In hypoxia, BH4 (100 mg/kg) reduced RVSP over 7 days (radiotelemetry, from 63±4 to 44.5±4 mmHg). At two weeks, BH4 (100 mg/kg) partially reversed RVH compared to placebo and BH4 (10 mg/d) (0.50±0.02, 0.58±0.03 and 0.57±0.1 respectively, $p<0.05$). Two weeks of hypoxia induced distal pulmonary vascular muscularization (from 15.6±2% to 79.2±7%, $p<0.01$). BH4 (100 mg/kg) induced partial reversal in vascular muscularization compared to placebo and BH4 (10 mg/kg) (65.2±6%, 81.4±9% and 74.3±10% respectively, $p<0.01$).
BH4 (100 mg/kg) increased eNOS enzymatic activity in lung homogenates (but not protein levels). BH4 moderately increased cGMP ($p>0.05$) and significantly reduced superoxide.
Conclusions: BH4 is a potential therapy which addresses the vasoconstrictive, hyperproliferative and hypertrophic nature of PH and warrants further investigation.

70. Treatments for pulmonary hypertension in human and experimental models

407
Pharmacological treatment with tetrahydrobiopterin in pulmonary hypertension
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Introduction: Tetrahydrobiopterin (BH4), by "recoupling" endothelial nitric oxide synthase (eNOS), increases NO bioavailability and decreases superoxide production. It is critical in maintaining pulmonary vascular homeostasis and may offer a treatment for pulmonary hypertension (PH).
Methods: BH4 administration was studied in both monocrotaline and hypoxia models of PH.
Results: In vivo BH4 administration (1mg bolus) caused minimal change in pulmonary artery pressure (PAP) in control rats (from 22.9 to 20 mmHg), but a significant acute reduction in the monocrotaline model (from 36 to 18 mmHg). In a Langendorff heart preparation, BH4 increased right ventricular systolic pressure (RVSP) in the hypertrophied right ventricle (RVH) compared to control (ΔRVSP=5.7±1.3 mmHg vs 0.7±1 mmHg respectively, $p<0.05$) as well as contractility (Δdp/dtmax=640±247 vs 261±173 mmHg/sec, $p<0.05$).

408
A role for ST2/IL-33 signalling in fibroblast proliferation utilising a novel transgenic mouse model of pulmonary hypertension
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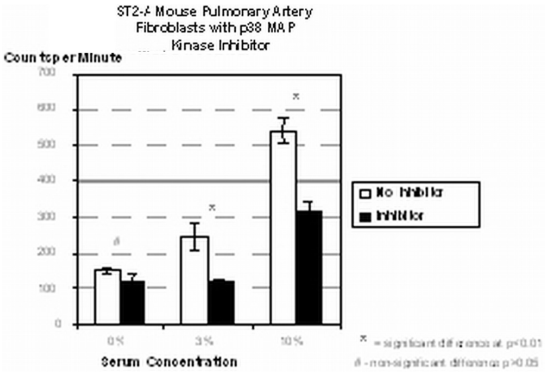
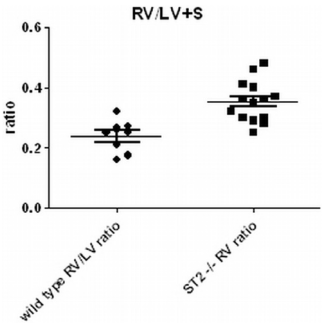
In this study, we determined if the ST2 receptor and its ligand, interleukin-33 (IL-33) was involved in mouse PAF proliferation and if ST2-/- mice displayed a pulmonary hypertensive phenotype (RVH).
Wild type (WT) and ST2 knockout mice (ST2-/-) were used. The effect of IL-33 on proliferation of WT cells was determined by incubation in hypoxia (35mmHg). p38 MAPK, which is involved in fibroblast proliferation, was detected by Western blotting. Right ventricular hypertrophy (RVH) was assessed by measuring the right ventricular wall (RV) and left ventricle with the septum (LV+S).
ST2-/- cells proliferated to a greater level compared to WT cells ($p<0.01$). Proliferation of ST2-/- cells could be reduced by p38 MAPK inhibition (Fig. 1).



p38 MAPK was detected in ST2-/- cells but not in WT cells. Proliferation to hypoxia in WT cells could be blocked by IL-33. The ST2-/- mice displayed right ventricular hypertrophy (Fig. 2, $p< 0.005$).



Excessive proliferation of fibroblasts from ST2-/- mice involves p38 MAPK. Proliferation by hypoxia in WT cells can be blocked by IL-33. ST2-/- mice display a pulmonary hypertensive phenotype.

