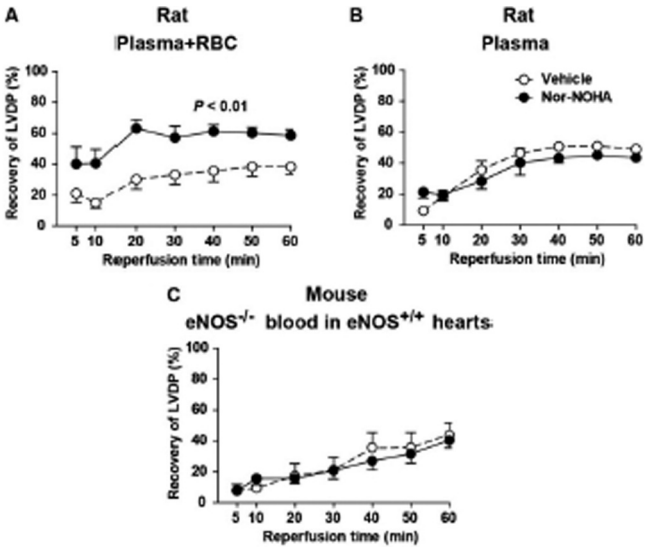

Figure 1. Effect of vehicle and the arginase inhibitor nor-NOHA on recovery of left ventricular develop pressure (LVDP) in rat (panel A and B) and mouse (panel C) hearts during reperfusion following global ischemia. Vehicle and nor-NOHA were administered to rat hearts in red blood cells (RBC) + plasma (panel A) or plasma only (panel B) at the onset of ischemia. In panel C blood from eNOS-/- mice were given with vehicle or nor-NOHA to wild type mouse hearts. Recovery of LVDP during reperfusion is expressd in percentage of baseline; n=6–8.

Conclusion: The present study demonstrates a novel role of arginase 1 in control of eNOS function in RBCs. Inhibition of arginase unravels an important functional effect of RBC-derived NO that mediates protection against myocardial ischemia-reperfusion injury. These observations provide evidence for an important regulatory role of RBCs in control of NO bioactivity with pathophysiological consequences in ischemia-reperfusion.

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Intracoronary injection of encapsulated antagomir-92a promotes angiogenesis and prevents adverse infarct remodeling in a pig model of myocardial infarction

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Background: Intravenous antagomir-92a based-therapy enhanced neoangiogenesis and improved left ventricular contractility in a mouse model of acute myocardial infarction (MI), but its effect on postMI remodeling is unknown and clinical translation limited by the need of repeated administration and potential systemic adverse effects (AE). We investigated whether a single intracoronary administration of antagomir-92a encapsulated in microspheres (Antag92aME) could prevent deleterious myocardial remodeling one month (1mo) after MI.
Methods and results: We developed poly-d,-lactide-co-glycolide 9 μm ME with 7-10% loads of 3 mg Antag92a. In a first phase, ME were injected in the LAD coronary artery of healthy pigs. Repetitive injections to a total dose of 240 mg were used (n=3) to rule out persistent effects in myocardial contraction (intramyocardial piezoelectric crystals), LAD flow (Doppler probe) or necrosis (histology), and fluorescence labeled ME injection (n=4) showed no ME content in myocardium outside the LAD territory or in lung, spleen or liver (fluorescence microscopy). Finally, miR-92a expression was quantified by RT-PCR in 3 pigs euthanized at 1, 3 and 10 days after Antag92aME injection demonstrating local and sustained miR-92a inhibition ($>8\times$ and $>4\times$ fold at 1 and 10 days). In a second phase, MI was induced inflating a 2.5/12 mm balloon (49 min) in LAD of 27 closed-chest minipigs, randomly to blind receive Antag92aME, placeboME or saline administration, 5 minutes after reperfusion. Intravascular echocardiography was performed during ischemia, reperfusion and repeated 1mo later immediately before the animals were euthanized and the hearts excised, ex-vivo MRI was performed (maximum necrotic wall thickness (Tmax), largest diameter (D) between remodeled and contralateral wall (DR) and its largest perpendicular D between unaffected walls (DN) were measured in short-axis transverse ventricular slices) and ventricle histologic sections were obtained. Mortality was 23% (1, 2, 3 died in Antag92aME, placeboME and saline). Antag92aME induced vessel growth (161.57$\pm$58.71 vessels/cm^2, 68.49$\pm$23.56, 73.91$\pm$24.97; p=0.001) reduced regional wall motion dysfunction (28.6% and 76.9% of dyskinesia in treated vs non-treated p=0.03) and prevented adverse remodeling 1mo after injury (Tmax: 9.01$\pm$0.6, 5.61$\pm$0.5, 6.07$\pm$0.9 p=0.006, DR/DN: 1.29$\pm$0.1, 2.02$\pm$0.2, 1.93$\pm$0.2 p=0.03).
Conclusions: Early intracoronary administration of Antag92aME in a pig model of reperfused MI prevents ventricular remodeling with no local or distant AE emerging as a promising therapeutic approach to translate to patients that suffer a large MI.

