

From novel discovery tools and biomarkers to precision medicine-basic cardiovascular science highlights of 2021/22

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1 **Basic Cardiovascular Science Highlights of 2021/2022 – from novel discovery**
2 **tools and biomarkers to precision medicine.**
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Paul C Evans^{^*}, Sean M. Davidson^{^*}, Johann Wojta^{^*}, Magnus Bäck^{^*}, Sveva Bollini, Mairi Brittan, Alberico L. Catapano, Bill Chaudhry, Matthijs Cluitmans, Massimiliano Gneccchi, Tomasz J Guzik, Imo Hofer, Rosalinda Madonna, João P Monteiro, Henning Morawietz, Elena Osto, Teresa Padró, Judith C. Sluimer, Carlo Gabriele Tocchetti, Kim Van der Heiden, Gemma Vilahur, Johannes Waltenberger, Christian Weber[^].

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6 Here we review the highlights of cardiovascular basic science in published in 2021 and early 2022 on
7 behalf of the European Society of Cardiology Council for Basic Cardiovascular Science. We begin
8 with non-coding RNAs which have emerged as central regulators cardiovascular biology, and then
9 discuss how technological developments in single-cell 'omics are providing new insights in
10 cardiovascular development, inflammation and disease. We also review recent discoveries on the
11 biology of extracellular vesicles in driving either protective or pathogenic responses. The Nobel Prize
12 in Physiology or Medicine 2021 recognised the importance of the molecular basis of mechanosensing
13 and here we review breakthroughs in cardiovascular sensing of mechanical force. We also
14 summarise discoveries in the field of atherosclerosis including the role of clonal haematopoiesis of
15 indeterminate potential, and new mechanisms of cross-talk between hyperglycemia, lipid mediators
16 and inflammation. The past 12 months also witnessed major advances in the field of cardiac
17 arrhythmia including new mechanisms of fibrillation. We also focus on inducible pluripotent stem cell
18 (iPSC) technology which has demonstrated disease causality for several genetic polymorphisms in
19 long QT syndrome and aortic valve disease, paving the way for personalized medicine approaches.
20 Finally, the cardiovascular community has continued to better understand COVID-19 with significant
21 advancement in our knowledge of cardiovascular tropism, molecular markers, the mechanism of
22 vaccine-induced thrombotic complications and new anti-viral therapies that protect the cardiovascular
23 system.
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1 **From novel discovery tools and biomarkers to precision medicine - basic**
2 **cardiovascular science highlights of 2021/2022**
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1 **ABSTRACT**

2 Here we review the highlights of cardiovascular basic science in published in 2021 and early 2022 on
3 behalf of the European Society of Cardiology Council for Basic Cardiovascular Science. We begin
4 with non-coding RNAs which have emerged as central regulators cardiovascular biology, and then
5 discuss how technological developments in single-cell 'omics are providing new insights in
6 cardiovascular development, inflammation and disease. We also review recent discoveries on the
7 biology of extracellular vesicles in driving either protective or pathogenic responses. The Nobel Prize
8 in Physiology or Medicine 2021 recognised the importance of the molecular basis of mechanosensing
9 and here we review breakthroughs in cardiovascular sensing of mechanical force. We also
10 summarise discoveries in the field of atherosclerosis including the role of clonal haematopoiesis of
11 indeterminate potential, and new mechanisms of cross-talk between hyperglycemia, lipid mediators
12 and inflammation. The past 12 months also witnessed major advances in the field of cardiac
13 arrhythmia including new mechanisms of fibrillation. We also focus on inducible pluripotent stem cell
14 (iPSC) technology which has demonstrated disease causality for several genetic polymorphisms in
15 long QT syndrome and aortic valve disease, paving the way for personalized medicine approaches.
16 Finally, the cardiovascular community has continued to better understand COVID-19 with significant
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18 vaccine-induced thrombotic complications and new anti-viral therapies that protect the cardiovascular
19 system.
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1 1. INTRODUCTION

2 The aim of this review from the European Society of Cardiology (ESC) Council for Basic
3 Cardiovascular Science is to highlight the most noteworthy developments over the past year, in the
4 field of cardiovascular basic science. The cited reports were selected as representative examples of
5 studies which provided robust evidence for particularly novel insights. *Cardiovascular Research*
6 previously reviewed the highlights of 2020 divided into vascular and cardiac topics,^{1 2} but here we
7 integrate both areas to generate the Basic Cardiovascular Science Highlights of 2021/2022.

9 2. CARDIOVASCULAR RNA UNIVERSE

10 2.1 Non-coding RNAs (nc RNAs)

11 In addition to the role of messenger RNA (mRNAs) in the 'central dogma' of molecular biology as a
12 template for protein synthesis, the RNA universe also contains multiple constellations of microRNAs
13 (miRNAs; miRs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) that control
14 fundamental processes of life. These RNA species adopt complex structures and interact with
15 nucleotides, proteins and lipids to control multiple functions including chromatin structure,
16 transcription, RNA splicing and stability, intracellular signalling and organelle dynamics. Research
17 reported in 2021 has provided further insight into the role of miRNAs, lncRNAs, and circRNAs in the
18 regulation of vascular remodeling and cardiac disease. Using both single-cell (sc) and bulk RNA-
19 sequencing to investigate transcriptional changes associated with endothelial-to-mesenchymal
20 transition (EndMT), *Monteiro et al* identified for the first time the genomic locus hosting the lncRNA
21 MIR503HG as necessary to maintain endothelial cell (EC) identity and function³. In a series of our
22 loss- and gain-of-function experiments the group demonstrated that loss of lncRNA is a causal event
23 in EndMT observed in pulmonary arterial hypertension (PAH) in association with vascular remodelling
24 (Figure 1). Further, located upstream from the vascular smooth muscle cell (vSMC)-associated miR-
25 143 and -145 cluster, the lncRNA CARMN (Cardiac Mesoderm Enhancer-associated Noncoding
26 RNA) was recently identified as key regulator of vSMC function and the pathophysiology of
27 atherosclerotic disease⁴. Crucially, while crosstalk between lncRNA host genes and coupled miRNAs
28 is often seen, CARMN was found to function independently from miR-143/-145 in regulating vSMC
29 and activating a pro-atherogenic proliferative state (Figure 1).

30
31 *Gong et al* identified in atherosclerotic mouse models a novel circRNA, circEsys2, involved in vascular
32 remodeling through the targeted inhibition of alternative mRNA splicing. By performing loss- and gain-
33 of-function mutation analyses in vascular smooth muscle cells, circEsys2 was shown to enhance cell
34 proliferation and migration and blunt apoptosis and differentiation. Furthermore, silencing of circEsys2

1 prevented neointima formation while circEsys2 overexpression enhanced neointimal hyperplasia in an
2 *in vivo* model of carotid artery injury.⁵ The role of miRNAs in atherosclerosis progression was
3 examined by Liu *et al* by describing the role of the Nuclear Factor of Activated T-cell isoform c3
4 (NFATc3)/miR-204 axis in the regulation of foam cell formation in atherosclerosis. Using genetically
5 modified mice, they showed that NFATc3 prevents macrophage foam cell formation and limits the
6 expression of scavenger receptors SR-A and CD36 by inducing expression of the microRNA miR-
7 204,⁶ suggesting the NFATc3/miR-204 axis as a potential therapeutic target to reduce plaque
8 formation. In a separate study involving macrophages, *Schober et al* illuminated the circadian
9 patterns of myocardial infarction (MI) by evaluating macrophage-related miRNAs. They evidence, in a
10 murine model of atherosclerosis, that macrophage miR-21 drives circadian regulation of macrophage
11 apoptosis by targeting proapoptotic Xaf1 (XIAP-associated factor 1), thereby regulating plaque
12 composition and susceptibility to rupture.⁷ Further studies in a murine model of pressure-overload
13 heart failure have also found a key role for macrophage miR-21 in modulating cardiac fibrosis by
14 regulating macrophage polarization towards a pro-inflammatory (M1) phenotype.⁸ In addition, *Hinkel*
15 *et al* identified a pivotal role of miR-132 in the mediation of pathologic cardiac hypertrophy in a novel
16 porcine model of percutaneous aortic constriction by stent implantation.⁹

