EXAC$DIABETES_KIDNEYS<-NULL

EXAC$DIABETES_LIMBS<-EXAC$`History of CV disease at study entry`<-EXAC$`Met
protocol CV entry criteria`<-NULL

EXAC$GLUC_CAT<-EXAC$BB_1<-EXAC$ACEIARB_1<-NULL
```

```
EXAC$ATENOLOL<-EXAC$BISOPROLOL<-EXAC$CLOPIDOGREL<-
EXAC$NEBIVOLOL<-EXAC$ASPIRIN<-NULL



EXAC_PS<-matchit(ASPIRIN~PAD+PREV_STROKE+

IHD_CAD_1,data=subset(EXAC,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPID
OGREL_1=="N"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="
N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&

ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&

SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&

TRIF=="0"&TIRO=="0"&TREPR=="0"),method="nearest",caliper=0.2,distance="logit")
summary(EXAC_PS,standardize=TRUE)
EXAC_PS$match.matrix
EXAC_PS$discarded


bal.tab(EXAC_PS,m.threshold=0.1,un=TRUE)
bal.tab(EXAC_PS,v.threshold=2)


EXAC_PS1<-match.data(EXAC_PS)
head(EXAC_PS1)


EXAC_PS1$CNSR_EXAC_TOTAL<-as.factor(EXAC_PS1$CNSR_EXAC_TOTAL)
EXAC_PS1$CNSR_EXAC_MOD<-as.factor(EXAC_PS1$CNSR_EXAC_MOD)
EXAC_PS1$CNSR_EXAC_SEV<-as.factor(EXAC_PS1$CNSR_EXAC_SEV)
EXAC_PS1$CNSR_CVCOMP<-as.factor(EXAC_PS1$CNSR_CVCOMP)
```

```
EXAC_TABLE<-
tableby(~AGE+SEX+TOTAL_EXAC+TRTPN+BMI+SMKBLN+PACK_YRS+RACE_CO
DE+DIABETES+

HYPERCHOLESTEROL+PAD+CAD+HYPERTENSION+PREV_MI+PREV_STROKE+

ALPHABETABLOCK_1+ACEI_1+ARB_1+ANTI_PLATE_1+ANTI_COAG_1+

CHOL_BILE_ABSORB_INHIB_1+CLASS3_1+DIHYDROPYRIDINE_1+DIR_RENIN_I
NHIB_1+

Fibrates_1+LONG_NITRATES_1+Loop_1+MRA_1+NON_DIHYDROPYRIDINE_1+

NON_SELEC_B_BLOCK_1+SELEC_B_BLOCK_1+ACEIARB_2+BB_2+SHORT_NITR
ATES_1+

STATINS_1+THIAZIDES_DIUR_1+BIGUANIDES_1+DPP4_1+GLPR_AGONIST_1+

GLUCOSIDASE_INHIB_1+INSULINS_1+MEGLITINIDES_1+SULFONYLUREAS_1+

THIAZOLIDINEDIONE_1+DPP4_BIGUANIDES_1+SODIUM_TRANSPORT_INHIB_1+
                SULFONYLUREAS_BIGUANIDES_1+GLUCOSE+PREVEXCT+FEV1+

IHD_CAD_1+HF+CNSR_EXAC_TOTAL+CNSR_EXAC_MOD+CNSR_EXAC_SEV+CN
SR_CVCOMP+ATENOLOL_1+BISOPROLOL_1+

NEBIVOLOL_1+CLOPIDOGREL_1+ASPIRIN,data=subset(EXAC_PS1,INTENTION_TO
_TREAT=="Y"&ASP_STAT_CLOPIDOGREL_1=="N"&

ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="
N"&

ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&

ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&

SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&
                                        TRIF=="0"&TIRO=="0"&TREPR=="0"))
```

```
summary(EXAC_TABLE,title="EXAC_TABLE")


EXAC_PS1$CNSR_EXAC_TOTAL<-as.numeric(EXAC_PS1$CNSR_EXAC_TOTAL)

EXAC_PS1%<>%mutate(CNSR_EXAC_TOTAL=case_when(CNSR_EXAC_TOTAL=="1
"~0,CNSR_EXAC_TOTAL=="2"~1,TRUE~NA_real_))

EXAC_PS1$CNSR_EXAC_MOD<-as.numeric(EXAC_PS1$CNSR_EXAC_MOD)

EXAC_PS1%<>%mutate(CNSR_EXAC_MOD=case_when(CNSR_EXAC_MOD=="1"~0,
CNSR_EXAC_MOD=="2"~1,TRUE~NA_real_))

EXAC_PS1$CNSR_EXAC_SEV<-as.numeric(EXAC_PS1$CNSR_EXAC_SEV)

EXAC_PS1%<>%mutate(CNSR_EXAC_SEV=case_when(CNSR_EXAC_SEV=="1"~0,CN
SR_EXAC_SEV=="2"~1,TRUE~NA_real_))

EXAC_PS1$CNSR_CVCOMP<-as.numeric(EXAC_PS1$CNSR_CVCOMP)

EXAC_PS1%<>%mutate(CNSR_CVCOMP=case_when(CNSR_CVCOMP=="1"~0,CNSR_
CVCOMP=="2"~1,TRUE~NA_real_))


coxph(Surv(TIME_EXAC_SEV,CNSR_EXAC_SEV)~AGE+SEX+BMI+SMKBLN+PACK
_YRS+TRTPN+SMKBLN+PACK_YRS+PREVEXCT+

      FEV1+ASPIRIN,


data=subset(EXAC_PS1,INTENTION_TO_TREAT=="Y"&ASP_STAT_CLOPIDOGREL_
1=="N"&


ASP_CLOPIDOGREL_1=="N"&STAT_CLOPIDOGREL_1=="N"&CLOPIDOGREL_1=="
N"&


ABCIXIMAB=="0"&ASPDIP=="0"&CARB=="0"&DIP=="0"&ETHICO=="0"&

            ILO=="0"&ICO=="0"&MESOGLYCAN=="0"&OZAGREL=="0"&

            SARPO=="0"&PRAS=="0"&PAI=="0"&TICA=="0"&TICLO=="0"&


TRIF=="0"&TIRO=="0"&TREPR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)
```

IMPACT CODE

```
library("survival","survminer")

library("tidyverse","dplyr")

library("tibble")
```

```
library(reshape2)
library(lubridate)
library(survival)
library(survminer)
library(forcats)
library(arsenal)
library("broom")
library("gtsummary")
library("MASS")
library("MatchIt")
library("Hmisc")
library("nnet")
library("tableone")
library("cobalt")
library("weights")
library("rbounds")
library("randomForest")
library(arsenal)
library("broom")
library("magrittr")
library("MatchThem")
library("mice")

#Load files

SLA<-gsk_116855_adsl_v02
LAB<-gsk_116855_adlb_v02
HIST<-gsk_116855_admh_v02
EVENTS<-gsk_116855_adadjud_v02
FEV<-gsk_116855_adpft_v02
ADEXAC<-gsk_116855_adexac_v02
MEDS<-gsk_116855_adcm_v02
```

```
ADEXACA<-gsk_116855_adexaca_v02

EVENTS2<-gsk_116855_adtte_v02

SMOKE<-gsk_116855_adsu_v02

ADV<-gsk_116855_adae_v02

MACE<-gsk_116855_admace_v02



SLA<-
dplyr::select(gsk_116855_adsl_v02,USUBJID,AAGE,SEX,ARMCD,ADTHDT,RACE,ITTF
L,RANDDT,BMIBL,CTRYGR1,W52ACTDT,

SMKBLN,PREVEX,DTHFL,DTHPHASE,PNEUHISN,TRTDUR,ACOUNTRY,AGEGR3)


SLA%<>%mutate(PREVEXCT=case_when(PREVEX>=2~'>=2',PREVEX==1~'1',PREVEX
==0~'0'))


colnames(SLA)[colnames(SLA)=="W52ACTDT"]<-"W52_END_DATE"

colnames(SLA)[colnames(SLA)=="RANDDT"]<-"RANDOMISATION_DATE"

colnames(SLA)[colnames(SLA)=="TRTDUR"]<-"TREATMENT_YEARS"

colnames(SLA)[colnames(SLA)=="CTRYGR1"]<-"REGION"

colnames(SLA)[colnames(SLA)=="BMIBL"]<-"BMI"

colnames(SLA)[colnames(SLA)=="ITTFL"]<-"INTENTION_TO_TREAT"

colnames(SLA)[colnames(SLA)=="PREVEXCT"]<-"PREV_EXAC"

colnames(SLA)[colnames(SLA)=="PNEUHISN"]<-"PNEUMONIA"


SLA%>%mutate(RANDOMISATION_DATE=ymd(RANDOMISATION_DATE),W52_EN
D_DATE=ymd(W52_END_DATE))

SLA$RANDOMISATION_DATE<-as.Date(SLA$RANDOMISATION_DATE)

SLA$W52_END_DATE<-as.Date(SLA$W52_END_DATE)

SLA%<>%mutate(os_yrs=as.duration(RANDOMISATION_DATE%-
-%W52_END_DATE)/dyears(1))
```