769 | BENCH
First approach to stimulate arteriogenesis using monoclonal antibodies: blocking the interferon-alpha/beta receptor subunit 1 stimulates restoration of perfusion in a murine hindlimb-ischemia model without affecting atherosclerosis

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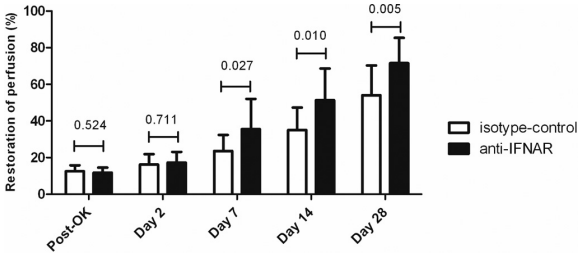
Background: Increased expression of interferon (IFN)-beta was shown in patients with insufficient coronary collaterals. Furthermore, mice treated with IFN-beta demonstrate inhibition of collateral artery growth (arteriogenesis). Interestingly, type I interferons (IFN-alpha and IFN-beta) have been identified as proatherosclerotic cytokines and in mouse models of atherosclerosis, IFN-beta treatment accelerated lesion formation and increased accumulation of macrophages in plaques. We hypothesized that arteriogenesis can be stimulated using monoclonal antibodies inhibiting IFN-beta signaling without accelerating atherosclerosis.
Methods: In an atherosclerotic murine hindlimb-ischemia model, LDLR-/- mice were treated during a 4-week period with monoclonal antibodies specific for mouse Interferon-alpha/beta Receptor subunit 1 (IFNAR-1) or murine IgG isotype as control. Hindlimb perfusion was measured using laser Doppler perfusion imaging (LDPI) directly after femoral artery ligation as well as at 2, 7, 14 and 28 days following ligation. We used a disease model to investigate effects of anti-IFNAR-1 on atherosclerosis, which was evaluated with histology to determine plaque area and composition.
Results: Hindlimb perfusion restoration after femoral artery ligation was improved in mice treated with anti-IFNAR-1 compared to controls as assessed by LDPI after 7, 14 and 28 days (treatment vs. control; 7 days: 35.6$\pm$16.5% vs. 23.6$\pm$8.7%, p=0.027; 14 days: 51.4$\pm$17.2% vs. 35.0$\pm$12.3%, p 0.010; 28 days 71.5$\pm$13.8% vs. 54.0$\pm$16.3%, p=0.005). Total plaque area (treatment vs. control: 118.8$\pm$46.1$\times$10^3 μm^2 vs. 139.3$\pm$46.5$\times$10^3 μm^2, p=0.275) as well as composition were unaffected.



Conclusion: Blocking IFNAR-1 using monoclonal antibodies stimulates collateral artery growth in mice and has a neutral effect on atherosclerosis.

770 | BENCH
Human Genetic Evidence that Common Variants near PIK3CG are Associated with Atherosclerotic Plaque Hemorrhage and Vessel Density

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Aim: Atherosclerotic plaque characteristics may vary among individuals, in part due to heritable factors. The genetic architecture of plaque phenotypes is largely unknown. A common variant (rs17398575-A) near PIK3CG on chromosome 7 has previously been associated with carotid plaque presence. Animal models suggest that PIK3CG may play a role in plaque formation through neovascularization. We hypothesized that the PIK3CG variant is associated with intraplaque hemorrhage and vessel density in human plaque tissue.
Methods: We collected 831 patients in the Athero-Express Biobank Study, all of whom underwent carotid endarterectomy and genotyped them using Affymetrix SNP 5.0. We tested PIK3CG variants for association to intraplaque hemorrhage and vessel density using logistic and linear regression model, respectively, correcting for age, gender and principal components. We used the BiKE cohort to assess the effect of PIK3CG variants on PIK3CG expression in circulating monocytes (N=95) and in carotid plaques (N=126).