17
18 ncRNAs have also continued to attract attention as biomarkers with prognostic and diagnostic
19 potential. A landmark study from *Blanco-Dominguez et al.* identified a novel miRNA with potential
20 diagnostic value in acute myocarditis. The authors performed miRNA microarray analyses in sorted
21 CD4+ T cells and type 17 helper T (Th17) cells after inducing experimental autoimmune myocarditis
22 or MI in mice and identified mmu-miR-72 as a differentially expressed miRNA. They further identified
23 the human homologue hsa-miR-Chr8:96 and demonstrated its potential to distinguish patients with
24 myocarditis from those with MI and healthy controls.¹⁰ Thus, miR-Chr8:96 has translational potential
25 as a novel biomarker to diagnose myocarditis. miR-133a is a well-established, diagnostic circulating
26 biomarker in patients with heart failure.¹¹ *Escate et al.* expanded on the diagnostic potential of this
27 miRNA by demonstrating that elevated plasma levels of miR-133a predict the future occurrence of
28 major adverse cardiovascular events (MACE) in patients with familial hypercholesterolaemia (FH).¹²
29 This observation supports the potential utility of miR-133a in improving risk stratification and
30 prognosis in high-risk patients. More broadly, an international consortium supporting collaboration
31 and research on ncRNAs in cardiovascular disease (CardioRNA Cost Action CA17129) published a
32 Position Paper on the pathophysiologic role of ncRNAs, and to provide recommendations to translate
33 this into clinical practice.¹³

1 Other studies have progressed ncRNA candidates with therapeutic potential towards clinical
2 translation.^{9, 14, 15} *Kay et al* examined the potential of targeting ncRNAs to promote cell-based
3 regenerative strategies for heart disease. Using an integrated approach, they identified CARMA
4 (CARdiomyocyte Maturation-Associated lncRNA), a conserved lncRNA controlling cardiomyocyte
5 differentiation and maturation in human embryonic stem cells. CARMA knockdown promoted
6 cardiogenic commitment and cardiomyocyte differentiation in embryonic stem cells, and is therefore a
7 novel target for improving human ESC-derived cardiomyocyte production in regenerative
8 cardiovascular medicine.¹⁴ On the other hand, *Modica et al* provided evidence for the effectiveness of
9 a novel nanotechnology-based approach for delivering exogenous synthetic miR-133a. The authors
10 demonstrated that intra-tracheal nebulization of miR-133a-nanoconstruct once-a-day on alternate
11 days for 4 consecutive weeks protects against heart failure progression (improved cardiac function
12 parameters and lower fibrosis) in a murine model. This improvement was associated with the
13 restoration of physiological levels of miR-133a in cardiomyocytes without significant accumulation in
14 other myocardial cells or organs.¹⁵

16 **2.2 Single cell approaches**

17 Single-cell RNA sequencing (scRNAseq) has emerged as a powerful tool to dissect transcriptional
18 profiles of the complex cardiovascular system at single-cell resolution. scRNAseq has been insightful
19 in our understanding of the earliest stages of cardiac development by identifying the epicardial
20 progenitor field, which is anatomically and transcriptionally distinct from the currently known first and
21 second heart fields.¹⁶ In the formed heart, scRNAseq and spatial transcriptomics were used to show
22 that dysregulation of TBX5, the mutated gene causing septal and conduction defects in patients with
23 Holt-Oram syndrome, leads to transcriptional consequences in specific cardiomyocyte subtypes.¹⁷
24 The study went on to show using cell-based analyses and mice that the stability of many gene
25 regulatory networks, including those that have been shown to be relevant to congenital heart disease,
26 are sensitive to TBX5 dosage.

27
28 At the level of the vasculature, the number of publications of atlas-type human or primate scRNAseq,
29 or Assay for Transposase-Accessible Chromatin (ATAC) datasets has steadily increased, which
30 provides a valuable, yet often descriptive resource.¹⁸⁻²¹ scRNA-seq has been used to identify
31 transcriptional changes upon conditional cell type-specific genetic deletion, otherwise obscured in
32 bulk tissue RNA sequencing.²² As for immune cells in atherosclerosis, the detection of different
33 subsets has culminated in a consensus on cell type markers,²³ yet to be achieved for the many
34 varieties of vSMCs identified using scRNAseq in recent years, i.e. fibromyocytes, proinflammatory or

1 modified vSMCs, SMC-derived intermediate cells.^{21, 24-26} scRNAseq has also progressed our
2 understanding of EC,^{27 28} with *Rodor et al* identifying CD74 as potential target in PAH and showing its
3 capacity to regulate barrier integrity.²⁸

4
5 At a cardiac level, the implementation of scRNAseq allowed the impact of heart failure on circulating
6 immune cells to be determined.²⁹ Furthermore, it demonstrated an exacerbated inflamed
7 transcriptome in circulating monocytes and a signature of T-cell activation in heart failure patients
8 harbouring clonal haematopoiesis-driver mutations in DNA methyltransferase DNMT3A, thereby
9 providing further insights into the potential effect of DNMT3A mutations in heart failure progression.³⁰
10 On the other hand, *Hesse et al.* have defined a high level of heterogeneity of epicardial stromal cells
11 following MI, similar to cardiac fibroblast heterogeneity, with evidence of regenerative capacity and
12 hypoxic signalling.³¹ *Tombor et al* used scRNAseq of endothelial-lineage traced mice to change the
13 dogma on EndMT in MI, showing this is a transient affair, often without a definite mesenchymal
14 endstage.³²

15
16 Moving forward, cardiovascular scientists will benefit greatly from the generation of multi-omics
17 reference atlases, including different layers of information on RNA, protein, spatial anatomy,
18 interactome and cell ontology.³³⁻³⁵ Overall, scientific progress can be expedited by open-access
19 science and data sharing. Thus, the integration of available datasets for mesenchymal cells,³⁶ as
20 previously carried out for immune cells in atherosclerosis,³⁷ and a web-based application by the *Miller*
21 lab (plaqview.com),³⁸ pave the way for new, meaningful discoveries in cardiovascular biology.

22 23 **3. CARDIOVASCULAR DEVELOPMENT**

24 2021 witnessed progress in several important aspects of heart development with implications for our
25 understanding of both congenital and acquired heart conditions. Genomic studies of congenital heart
26 malformations now allow the analysis of variants within the context of gene networks. A good
27 example of this is the recent genomic study on hypoplastic left heart syndrome (HLHS),³⁹ where
28 whole-exome sequencing, coupled to nuclear transcriptomics and scRNAseq identified genetic
29 heterogeneity in HLHS that converges to alter fundamental processes (e.g. autophagy, apoptosis,
30 proliferation) in myogenesis.

31
32 Despite the relative ease in differentiating functional, if immature, cardiomyocytes from iPSC, it has
33 proven remarkably difficult to create organoids resembling the cellular and structural complexity of the
34 vertebrate heart *in vitro*. However, *Lewis-Israeli et al*⁴⁰ described a robust protocol for producing

1 cardiac organoids from iPSC using a three-step Wnt signaling modulation strategy. These organoids
2 develop a broad range of cardiac cell types, including those that are induced through interactions
3 between distinct primary cardiac cell types, and develop cavities that superficially resemble the lumen
4 of the chambers. Moreover, they are vascularised and display regular beating. Importantly, the
5 transcriptome of the organoids more closely resembles foetal hearts than monolayer cardiomyocytes.
6 This method is an important step on the path to developing a robust human-based *in vitro* model of
7 the heart.

8
9 It is increasingly apparent that the majority of valve malformations and dysfunction arise from
10 abnormal development, and yet the mechanisms of valve development are incompletely understood.
11 The study by *Fukui* et al focussed on the role of mechanical factors using zebrafish embryos.⁴¹ They
12 identified a critical role for shear stress by showing that ectopic activation of wall shear stress, using
13 agarose beads implanted into the atrium of the early zebrafish heart, resulted in the formation of
14 valve-like structures that expressed the characteristic molecular signature of primitive valves,
15 including the activation of NFATc and *klf2a*. Downstream of this, they ruled out a number of well-
16 known mechanosensitive pathways, and instead identified adenosine tri-phosphate (ATP) signalling
17 as a mediator of Ca^{2+} oscillations that were essential for specifying valve cell identity. Overall, the
18 convergence of large-scale genomic network analyses, scRNAseq and spatial transcriptomics and
19 experimental developmental biology is coming close to explaining the mechanisms underlying heart
20 malformations presenting at birth and in adulthood.

21 22 **4. VASCULAR DISEASE AND REPAIR**

23 **4.1 Mechanosensing**

24 The Nobel Prize in Physiology or Medicine 2021 was awarded to *David Julius* from the University of
25 California San Francisco and *Ardem Patapoutian* from The Scripps Research Institute La Jolla for
26 explaining the molecular basis for sensing heat, cold and mechanical force.⁴² *Ardem Patapoutian*
27 identified PIEZO 1 and 2 as ion channels activated by mechanical force,⁴³ and they are central
28 responders of arterial responses to flow.⁴⁴ Recently, the protein kinase N2 (PKN2) has been shown to
29 be activated by flow through the mechanosensitive ion channel PIEZO1 and mediate flow-induced
30 endothelial NO synthase activation and vascular tone regulation⁴⁵ (Figure 2). As another important
31 mechanosensor, the glycocalyx modulates the endothelial redox state in response to shear stress
32 and could mediate an atheroprotective synergism between glycocalyx sialic acids and nuclear factor
33 erythroid 2-related factor (NRF2) antioxidant signaling.⁴⁶ The regulation of NRF2 plays also a major
34 role in the reduced endothelial cell viability and wound healing in response to cigarette smoke

1 extracts under atherogenic low flow conditions.⁴⁷ The concept of disturbed flow as an initial stimulus
2 for the development of atherosclerotic plaques has led to exciting new therapies to target
3 mechanosensitive genes like *TWIST1*, *GATA4*, and bone morphogenic proteins (*BMPs*) using siRNA-
4 based technologies in an attempt to slow down the progression of atherosclerosis.^{48, 49}

6 **4.2. Atherosclerosis risk factors**

7 The metabolic syndrome – in concert with inflammation - plays a central role in atherosclerosis. In
8 particular, the causal role low-density lipoprotein (LDL) in atherosclerosis is indisputably supported by
9 multiple lines of evidence such as epidemiological studies, Mendelian randomization and genetic
10 analyses, as well as randomized clinical trials and animal model experimentation.