```
LAB<-dplyr::select(gsk_116855_adlb_v02,USUBJID,PARAMLBL,AVISIT,AVAL)

LAB[1,]

colnames(LAB)[colnames(LAB)=="AVAL"]<-"GLUCOSE"

LAB<-subset(LAB,PARAMLBL=="Glucose (mmol/L)")

LAB<-subset(LAB,AVISIT=="Screening")

LAB$PARAMLBL<-LAB$AVISIT<-NULL



HIST<-dplyr::select(gsk_116855_admh_v02,USUBJID,ACAT,MHOCCUR)

HIST[1,]

HIST%<>%filter(ACAT=="Angina Pectoris"|ACAT=="Arrhythmia"|ACAT=="Congestive
Heart Failure"|ACAT=="Coronary Artery Disease"|

        ACAT=="Myocardial Infarction"|ACAT=="Diabetes Mellitus"|

        ACAT=="Hypercholesterolemia"|ACAT=="Cerebrovascular
Accident"|ACAT=="Hypertension"|ACAT=="Carotid or Aorto-femoral Vascular Disease")

HIST$MHOCCUR[HIST$MHOCCUR==""]=NA

HIST$MHOCCUR=droplevels(HIST$MHOCCUR)

HIST<-reshape2::dcast(HIST,USUBJID~ACAT,value.var="MHOCCUR") #long to wide
format



MACE<-dplyr::select(gsk_116855_admace_v02,USUBJID,AVAL,PARAM)

colnames(MACE)[colnames(MACE)=="AVAL"]<-"MACE_COUNT"

MACE$PARAM<-as.character(MACE$PARAM)

MACE<-
reshape2::dcast(MACE,USUBJID~PARAM,value.var="MACE_COUNT",fun.aggregate=su
m)

colnames(MACE)[colnames(MACE)=="Adjudicated On-trt. CV deaths"]<-"CVDEATH"

colnames(MACE)[colnames(MACE)=="Non-fatal On-trt. acute MI PT"]<-"AMIPT"

colnames(MACE)[colnames(MACE)=="Non-fatal On-trt. CNS haem./CBV SMQ"]<-"CNS"

colnames(MACE)[colnames(MACE)=="Non-fatal On-trt. heart disease SMQ"]<-
"HEARTDIS"
```

```
colnames(MACE)[colnames(MACE)=="Non-fatal On-trt. MI PT"]<-"MIPT"

colnames(MACE)[colnames(MACE)=="Non-fatal On-trt. MI SMQ"]<-"MISMQ"

MACE%<>%mutate(test=CVDEATH+AMIPT)

MACE%<>%mutate(test1=test+CNS)

MACE%<>%mutate(test2=test1+HEARTDIS)

MACE%<>%mutate(test3=test2+MIPT)

MACE%<>%mutate(TOTAL_MACE=test3+MISMQ)

MACE$test<-MACE$test1<-MACE$test2<-MACE$test3<-NULL



EVENTS<-
dplyr::select(gsk_116855_adadjud_v02,USUBJID,AVALC,APHASE,PARAM,ADY)

EVENTS[1,]



ADV<-dplyr::select(gsk_116855_adae_v02,USUBJID,AELLT,ASTDY)

ADV%<>%filter(AELLT=="Upper gastrointestinal hemorrhage"|AELLT=="Gastrointestinal
bleeding"|AELLT=="Duodenal ulcer"|AELLT=="Duodenal ulcer aggravated"|

        AELLT=="Gastric ulcer"|AELLT=="Acute duodenal ulcer"|

            AELLT=="Lower gastrointestinal hemorrhage"|AELLT=="Bleeding gastric
ulcer"|AELLT=="Peptic ulcer disease"|

        AELLT=="Cerebral hemorrhage"|AELLT=="Perforated gastric ulcer"|

            AELLT=="Acute hemorrhage"|AELLT=="Intracranial
hemorrhage"|AELLT=="Cerebral hemorrhage")


colnames(ADV)[colnames(ADV)=="AELLT"]<-"ADVERSE_BLEED"

colnames(ADV)[colnames(ADV)=="ASTDY"]<-"ADY_BLEED"

ADV=ADV[order(ADV[,'USUBJID'],ADV[,'ADY_BLEED']),]

ADV=ADV[!duplicated(ADV$USUBJID),]


ADV%<>%mutate(BLEED_STATUS=ADY_BLEED)

ADV$BLEED_STATUS[ADV$BLEED_STATUS>0]<-1
```

```
ADV$BLEED_STATUS[is.na(ADV$BLEED_STATUS)]<-0
ADV$BLEED_STATUS[ADV$USUBJID=="6232"]<-NA
ADV$ADY_BLEED[ADV$USUBJID=="6232"]<-NA
ADV$BLEED_STATUS[ADV$USUBJID=="13142"]<-NA
```

```
ADI<-dplyr::select(gsk_116855_adae_v02,USUBJID,AELLT,ASTDY)
ADI%<>%filter(AELLT=="Influenza")
colnames(ADI)[colnames(ADI)=="AELLT"]<-"INFLUENZA"
colnames(ADI)[colnames(ADI)=="ASTDY"]<-"ADY_INFLUENZA"
ADI=ADI[order(ADI[,'USUBJID'],ADI[,'ADY_INFLUENZA']),]
ADI=ADI[!duplicated(ADI$USUBJID),]
ADI%<>%mutate(INFLUENZA_STATUS=ADY_INFLUENZA)
ADI$INFLUENZA_STATUS[ADI$INFLUENZA_STATUS>0]<-1
ADI$INFLUENZA_STATUS[is.na(ADI$INFLUENZA_STATUS)]<-0
```

```
FEV<-dplyr::select(gsk_116855_adpft_v02,USUBJID,ATPT,AVISIT,PARAM,AVAL)
FEV[1,]
FEV%<>%filter(PARAM=="Percent Predicted FEV1 (%)")
FEV%<>%filter(ATPT=="Post-bronchodilator")
FEV%<>%filter(AVISIT=="Screening")
FEV$AVISIT<-FEV$ATPT<-FEV$PARAM<-NULL
colnames(FEV)[colnames(FEV)=="AVAL"]<-"FEV1"
```

```
ADEXAC<-
dplyr::select(gsk_116855_adexac_v02,USUBJID,ADURN,ASEV,ASTDT,ASTDY,APHAS
E)

ADEXAC[1,]

colnames(ADEXAC)[colnames(ADEXAC)=="ASEV"]<-"SEVERITY"

ADEXAC%<>%filter(APHASE=="On-treatment")

ADEXAC%<>%distinct(USUBJID,ASTDY,.keep_all=TRUE)

ADEXAC$ADURN<-ADEXAC$ASTDT<-ADEXAC$ASTDY<-ADEXAC$APHASE<-
NULL

ADEXAC$VALUE<-1

ADEXAC<-
reshape2::dcast(ADEXAC,USUBJID~SEVERITY,value.var="VALUE",fun.aggregate=sum)

ADEXAC%<>%mutate(TOTAL_EXAC=Moderate+Severe)
```

```
ADEXACA<-dplyr::select(gsk_116855_adexaca_v02,USUBJID,AVAL,PARAM)

ADEXACA[1,]

ADEXACA%<>%filter(PARAM=="Number of On-trt. Moderate
Exac."|PARAM=="Number of On-trt. Severe Exac."|

        PARAM=="Number of On-trt. Mod/Sev Exac."|PARAM=="Rate of On-trt.
Mod/Sev Exac.")

ADEXACA$PARAM=droplevels(ADEXACA$PARAM)

ADEXACA$AVAL<-as.integer(ADEXACA$AVAL)

ADEXACA<-reshape2::dcast(ADEXACA,USUBJID~PARAM,value.var="AVAL") # long
to wide
```

```
EVENTS2<-dplyr::select(gsk_116855_adtte_v02,USUBJID,AVAL,CNSR,PARAM)
EVENTS2[1,]
EVENTS2%<>%filter(PARAM=="On-trt All Cause Mortality (days)")
colnames(EVENTS2)[colnames(EVENTS2)=="AVAL"]<-"TIME_ACM"
colnames(EVENTS2)[colnames(EVENTS2)=="CNSR"]<-"CNSR_ACM"
EVENTS2$PARAM<-NULL
EVENTS2%<>%distinct(USUBJID,TIME_ACM,.keep_all=TRUE)

EVENTS3<-dplyr::select(gsk_116855_adtte_v02,USUBJID,AVAL,CNSR,PARAM)
EVENTS3[1,]
EVENTS3%<>%filter(PARAM=="On-trt Cardiovascular AESI (days)")
colnames(EVENTS3)[colnames(EVENTS3)=="AVAL"]<-"TIME_CVCOMP"
colnames(EVENTS3)[colnames(EVENTS3)=="CNSR"]<-"CNSR_CVCOMP"
EVENTS3$PARAM<-NULL
EVENTS3%<>%distinct(USUBJID,TIME_CVCOMP,.keep_all=TRUE)

