

## BTS/BLF/BALR Early Career Investigator of the Year

### T1 HYPOXIA-INDUCIBLE FACTOR 2 $\alpha$ REGULATES NEUTROPHILIC INFLAMMATION IN HUMANS, MICE AND ZEBRAFISH

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Neutrophilic inflammation plays an important role in inflammatory lung diseases but therapeutic targeting of neutrophil (PMN) persistence is lacking. PMN lifespan and function is regulated by hypoxia, a characteristic feature of inflamed tissues, via the HIF/VHL/hydroxylase pathway, specifically hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and prolyl hydroxylase-3 (PHD3). Targeting HIF-1 $\alpha$  in myeloid cells impaired immune function, but PHD3 regulated PMN lifespan without affecting function. Given that PHD3 preferentially regulates HIF-2 $\alpha$ , we investigated the role of HIF-2 $\alpha$  in PMN-mediated inflammation.

Peripheral blood PMNs isolated from healthy volunteers and mice expressed HIF-2 $\alpha$  and expression was enhanced by heat-killed bacteria. Using PMNs isolated from patients with active inflammatory arthritis (IA) we demonstrated significant upregulation of *HIF2A* mRNA (IA 92.9 $\pm$ 30.3 vs. control 4.3 $\pm$ 0.9 AU relative to *ACTB*,  $P < 0.05$ ) and protein (IA 0.26 $\pm$ 0.05 vs. control 0.01 $\pm$ 0.01 OD relative to P38,  $P < 0.01$ ) in circulating inflammatory PMNs. PMNs recruited to the airways of patients with COPD also displayed strong HIF-2 $\alpha$  staining. The consequences of HIF-2 $\alpha$  upregulation were examined using human PMNs from patients with gain-of-function mutations in the *HIF2A* gene. Neutrophils isolated from these patients had reduced rates of constitutive apoptosis. Recapitulation of the human *HIF2A* mutations in the orthologous *HIF2A* gene, *epas1a*, in zebrafish delayed resolution of inflammation in a tail injury model (24 hrs post injury, *epas1a* 12.7 $\pm$ 1.4 vs. ctrl 5.2 $\pm$ 0.5 PMNs,  $p < 0.001$ ) with an associated reduction in PMN apoptosis (*epas1a* 1.0% vs. ctrl 1.6%,  $p < 0.05$ ). Mice with myeloid-specific deletion of *Hif2a* had normal PMN survival in response to hypoxia and the cells showed no functional defect *in vitro*. Importantly, in a PMN-mediated acute lung injury model, myeloid-specific deficiency of HIF-2 $\alpha$  markedly enhanced resolution of inflammation (BAL PMN count 48 hours following nebulised LPS, WT 2.13 $\times 10^6 \pm 0.08$  vs. KO 1.39 $\times 10^6 \pm 0.24$ ,  $p < 0.05$ ) and reduced lung injury (BAL IgM at 48 hours, WT 211 $\pm$ 22.8 vs. KO 74.7 $\pm$ 27.2 ng/ml), implicating HIF-2 $\alpha$  in PMN persistence in inflamed lung tissue.

These data support a critical and selective role for HIF-2 $\alpha$  in the resolution of inflammation through the maintenance of PMN survival, and provide a platform to dissect the therapeutic utility of targeting HIF-2 $\alpha$  in chronic inflammatory diseases.

### T2 MSCTRAIL AS A NOVEL CELLULAR THERAPY FOR MALIGNANT MESOTHELIOMA

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**Background** Malignant pleural mesothelioma (MPM) is an aggressive fatal cancer with no effective treatments. Mesenchymal stem

cells (MSCs) migrate and incorporate into tumour stroma making them good vehicles for the delivery of anti-cancer therapies. TNF-related apoptosis inducing ligand (TRAIL) selectively induces apoptosis in malignant cells without affecting healthy tissues. This study aimed to test whether MSCs modified to express TRAIL (MSC-TRAIL) could be a successful cell therapy for MPM.

**Methods** Human MSCs were transduced with a lentiviral vector containing TRAIL IRES-GFP under the control of a tetracycline dependent promoter. Successful transduction was measured using flow cytometry and TRAIL expression was confirmed by immunoblotting and ELISA. The biological activity of MSC-TRAIL was determined using co-culture experiments. DiI stained MPM cells were plated in a 1:1 ratio with MSC-TRAIL cells. TRAIL production was activated and cells were treated for 48 hours. Both cells and supernatant were collected and stained with Annexin V and DAPI to detect apoptosis and death respectively on flow cytometry.

To test the effect of MSC-TRAIL *in vivo* a bioluminescent tumour model was established. Plasmid containing firefly luciferase and YFP was expanded and the correct sequences confirmed by restriction digest. Lentivirus was produced, viral titres were determined using flow cytometry and MPM cells were transduced (MPMLuc). A pure population was generated using hygromycin selection. 80,000 MPMLuc cells were injected into the pleural cavity of NOD/SCID mice and their growth was assessed using an IVIS Lumina system to detect bioluminescence. 1 million MSC-TRAIL cells were delivered via tail vein injections on days 5, 9, 12, 15 and 18 post tumour inoculation and bioluminescence was measured twice weekly.

**Results** MSCs were successfully transduced with TRAIL with 96% efficiency and TRAIL production was confirmed by ELISA. Seven human MPM cell lines were tested with 6/7 (86%) being sensitive to MSC-TRAIL. *In vivo* delivery of MSC-TRAIL resulted in a significant reduction in MPM tumour growth.

**Conclusions** Delivery of TRAIL via MSCs causes a significant reduction in MPM tumour growth and is a potential novel cellular therapy for this currently incurable disease.

### T3 ACUTE MUSCLE LOSS IN THE CRITICALLY ILL: FROM BEDSIDE TO BENCH

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**Background** Critical illness survivors demonstrate skeletal muscle wasting with associated functional impairment. We prospectively characterised this process, and defined the pathogenic roles of altered protein synthesis and degradation.

**Methods** Critically ill patients (n=63, 59% male, age 54.7 $\pm$ 18.0 years, APACHE II score 23.5 $\pm$ 6.5) were recruited <24 hours following Intensive Care Unit (ICU) admission. Muscle loss trajectory was determined through serial ultrasound measurement of rectus femoris cross-sectional area (RF<sub>CSA</sub>) and, in a subset (n=28), quantification of myofibre area (Fibre<sub>CSA</sub>) and protein/DNA ratio in vastus lateralis biopsies. Histopathological analysis was also performed. Muscle protein synthesis and breakdown rates were determined ([1,2-<sup>13</sup>C<sub>2</sub>]-Leucine incorporation and D<sub>5</sub>-Phenylalanine dilution, n=11), and respective signalling pathways examined (Luminex technology and Western Blotting, n=33).