11
12 Traditional lipid-lowering drugs such as statins aim to reduce lipid uptake and/or cholesterol synthesis
13 and are still widely used. However, the availability of genetic data and the identification of the genetic
14 cause for rare diseases linked to dyslipidaemias has prompted spectacular advances in the
15 identification of pharmacological targets for the treatment of dyslipidaemias (Figure 3). The most
16 recent advances in lipid-lowering relate to the inhibition of proprotein convertase subtilisin kexin 9
17 (*PCSK9*), angiotensin-like 3 (*ANGPTL3*) and lipoprotein (a) (*Lp(a)*). Besides monoclonal antibodies,
18 additional options to inhibit *PCSK9* are emerging, including gene silencing with an siRNA or gene
19 editing employing the CRISPR/Cas system. Inclisiran, a siRNA conjugated with *N*-
20 acetylgalactosamine residues ensuring hepatic selectivity, decreases *PCSK9* production by
21 promoting the degradation of its mRNA. This approach allows for twice-yearly dosing, with long-term
22 lowering of LDL-C (~50%), potentially enhancing patient compliance compared with other cholesterol-
23 lowering drugs.^{50, 51} Along the same line of RNA interference, *Lp(a)*-reducing drugs are being
24 investigated in phase 2-3 trials.⁵² At earlier stages of development are gene-editing technologies,
25 which introduce permanent genomic changes to alter gene function. A single treatment with *PCSK9*
26 gene or base editors has been shown to confer durable LDL-C reduction in primates⁵³. Evinacumab is
27 a monoclonal antibody targeting *ANGPTL3*. It reduces significantly triglycerides (TG) by up to 80% in
28 hypertriglyceridaemic subjects⁵⁴ and it is highly effective in reducing LDL-C levels in patients with
29 homozygous FH carrying null *LDLR* mutations⁵⁵ providing a new pharmacological tool. In a recent
30 study, membrane type 1 matrix metalloproteinase (MT1-MMP), in addition to activating MMP-2, was
31 shown to regulate LDL-receptor (*LDLR*) shedding, affecting circulating lipid concentrations and
32 atherosclerosis.⁵⁶

1 The past year has further blurred the borders between traditional risk factors and the role of
2 inflammation in atherosclerosis as their connections and interplay become more evident. Diabetes
3 mellitus elevates cardiovascular risk, and hyperglycaemia contributes strongly to metabolic
4 syndrome. Besides these known effects, *Edgar et al* elucidated a pro-inflammatory and pro-
5 atherogenic switch in macrophages from diabetic mice persisting even when cultured under
6 normoglycaemic conditions.⁵⁸ This persevering effect of earlier hyperglycaemia may explain the
7 relatively low degree of risk reduction upon glucose level normalisation in diabetics. The inseparable
8 connection between cholesterol and inflammation and atherosclerosis is further supported by a recent
9 study that showed how sensing of cholesterol crystals by macrophages induces complement
10 component C5aR1 signaling on mitochondrial membranes and results in interleukin (IL)-1 β production
11 and sterile inflammation.⁵⁹ Hence, intracellular C5aR1 targeting may be used to normalize
12 mitochondrial function and reduce IL-1 β release. This has translational relevance since inhibition of
13 IL-1 β production through targeting the inflammasome has been identified as a target in cardiovascular
14 disease previously. Another old acquaintance in cardiovascular disease therapy, rivaroxaban, a direct
15 oral anticoagulant, not only targets factor Xa activity, but may also reduce inflammasome formation.
16 In mice treated with rivaroxaban, macrophage autophagocytic activity increased significantly, which
17 the authors were able to trace back to the Xa-PAR2 axis.⁵⁷

18
19 Recent studies show the complex intertwinement between traditional risk factors, vascular biology
20 and immunology. Cardiovascular risk factors can affect haematopoiesis through defective
21 angiogenesis in the bone marrow towards generation of inflammatory leukocytes, thereby creating a
22 self-energizing circle of cardiovascular risk factors – defective angiogenesis – release of inflammatory
23 cells – cardiovascular disease exacerbation.⁶⁰ Sakic et al emphasised crosstalk between vSMCs and
24 vascular inflammation by demonstrating that S100A4 induces vSMC change towards a
25 proinflammatory phenotype to drive features of plaque instability⁶¹. Together, these studies call for an
26 integrated and unprejudiced approach in atherosclerosis research to link traditional risk factors with
27 novel molecular mechanisms.

28 29 **4.3 Inflammation in Atherosclerosis**

30 The immune response is critical throughout the development of atherosclerotic lesions, during
31 disease initiation, as a trigger for episodic plaque progression, and a contributor to thrombotic
32 complications.⁶² A failure in the resolution of inflammation can prevent healing and repair of the
33 vascular wall.⁶²⁻⁶⁴ This concept was advanced by *Arnardottir et al* who found that lipid-specialized,
34 pro-resolving mediators (SPM) signalling through G-protein coupled receptor (GPR)-32,

1 Is critical for inflammatory resolution and atheroprotection.⁶⁴

2
3 The proposal that macrophage uptake mechanisms are decisive for the turning point that leads either
4 to inflammation resolution or to chronic inflammation and plaque progression has received further
5 support from analysis pro-resolving pathways⁶⁴ or phagocytic immune checkpoints in murine
6 models.⁶⁵ Focussing on the CD47- signal-regulatory protein (SIRP) α immune checkpoint, loss of
7 SIRP α in macrophages stimulated efferocytosis, attenuated oxidized LDL-induced inflammation and
8 induced an M2 macrophage phenotype.⁶⁵ These findings may pave the way for novel interventions to
9 promote inflammatory resolution through macrophage uptake mechanisms and phenotypic transitions
10 to protect the vasculature.

11
12 Adaptive immune responses are critical regulators of atherosclerosis. On a systemic level, pro-
13 inflammatory and cytotoxic T-lymphocytes prevail in atherosclerosis, as demonstrated by a
14 preferential expansion and function of CD28^{null} T lymphocytes after *ex vivo* IL-7 and IL-15 stimulation
15 of high-purity sorted CD4+ cells isolated from patients with acute coronary syndrome.⁶⁶ The local
16 recruitment of regulatory T lymphocytes (T_{reg}) is critical for the control of atherosclerotic lesion
17 inflammation and is, in part, regulated by cellular metabolism.⁶⁷ As an approach to use T_{reg}
18 recruitment as a therapeutic strategy to selectively target adaptive immune regulation in the
19 atherosclerotic plaque, adoptive transfer of the fractalkine receptor CX3CR1 overexpressing T_{reg} was
20 shown to increase their recruitment to atherosclerotic lesions and decreased atherosclerosis
21 burden.⁶⁸

22
23 However, inhibition of some immune checkpoints can lead to enhanced atherosclerosis. This is
24 exemplified by *Poels et al.* who found that short-term immune checkpoint inhibitors (ICIs) therapy
25 aggravates T cell-mediated plaque inflammation and drives plaque progression in mice.⁶⁹ Also, ICIs
26 used to treat cancer, such as monoclonal antibodies targeting CTLA-4, PD-1, and PD-L1, have been
27 associated with adverse cardiovascular events.⁷⁰ For example, *Michel et al.* discovered that anti-PD1
28 therapy in a mouse model of melanoma led to impaired left ventricular function and promoted
29 myocardial infiltration with CD4+ and CD8+ T cells via a TNF-dependent mechanism.^{71, 72} Therefore,
30 the use of ICIs in the treatment of cancer provides exciting new opportunities for therapies but should
31 be pursued with caution.

