specificity and the sensitivity to quantify biological processes in vivo. However, PET tracer uptake does not usually provide sufficiently detailed anatomical structure to accurately allocate receptor activity to precise tissue regions. This may be overcome by simultaneous imaging with magnetic resonance imaging (MRI). The aim of the study was to evaluate the combined PET/MRI scanner for the investigation of biological processes in rodents. N-(5-fluoro-2-phenoxyphenyl)-N-(2-[18F]-fluoroethyloxy-5-methoxybenzyl)acetamide ([18F]FEDAA1106) binds the translocator protein (TSPO) which is up-regulated in activated macrophages and may quantify vascular inflammatory pathologies. Dynamic in vivo imaging was carried out using a modified Focus F120 microPET incorporated into a bespoke 1 Tesla MR magnet (1). The pharmacokinetic profile of [18F]-FEDAA was characterised in mice. Simultaneously acquired PET and MRI reconstructed images were aligned together and the combined images revealed rapid uptake of [18F]-FEDAA into the heart, liver, lungs, kidneys and brain. Time activity curves were constructed using regions of interest delineated by the MR images and showed the expected pharmacokinetic profile. Thus, the results show that the fused anatomical-functional image not only provides anatomical context to the PET data, but can also allow improved quantification by more accurately defining the region of radioactive emission.

(1) Hawkes RC, et al. Technol Cancer Res Treat. 2010; 9(1):53–60.

## 08 RAS-ASSOCIATION DOMAIN FAMILY 1 ISOFORM A (RASSF1A) IS A NOVEL REGULATOR OF TNF-ALPHA SIGNALLING IN CARDIOMYOCYTES

doi:10.1136/heartjnl-2012-302951.008

Presenter: Tamer Mohamed

TMA Mohamed, M Zi, A Maqsood, S Prehar, L Neyses, D Oceandy. *Cardiovascular Medicine Research Group, School of Biomedicine, University of Manchester*

Tumour necrosis factor-alpha; (TNF-alpha) plays key roles in the pathogenesis of heart failure. Cardiomyocytes express the TNF-alpha; receptor (TNFR), however, the mechanism of TNF-alpha; signal transmission in cardiomyocytes is not completely understood. Recent studies showed that in cancer cells TNFR is regulated by Ras-association domain family 1 isoformA (RASSF1A). Therefore, we investigated whether RASSF1A modulates TNF-alpha; signalling in cardiomyocytes. We used RASSF1A knockout (KO) mice and wild type (WT) controls and stimulated them with TNF-alpha; (10μg/kg i.v.). In WT mice acute treatment with low dose of TNF-alpha increased cardiac contractility and intracellular calcium transient amplitude, which is consistent with previously published data (Circulation 2004; 109:406-411). However, KO mice showed a blunted contractile response following acute TNF-alpha treatment as indicated by the change in end systolic elastance (in vivo) and intracellular calcium transient amplitude (isolated adult cardiomyocytes). We also found that RASSF1A formed a molecular complex with TNF-alpha; receptor in cardiomyocytes and this interaction was essential in the recruitment of TRADD and TRAF2, the major downstream effectors of TNF-alpha; signalling. By mapping the interaction domain we found that the C-terminal region of RASSF1A was responsible for the formation of TNF-alpha; receptor complex. Furthermore, using an adenoviral-mediate d shRNA construct we found that cardiomyocytes lacking RASSF1A exhibited reduced activation of NFkB, a downstream target of TNF-alpha. Overall, our data indicate an essential role of RASSF1A in regulating TNF-alpha; signalling in cardiomyocytes, with RASSF1A being key in the formation of TNF receptor complex and in the signal transmission to the downstream targets.

## 09 BIOMARKERS FOR DETECTION OF ACTIVATED MACROPHAGES IN ATHEROSCLEROSIS

doi:10.1136/heartjnl-2012-302951.009

Presenter: Joseph Bird

[1]JLE Bird,[1]L Burzynski, [2]R Manavaki, [3]JHF Rudd, [4]EA Warburton, [1]AP Davenport. *[1]Clinical Pharmacology Unit; [2]Department of Medicine; [3]Division of Cardiovascular Medicine; [4]Department of Clinical Neurosciences, University of Cambridge, Addenbrooke's Hospital Cambridge CB2 2QQ, UK*