EVENTS4<-dplyr::select(gsk_116855_adtte_v02,USUBJID,AVAL,CNSR,PARAM)
EVENTS4[1,]
EVENTS4%<>%filter(PARAM=="On-trt Moderate/Severe Exac. (days)")
colnames(EVENTS4)[colnames(EVENTS4)=="AVAL"]<-"TIME_EXAC_TOTAL"
colnames(EVENTS4)[colnames(EVENTS4)=="CNSR"]<-"CNSR_EXAC_TOTAL"
EVENTS4$PARAM<-NULL
EVENTS4%<>%distinct(USUBJID,TIME_EXAC_TOTAL,.keep_all=TRUE)

EVENTS5<-dplyr::select(gsk_116855_adtte_v02,USUBJID,AVAL,CNSR,PARAM)
EVENTS5[1,]
EVENTS5%<>%filter(PARAM=="On-trt Moderate Exac. (days)")
colnames(EVENTS5)[colnames(EVENTS5)=="AVAL"]<-"TIME_EXAC_MOD"
colnames(EVENTS5)[colnames(EVENTS5)=="CNSR"]<-"CNSR_EXAC_MOD"
EVENTS5$PARAM<-NULL
```

```
EVENTS5%<>%distinct(USUBJID,TIME_EXAC_MOD,.keep_all=TRUE)



EVENTS6<-dplyr::select(gsk_116855_adtte_v02,USUBJID,AVAL,CNSR,PARAM)
EVENTS6[1,]
EVENTS6%<>%filter(PARAM=="On-trt Severe Exac. (days)")
colnames(EVENTS6)[colnames(EVENTS6)=="AVAL"]<-"TIME_EXAC_SEV"
colnames(EVENTS6)[colnames(EVENTS6)=="CNSR"]<-"CNSR_EXAC_SEV"
EVENTS6$PARAM<-NULL
EVENTS6%<>%distinct(USUBJID,TIME_EXAC_SEV,.keep_all=TRUE)



SMOKE<-dplyr::select(gsk_116855_adsu_v02,USUBJID,AVAL,PARAM)
SMOKE[1,]
SMOKE%<>%filter(PARAM=="Number of pack years")
colnames(SMOKE)[colnames(SMOKE)=="AVAL"]<-"PACK_YRS"
SMOKE%<>%distinct(USUBJID,PACK_YRS,.keep_all=TRUE)
SMOKE$PARAM<-NULL



MEDS<-dplyr::select(gsk_116855_adcm_v02,USUBJID,CMBASE,DCL2T)
MEDS[1,]
MEDS%<>%filter(DCL2T=="DRUGS USED IN DIABETES")
MEDS%<>%distinct(USUBJID,CMBASE,.keep_all=TRUE)
MEDS$CMBASE=droplevels(MEDS$CMBASE)
MEDS%<>%mutate(CLASS=case_when(CMBASE=="ACARBOSE"~"GLUCOSIDASE_I
NHIB",
                               CMBASE=="MIGLITOL"~"GLUCOSIDASE_INHIB",
                               CMBASE=="VOGLIBOSE"~"GLUCOSIDASE_INHIB",
                               CMBASE=="INSULIN ASPART"~"INSULINS",
```

CMBASE=="INSULIN ASPART PROTAMINE"~"INSULINS",

CMBASE=="INSULIN DETEMIR"~"INSULINS",

CMBASE=="INSULIN GLARGINE"~"INSULINS",

CMBASE=="INSULIN GLULISINE"~"INSULINS",

CMBASE=="INSULIN HUMAN"~"INSULINS",

CMBASE=="INSULIN HUMAN SEMISYNTHETIC"~"INSULINS",

CMBASE=="INSULIN INJECTION, BIPHASIC ISOPHANE"~"INSULINS",

CMBASE=="INSULIN ISOPHANE, HUMAN BIOSYNTHETIC"~"INSULINS",

CMBASE=="INSULIN LISPRO"~"INSULINS",

CMBASE=="INSULIN LISPRO PROTAMINE"~"INSULINS",

CMBASE=="INSULIN NOS"~"INSULINS",

CMBASE=="INSULIN PORCINE"~"INSULINS",

CMBASE=="INSULIN, HUMAN BIOSYNTHETIC"~"INSULINS",

CMBASE=="INSULIN DEGLUDEC"~"INSULINS",

CMBASE=="HUMAN BIOSYNTHETIC INSULIN"~"INSULINS",

CMBASE=="ISOPHANE INSULIN"~"INSULINS",

CMBASE=="BUFORMIN"~"BIGUANIDES",

CMBASE=="METFORMIN"~"BIGUANIDES",

CMBASE=="PHENFORMIN"~"BIGUANIDES",

CMBASE=="GLUCOMET (NOS)"~"BIGUANIDES",

CMBASE=="GLUCONORM (NOS)"~"SULFONYLUREAS_BIGUANIDES",

CMBASE=="GLUCORED NOS"~"SULFONYLUREAS_BIGUANIDES",

CMBASE=="ZOMARIST (NOS)"~"DPP4_BIGUANIDES",

CMBASE=="GLUCONORM"~"SULFONYLUREAS_BIGUANIDES",

CMBASE=="ALOGLIPTIN"~"DPP4",

CMBASE=="GALVUS (NOS)"~"DPP4",

CMBASE=="GEMIGLIPTIN"~"DPP4",

CMBASE=="LINAGLIPTIN"~"DPP4",

```
                CMBASE=="SAXAGLIPTIN"~"DPP4",
                CMBASE=="SITAGLIPTIN"~"DPP4",
                CMBASE=="VILDAGLIPTIN"~"DPP4",
            CMBASE=="TENELIGLIPTIN"~"DPP4",
                CMBASE=="EXENATIDE"~"GLPR_AGONIST",
            CMBASE=="DULAGLUTIDE"~"GLPR_AGONIST",
                CMBASE=="LIRAGLUTIDE"~"GLPR_AGONIST",
                CMBASE=="LIXISENATIDE"~"GLPR_AGONIST",
                CMBASE=="CHLORPROPAMIDE"~"SULFONYLUREAS",
                CMBASE=="DIABETA (NOS)"~"SULFONYLUREAS",
                CMBASE=="GLIBENCLAMIDE"~"SULFONYLUREAS",
                CMBASE=="GLIBETIC (NOS)"~"SULFONYLUREAS",
                CMBASE=="GLICLAZIDE"~"SULFONYLUREAS",
                CMBASE=="GLIM (NOS)"~"SULFONYLUREAS",
                CMBASE=="GLIMEL (NOS)"~"SULFONYLUREAS",
                CMBASE=="GLIMEPIRIDE"~"SULFONYLUREAS",
                CMBASE=="GLIPID NOS"~"SULFONYLUREAS",
                CMBASE=="GLIPIZIDE"~"SULFONYLUREAS",
                CMBASE=="GLIQUIDONE"~"SULFONYLUREAS",
                CMBASE=="GLYCRON (NOS)"~"SULFONYLUREAS",
                CMBASE=="TOLBUTAMIDE"~"SULFONYLUREAS",

CMBASE=="CANAGLIFLOZIN"~"SODIUM_TRANSPORT_INHIB",
            CMBASE=="IPRAGLIFLOZIN"~"SODIUM_TRANSPORT_INHIB",
            CMBASE=="EMPAGLIFLOZIN"~"SODIUM_TRANSPORT_INHIB",

CMBASE=="DAPAGLIFLOZIN"~"SODIUM_TRANSPORT_INHIB",
                CMBASE=="MITIGLINIDE"~"MEGLITINIDES",
                CMBASE=="NATEGLINIDE"~"MEGLITINIDES",
                CMBASE=="REPAGLINIDE"~"MEGLITINIDES",
                CMBASE=="LOBEGLITAZONE"~"THIAZOLIDINEDIONE",
                CMBASE=="PIOGLITAZONE"~"THIAZOLIDINEDIONE",
                CMBASE=="ROSIGLITAZONE"~"THIAZOLIDINEDIONE",
```

```
                    CMBASE=="THIAZOLIDINEDIONE
(NOS)"~"THIAZOLIDINEDIONE",
                    CMBASE=="CINNAMOMUM VERUM  EXTRACT"~"OTHER",
                CMBASE=="CINNAMOMUM CASSIA"~"OTHER",
                    CMBASE=="COLESEVELAM"~"OTHER",
                    CMBASE=="DIABETOL (NOS)"~"OTHER",
                CMBASE=="MOMORDICA CHARANTIA"~"OTHER",
                CMBASE=="DIABETA (NOS)"~"OTHER",
                CMBASE=="DIABESIN (NOS)"~"OTHER",
                    CMBASE=="EPALRESTAT"~"OTHER",
                    CMBASE=="ORAL HYPOGLYCEMICS NOS"~"OTHER",
                    CMBASE=="PRAMLINTIDE"~"OTHER",
                    CMBASE=="THIOCTIC
ACID"~"OTHER",TRUE~NA_character_))


MEDS[1,]
MEDS$DCL2T<-MEDS$CMBASE<-NULL
MEDS%<>%distinct(USUBJID,CLASS,.keep_all=TRUE)
MEDS$VALUE<-1
MEDS<-reshape2::dcast(MEDS,USUBJID~CLASS,value.var="VALUE")
MEDS[is.na(MEDS)]<-0