4.4 Haematopoiesis of Indeterminate Potential

Clonal haematopoiesis of indeterminate potential (CHIP) has recently emerged as an exciting topic in cardiovascular medicine and biology. CHIP is defined as positive selection of specific somatic mutations in haematopoietic stem cells that provide a proliferative advantage and finally result in a clonal population carrying the mutation. Besides being associated with a 0.5 to 1% risk per year to develop leukaemia, CHIP is also associated with ageing, smoking, obesity and type 2 diabetes mellitus, chronic inflammation, infections, sleep deprivation, stress, hyperlipidaemia and atherosclerosis. Most mutations identified in CHIP affect the epigenetic regulators DNA (cytosine-5)-methyltransferase 3A (*DNMT3A*), tet methylcytosine dioxygenase 2 (*TET2*) and ASXL transcriptional regulator 1 (*ASXL1*) and the tyrosine kinase janus kinase 2 (*JAK2*) which result in a pro-inflammatory state that offers a possible explanation for the association of CHIP with a two-fold increase in risk to develop cardiovascular disease.^{73, 74} Using mice that express the *JAK2*^{V617F} variant exclusively in macrophages, *Fidler et al* reported increased proliferation of macrophages in atherosclerotic lesions and greater necrotic cores. These effects were ameliorated when caspase 1 and 11, which are key components of the inflammasome or gasdermin D, which plays a major role in pyroptosis, were deleted. The authors also noted increased lesional expression of absent in melanoma 2 (*AIM2*) and found that atherosclerosis was reduced in mice deficient in *Aim2*. The authors concluded that enhanced proliferative stress caused by *JAK2*^{V617F} leads to DNA damage and to activation of the *AIM2* inflammasome resulting in IL-1 β activation, which then in turn starts a feed forward loop resulting in even more macrophage proliferation thereby aggravating atherosclerosis.⁷⁵

A new perspective to the field added *Heyde et al* who recently showed by mathematical modeling and murine models that increased proliferation of haematopoietic stem cells occurs in individuals suffering from atherosclerosis thereby increasing the risk to develop clonal haematopoiesis by the age of 70 3.5-fold. Based on their findings the authors propose a vicious cycle in which atherosclerosis leads to clonal haematopoiesis, which in turn aggravates atherosclerosis.⁷⁶

5. CARDIAC DISEASE AND REPAIR

5.1 Extracellular vesicles and nanoparticles

2021 was another exciting year in the field of extracellular vesicle (EV) biology for regenerative medicine, including cardiac repair and regeneration (Figure 4). There was increasing interest in understanding the mechanism of EV-based intercellular communication within the myocardium during ventricular remodeling after acute MI. In terms of the role of EVs in cardiac fibrosis after MI, however, findings differ. For example, *Li et al* showed that miR-30d is mainly secreted in EVs by

1 cardiomyocytes and inhibits fibroblast proliferation by acting on integrin $\alpha 5$ via paracrine signaling.⁷⁷
2 Counterbalancing this view, *Wang et al* evidenced, in a mouse model of MI, that EVs released by
3 myocardial M2 macrophages exacerbate migration, proliferation and myofibroblastic transformation of
4 cardiofibroblasts.⁷⁸ By performing mechanistic studies in cocultured primary cardiofibroblasts and M2
5 macrophages, the authors linked these effects to activation of miR-138-5p/RhoC signaling after
6 delivery of the M2 macrophage-derived EVs containing circular RNACirCUBE3a into the
7 cardiofibroblasts.⁷⁸ These findings may offer an additional therapeutic target to optimize the
8 endogenous mechanism of cardiac repair but suggest that EV function may depend on cell of origin.

9
10 There is great interest in the potential for EVs prepared from stem or progenitor cells to enhance
11 cardiac repair. Increasing evidence suggests the mechanism may involve the resolution of
12 inflammation. For example, *Correa et al* reported that EVs secreted from human iPSC-derived
13 cardiovascular progenitor cells (CPC) can trigger a pro-resolving immune response in preclinical
14 murine models of either chronic or acute heart failure. Similar results were confirmed *in vitro* on
15 human inflammatory cells, suggesting that this EV formulation can instruct the immune cell response
16 towards a pro-resolving phenotype.⁷⁹ *Patil et al* showed a similar pro-resolving effect of mesenchymal
17 stem cell (MSC)-derived small EVs, which they attributed to the EVs both enhancing opsonisation of
18 dead cells and activating phagocytic signaling, thereby augmenting removal of apoptotic cells,
19 resolution of inflammation, and improving cardiac recovery after injury.⁸⁰

20
21 In order to investigate a clinically feasible translational approach, *Katsur et al* assessed whether
22 cardioprotection could be achieved using a reproducible, clinical-grade preparation of small EVs
23 obtained from the CTX0E03 human neural stem cell line. Systemic administration of small EVs from
24 differentiating CTX0E03 reduced infarct size in mice and prevented *in vitro* cardiomyocyte
25 mitochondrial permeability transition pore opening, which is responsible for cardiomyocyte death
26 during reperfusion injury. These findings provide evidence for considering non-cardiovascular, yet
27 stabilised, cell lines as additional candidate source of therapeutic EVs.⁸¹ Interestingly, however, EVs
28 from proliferating CTX0E03 cells were not cardioprotective, which suggests that the status of cells of
29 origin can impact their secreted EV activity.⁸¹ Further evidence of this is provided by a study showing
30 that systemic administration of serum small EVs from young rats into aged ischaemic rats improved
31 functional outcomes after ischemic stroke, in contrast to small EVs from aged rats that worsened
32 outcome.⁸² This provides further evidence that EV function is altered in disease, and further suggests
33 that EV-miR-mediated vascular intercellular communication is altered in patients with chronic kidney
34 disease and coronary artery disease.

1
2 A major goal in cardiac regenerative medicine is to identify novel methods to reinstate cardiomyocyte
3 renewal. In such a scenario, EVs released from cardiac progenitors have been widely investigated,
4 given the role of cardiac stromal cells such as the epicardium-derived progenitor cells play in cardiac
5 muscle growth during embryonic development, and in heart regeneration in zebrafish and in neonatal
6 mice. *Villa del Campo et al* reported that epicardial EVs isolated from the secretome of both mouse
7 and human progenitors enhanced the proliferative activity of neonatal murine cardiomyocytes *in vitro*
8 and promoted cell cycle re-entry when injected into the injured area of infarcted neonatal hearts.
9 These EVs also enhanced regeneration in cryoinjured engineered human myocardium constructs, as
10 a novel model of human myocardial injury. Notably, the epicardial EV cargo was found enriched with
11 specific miRNAs, including miR-30a, miR-100, miR-27a, and miR-30e, which recapitulated the EV
12 regenerative influence on human stem cell-derived cardiomyocytes and cryoinjured cardiac
13 constructs *in vitro*.⁸³
14

15 The relevance of the content of cardiovascular cell-derived EVs was highlighted by publications
16 showing that miRNAs of the miR-106a-363 cluster,⁸⁴ periostin⁸⁵ and mitochondrial cargoes⁸⁶ can act
17 as effectors of cardiac repair. While such encouraging evidence supports the exploitation of
18 stem/progenitor cell-EVs as candidate therapeutics to promote adult cardiomyocyte proliferation, a
19 general consensus has not been reached yet on their mechanism of action. In fact, *Lima Correa et al*
20 recently showed that EVs obtained from human iPSC-derived cardiac progenitor cells failed to trigger
21 the generation of new cardiomyocytes in chronically infarcted hearts in mouse models. Despite this
22 negative result, the authors confirmed that EVs from cardiac progenitor cells remained capable of
23 significantly improving cardiac function by non-regenerative mechanisms.⁸⁷
24

25 These findings suggest that further analyses and accurate lineage tracing are required to better
26 understand the regenerative potential of cardiac EVs. At present, the rapid clearance of EVs from
27 circulation is a limitation for their clinical application. During 2021, a number of studies aimed to
28 overcome this barrier by constructing specific nanoparticles and genetically modifying cells to improve
29 retention time of the cell-derived EVs. Thus, *Wei et al* demonstrated that intravenously-injected EV
30 derived from modified mouse bone marrow MSC overexpressing CD47, a transmembrane protein
31 known to elicit blockade of the mononuclear cell phagocytosis, have prolonged retention in the
32 circulation and accumulate at greater levels in the ischemic heart.⁸⁸
33
34

5.2 Cardiotoxicity and regeneration

A wide range of drugs, including but not limited to antineoplastic chemotherapeutic agents, can cause heart electrophysiology dysfunction, muscle damage and other cardiovascular pathologies. For example, anthracyclines such as doxorubicin (DOX) are a cornerstone for the treatment of many cancers, but their use is complicated by cardiotoxicity, especially left ventricular dysfunction.