Atherosclerosis is the pathogenic mechanism underlying the majority of strokes, heart attacks and peripheral vascular disease. These are the principal causes of morbidity and mortality in the developed nations. The atherosclerotic lesion (or plaque) is characterised by a build up of lipid and inflammatory cells in the artery wall. Arterial stenosis is the current clinical predictor for endarterectomy, although macrophage burden in the lesion correlates strongly with likelihood of rupture and could be a better proxy of plaque vulnerability. Activated macrophages have up-regulated expression of translocator protein (TSPO), which is readily quantified by the positron emission tomography (PET)-based ligands (1) such as [11C](R)-1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline-carboxamide ([11C](R)-PK11195). Studies examining TSPO plaque biodistribution frequently use CD68, a pan-phenotype marker, for identification of macrophage presence. However, this biomarker demonstrates some inconsistencies in these analyses (1). We hypothesise that more specific macrophage phenotype markers may perform better as correlates for TSPO PET-ligand binding. In this study, we examined the expression of phenotype-restricted macrophage markers in endarterectomy tissue, and correlated these with the distribution of [3H](R)-PK11195 using autoradiographic, immunohistochemical and immunofluorescence techniques. Autoradiographic and immunohistochemical images were digitised and co-registered to provide more accurate correlation of expression. We identified several markers of activated macrophage phenotype which have better correlation with [3H](R)-PK11195 binding patterns compared with CD68. These data indicate that TSPO PET ligands bind principally to activated macrophage phenotypes, and the phenotype markers have the potential to more accurately identify PET-ligand binding macrophages in ex vivo analysis.

1) Bird JL et al. Atherosclerosis, (2010), 210(2): 388–91.

## 10 REGULATION OF MONOCYTE-ENDOTHELIAL CELL INTERACTIONS BY NEUTROPHIL-DERIVED MICROPARTICLES

doi:10.1136/heartjnl-2012-302951.010

Presenter: Amanda Burnett

A Burnett, P Hellewell, V Ridger. *Department of Cardiovascular Science, University of Sheffield, Sheffield, S10 2RX*

**Rationale** Monocytes play a major role in atherosclerosis progression through migration into the arterial wall. Neutrophil involvement in atherosclerosis was previously only thought to be via enzymatic weakening of the fibrous cap. However, neutrophil depletion can delay atherogenesis and conversely increasing circulating neutrophil enhance plaque progression in mice. Lack of evidence for the presence of neutrophils in atherosclerotic plaques makes their role in disease progression less clear. We have found neutrophil-derived microparticles increase migration of neutrophils. Our hypothesis: neutrophil-derived microparticles increase endothelial cell-monocyte interactions and facilitate monocyte transendothelial migration.

**Methodology** Neutrophils were incubated with various agents to stimulate microparticle formation. Microparticles were characterised and quantified using a novel, standardised flow cytometry

method and electron microscopy. Monocytes were migrated to MCP-1 across CAEC incubated with neutrophil-derived microparticles using a transendothelial migration assay. Cytokine release and adhesion molecule expression by hCAEC was investigated using a cytometric bead array and fluorescent antibody binding respectively. Microparticle adhesion to hCAECs was investigated under static and flow conditions.

**Findings** Neutrophil-derived microparticles were between 0.5 and 0.9μm. The most abundant surface markers were Annexin V with 20-30% positive microparticles, CD66c (10–15%) and CD18 (5–15%). Low levels of CD11b, CXCR2, and L-selectin were observed. More microparticles were produced in response to fMLP and aLDL compared to PBS. Microparticles bind to and induce MCP-1 release from hCAEC. Monocyte migration increased upon hCAEC incubation with neutrophil-derived microparticles and the extent of the increase was dependent on the stimulus used with aLDL>fMLP>PBS. This highlights a role for microparticles in endothelial activation.

## 11 THE MAMMALIAN STE20-LIKE KINASE 2 (MST2) MODULATES PATHOLOGICAL HYPERTROPHY BY ACTIVATING THE PROTO-ONCOGENE RAF1

doi:10.1136/heartjnl-2012-302951.011

Presenter: Min Zi

Zi M*, S Prehar, A Maqsood, Y Reynolds-Khan, L Neyses, E Cartwright, D Oceandy. *Cardiovascular Medicine Research Group, School of Biomedicine, University of Manchester*

The novel idea that the molecular regulation of cardiac hypertrophy is closely related to tumour/cancer growth has emerged recently. One key finding was the identification of tumour suppressor RASSF1A as a powerful inhibitor of pathological hypertrophy. The mammalian STE20-like kinase 2 (Mst2) forms a molecular complex with RASSF1A and is also implicated in the development of various tumours. In contrast to Mst1, the role of Mst2 in the heart has not been precisely elucidated.

We used Mst2 knockout mice and isolated neonatal rat cardiomyocytes (NRCM) with an adenoviral mediated overexpression of Mst2 to investigate the role of Mst2 in the heart. Mst2-/- mice exhibited a significant reduction of hypertrophy in response to transverse aortic constriction (30% elevation in heart weight/tibia length ratio in Mst2-/- mice compared to 50% in wild type (WT), n=8, $P<0.05$). In agreement with the in vivo data, overexpression of Mst2 in NRCM significantly enhanced phenylephrine-induced cellular hypertrophy as indicated by cell size measurements and expression of the hypertrophic marker BNP. Since Mst2 physically interacts with Raf1, we investigated the activation of Raf1 and its downstream effector ERK1/2 in NRCM. Overexpression of Mst2 significantly increased the activation of Raf1 and ERK1/2 indicating that Mst2 positively regulates the pro-hypertrophic and pro cancer Raf1-ERK1/2 pathway. In conclusion, our data provide a key evidence of novel role of Mst2 as a positive regulator of cardiac hypertrophy by modulating the Raf1-ERK1/2 pathway. It also reinforces the notion that genes that are implicated in tumour growth/cancer are also important in myocardial hypertrophy.