409
Safety and efficacy of bosentan in combination with sildenafil in pulmonary arterial hypertension: The COMPASS-3 study
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Introduction: Combination therapy may improve outcomes in patients with severe PAH. However, the appropriate time and functional thresholds to initiate such therapy are ill-defined.
Aim and objectives: COMPASS-3 assessed the benefits of a bosentan-based stepped approach using a 6-minute walk distance (6MWD) of 380 meters as the functional threshold.
Methods: Treatment-naïve PAH patients with a baseline (BL) 6MWD of 150-360 meters were enrolled. Patients received bosentan for 16 weeks and, based on achievement of a 380 meter 6MWD, continued on monotherapy (125 mg bid) or combination therapy (bosentan 125 mg bid + sildenafil 20 mg tid) for an additional 12 weeks.
Results: 100 patients were enrolled (mean age 56 years, mean BMI 29.8 kg/m^2). At BL, mean ± SE 6MWD was 273±5.6 meters and 79% of patients were functional class (FC) III. By study end, 31 patients (31%) reached the 380 meter threshold, either at Week 16 (monotherapy, n = 16) or Week 28 (combination, n = 15). 6MWD improvement from BL was 22±7.8 meters at Week 16 and 45±11.1 meters at Week 28. At Week 16, 21/93 patients (22.6%) improved ≥ 1 FC from BL. At Week 28, 85 patients had FC evaluated and 34% improved ≥ 1 FC from BL. Both monotherapy and combination therapy were well tolerated. Most common reported adverse events were peripheral edema, dyspnea, headache, dizziness, anemia, and abnormal liver function tests.
Conclusions: This study demonstrates that a bosentan-based stepped approach is safe and efficacious, indicating the potential positive effect of combining the two most commonly used oral PAH therapies, bosentan and sildenafil, on functional ability in severe PAH patients.

410

PDGF and Src tyrosine kinases in pulmonary arterial smooth muscle cells: Effects of dasatinib and nilotinib on pulmonary vascular remodeling

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Introduction: Pulmonary arterial hypertension (PAH) is a progressive pulmonary vascular disorder with high morbidity and mortality. Compelling evidence suggests that receptor tyrosine kinases, such as platelet-derived growth factor (PDGF) are closely involved in the pathogenesis of PAH. We intend to study effects of 2 novel PDGF inhibitors, Nilotinib/AMN107 (abl kinases/PDGFR inhibitor) and Dasatinib/BMS-354825 (abl kinases/PDGFR/Src inhibitor) on pulmonary arterial smooth muscle cells (PASMCs) proliferation and migration in vitro and on reverse-remodeling potential in vivo in experimental PAH and to evaluate expression and regulation of Src family kinases in PAH.
Methods and results: Human PASMCs were stimulated by PDGF- or growth factor (FCS, EGF, FGF, Insulin) cocktail- to induce proliferation and migration in vitro. Dasatinib (30nM), Nilotinib (300nM) and Imatinib (1μM) potently inhibited PDGF-induced Stat3, Akt and Erk1 phosphorylation. All 3 inhibitors decreased PDGF-induced proliferation, cell cycle gene regulation and migration. In contrast, only Dasatinib inhibited growth factor cocktail-induced Src phosphorylation and proliferation. However, combination of Src inhibitors (PP1, PP2) to Imatinib or Nilotinib reduced growth factor cocktail-induced proliferation to similar extent of inhibition observed by Dasatinib. Importantly, Src phosphorylation is increased in PAH-PASMCs as compared to control-PASMCs. In vivo, Dasatinib (15mg/kg/bw) treatment caused a complete reversal of pulmonary vascular remodeling and achieved efficacy similar to Imatinib (100mg/kg/bw) in monocrotaline-induced PAH rats.
Conclusions: We suggest that dual inhibition of PDGFR and Src kinases potently inhibits mitogenic and motogenic responses to growth factors in PASMCs and pulmonary vascular remodeling in vivo thus can be an attractive therapeutic option for PAH.