An interesting 2021 paper reported that transcutaneous vagal nerve stimulation prevented DOX-induced cardiotoxicity in rats by rebalancing autonomic tone, ameliorating cardiac dysfunction and remodelling. It was hypothesized that the mechanism involved crosstalk between autonomic neuromodulation, innate immune cells such as macrophages and chemokines.⁸⁹ Indeed, there are multiple mechanisms responsible for anthracycline cardiotoxicity.^{70, 90, 91} *Chan et al.* found that two orally available MMP inhibitors ameliorated DOX cardiotoxicity by attenuating intracellular and extracellular matrix remodelling, suggesting that they may be a potential prophylactic strategy to prevent heart injury during chemotherapy.⁹⁰ Remote ischaemic preconditioning can ameliorate DOX-induced cardiotoxicity by preserving mitochondrial integrity⁹² and this is currently the subject of the RESILIENCE clinical trial.⁹³

Other recent studies (discussed in⁹⁴) have identified harmful effects of anticancer therapies on the ability of stem/progenitor cells to repair cardiac damage, through a reduction of stem cell viability and paracrine activity. Thus numerous animal and clinical studies have demonstrated that local or systemic administration of mesenchymal stem cells significantly improve cardiac function, through a reduction in inflammatory responses and myocardial fibrosis.⁹⁵ Antivirals can also induce cardiotoxicity, including the only FDA-approved treatment for hospitalized COVID-19 patients, remdesivir which can induce toxicity in human iPSC-derived cardiomyocytes through mitochondrial fragmentation, electrophysiological alterations and sarcomere disarray.⁹⁶

5.3 Cardiac arrhythmias

Several key insights into fibrillation and re-entrant arrhythmias were obtained in 2021 (Figure 5). *Handa et al* revealed that the degree of gap junction coupling as well as the pattern of fibrosis influences mechanisms sustaining ventricular fibrillation.⁹⁷ Differentiating between these underlying mechanisms of maintenance of fibrillation may help to guide therapy. Re-entrant arrhythmias may also initiate in the absence of structural abnormalities, shown recently in a study on the spatiotemporal interaction between trigger and electrical substrate in the context of unexplained sudden cardiac arrest (SCA).⁹⁸ Analysis of explanted hearts and observations in survivors of

1 unexplained SCA, identified key elements required for re-entry initiation including the occurrence of
2 an early premature beat from an early repolarizing region of the ventricles, which may block against a
3 steep repolarization time (RT) gradient to start re-entry. They also showed that detection of the origin
4 of premature beats and their relation to RT gradients in patients is possible with non-invasive
5 electrocardiographic imaging (ECGI) and may provide targets for therapy. ECGI was also employed
6 by *Leong et al* in survivors of SCA to show that not only repolarization abnormalities, but also
7 underlying conduction abnormalities play a role in the initiation of SCA.⁹⁹ A similar mechanistic
8 reasoning extends to atrial arrhythmias.¹⁰⁰ Bringing these studies together highlights that any cause
9 of steep excitability dispersion – whether resulting from local changes in gap junction coupling,
10 fibrosis, local conduction slowing, or inherent repolarization duration heterogeneity – play a critical
11 role in the initiation and maintenance of re-entry and fibrillation.

12
13 New tools are essential to obtain mechanistic insights and recent reports highlight how the field of
14 atrial fibrillation research should transition from a translational approach to an integrative research
15 approach¹⁰¹ and how personalized computer models may provide more individualised insights in
16 disease and guide therapy.¹⁰² Application of novel therapeutic tools also brings new mechanistic
17 insights. Non-invasive radiation therapy for cardiac arrhythmias was initially thought to induce fibrosis,
18 similar to invasive catheter-based therapy.¹⁰³ However, *Zhang et al* found that transmural fibrosis
19 does not develop in the hearts of patients receiving radiation therapy within the timeframe of its
20 ventricular tachycardia-reducing effects.¹⁰⁴ Interestingly, they showed that irradiating murine hearts
21 results in a persistent supraphysiologic electrical phenotype, mediated by increases in sodium
22 channel function and gap junction function. This functional restoration was confirmed by a shortening
23 of QRS duration in patients receiving radiation therapy, highlighting that radiation-induced
24 reprogramming of cardiac conduction is the potential mechanism beyond the initial success of
25 radiation therapy for refractory ventricular tachycardia. This holds promise for extending the use of
26 non-invasive radiation therapy to other applications, as for example recently demonstrated in heart
27 failure with reduced ejection fraction.¹⁰⁵

28 29 **6. CARDIOVASCULAR PRECISION MEDICINE AND iPSC**

30 Precision medicine aims to improve risk stratification and customize the management and therapy of
31 patients based on their clinical and genetic characteristics, on datasets of large populations and the
32 use of advanced technologies.¹⁰⁶ Genome-wide association studies (GWAS) has progressed through
33 advances in genome-wide genotyping technology and large population and patient datasets to
34 explore the role of common variants on phenotypic traits and disease susceptibility. According to the

1 GWAS catalogue database, there are known to be 1329 polymorphism-cardiovascular trait
2 associations. This growing catalogue of genome-wide and nominally significant variants has also
3 opened the door to creating polygenic risk scores that could identify individuals at risk of developing
4 specific cardiovascular diseases or sub-groups of patients with a more severe prognosis.¹⁰⁷ However,
5 this approach must consider numerous confounding factors such as epigenetic and transcriptomic
6 data that may correlate with genetic variants. *Boix et al* undertook a *tour de force* to create EpiMap, a
7 compendium comprising 10,000 epigenomic maps across 800 samples, which were used to define
8 chromatin states, high-resolution enhancers, enhancer modules, upstream regulators, and
9 downstream target genes.¹⁰⁸ This resource allowed the annotation of 30,000 genetic loci associated
10 with 540 traits, predicting trait-relevant tissues, putative causal nucleotide variants in enriched tissue
11 enhancers and candidate tissue-specific target genes for each of them. These different data
12 integration layers could be essential for understanding the genetic architecture underlying the broad
13 phenotypic traits encountered in common and complex cardiovascular diseases such as coronary
14 artery disease. For instance, while “only” 56 ‘unifactorial’ traits were enriched in the case of long QT
15 syndrome (LQTS), a total of 192 ‘multifactorial’ traits were enriched in an average of five different
16 tissues, and in the case of coronary artery disease, 26 ‘polyfactorial’ traits were enriched in 14
17 tissues. The study by *Boix et al* is at the same time a rich scientific resource, but also a lesson
18 regarding the profound and magnificent complexity of the human genome and the causal basis of
19 common diseases like coronary artery disease.

20
21 The GENMED consortium conducted a large GWAS study focused on dilated cardiomyopathy
22 (DCM), enrolling 2719 cases and 4440 controls.¹⁰⁹ They identified and replicated two new DCM-
23 associated loci on chromosome 3p25.1 and chromosome 22q11.23. *In silico* annotation and
24 functional 4C-sequencing analyses on cardiomyocytes derived from iPSC-derived cardiomyocytes
25 identified SLC6A6, a gene encoding a taurine, as the most likely DCM candidate at the 3p25.1 locus,
26 and SMARCB1 as the candidate culprit gene at the 22q11.23 locus. The consortium also constructed
27 a genetic risk score for DCM.

28
29 In another important study, exome sequencing data from 811 probands with tetralogy of Fallot (TOF)
30 were used to identify rare loss-of-function and other likely pathogenic variants in genes associated
31 with congenital heart disease.¹¹⁰ The role of some likely pathogenic variants was confirmed and
32 multiple loss-of-function variants provided support for 3 emerging congenital heart disease/TOF
33 candidate genes: *KDR*, *IQGAP1*, and *GDF1*. Moreover, using composite genes in a STRING protein

1 interaction enrichment analysis, a biologically relevant network was revealed, with vascular
2 endothelial growth factor receptor 2 (VEGFR2) and NOTCH1 representing central nodes.

3
4 The use of iPSC technology for disease modelling and drug testing is increasingly used for
5 cardiovascular precision medicine. Last year, for the first time, the combination of patient-specific
6 iPSC-derived cardiomyocytes, genetics and genome editing unveiled the mechanisms of action of
7 modifier genes in subsets of patients affected by long QT syndrome (LQTS).^{111, 112} By comparing
8 patient-specific iPSC-CMs derived from symptomatic and asymptomatic LQT1 carriers of the same
9 mutation, it was shown that genetic variants of *MTMR4*, an upstream regulator of neural precursor
10 cell expressed developmentally downregulated gene 4-like (NEDD4L), control potassium channel
11 turnover, thus influencing the clinical manifestations of the disease. iPSC technology has also been
12 used to gain insights into the molecular mechanisms of atrial septum defect (ASD), a form of
13 congenital heart disease, by implicating a mutation in *GATA4* that modifies *FGF16* induction.¹¹³

14
15 Pioneering work from *Srivastava* and collaborators developed a machine-learning approach to
16 identify small molecules that broadly correct gene networks dysregulated in an iPSC model of aortic
17 valve (AV) disease.¹¹⁴ Correction of the gene network by the most effective therapeutic candidate,
18 XCT790, was sufficient to prevent and treat AV disease *in vivo* in a mouse model. This strategy,
19 made possible by combining iPSC technology, network analytics and machine learning, may can
20 represent an effective path to discovering new therapies.