Results: The reported PIK3CG variant, rs17398575 (risk allele A, frequency = 0.72), was nominally significantly associated with intraplaque hemorrhage (OR=1.40 [1.10-1.69 95% CI], p=0.0271) and vessel density (β=0.095 [0.0415 s.e.m.], p=0.0221). We also report another nearby variant, rs849429 (risk allele A, frequency=0.49), but uncorrelated to rs17398575, associated with intraplaque vessel density (β=-0.164 [0.0361 s.e.m.], $p=6.70\times10^{-6}$). The SNP dependent PIK3CG mRNA expression demonstrated a differential effect in the vascular wall (p=0.783 for rs17398575; p=0.0198 for rs849429) compared to circulating monocytes (p=0.0261 for rs17398575; p=0.350 for rs849429).
Conclusion: To our knowledge this is the first report involving the association of common genetic variants to histological plaque phenotypes. These results require further replication in independent cohorts. Further research should focus on elucidating the underlying mechanisms leading to plaque vessel formation and intraplaque hemorrhage, as both have been demonstrated to associate with secondary cardiovascular disease.

771 | BENCH
Induction of angiogenesis and prevention of apoptosis by implantation of adipose tissue-derived mesenchymal stromal cells on VEGF-releasing PLGA microspheres: A combined growth factor therapy cell transplantation approach

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Background: The success of tissue engineering implant relies on the ability of the scaffold to guide cell engraftment and vessel ingrowth in the ischemic tissue. Transplanted cells must be kept surviving in the harmful microenvironment to support tissue regeneration. Vascular endothelial growth factor (VEGF) is a potent angiogenic and anti-apoptotic factor, which may facilitate the engraftment of transplanted cells and guide angiogenesis.
Aim: To realize poly(lactide-co-glycolide) (PLGA) pharmacologically active microspheres (PAM) able to release VEGF and assess their ability to promote angiogenesis and prevent apoptosis of adipose tissue-derived mesechymal stromal cells (AT-MSCs).
Methods and results: Non-functionalized (empty) PAM or VEGF-loaded PAM were produced and coated with MSCs isolated from rat periepididimal AT at increasing cell:PAM concentrations. The release of VEGF from PAM occurred at a maximum rate of 0.7 ng/day per mg of PAM. The best ratio of adhesion was 4×10^4 cells per 0.5 mg of PAM. Cell-proliferation increased three fold after coating with VEGF-loaded PAM compared with AT-MSCs alone and AT-MSCs coated on empty PAM, while the adipogenic and osteogenic differentiation of AT-MSCs was unchanged. MTT analysis and cleaved caspase-3 expression (immunoblotting) revealed that AT-MSCs alone, and to a higher extent the AT-MSCs coated on VEGF-loaded PAM, exhibited high resistance toward H2O2-induced apoptosis, and this effect was dependent by VEGF/Akt axis since reverted by pre-incubation with Akt inhibitor LY294002 or anti-VEGF receptor antibody (Figure 1, panel A-B). PAM-VEGF enhanced AT-MSC tubulization in a Matrigel assay as compared with AT-MSCs alone or AT-MSCs coated on empty PAM (n=3 independent experiments, P<0.01 vs controls, Figure 1, panel C).

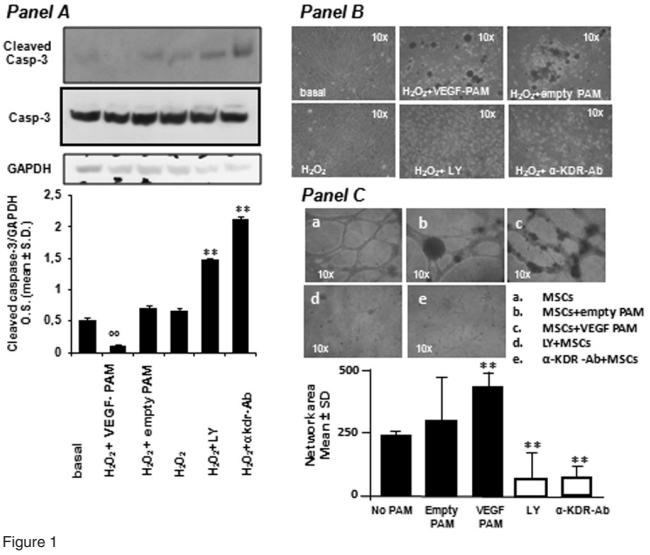

Figure 1

Conclusions: VEGF-loaded PAM coated with AT-MSCs may have therapeutic applications for enhancing angiogenesis and AT-MSC survival in harmful microenvironment of post-ischemic tissues.