MEDS2<-dplyr::select(gsk_116855_adcm_v02,USUBJID,DCL4T)
MEDS2[1,]
MEDS2%<>%filter(DCL4T=="ALPHA AND BETA BLOCKING
AGENTS"|DCL4T=="FIBRATES"|DCL4T=="ACE INHIBITORS,
PLAIN"|DCL4T=="HMG COA REDUCTASE INHIBITORS"|
        DCL4T=="THIAZIDES, PLAIN"|DCL4T=="ANGIOTENSIN II
ANTAGONISTS, PLAIN"|DCL4T=="BETA BLOCKING AGENTS,
SELECTIVE"|DCL4T=="BETA BLOCKING AGENTS, NON-
SELECTIVE"|DCL4T=="ANTIARRHYTHMICS, CLASS III")
MEDS2%<>%distinct(USUBJID,DCL4T,.keep_all=TRUE)
```

```
MEDS2$DCL4T=droplevels(MEDS2$DCL4T)

MEDS2$VALUE<-1

MEDS2<-reshape2::dcast(MEDS2,USUBJID~DCL4T,value.var="VALUE")

MEDS2[is.na(MEDS2)]<-0


MEDS3<-dplyr::select(gsk_116855_adcm_v02,USUBJID,ADECOD,DCL4T)

MEDS3%<>%filter(DCL4T=="PLATELET AGGREGATION INHIBITORS EXCL. HEPARIN")

MEDS3%<>%distinct(USUBJID,ADECOD,.keep_all=TRUE)


MEDS3%<>%mutate(ANTIPLATE=case_when(ADECOD=="ACETYLSALICYLIC ACID"~"ASPIRIN",

                ADECOD=="ACETYLSALICYLATE LYSINE"~"ASPIRIN",

                ADECOD=="ACETYLSALICYLIC ACID + ALUMINIUM GLYCINATE + MAGNESIUM CARBONATE"~"ASPIRIN",

                ADECOD=="ACETYLSALICYLIC ACID + LANSOPRAZOLE"~"ASPIRIN",

                ADECOD=="ACETYLSALICYLATE CALCIUM"~"ASPIRIN",

                ADECOD=="ACETYLSALICYLIC ACID + MAGNESIUM HYDROXIDE"~"ASPIRIN",ADECOD=="CLOPIDOGREL"~"CLOPIDOGREL",

                ADECOD=="CLOPIDOGREL BISULFATE"~"CLOPIDOGREL",ADECOD=="CARBASALATE CALCIUM"~"CARBASALATE",


ADECOD=="FONDAPARINUX"~"FONDAPARINUX",ADECOD=="TICAGRELOR"~"TICAGRELOR",


ADECOD=="CILOSTAZOL"~"CILOSTAZOL",ADECOD=="DIPYRIDAMOLE"~"DIPYRIDAMOLE",

                ADECOD=="LIMAPROST ALFADEX"~"LIMAPROST ALFADEX",ADECOD=="BERAPROST SODIUM"~"BERAPROST SODIUM",

                ADECOD=="SARPOGRELATE HYDROCHLORIDE"~"SARPOGRELATE HYDROCHLORIDE",

                ADECOD=="ETHYL ICOSAPENTATE"~"ETHYL ICOSAPENTATE",ADECOD=="ICOSAPENT"~"ICOSAPENT",


ADECOD=="CARBASALATE"~"CARBASALATE",ADECOD=="RESVERATROL"~"RESVERATROL",
```

```
ADECOD=="OZAGREL SODIUM"~"OZAGREL SODIUM",ADECOD=="FONDAPARINUX SODIUM"~"FONDAPARINUX",

ADECOD=="TRIFLUSAL"~"TRIFLUSAL",ADECOD=="VORAPAXAR SULFATE"~"VORAPAXAR SULFATE",

ADECOD=="TICLOPIDINE HYDROCHLORIDE"~"TICLOPIDINE HYDROCHLORIDE",

ADECOD=="PRASUGREL"~"PRASUGREL",ADECOD=="CILOSTAZOL + GINKGO BILOBA EXTRACT"~"CILOSTAZOL + GINKGO BILOBA EXTRACT",

ADECOD=="ACETYLSALICYLIC ACID + DIPYRIDAMOLE"~"ACETYLSALICYLIC ACID + DIPYRIDAMOLE",

ADECOD=="CLOPIDOGREL BESYLATE"~"CLOPIDOGREL",ADECOD=="ACETYLSALICYLIC ACID + CLOPIDOGREL"~"ACETYLSALICYLIC ACID + CLOPIDOGREL",

ADECOD=="ACETYLSALICYLIC ACID + CLOPIDOGREL BISULFATE"~"ACETYLSALICYLIC ACID + CLOPIDOGREL",

ADECOD=="EPTIFIBATIDE"~"EPTIFIBATIDE",ADECOD=="MESOGLYCAN"~"MESOGLYCAN",ADECOD=="ANAGRELIDE"~"ANAGRELIDE",

ADECOD=="GINKGO BILOBA EXTRACT + TICLOPIDINE HYDROCHLORIDE"~"GINKGO BILOBA EXTRACT + TICLOPIDINE HYDROCHLORIDE",TRUE~NA_character_))


MEDS3$DCL4T<-MEDS3$ADECOD<-NULL

MEDS3$VALUE<-1

MEDS3%<>%distinct(USUBJID,ANTIPLATE,.keep_all=TRUE)

MEDS3<-reshape2::dcast(MEDS3,USUBJID~ANTIPLATE,value.var="VALUE")

MEDS3[is.na(MEDS3)]<-0




MEDS2<-merge(MEDS2,MEDS3,by="USUBJID",all.x=TRUE,all.y=TRUE)

MEDS2[is.na(MEDS2)]<-0



#MERGING
```

```
U<-merge(SLA,LAB,by="USUBJID",all.x=TRUE,all.y=TRUE)
V<-merge(U,HIST,by="USUBJID",all.x=TRUE,all.y=TRUE)
X<-merge(V,FEV,by="USUBJID",all.x=TRUE,all.y=TRUE)
Y<-merge(X,ADEXACA,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z<-merge(Y,EVENTS2,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z1<-merge(Z,EVENTS3,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z2<-merge(Z1,EVENTS4,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z3<-merge(Z2,EVENTS5,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z4<-merge(Z3,EVENTS6,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z5<-merge(Z4,SMOKE,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z6<-merge(Z5,MEDS,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z7<-merge(Z6,MEDS2,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z7<-merge(Z7,ADV,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z7<-merge(Z7,ADI,by="USUBJID",all.x=TRUE,all.y=TRUE)
Z7<-merge(Z7,MACE,by="USUBJID",all.x=TRUE,all.y=TRUE)


colnames(Z7)[colnames(Z7)=="ALPHA AND BETA BLOCKING AGENTS"]<-
"ALPHABETABLOCK"
colnames(Z7)[colnames(Z7)=="ACE INHIBITORS, PLAIN"]<-"ACEI"
colnames(Z7)[colnames(Z7)=="ANGIOTENSIN II ANTAGONISTS, PLAIN"]<-"ARB"
colnames(Z7)[colnames(Z7)=="HMG COA REDUCTASE INHIBITORS"]<-"STATINS"
colnames(Z7)[colnames(Z7)=="ANTIARRHYTHMICS, CLASS III"]<-"CLASS3"
colnames(Z7)[colnames(Z7)=="THIAZIDES, PLAIN"]<-"THIAZ"
colnames(Z7)[colnames(Z7)=="BETA BLOCKING AGENTS, NON-SELECTIVE"]<-
"NON_SELEC_B_BLOCK"
colnames(Z7)[colnames(Z7)=="BETA BLOCKING AGENTS, SELECTIVE"]<-
"SELEC_B_BLOCK"
colnames(Z7)[colnames(Z7)=="Number of On-trt. Severe Exac."]<-"SEVEXAC"
colnames(Z7)[colnames(Z7)=="Number of On-trt. Moderate Exac."]<-"MODEXAC"
colnames(Z7)[colnames(Z7)=="Number of On-trt. Mod/Sev Exac."]<-"MODSEVEXAC"
colnames(Z7)[colnames(Z7)=="AAGE"]<-"AGE"
```

```
colnames(Z7)[colnames(Z7)=="Myocardial Infarction"]<-"PREV_MI"
colnames(Z7)[colnames(Z7)=="Cerebrovascular Accident"]<-"PREV_STROKE"
colnames(Z7)[colnames(Z7)=="Congestive Heart Failure"]<-"PREV_HF"
colnames(Z7)[colnames(Z7)=="Coronary Artery Disease"]<-"PREV_CAD"
colnames(Z7)[colnames(Z7)=="Diabetes Mellitus"]<-"PREV_DIABETES"
colnames(Z7)[colnames(Z7)=="Hypercholesterolemia"]<-"PREV_HYPERCHOL"
colnames(Z7)[colnames(Z7)=="Hypertension"]<-"PREV_HYPERTENS"
colnames(Z7)[colnames(Z7)=="Arrhythmia"]<-"PREV_ARR"
colnames(Z7)[colnames(Z7)=="Angina Pectoris"]<-"PREV_ANG"
colnames(Z7)[colnames(Z7)=="PNEUMONIA"]<-"PREV_PNEU"
colnames(Z7)[colnames(Z7)=="Carotid or Aorto-femoral Vascular Disease"]<-"PREV_PAD"