## 12 PHARMACOLOGY OF HUMAN ETA AND ETB RECEPTOR SIGNALLING VIA G-PROTEIN AND BETA-ARRESTIN PATHWAYS

doi:10.1136/heartjnl-2012-302951.012

Presenter: Janet Maguire

JJ Maguire, RE Kuc, VR Pell , AP Davenport. *Clinical Pharmacology Unit, University of Cambridge, Level 6 ACCI, Box 110 Addenbrooke's Hospital, Cambridge CB2 0QQ UK*

The endothelin (ET) system is altered in cardiovascular diseases and ET receptor antagonists are licenced for treatment of pulmonary arterial hypertension (PAH). Endothelins act via two G-protein-coupled receptors (GPCRs), ETA and ETB. However, it is recognised that GPCRs may also activate signalling pathways in a G-protein-independent manner via beta-arrestin. Ligand-specific pathway modulation (biassed agonism/antagonism) may have therapeutic application and therefore we have investigated the potential for pathway bias of ET agonists and antagonists. Concentration-response curves were constructed to ET agonists using ETA and ETB beta-arrestin assays. ET receptor antagonists were tested for their ability to block ET-1 responses in each assay and agonist-dependence of the ETA selective antagonist BQ123 was investigated. These data were compared to results from binding experiments in human heart (that expresses both subtypes) and to ETA-mediated vasoconstrictor experiments in human saphenous vein. The relative potency of ET peptides in the ETA and ETB beta-arrestin assays was as expected. Interestingly, for ETA, compared to ET-1, all other agonists tested were partial agonists. Differences from the known pharmacology of antagonists were also revealed in the beta-arrestin assays. Specifically, BQ123 behaved as a negative allosteric modulator in the ETA assay, exhibiting agonist-dependent affinities. Bosentan was a potent ETA-selective antagonist in the beta-arrestin assay in contrast to its non-selective profile determined in human heart. The apparent ETA beta-arrestin bias of bosentan may contribute to its clinical effectiveness in PAH but further investigation of a role for functional selectivity and biassed signalling via the ET receptors in cardiovascular disease is required.

## 13 FLOW INDUCED VESSEL REMODELLING IN THE CHICKEN EMBRYO IS ASSOCIATED WITH A SPECIFIC GENE EXPRESSION PROFILE

doi:10.1136/heartjnl-2012-302951.013

Presenter: Emily Hoggar

[1]EC Hoggar, [1]M Placzek, [2]T J Chico. *[1]MRC Centre for Biomedical and Developmental Genetics, The University of Sheffield, Sheffield, United Kingdom; [2]NIHR Cardiovascular Biomedical Research Unit, Sheffield Teaching Hospitals, Sheffield, United Kingdom*

Collateral vessel development occurs by remodelling of pre-existing endothelial communications between occluded and neighbouring vascular territories to restore blood flow following occlusion of a large conducting artery. We have identified the genes involved in blood vessel remodelling in the chick embryo model of collateral vessel formation. In ovo ligation of the vitelline artery of E3 chick embryos occluded blood flow to the right side of the extra-embryonic vascular network. Collaterals arose by enlargement of pre-existing vessels and demonstrated active remodelling, peaking in number at 12h post ligation, followed by selection of a more efficient haemodynamic configuration of fewer, larger vessels over 48h. We characterised the global transcriptional changes at 4 and 12h post ligation and found 164 differentially expressed genes, unique to developing collateral vessels and therefore suggested to be driven by shear stress. Phosphodiesterase 10A (PDE10A) was highly up-regulated at 4h post-ligation. Local application of the PDE10A inhibitor Papaverine Hydrochloride had no effect on normal vessel diameter (ctrl 466±34μm, Papaverine [20uM] 600±90μm) but significantly impaired collateral vessel formation at 24h post-ligation (ctrl 109±9μm, Papaverine [20uM] 49±5 μm $P<0.0001$). Time course micrographs revealed significantly reduced collateral development from 6h post-ligation. In vitro proliferation assays using explanted collateral vessels, showed that flow-sensitised endothelial cells treated with papaverine, had a significantly lower proliferation index than controls at 12h post-ligation (ctrl 8.5%±0.5, Papaverine [20uM]) 2.5%±1.5 $P=0.04$). We conclude that developing collateral vessels demonstrate a unique gene expression profile. PDE10A is up-regulated during flow induced remodelling and pharmacologic inhibition significantly impairs this process.