21 22 **7. COVID-19**

23 **7.1 Cardiovascular tropism and molecular markers**

24 The aetiology of myocarditis caused by cardiotropic viruses has become a major topic of interest
25 during the COVID-19 pandemic.^{115, 116} A comparative study revealed that while myocardial injury
26 occurred with a similar frequency in infection with influenza and SARS-CoV-2, the mortality was
27 almost 4-fold higher in COVID-19 compared with influenza.¹¹⁷ Evidence of viral infection was seen
28 mainly in endothelium and rarely in cardiomyocytes,¹¹⁸ however, evidence for stromal cells infection
29 by SARS-CoV-2 has been found.¹¹⁹ Endothelial-dependent dilation in human arterioles is impaired for
30 months after SARS-CoV-2 exposure, and could contribute to long-lasting symptoms of post-COVID-
31 19 infection.¹²⁰ Consistently, *Bräuninger et al* performed massive analysis of cDNA ends–RNAseq in
32 myocardial tissue from fatal COVID-19 cases with and without cardiac infection to reveal potential
33 SARS-CoV-2-related pro-inflammatory transcriptomic alterations in EC, while no differences were
34 detected in immune cell infiltrations.¹²¹ Interestingly, the levels of several known cardiometabolic

1 biomarkers are associated with COVID-19 severity and mortality, particularly myocyte-derived miR-
2 133a and liver-derived miR-122.¹²² The potential for the use of cardiovascular RNA markers and
3 artificial intelligence in the setting of COVID-19 has been reviewed in.¹²³ In a study of 95 SARS-CoV-
4 2-positive autopsy tissue, cardiac SARS-CoV-2 infection was shown to increase transcription of
5 interferon pathways, originating predominantly from EC.¹¹⁸ The ESC has provided guidance for the
6 diagnosis and management of cardiovascular disease during the COVID-19 pandemic^{124, 125} and
7 recommendations for future research.¹²⁶

9 **7.2 Virus- and vaccine-induced thrombotic complications and COVID-19**

10 Accumulating evidence suggests that patients suffering from COVID-19 have an increased risk to
11 experience thrombotic events such as microthrombosis, venous thromboembolism and ischaemic
12 stroke (for a review see¹²⁷). Two recent studies have found microthrombi in the hearts of patients who
13 succumbed to SARS-CoV-2 infections. *Pellegrini et al* identified microthrombi as a cause of myocyte
14 necrosis. Interestingly these microthrombi contained more fibrin and more of the complement
15 components C5b-9 than thrombi isolated from the myocardium of patients of COVID-19 negative
16 patients and coronary thrombi aspirated from COVID-19 negative and positive patients with ST-
17 *elevation* MI.¹²⁸ *Bois et al* found nonocclusive microthrombi in myocardial arterioles in 12 out of 15
18 patients who died from SARS-CoV-2 infections. However, no evidence of acute ischaemic injury of
19 the heart was detected in this study.¹²⁹ When tissue factor (TF)-bearing microvesicles isolated from
20 the plasma of 100 patients with moderate and severe COVID-19 and from the plasma of 28 healthy
21 subjects were studied, the authors found that TF-activity on such microvesicles, which is indicative of
22 a procoagulatory state, was increased in patients suffering from COVID-19 and is significantly linked
23 to disease severity and mortality.¹³⁰

24
25 Thrombotic complications have been reported in 1 per 100 000 adenoviral COVID-19 vaccinated
26 irrespective of age, rising to 1 in 50 000 above 50 years vaccinated with ChAdOx1.¹³¹ This is referred
27 to as vaccine-induced immune thrombotic thrombocytopenia (VITT).^{131, 132} Fibrinogen, Age, Platelet
28 count, and the presence of Intracranial haemorrhage, and Cerebral venous sinus thrombosis (the
29 *FAPIC* score) are significantly associated with mortality in cases of VITT.¹³³ Increased levels of anti-
30 PF4 antibodies post-vaccination unrelated to previous heparin exposure implicates an augmentation
31 of the antibody response by unknown PF4 co-factors.¹³² The antigenic component with PF4 may be
32 vaccine constituents but remains an unsolved critical question in VITT pathophysiology.¹³² The
33 immune complexes transduce platelet activation through the Fcγ receptor IIA (FcγRIIA) resulting in
34 thrombosis with concomitant thrombocytopenia accompanied by a fulminant immune activation.¹³⁴

1 Among novel therapeutic options for VITT, inhibitors of Bruton tyrosine kinase (Btk), which is used for
2 B-cell malignancies, have been explored for their ability to block FcγRIIA for preventing the
3 downstream platelet activation and aggregation. The Btk inhibitors ibrutinib and fenebrutinib
4 prevented platelet aggregation induced by serum obtained from patients with VITT.¹³⁵ Additional
5 possibly favourable effects of Btk inhibition in VITT are blocking of neutrophil-platelet complexes and
6 reduced NET release,¹³⁶ which are part of the massive immune activation during VITT.¹³⁴

7 8 **7.3 Cardiovascular drugs and COVID-19**

9 In the beginning of the COVID-19 pandemic, the interactions with cardiovascular drugs were focused
10 on ACE-inhibition and anti-thrombotic treatments¹³⁷ and more recently extended to lipid-modulating
11 agents.¹³⁸ In the latter context, omega-3 fatty acids may provide beneficial cardiovascular effects
12 through immunomodulation, anti-thrombosis and improved endothelial function.¹³⁹ Specific cytokine
13 antibodies to dampen the inflammatory storm in COVID-19 exhibit anti-inflammatory strategies
14 explored for cardiovascular prevention and have shown some success in improving survival and
15 clinical outcomes.¹⁴⁰ The RECOVERY trial tested multiple different therapeutic approaches including
16 antiviral, immunomodulatory and antithrombotic treatments, in a multi-arm factorial design inspired by
17 the International Study of Infarct Survival (ISIS) trials of the 1980s, and demonstrated benefit with
18 tocilizumab and dexamethasone, but not hydroxychloroquine, convalescent plasma or other tested
19 approaches.¹⁴¹ In a separate study, anticoagulation with low-molecular-weight heparin (LMWH) may
20 curtail viral persistence and reduce mortality.¹⁴²

21 22 **Perspectives**

23 The substantial progress of basic cardiovascular science during the past year has revealed a plethora
24 of novel therapeutic and diagnostic possibilities. Non-coding RNA, scRNAseq, and iPSC are
25 examples of discovery tools to widen the understanding of cardiac and vascular pathophysiology.
26 Through the integration cardiovascular risk factors, genetics, and biomarkers, the basic
27 cardiovascular science field is expanding towards applications in precision medicine. The year was
28 still marked by the COVID-19 pandemic and several important contributions have increased our
29 knowledge of the cardiac and thrombotic effects of SARS-CoV-2, and the underlying pathways
30 behind reported vaccinal complications. Finally, the mechanistic insights from *in vitro* and *in vivo*
31 basic science models have deepened our understanding of inflammation, CHIP, EVs, regeneration,
32 and mechanosensing in cardiovascular disease.

Conflicts of interest

CGT has received funding from Amgen, and personal fees from VivaLyfe, and is listed as an inventor on 2 heart failure patents.

FIGURE LEGENDS

Figure 1. Novel insights into the role of ncRNAs.

Several complex loci composed of lncRNA and miRNA clusters have been identified throughout the genome. Nonetheless, despite their genomic and often transcriptional overlap, they have been found to have distinct functional and regulatory targets. The X-linked lncRNA MIR503HG maintains endothelial cell (EC) identity by interacting with the RNA splicing regulatory protein PTBP1, with decreased expression leading to broad changes associated with EndMT. Importantly, these phenotypic changes seem to be independent of miR-424 and miR-503 expression, which overlap the lncRNA locus³. Similarly, loss of the Cardiac Mesoderm Enhancer-associated Non-coding RNA (CARMN) primes vascular smooth muscle cells (vSMCs) into a pro-atherogenic proliferative state, while migration or dedifferentiation are regulated through the modulation of the overlapping miR-143 and miR-145⁴.

Figure 2. Recent findings on cardiovascular mechanosensing.

Newly discovered flow-stimulated mechanosensitive signalling pathways. Flow-activated PIEZO1 was shown to activate the protein kinase N2 (PKN2) via PKD1, resulting in phosphorylation of Akt and eNOS, with subsequent vascular tone regulation via NO.⁴⁵ The glycocalyx component sialic acid, was shown to activate NRF2 antioxidant signalling, via phosphorylation of AKT⁴⁶, whereby modulating the endothelial redox state in response to shear stress. The pathways are likely to be interconnected as both result in phosphorylation of AKT and eNOS and as NRF2-induced antioxidant signalling is likely to affect NO bioavailability.

Figure 3. New insights and interventions in lipid biology.