Z7$PREV_PNEU<-as.factor(Z7$PREV_PNEU)
Z7$SMKBLN<-as.factor(Z7$SMKBLN)
Z7$PREV_ANG<-as.factor(Z7$PREV_ANG)
Z7$PREV_ARR<-as.factor(Z7$PREV_ARR)
Z7$PREV_STROKE<-as.factor(Z7$PREV_STROKE)
Z7$PREV_PAD<-as.factor(Z7$PREV_PAD)
Z7$PREV_HF<-as.factor(Z7$PREV_HF)
Z7$PREV_CAD<-as.factor(Z7$PREV_CAD)
Z7$PREV_DIABETES<-as.factor(Z7$PREV_DIABETES)
Z7$PREV_HYPERCHOL<-as.factor(Z7$PREV_HYPERCHOL)
Z7$PREV_HYPERTENS<-as.factor(Z7$PREV_HYPERTENS)
Z7$PREV_MI<-as.factor(Z7$PREV_MI)
Z7$CNSR_ACM<-as.factor(Z7$CNSR_ACM)
Z7$CNSR_CVCOMP<-as.factor(Z7$CNSR_CVCOMP)
Z7$CNSR_EXAC_TOTAL<-as.factor(Z7$CNSR_EXAC_TOTAL)
Z7$CNSR_EXAC_MOD<-as.factor(Z7$CNSR_EXAC_MOD)
Z7$CNSR_EXAC_SEV<-as.factor(Z7$CNSR_EXAC_SEV)
Z7$BIGUANIDES<-as.factor(Z7$BIGUANIDES)
Z7$DPP4<-as.factor(Z7$DPP4)
```

```
Z7$DPP4_BIGUANIDES<-as.factor(Z7$DPP4_BIGUANIDES)
Z7$GLPR_AGONIST<-as.factor(Z7$GLPR_AGONIST)
Z7$GLUCOSIDASE_INHIB<-as.factor(Z7$GLUCOSIDASE_INHIB)
Z7$INSULINS<-as.factor(Z7$INSULINS)
Z7$MEGLITINIDES<-as.factor(Z7$MEGLITINIDES)
Z7$OTHER<-as.factor(Z7$OTHER)
Z7$SODIUM_TRANSPORT_INHIB<-as.factor(Z7$SODIUM_TRANSPORT_INHIB)
Z7$SULFONYLUREAS<-as.factor(Z7$SULFONYLUREAS)
Z7$THIAZOLIDINEDIONE<-as.factor(Z7$THIAZOLIDINEDIONE)
Z7$ACEI<-as.factor(Z7$ACEI)
Z7$ALPHABETABLOCK<-as.factor(Z7$ALPHABETABLOCK)
Z7$ARB<-as.factor(Z7$ARB)
Z7$CLASS3<-as.factor(Z7$CLASS3)
Z7$NON_SELEC_B_BLOCK<-as.factor(Z7$NON_SELEC_B_BLOCK)
Z7$SELEC_B_BLOCK<-as.factor(Z7$SELEC_B_BLOCK)
Z7$FIBRATES<-as.factor(Z7$FIBRATES)
Z7$STATINS<-as.factor(Z7$STATINS)
Z7$THIAZ<-as.factor(Z7$THIAZ)
Z7$ASPIRIN<-as.factor(Z7$ASPIRIN)
Z7$CLOPIDOGREL<-as.factor(Z7$CLOPIDOGREL)

Z7$ARMCD[Z7$ARMCD==""]=NA
Z7$ARMCD=droplevels(Z7$ARMCD)
Z7%<>%filter(ARMCD=="FFVI"|ARMCD=="UMECVI"|ARMCD=="FFUMECVI")
Z7$ARMCD=droplevels(Z7$ARMCD)
Z7$SEX[Z7$SEX==""]=NA
Z7$SEX=droplevels(Z7$SEX)

Z7$RACE[Z7$RACE==""]=NA
Z7%<>%mutate(RACE_CODE=case_when(RACE=="AMERICAN INDIAN OR ALASKA
NATIVE"~"OTHER",
                    RACE=="ASIAN"~"ASIAN",
```

```
                RACE=="BLACK OR AFRICAN AMERICAN"~"OTHER",
                RACE=="MULTIPLE"~"OTHER",
                RACE=="NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER"~"OTHER",
                RACE=="WHITE"~"WHITE",TRUE~NA_character_))



Z7$ANG_CAD<-paste(Z7$PREV_ANG,Z7$PREV_CAD)
Z7$IHD_CAD<-paste(Z7$ANG_CAD,Z7$PREV_MI)
Z7%<>%mutate(IHDCAD=case_when(IHD_CAD=="N N N"~"N",IHD_CAD=="N N NA"~"N",
                IHD_CAD=="N N Y"~"Y",IHD_CAD=="N NA N"~"N",IHD_CAD=="N Y N"~"Y",
                IHD_CAD=="N Y Y"~"Y",IHD_CAD=="NA N N"~"N",IHD_CAD=="NA NA N"~"N",IHD_CAD=="NA NA NA"~"NA",
                IHD_CAD=="NA NA Y"~"Y",IHD_CAD=="Y N N"~"Y",IHD_CAD=="Y N NA"~"Y",IHD_CAD=="Y N Y"~"Y",
                IHD_CAD=="Y NA N"~"Y",IHD_CAD=="Y Y N"~"Y",IHD_CAD=="Y Y Y"~"Y",TRUE~NA_character_))

Z7$IHDCAD[Z7$IHDCAD=="NA"]<-NA
Z7$IHDCAD<-as.factor(Z7$IHDCAD)
Z7$ANG_CAD<-Z7$IHD_CAD<-NULL

Z7$BIGUANIDES[is.na(Z7$BIGUANIDES)]<-0
Z7$DPP4[is.na(Z7$DPP4)]<-0
Z7$DPP4_BIGUANIDES[is.na(Z7$DPP4_BIGUANIDES)]<-0
Z7$GLPR_AGONIST[is.na(Z7$GLPR_AGONIST)]<-0
Z7$GLUCOSIDASE_INHIB[is.na(Z7$GLUCOSIDASE_INHIB)]<-0
Z7$INSULINS[is.na(Z7$INSULINS)]<-0
Z7$MEGLITINIDES[is.na(Z7$MEGLITINIDES)]<-0
Z7$OTHER[is.na(Z7$OTHER)]<-0
Z7$SODIUM_TRANSPORT_INHIB[is.na(Z7$SODIUM_TRANSPORT_INHIB)]<-0
```

```
Z7$SULFONYLUREAS[is.na(Z7$SULFONYLUREAS)]<-0

Z7$THIAZOLIDINEDIONE[is.na(Z7$THIAZOLIDINEDIONE)]<-0

Z7$ACEI[is.na(Z7$ACEI)]<-0

Z7$ALPHABETABLOCK[is.na(Z7$ALPHABETABLOCK)]<-0

Z7$ARB[is.na(Z7$ARB)]<-0

Z7$CLASS3[is.na(Z7$CLASS3)]<-0

Z7$NON_SELEC_B_BLOCK[is.na(Z7$NON_SELEC_B_BLOCK)]<-0

Z7$SELEC_B_BLOCK[is.na(Z7$SELEC_B_BLOCK)]<-0

Z7$FIBRATES[is.na(Z7$FIBRATES)]<-0

Z7$STATINS[is.na(Z7$STATINS)]<-0

Z7$THIAZ[is.na(Z7$THIAZ)]<-0

colnames(Z7)[colnames(Z7)=="ACETYLSALICYLIC ACID + CLOPIDOGREL"]<-
"ASPCLOP"

Z7$ASPCLOP[is.na(Z7$ASPCLOP)]<-0

colnames(Z7)[colnames(Z7)=="ACETYLSALICYLIC ACID + DIPYRIDAMOLE"]<-
"ASPDIP"

Z7$ASPDIP[is.na(Z7$ASPDIP)]<-0

Z7$ANAGRELIDE[is.na(Z7$ANAGRELIDE)]<-0

Z7$ASPIRIN[is.na(Z7$ASPIRIN)]<-0

colnames(Z7)[colnames(Z7)=="BERAPROST SODIUM"]<-"BERA"

Z7$BERA[is.na(Z7$BERA)]<-0

Z7$CARBASALATE[is.na(Z7$CARBASALATE)]<-0

Z7$CILOSTAZOL[is.na(Z7$CILOSTAZOL)]<-0

colnames(Z7)[colnames(Z7)=="CILOSTAZOL + GINKGO BILOBA EXTRACT"]<-
"CILOGIN"