RAPID FIRE – PROTEIN CHANGES: METABOLISM, INJURY AND PROTECTION

773 | BENCH
Calpastatin overexpression favors cardiac rupture and aggravates left ventricular dysfunction in mice after myocardial infarction

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Rationale: Inhibition of calpains, ubiquitous cysteine proteases that require Ca2+ for activity, preserves left ventricular (LV) structure and function at short term post myocardial infarction (MI). However long term effects of this inhibition are still unclear.
Objective: The purpose of this study was to examine the effects of calpain inhibition by ubiquitous calpastatin overexpression at long term post MI.
Methods and results: Myocardial infarction was generated in transgenic (TG) male mice constitutively overexpressing calpastatin and wild-type (WT) controls. Acute phase mortality (<48h) was comparable between groups. All-cause mortality from 48h to 6w post MI was 43% in TG-MI and 20% WT-MI (log-rank P<0.05). Mortality by LV rupture was 31% in TG-MI and 11% in WT-MI (P<0.05). At 6w post MI, infarct size and LV dilation were comparable, while LV contractility was reduced (+dP/dt 7635±782 vs. 9110±1292mmHg/s; P<0.05), LV end-diastolic pressure was increased (6.7±1.8 vs. 4.9±2.2mmHg, P<0.05), and wet pulmonary weight was more important (185.8±14.4 vs. 153.0±8.6mg, P<0.05) in TG-MI as compared to WT-MI survivors. At that time, remote myocardium (RM) of TG-MI showed more fibrosis (12.8±0.9 vs. 8.8±0.4% in WT-MI, P<0.01) and more cardiac myocyte hypertrophy (539.6±9.4 vs. 463.2±20.2μm^2 in WT-MI, P<0.05). To identify underlying mechanisms that might contribute to the worse outcome in TG-MI, a subgroup of animals was sacrificed 5d post MI. Infarct size was comparable between TG-MI and WT-MI. In the peri-infarct myocardium (PIM) of TG-MI mice, fibrosis was less pronounced, microvascularization was reduced, and macrophage and CD4+ T lymphocyte infiltration was impaired as compared to WT-MI (57%, P<0.01; and 79%; P<0.001, respectively).
Conclusions: Calpastatin overexpression is deleterious at long term post MI with more severe LV failure and increased mortality owing to cardiac rupture. This is primarily due to an insufficiency in wound healing of the myocardium associated with reduced capillary repair, inflammatory response and extracellular matrix synthesis in the PIM.

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Blood cell NOS3 improves cardiac function in an acute murine model of myocardial ischemia/reperfusion

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Purpose: Nitric oxide (NO) plays a protective role in myocardial ischemia and reperfusion (IR) and is constitutively produced within the endothelium by the isoform 3 of NO synthase (NOS3). All blood cells subpopulations carries an active NOS3, including red blood cells as recently demonstrated by us. We hypothesized that circulating blood cell NOS3 exert a protective role in an acute murine model of myocardial ischemia/reperfusion.
Methods: To analyze the role of blood cell NOS3 expression in an acute model of myocardial I/R, we generated chimera mice carrying NOS3 in blood cells (BC) only but not within the endothelium (EC) by transplanting the bone marrow of WT mice into irradiated NOS3-/- mice (BC+/EC-). Viceversa, mice lacking blood cell NOS3 only were created by transplanting bone marrow from NOS3-/- mice into irradiated WT mice (BC-/EC+). WT bone marrow transplantation into WT mice (BC+/EC+) or NOS3-/- bone marrow transplantation into NOS3-/- (BC-/EC-) were applied to generate the respective controls. All four groups underwent a 60-minute coronary occlusion in a closed-chest model of myocardial I/R. After 24 hours of reperfusion cardiac function was assessed via high-resolution echocardiography (Vevo 2100, VisualSonics). Blood pressure was measured invasively using a pressure catheter. To analyze area at risk (AAR) and infarct size (IS), 2,3,5-Triphenyltetrazolium chloride (TTC) staining was applied. The levels of NO metabolites in plasma and heart tissue were measured by high pressure liquid chromatography.
Results: After 24 hours of reperfusion systolic left ventricular function was impaired with reduced ejection fraction and increased endsystolic volume in BC-/EC+ compared to BC+/EC+ and BC-/EC- compared to BC+/EC-. In BC-/EC+ infarct sizes were significantly increased compared to BC+/EC+ (BC-/EC+: 26,59±6,98% vs. BC+/EC+: 14,22±5,31%, p< 0,01, n= 6-9 per group). Similarly, in BC-/EC- infarct size was significantly increased compared to BC+/EC- (BC-/EC-: 26,97±5,13% vs. BC+/EC-: 20,54±5,25%, p<0,05, n=6-10 per group, AAR per LV did not differ between the groups). Blood pressure was progressively elevated in BC-/EC+, BC+/EC- and BC-/EC- compared to BC+/EC+, while BC-/EC- exhibited the maximum blood pressure of the four types of chimera generated. Nitrite and nitrate levels were reduced in BC-/EC+ plasma at baseline and 24 hours of reperfusion compared to BC+/EC+.
Conclusion: Reduced infarct size, preserved cardiovascular function and im-