Gene silencing with small interfering RNA (siRNA) like inclisiran or gene editing are becoming additional options to monoclonal antibodies for the inhibition of proprotein convertase subtilisin kexin 9 (PCSK9) leading to long-lasting circulating LDL-Cholesterol (LDL-C) decrease. Lipoprotein(a)

1 (Lp(a))-reducing drugs by RNA interference, via antisense oligonucleotide (ASO), like Pelacarsen or
2 siRNA, like Olpasiran are holding promising results in clinical trials. The inhibition of angiotensin-like
3 3 (ANGPTL3) via evinacumab, a monoclonal antibody or Vupanorsen, a GalNAc-conjugated ASO
4 markedly reduces circulating triglyceride-rich lipoprotein (TGRL) levels. N-acetylgalactosamine
5 (GalNAc) ligands conjugated with siRNAs or ASOs allow its hepatocyte-targeted delivery lowering
6 incidence and severity of off-target effects, commonly observed with the first generation RNA
7 interference.

9 **Figure 4. Source of EVs affects their function**

10 Several thought-provoking studies published in 2021/2022 demonstrated that the cardiovascular
11 effects of extracellular vesicles (EVs) can depend upon their origin. For example, EVs originating from
12 different cell types (cardiomyocytes vs M2 macrophages), different cellular states (proliferating vs
13 differentiated), different ages (young vs old serum) or different health states (chronic kidney disease
14 and coronary artery disease [CKD+CAD] vs healthy) can have opposite effects.

16 **Figure 5. Novel mechanisms of arrhythmia.**

17 Recent publications (top left) and accepted concepts (top right) on the mechanisms leading to re-
18 entry may be combined to arrive at a generalized theory of the spatiotemporal interaction between
19 triggers and substrate leading to re-entry arrhythmias (bottom). The generalized hypothesis highlights
20 that re-entry can only initiate when there is a local dispersion of excitability, with some tissue excitable
21 whereas other tissue is (still, or always) refractory at the time when the trigger occurs. The trigger
22 should originate from the excitable tissue, may block and travel around (relatively large) refractory
23 tissue before it arrives at the previously excited tissue again.

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Figure 1. Novel insights into the role of ncRNAs.

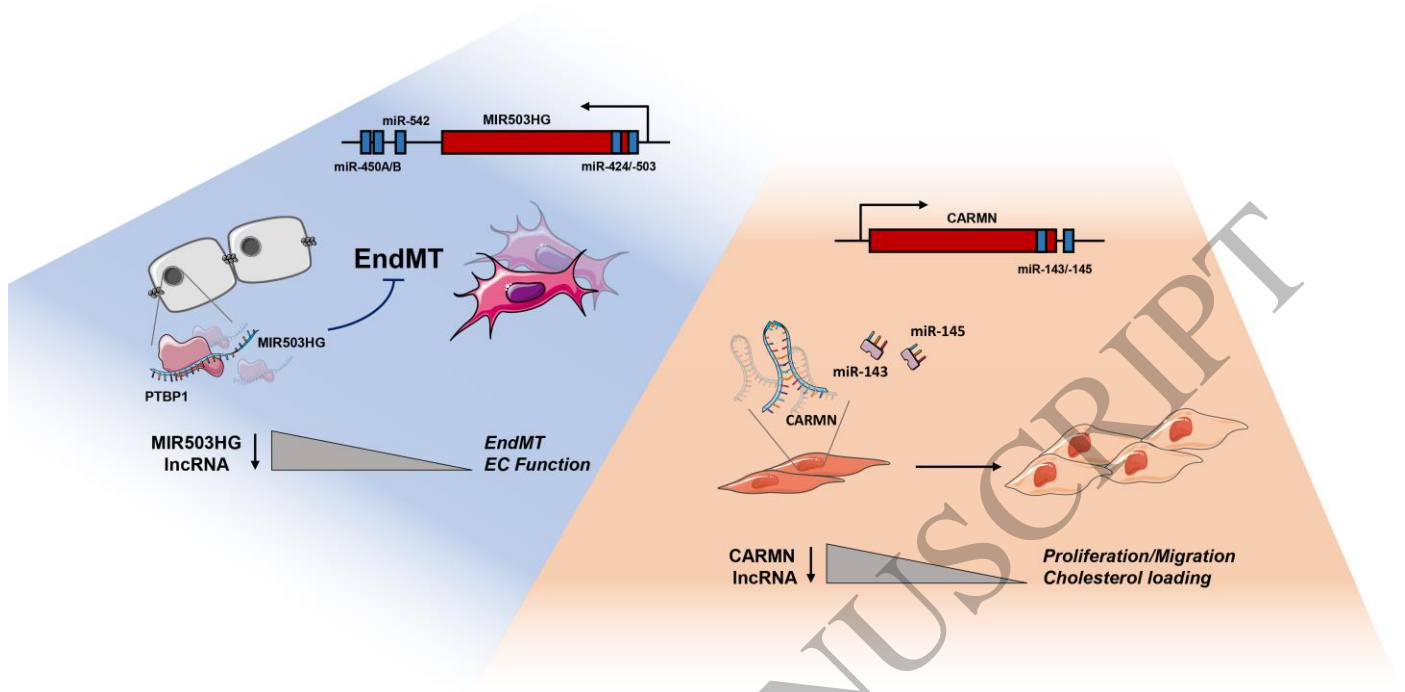


Figure 1
185x103 mm (x DPI)

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ACCEPTED MANUSCRIPT

Figure 2. Progress in mechanosensing.

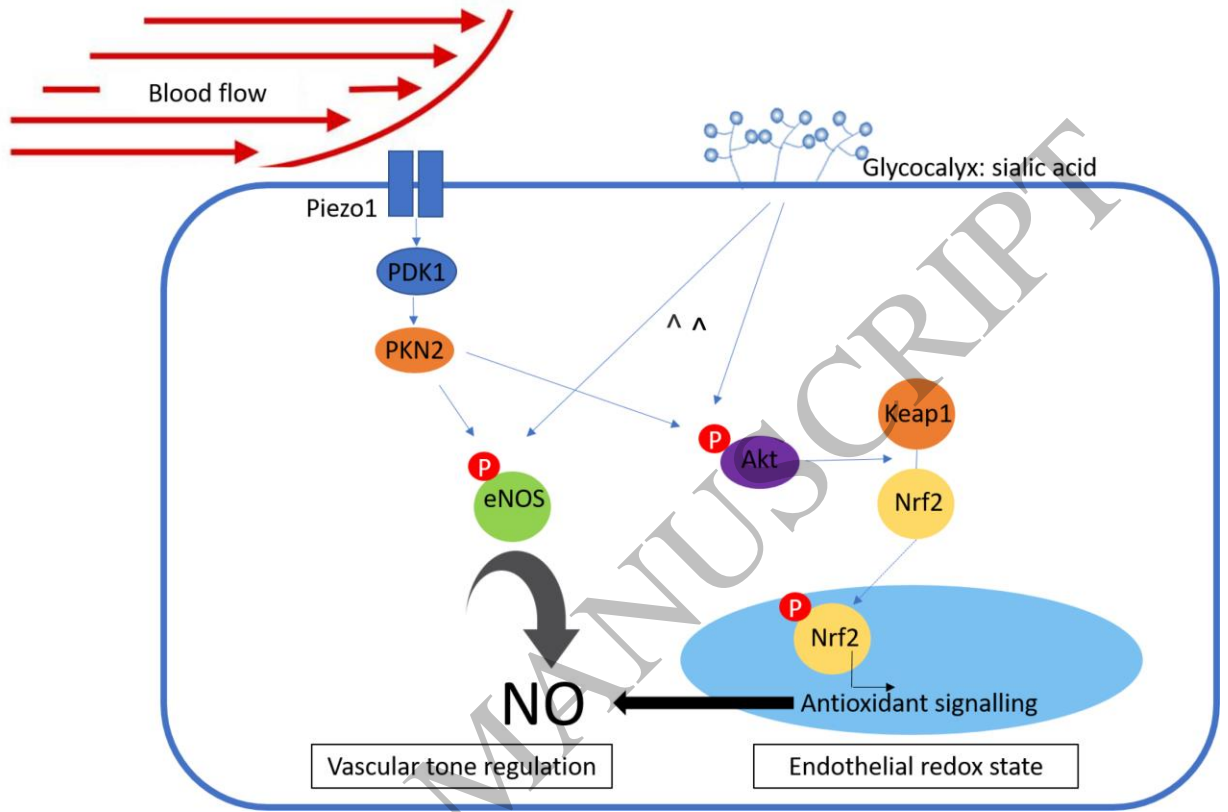


Figure 2
185x129 mm (x DPI)

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Figure 3. New insights in lipid biology and cross-talk with inflammation