Z7$CILOGIN[is.na(Z7$CILOGIN)]<-0

Z7$CLOPIDOGREL[is.na(Z7$CLOPIDOGREL)]<-0

Z7$DIPYRIDAMOLE[is.na(Z7$DIPYRIDAMOLE)]<-0

Z7$EPTIFIBATIDE[is.na(Z7$EPTIFIBATIDE)]<-0

colnames(Z7)[colnames(Z7)=="ETHYL ICOSAPENTATE"]<-"ETHICO"

Z7$ETHICO[is.na(Z7$ETHICO)]<-0

Z7$FONDAPARINUX[is.na(Z7$FONDAPARINUX)]<-0
```

```
colnames(Z7)[colnames(Z7)=="GINKGO BILOBA EXTRACT + TICLOPIDINE HYDROCHLORIDE"]<-"GINTIC"

Z7$GINTIC[is.na(Z7$GINTIC)]<-0

Z7$ICOSAPENT[is.na(Z7$ICOSAPENT)]<-0

colnames(Z7)[colnames(Z7)=="LIMAPROST ALFADEX"]<-"LIM"

Z7$LIM[is.na(Z7$LIM)]<-0

Z7$MESOGLYCAN[is.na(Z7$MESOGLYCAN)]<-0

colnames(Z7)[colnames(Z7)=="OZAGREL SODIUM"]<-"OZ"

Z7$OZ[is.na(Z7$OZ)]<-0

Z7$PRASUGREL[is.na(Z7$PRASUGREL)]<-0

Z7$RESVERATROL[is.na(Z7$RESVERATROL)]<-0

colnames(Z7)[colnames(Z7)=="SARPOGRELATE HYDROCHLORIDE"]<-"SARP"

Z7$SARP[is.na(Z7$SARP)]<-0

Z7$TICAGRELOR[is.na(Z7$TICAGRELOR)]<-0

colnames(Z7)[colnames(Z7)=="TICLOPIDINE HYDROCHLORIDE"]<-"TIC"

Z7$TIC[is.na(Z7$TIC)]<-0

Z7$TRIFLUSAL[is.na(Z7$TRIFLUSAL)]<-0

colnames(Z7)[colnames(Z7)=="VORAPAXAR SULFATE"]<-"VOR"

Z7$VOR[is.na(Z7$VOR)]<-0


Z7%<>%mutate(os.days=Z7$os_yrs*365.25)

Z7$os.days<-as.integer(Z7$os.days)

Z7%<>%mutate(ADY_BLEED_FULL=case_when(os.days>ADY_BLEED~ADY_BLEED,
TRUE~os.days))

Z7$BLEED_STATUS[is.na(Z7$BLEED_STATUS)]<-0

Z7%<>%mutate(ADY_INFLUENZA_FULL=case_when(os.days>ADY_INFLUENZA~AD
Y_INFLUENZA,TRUE~os.days))

Z7$INFLUENZA_STATUS[is.na(Z7$INFLUENZA_STATUS)]<-0




Z7$ADY_BLEED_FULL[Z7$USUBJID=="13142"]<-NA

Z7$ADY_BLEED_FULL[Z7$USUBJID=="6232"]<-NA
```

```
Z7%<>%mutate(CNSR_ACM=case_when(CNSR_ACM=="0"~1,CNSR_ACM=="1"~0,TR
UE~NA_real_))

Z7%<>%mutate(CNSR_CVCOMP=case_when(CNSR_CVCOMP=="0"~1,CNSR_CVCOM
P=="1"~0,TRUE~NA_real_))

Z7%<>%mutate(CNSR_EXAC_TOTAL=case_when(CNSR_EXAC_TOTAL=="0"~1,CNS
R_EXAC_TOTAL=="1"~0,TRUE~NA_real_))

Z7%<>%mutate(CNSR_EXAC_MOD=case_when(CNSR_EXAC_MOD=="0"~1,CNSR_E
XAC_MOD=="1"~0,TRUE~NA_real_))

Z7%<>%mutate(CNSR_EXAC_SEV=case_when(CNSR_EXAC_SEV=="0"~1,CNSR_EXA
C_SEV=="1"~0,TRUE~NA_real_))


Z7$CNSR_ACM<-as.factor(Z7$CNSR_ACM)

Z7$CNSR_CVCOMP<-as.factor(Z7$CNSR_CVCOMP)

Z7$CNSR_EXAC_TOTAL<-as.factor(Z7$CNSR_EXAC_TOTAL)

Z7$CNSR_EXAC_MOD<-as.factor(Z7$CNSR_EXAC_MOD)

Z7$CNSR_EXAC_SEV<-as.factor(Z7$CNSR_EXAC_SEV)


Z7$TOTAL_MACE<-as.character(Z7$TOTAL_MACE)


IMPACT<-
tableby(~AGE+SEX+ARMCD+BMI+SMKBLN+PACK_YRS+RACE_CODE+PREV_EXA
C+

                PREV_PNEU+TREATMENT_YEARS+GLUCOSE+PREV_ARR+

PREV_STROKE+PREV_HF+PREV_PAD+PREV_DIABETES+PREV_HYPERCHOL+

                PREV_HYPERTENS+FEV1+MODSEVEXAC+MODEXAC+SEVEXAC+

CNSR_ACM+CNSR_CVCOMP+CNSR_EXAC_TOTAL+CNSR_EXAC_MOD+

CNSR_EXAC_SEV+ACEI+ARB+NON_SELEC_B_BLOCK+SELEC_B_BLOCK+

STATINS+ASPIRIN+CLOPIDOGREL+PREV_MI+PREV_CAD+PREV_ANG+IHDCAD+

        TOTAL_MACE,data=subset(Z7,INTENTION_TO_TREAT=="Y"))
```

```
summary(IMPACT,title="IMPACT")

Z7$TOTAL_MACE<-as.numeric(Z7$TOTAL_MACE)


Z7$SMKBLN<-relevel(Z7$SMKBLN,ref="2")

Z7$PREV_PNEU<-relevel(Z7$PREV_PNEU,ref="2")

Z7$PREV_EXAC<-as.factor(Z7$PREV_EXAC)

Z7$PREV_EXAC<-relevel(Z7$PREV_EXAC,ref="0")

Z7$IHDCAD<-relevel(Z7$IHDCAD,ref="N")



Z7$CNSR_ACM<-as.numeric(Z7$CNSR_ACM)

Z7%<>%mutate(CNSR_ACM=case_when(CNSR_ACM=="1"~0,CNSR_ACM=="2"~1,TR
UE~NA_real_))


Z7$CNSR_CVCOMP<-as.numeric(Z7$CNSR_CVCOMP)

Z7%<>%mutate(CNSR_CVCOMP=case_when(CNSR_CVCOMP=="1"~0,CNSR_CVCOM
P=="2"~1,TRUE~NA_real_))


Z7$CNSR_EXAC_TOTAL<-as.numeric(Z7$CNSR_EXAC_TOTAL)

Z7%<>%mutate(CNSR_EXAC_TOTAL=case_when(CNSR_EXAC_TOTAL=="1"~0,CNS
R_EXAC_TOTAL=="2"~1,TRUE~NA_real_))


Z7$CNSR_EXAC_MOD<-as.numeric(Z7$CNSR_EXAC_MOD)

Z7%<>%mutate(CNSR_EXAC_MOD=case_when(CNSR_EXAC_MOD=="1"~0,CNSR_E
XAC_MOD=="2"~1,TRUE~NA_real_))


Z7$CNSR_EXAC_SEV<-as.numeric(Z7$CNSR_EXAC_SEV)

Z7%<>%mutate(CNSR_EXAC_SEV=case_when(CNSR_EXAC_SEV=="1"~0,CNSR_EXA
C_SEV=="2"~1,TRUE~NA_real_))


coxph(Surv(TIME_ACM,CNSR_ACM)~AGE+SEX+BMI+ARMCD+SMKBLN+
```

```
PACK_YRS+FEV1+PREV_ARR+PREV_STROKE+PREV_HF+PREV_HYPERCHOL+PR
EV_HYPERTENS+IHDCAD+PREV_PAD+PREV_DIABETES+ASPIRIN,
    data=subset(Z7,INTENTION_TO_TREAT=="Y"&BERA=="0"&

CARBASALATE=="0"&CILOSTAZOL=="0"&CILOGIN=="0"&CLOPIDOGREL=="0"&
            DIPYRIDAMOLE=="0"&ASPCLOP=="0"&ASPDIP=="0"&ETHICO=="0"&
            ANAGRELIDE=="0"&EPTIFIBATIDE=="0"&FONDAPARINUX=="0"&

GINTIC=="0"&ICOSAPENT=="0"&LIM=="0"&MESOGLYCAN=="0"&OZ=="0"&

PRASUGREL=="0"&RESVERATROL=="0"&SARP=="0"&TICAGRELOR=="0"&

TIC=="0"&TRIFLUSAL=="0"&VOR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)



coxph(Surv(ADY_INFLUENZA_FULL,INFLUENZA_STATUS)~AGE+SEX+ARMCD+B
MI+ASPIRIN,
    data=subset(Z7,INTENTION_TO_TREAT=="Y"&BERA=="0"&

