**Introduction:** A third of patients with critical limb ischaemia (CLI) eventually require amputation. In spite of clinically successful revascularisation patients rarely return to their pre-morbid status, and often report no improvement in their functional outcomes, which may be due to an underlying musculopathy. Non-haematopoietic EPO-derivatives have been designed to retain only tissue-protective functions of EPO. We hypothesised that ARA-290 (EPO-derivative) may have tissue-protective potential that would represent a novel therapeutic adjunct in patients with CLI.

**Methods:** The effect of EPO and ARA-290 in mediating cytoprotection was assessed firstly in vitro using skeletal myoblasts isolated from CLI and control donors, and a model of simulated ischaemia. Characterisation of CLI myoblasts was also performed, to assess their contractile, migratory and proliferative ability. Subsequently, an in vivo murine model of hindlimb ischaemia, which recapitulates the muscular pathology observed in CLI patients, was used to assess the potential of ARA-290 to improve functional, histological and perfusion outcomes.

**Results:** Skeletal myoblasts were successfully isolated from CLI patients for the first time. CLI myoblasts and myotubes exhibited increased proliferative capacity but reduced migratory and contractile function and importantly a reduced susceptibility to a second ischaemic-insult compared with control myoblasts and myotubes. EPO and ARA-290 treatment led to significant improvements in myoblasts and myotube function and survival via the JAK2/STAT3, PI3k/Akt and NFκB signalling pathways. In vivo, animals treated with EPO and ARA-290 demonstrated improved functional, histological and perfusion outcomes compared to vehicle-control treated animals.

**Conclusion:** These studies demonstrate the potential of EPO and its derivatives to protect tissues and cells from ischaemic-injury and encourages the development of novel pharmacological therapies for use in patients with “no option” CLI or severe functional deficit.

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VEGFR2 Blockade in Murine Vein Graft Results in Reduced Intra-Plaque Hemorrhage and Stable Atherosclerotic Lesions

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**Introduction:** Immature plaque neovessels contribute to atherosclerotic plaque instability and intra-plaque hemorrhage by leaking erythrocytes and leukocytes in the plaque. Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), together with the angiopoietin (Ang)-Tie2 system, regulates the maturation of growing neovessels. We have previously shown that murine vein graft lesions exhibit massive plaque neovascularization and that leaky vessels and intra-plaque hemorrhage contribute to lesion growth. We hypothesized that blockade of VEGFR2 results in more mature plaque microvessels and less intra-plaque hemorrhage.

**Methods:** Donor caval veins were engrafted in carotid arteries of recipient hypercholesterolemic ApoE3-Leiden mice (n = 14/group). Mice were treated at day 14, 17, 21 and 25 with VEGFR2 blocking antibodies (DC101) or control IgG antibodies (10 mg/kg). At day 28 mice were sacrificed for histological analysis of the vein grafts.

**Results:** Morphometric analysis revealed a striking 50% decrease in vein graft segments that expressed intra-plaque haemorrhage in the form of leaky vessels in the DC101 treated group. This was accompanied by a significant 25-fold decrease in extravasated erythrocytes. Furthermore, lesions that exhibit intra-plaque hemorrhage showed a strong increase in Ang-2, indicative for immature neo-vessels. VEGFR2 blockade however, did not affect the neo-vessel density in the lesions (control 52 ± 19 neovessels/section; DC101 63 ± 25 neovessels/section). Interestingly, the vein graft lesion area in the DC101 group was significantly reduced with 32% compared to the control group. Moreover, plaque stability was clearly increased in DC101 treated mice, determined by a 25% reduction in macrophage content, a 50% increase in collagen content and a 120% increase in SMC content.

**Conclusion:** Blockade of VEGFR2 leads to reduced intra-plaque hemorrhage, decreased vein graft lesion area and increased plaque stability. This identifies plaque neovascularization as an attractive target for the treatment of unstable atherosclerotic diseases.

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TLR4 Accessory Molecule RP105 (CD180) Regulates Arteriogenesis and Angiogenesis


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**Introduction:** TLR4-mediated mobilisation and activation of pro-inflammatory Ly6Chi monocytes is crucial for effective post-ischemic neovascularisation, i.e. arteriogenesis and angiogenesis. Therefore, we aimed to investigate the role of the TLR4-accessory molecule RP105 (CD180) in neovascularisation. RP105 has been identified as a negative regulator of TLR4 signalling in monocytes. Using immunohistochemical analyses, we found that RP105+ monocytes are present in the perivascular space of remodelling collateral arterioles. As RP105 inhibits TLR4 signalling, we hypothesized that RP105 deficiency would lead to an unrestrained TLR4-mediated inflammatory response and hence to enhanced neovascularisation and blood flow recovery after ischemia.

**Methods:** RP105−/− and wildtype mice were subjected to hind limb ischemia and blood flow recovery was followed by Laser Doppler Perfusion imaging during four weeks.