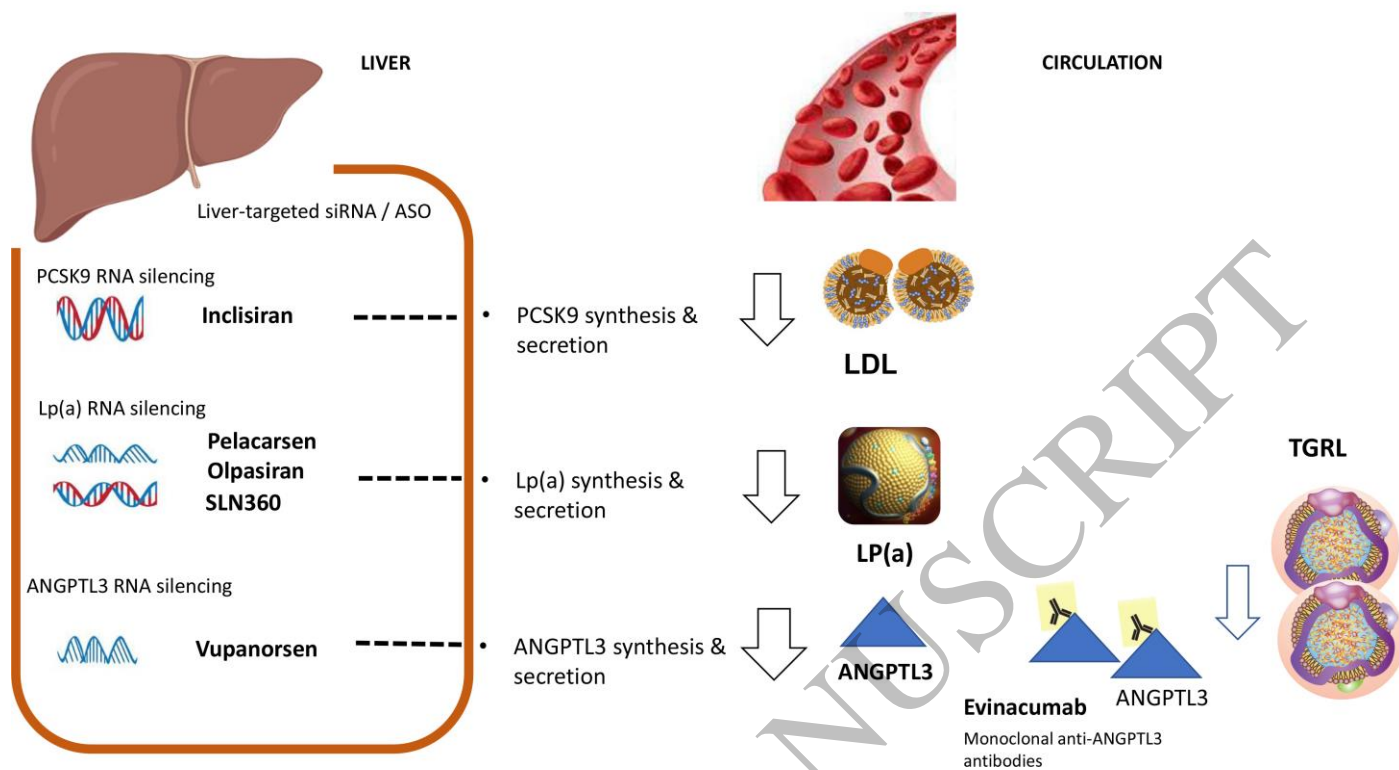


Figure 3
185x107 mm (x DPI)

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Figure 4. Source of EVs affects their function.

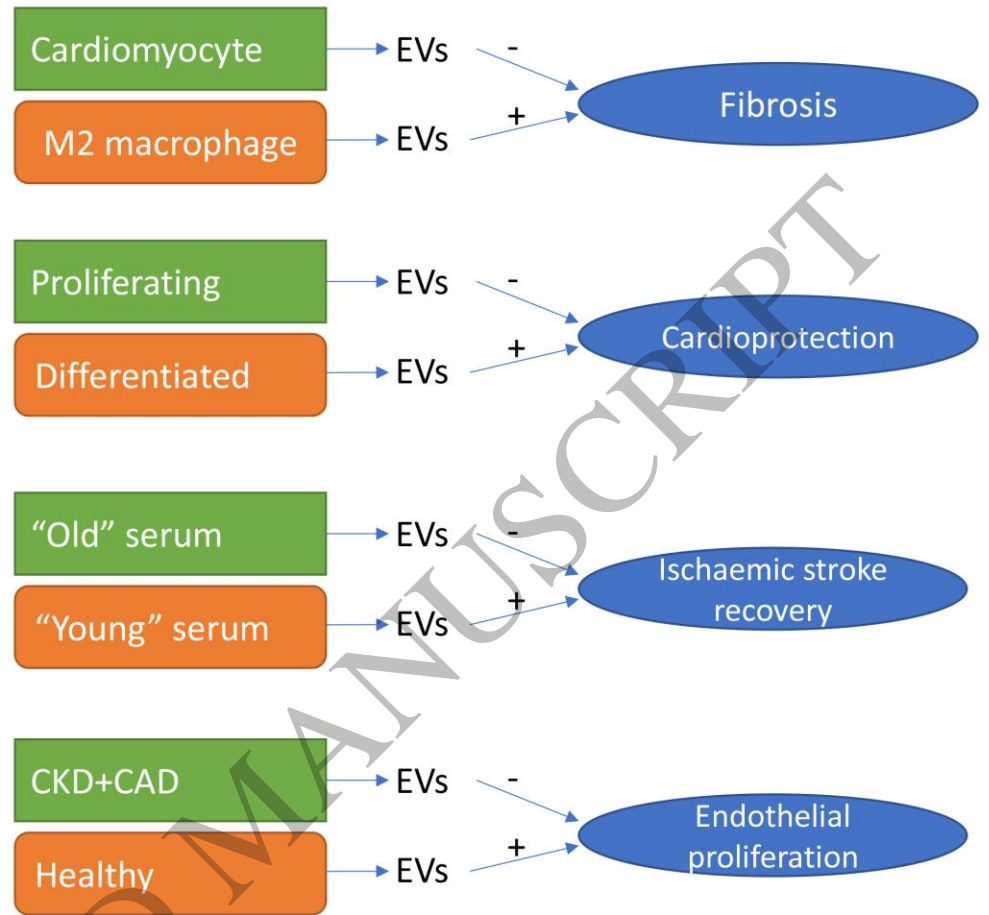


Figure 4
185x135 mm (x DPI)

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Figure 5. Novel mechanisms of arrhythmia.

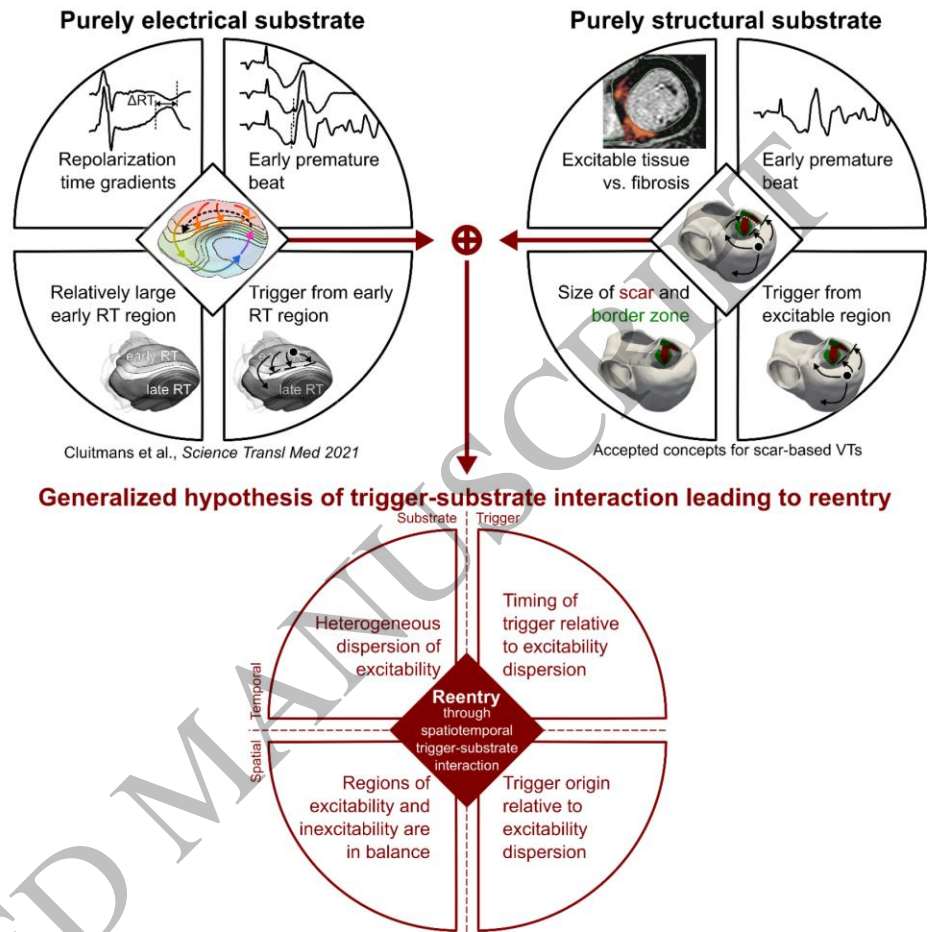


Figure 5
185x143 mm (x DPI)

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