CARBASALATE=="0"&CILOSTAZOL=="0"&CILOGIN=="0"&CLOPIDOGREL=="0"&
            DIPYRIDAMOLE=="0"&ASPCLOP=="0"&ASPDIP=="0"&ETHICO=="0"&
            ANAGRELIDE=="0"&EPTIFIBATIDE=="0"&FONDAPARINUX=="0"&

GINTIC=="0"&ICOSAPENT=="0"&LIM=="0"&MESOGLYCAN=="0"&OZ=="0"&

PRASUGREL=="0"&RESVERATROL=="0"&SARP=="0"&TICAGRELOR=="0"&

TIC=="0"&TRIFLUSAL=="0"&VOR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)



Z7%<>%mutate(TREATMENT_YEARS_1=(Z7$TREATMENT_YEARS)+1)
Z7%<>%mutate(TREATMENT_YEARS_LOG=log(Z7$TREATMENT_YEARS_1))
Z7$TOTAL_MACE[is.na(Z7$TOTAL_MACE)]<-0
```

```
MACE_RATE<-glm(TOTAL_MACE~AGE+SEX+BMI+ARMCD+SMKBLN+
        PACK_YRS+PREV_ARR+
        PREV_STROKE+PREV_HF+PREV_HYPERCHOL+
        PREV_HYPERTENS+IHDCAD+PREV_PAD+

PREV_DIABETES+PREV_EXAC+ASPIRIN+offset(TREATMENT_YEARS_LOG),data=s
ubset(Z7,INTENTION_TO_TREAT=="Y"&BERA=="0"&

CARBASALATE=="0"&CILOSTAZOL=="0"&CILOGIN=="0"&CLOPIDOGREL=="0"&

DIPYRIDAMOLE=="0"&ASPCLOP=="0"&ASPDIP=="0"&ETHICO=="0"&

ANAGRELIDE=="0"&EPTIFIBATIDE=="0"&FONDAPARINUX=="0"&

GINTIC=="0"&ICOSAPENT=="0"&LIM=="0"&MESOGLYCAN=="0"&OZ=="0"&

PRASUGREL=="0"&RESVERATROL=="0"&SARP=="0"&TICAGRELOR=="0"&

TIC=="0"&TRIFLUSAL=="0"&VOR=="0"),family="poisson")


summary(MACE_RATE)
exp(coef(MACE_RATE))
cbind(RR=exp(coef(MACE_RATE)))
round(cbind(RR=exp(coef(MACE_RATE))),digits=2)


fct_explicit_na(Z7$PREV_PAD,na_level="N")
Z7%<>%mutate(PREV_PAD=fct_explicit_na(PREV_PAD,na_level="N"))
fct_explicit_na(Z7$PREV_STROKE,na_level="N")
Z7%<>%mutate(PREV_STROKE=fct_explicit_na(PREV_STROKE,na_level="N"))
fct_explicit_na(Z7$IHDCAD,na_level="N")
Z7%<>%mutate(IHDCAD=fct_explicit_na(IHDCAD,na_level="N"))


Z7_PS<-matchit(ASPIRIN~PREV_PAD+PREV_STROKE+
            IHDCAD,data=subset(Z7,INTENTION_TO_TREAT=="Y"&BERA=="0"&
```

```
CARBASALATE=="0"&CILOSTAZOL=="0"&CILOGIN=="0"&CLOPIDOGREL=="0"&

DIPYRIDAMOLE=="0"&ASPCLOP=="0"&ASPDIP=="0"&ETHICO=="0"&

ANAGRELIDE=="0"&EPTIFIBATIDE=="0"&FONDAPARINUX=="0"&

GINTIC=="0"&ICOSAPENT=="0"&LIM=="0"&MESOGLYCAN=="0"&OZ=="0"&

PRASUGREL=="0"&RESVERATROL=="0"&SARP=="0"&TICAGRELOR=="0"&

TIC=="0"&TRIFLUSAL=="0"&VOR=="0"),method="nearest",caliper=0.2,distance="logit")
summary(Z7_PS,standardize=TRUE)
Z7_PS$match.matrix
Z7_PS$discarded

bal.tab(Z7_PS,m.threshold=0.1,un=TRUE)
bal.tab(Z7_PS,v.threshold=2)

Z7_PS1<-match.data(Z7_PS)
head(Z7_PS1)

Z7_PS1$CNSR_EXAC_TOTAL<-as.factor(Z7_PS1$CNSR_EXAC_TOTAL)
Z7_PS1$CNSR_EXAC_MOD<-as.factor(Z7_PS1$CNSR_EXAC_MOD)
Z7_PS1$CNSR_EXAC_SEV<-as.factor(Z7_PS1$CNSR_EXAC_SEV)
Z7_PS1$CNSR_ACM<-as.factor(Z7_PS1$CNSR_ACM)

Z7_TABLE<-
tableby(~AGE+SEX+ARMCD+BMI+SMKBLN+PACK_YRS+RACE_CODE+PREV_EXA
C+
            PREV_PNEU+TREATMENT_YEARS+GLUCOSE+PREV_ARR+

PREV_STROKE+PREV_HF+PREV_PAD+PREV_DIABETES+PREV_HYPERCHOL+
            PREV_HYPERTENS+FEV1+MODSEVEXAC+MODEXAC+SEVEXAC+

CNSR_ACM+CNSR_CVCOMP+CNSR_EXAC_TOTAL+CNSR_EXAC_MOD+
```

```
CNSR_EXAC_SEV+ACEI+ARB+NON_SELEC_B_BLOCK+SELEC_B_BLOCK+

STATINS+ASPIRIN+CLOPIDOGREL+PREV_MI+PREV_CAD+PREV_ANG+IHDCAD+

TOTAL_MACE,data=subset(Z7_PS1,INTENTION_TO_TREAT=="Y"&BERA=="0"&

CARBASALATE=="0"&CILOSTAZOL=="0"&CILOGIN=="0"&CLOPIDOGREL=="0"&

DIPYRIDAMOLE=="0"&ASPCLOP=="0"&ASPDIP=="0"&ETHICO=="0"&

ANAGRELIDE=="0"&EPTIFIBATIDE=="0"&FONDAPARINUX=="0"&

GINTIC=="0"&ICOSAPENT=="0"&LIM=="0"&MESOGLYCAN=="0"&OZ=="0"&

PRASUGREL=="0"&RESVERATROL=="0"&SARP=="0"&TICAGRELOR=="0"&
                        TIC=="0"&TRIFLUSAL=="0"&VOR=="0"))



summary(Z7_TABLE,title="Z7_TABLE")


Z7_PS1$CNSR_EXAC_TOTAL<-as.numeric(Z7_PS1$CNSR_EXAC_TOTAL)

Z7_PS1%<>%mutate(CNSR_EXAC_TOTAL=case_when(CNSR_EXAC_TOTAL=="1"~0,
CNSR_EXAC_TOTAL=="2"~1,TRUE~NA_real_))

Z7_PS1$CNSR_EXAC_MOD<-as.numeric(Z7_PS1$CNSR_EXAC_MOD)

Z7_PS1%<>%mutate(CNSR_EXAC_MOD=case_when(CNSR_EXAC_MOD=="1"~0,CNS
R_EXAC_MOD=="2"~1,TRUE~NA_real_))

Z7_PS1$CNSR_EXAC_SEV<-as.numeric(Z7_PS1$CNSR_EXAC_SEV)

Z7_PS1%<>%mutate(CNSR_EXAC_SEV=case_when(CNSR_EXAC_SEV=="1"~0,CNSR
_EXAC_SEV=="2"~1,TRUE~NA_real_))

Z7_PS1$CNSR_ACM<-as.numeric(Z7_PS1$CNSR_ACM)

Z7_PS1%<>%mutate(CNSR_ACM=case_when(CNSR_ACM=="1"~0,CNSR_ACM=="2"~
1,TRUE~NA_real_))


coxph(Surv(TIME_ACM,CNSR_ACM)~AGE+SEX+BMI+SMKBLN+PACK_YRS+ARM
CD+
```

```
FEV1+PREV_ARR+PREV_HF+PREV_HYPERCHOL+PREV_HYPERTENS+PREV_DIA
BETES+ASPIRIN,
    data=subset(Z7_PS1,INTENTION_TO_TREAT=="Y"&BERA=="0"&

CARBASALATE=="0"&CILOSTAZOL=="0"&CILOGIN=="0"&CLOPIDOGREL=="0"&
            DIPYRIDAMOLE=="0"&ASPCLOP=="0"&ASPDIP=="0"&ETHICO=="0"&
            ANAGRELIDE=="0"&EPTIFIBATIDE=="0"&FONDAPARINUX=="0"&