411

Effects of riociguat in severe experimental pulmonary hypertension

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Introduction: The NO-sGC-cGMP signaling pathway is impaired in different cardiovascular diseases, including pulmonary hypertension (PH). Riociguat is the first of a new class of drugs, the sGC stimulators. Riociguat has a dual mode of action: it sensitizes sGC to the body's own NO and can also increase sGC activity in the absence of NO, causing vasorelaxation, anti-proliferation and anti-fibrotic effects.
Aim: The aim of the study was to investigate the effects of riociguat as compared to the PDE5 inhibitor sildenafil on pulmonary vascular remodeling in severe experimental PH.
Methods: Angioproliferative PH was induced in rats by combined exposure to the VEGFr antagonist SU5416 and hypoxia. 21 days thereafter, rats were randomized for treatment with riociguat (10 mg/kg), sildenafil (50 mg/kg) or vehicle for the next 14 days. Echocardiography and invasive hemodynamic measurements were performed. Pulmonary vascular remodeling was assessed by histomorphomety.
Results: In rats with established PH, RVSP was significantly decreased by treatment with riociguat to 73±4 mmHg ($p<0.01$) and sildenafil to 80±3 mmHg ($p<0.05$) as compared to placebo (89±3 mmHg). No significant difference in systemic arterial pressure was detected between placebo and treated animals. Both compounds significantly decreased RV hypertrophy and improved RV function by normalization of TAPSE and Tei index, but effects of riociguat were more pronounced. Riociguat significantly reduced the proportion of occluded arteries, increased proportion of opened arteries and decreased neointima/media ratio.
Conclusion: We demonstrated that riociguat effectively suppresses pulmonary vascular remodeling and significantly improves RV function in an experimental model of PH.

412

Inhibition of microRNA-17 improves lung and heart function in experimental pulmonary hypertension

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MicroRNAs (miRs) control various cellular processes in tissue homeostasis and disease by regulating gene expression on the posttranscriptional level. Recently, it was demonstrated that the expression of miR-21 and members of the miR-17-92 cluster was significantly altered in experimental hypertension (PH). We evaluated whether inhibition of these miRs may provide a novel therapeutic option.
We first tested the effects of miR inhibitors (antagomirs), which were specifically designed to block miR-17 (A-17), -21 (A-21) and -92a (A-92a) in chronic hypoxia-induced PH in mice. A-17 and A-21 reduced right ventricular systolic pressure (RVSP) and all antagomirs decreased pulmonary arterial muscularization. However, only A-17 reduced hypoxia-induced right ventricular hypertrophy and improved pulmonary artery acceleration time (PAAT). Therefore, we additionally tested the effects of A-17 in monocrotaline-induced PH in rats. A-17 treatment significantly decreased RVSP and total pulmonary vascular resistance index, increased PAAT, normalized cardiac output and decreased pulmonary vascular remodelling. Among the tested miR-17 targets, the cyclin-dependent kinase inhibitor 1A (p21) was upregulated in lungs undergoing A-17 treatment. Likewise, in human pulmonary artery smooth muscle cells, A-17 increased p21. Overexpression of miR-17 significantly reduced p21 expression and increased proliferation of smooth muscle cells. Our data demonstrate that A-17 improves heart and lung function in experimental PH by interfering with lung vascular and right ventricular remodeling. Thus, inhibition of miR-17 may represent a novel therapeutic concept to ameliorate disease state in PH.

413

Imatinib in pulmonary arterial hypertension, a randomized, efficacy study (IMPRES)

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Pulmonary arterial hypertension (PAH) results in progressive increases in pulmonary vascular resistance (PVR), right ventricular failure and death. There is currently no pharmacological cure for PAH, which is associated with low survival rates if patients do not receive effective specific therapy. Current treatment approaches focus on vasodilator outcomes. Imatinib has been associated with

beneficial effects in some patients in a proof-of-concept study. The phase III, multinational, multicenter, double-blind, parallel-group IMPRES study evaluated the efficacy and safety and tolerability of imatinib to confirm these preliminary findings. Patients enrolled in IMPRES had severe symptomatic PAH on at least two PAH-specific therapies and PVR $\geq$800 dynes sec cm^{-5} and were randomized to imatinib or placebo. Treatment was initiated at a dose of 200 mg once daily, which was increased to 400 mg once daily after 2 weeks if well tolerated. Doses could then be reduced to 200 mg once daily if treatment was not well tolerated. The primary objective was to evaluate the efficacy of imatinib versus placebo for the change in 6-minute walk distance (6MWD) from baseline to week 24. Secondary objectives included evaluation of time to clinical worsening (all cause mortality, hospitalization for worsening PAH, worsening of World Health Organization [WHO] functional class or a 15% drop in 6MWD), safety and tolerability (including adverse events, laboratory data and vital signs), changes in pulmonary hemodynamics, changes in Borg dyspnea scores and pharmacokinetics. The study has enrolled a total of 202 patients. Findings from the IMPRES study will be available during 2011 and will be presented.