GINTIC=="0"&ICOSAPENT=="0"&LIM=="0"&MESOGLYCAN=="0"&OZ=="0"&

PRASUGREL=="0"&RESVERATROL=="0"&SARP=="0"&TICAGRELOR=="0"&

TIC=="0"&TRIFLUSAL=="0"&VOR=="0"))%>%gtsummary::tbl_regression(exp=TRUE)
```

Appendix B

Table 5: QUADAS-2 criteria

		Quality assessment score
1.	Study design (cohort or case controlled)	/1
2.	Quality of inclusion criteria for selecting representative population *and* clear disclosure of inclusion criteria	/2
3.	Quality of exclusion criteria for excluding non-representative participants *and* disclosure of exclusion criteria listed	/2
4.	Quality points for cross-sectional matched control defined for datasets that reported patient and control samples. 2 characteristics=2 **OR** Quality points for cohort study of population representing based on demographic characteristics (age, gender, BMI, disease definition, comorbidities)	/2
5	CVD clearly defined. Points assigned for how defined (diagnostic result, clinical code) and transparency in data collection and reporting	/2
6	Method for measurement of biomarker *and* analysis method for biomarker level fully disclosed (i.e log, mean/median SD/range values)	/2
7	Quality points for time points of study: cross-sectional time-point of test identical for all participants (1), related to index test if appropriate and reported (1). **OR** Time point for longitudinal study, >=12 months FU=2, 6-12 months=1, <6 months =0	/2
8	Representative sample. Points assigned for transparency of sample selection methods and applicability of the population representing. E.g. if heart failure, only those with severe (NHYA III-IV) so not representative of all heart failure patients so would lose a point.	/2
	Total	/15

Appendix C

The table of studies in the systematic review and meta-analysis is too large for an A4 format. The table can be found in the S3 file of the published study ‘Role of the IL-33/ST2 axis in cardiovascular disease: A systematic review and meta-analysis’.

URL: (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0259026).

Appendix D

Table 6: SUMMIT propensity matched groups

Variable	Aspirin user (n=5038)	Aspirin non-user (n=5038)
Age (mean years)	65.222	64.580
Sex	3741 (74.3%) male	3678 (73.0%) male
BMI (mean)	28.500	27.690
Race	4411 (87.6%) white	3999 (79.4%) white
Smoking Status	2422 (48.1%) current smoker	2344 (46.5%) current smoker
Pack Years (smoking, mean)	42.822	37.709
FEV1%	59.024	58.958
Previous exacerbations prior to study entry (0, 1, >=2)	0 (60.4%) 1 (24.7%) >=2 (14.8%)	0 (57.9%) 1 (26.2%) >=2 (15.9%)
Clinical History (Yes)		
Diabetes	1206 (23.9%)	1224 (24.3%)
Hypercholesterolemia	3164 (62.8%)	2560 (50.8%)
Hypertension	4386 (87.1%)	4197 (83.3%)
Heart Disease	2645 (52.5%)	2645 (52.5%)
Stroke	523 (10.4%)	523 (10.4%)
HF	1166 (23.3%)	1108 (22.2%)
Peripheral Artery Disease	1045 (20.7%)	1045 (20.7%)

Table 7: IMPACT propensity matched groups

Variable	Aspirin user (n=2037)	Aspirin non-user (n=2037)
Age (mean years)	66.795	65.196
Sex	1362 (66.9%) male	1362 (66.9%) male
BMI (mean)	28.002	26.428
Race	1779 (87.3%) white	1537 (75.5%) white
Smoking Status	711 (34.9%) current smoker	686 (33.7%) current smoker
Pack Years (smoking, mean)	49.937	45.686
FEV1%	45.196	45.727
Previous exacerbations prior to study entry (0, 1, >=2)	0 (0.0%) 1 (46.5%) >=2 (53.4%)	0 (0.1%) 1 (44.7%) >=2 (55.2%)
Clinical History (Yes)		
Diabetes	486 (24.1%)	269 (13.3%)
Hypercholesterolemia	1061 (52.5%)	633 (31.5%)
Hypertension	1406 (69.3%)	1075 (53.0%)
Heart Disease	550 (27.0%)	550 (27.0%)
Arrhythmia	222 (10.9%)	212 (10.4%)
Stroke	149 (7.3%)	149 (7.3%)
HF	178 (8.8%)	123 (6.0%)
Peripheral Artery Disease	113 (5.5%)	113 (5.5%)

Appendix E

Table 8: PRISMA 2009 checklist

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

Appendix F

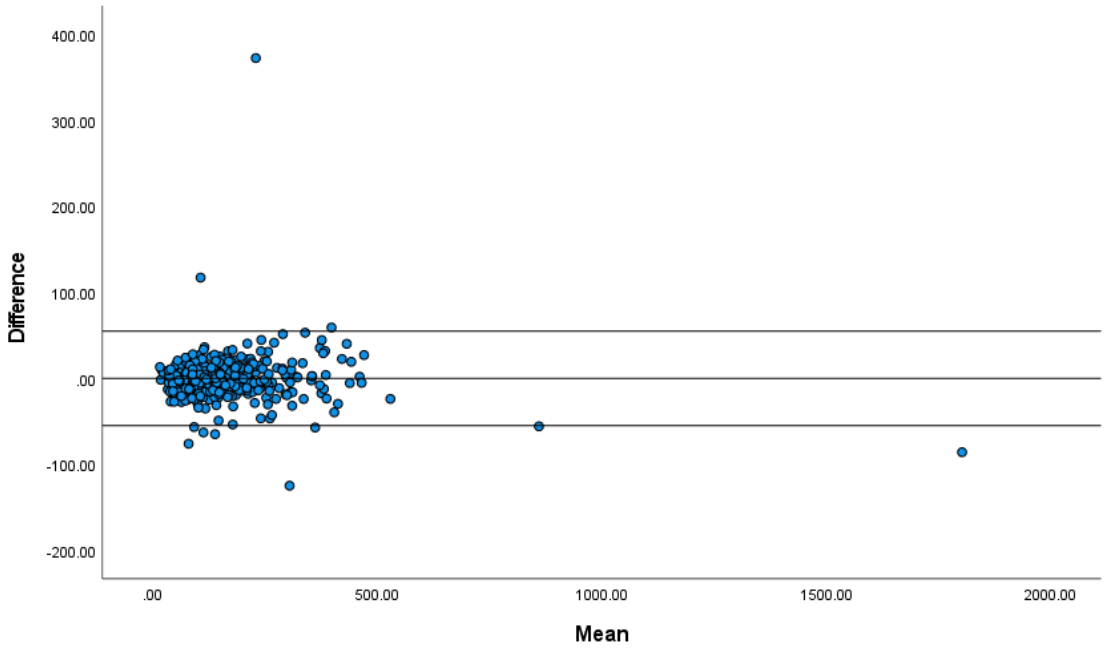

Figure 20: Bland-Altman plot showing differences between measurements of RvD1

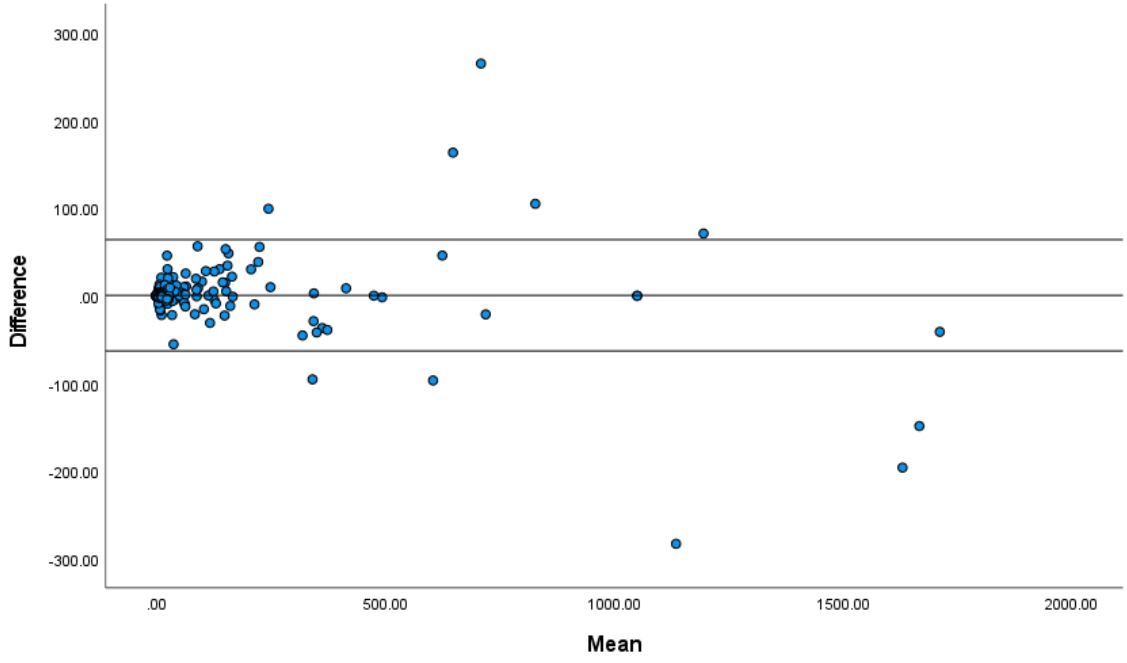

Figure 21: Bland-Altman plot showing differences between measurements of Del-1