3061 – PU.1 ENFORCES QUIESCENCE AND LIMITS HEMATOPOIETIC STEM CELL EXPANSION DURING CHRONIC INFLAMMATION

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Hematopoietic stem cell (HSC) quiescence supports lifelong blood regeneration and guards against pre-leukemic clonal expansion. Acute exposure to the pro-inflammatory cytokine interleukin (IL)-1 drives myeloid cell production and HSC cell cycle entry. However, HSC return to a quiescent state suggesting the presence of a 'braking' mechanism that limits HSC proliferative capacity during chronic inflammation. To identify mechanism(s) regulating HSC cell cycle activity, we injected mice with IL-1 for 20 days modeling chronic inflammation in vivo. RNA-seq analysis of HSC following IL-1 exposure revealed repression of cell cycle and protein synthesis genes, suggesting the activation of a 'growth arrest' gene program. This gene program coincided with increased PU.1 expression, and ChIP-seq analysis identified PU.1 binding on nearly all repressed genes, suggesting PU.1 enforces HSC quiescence during chronic inflammation. Strikingly, HSC from IL-1 treated PU.1-deficient mice exhibited loss of quiescence associated with aberrant myeloid expansion in the bone marrow and spleen. Together, our results suggest PU.1 induction maintains HSC quiescence under chronic inflammatory stress. They also suggest IL-1 may confer a competitive advantage to HSC with impaired PU.1 function, triggering aberrant proliferation and myeloid expansion. Several oncogenic mutations found in acute myelogenous leukemia (AML) impair the expression and/or function of PU.1. As AML pre-dominantly affects elderly individuals and leukemogenesis is often associated with chronic inflammation, our data supports a model where chronic inflammation triggers the selective expansion of HSC harboring oncogenic mutations, leading to a pre-leukemic state. Thus, blockade of inflammation may be a tractable approach to delay and/or prevent leukemia.

3062 – ASSESSING COMPATIBILITY OF ANTIMICROBIAL VIOLET-BLUE LIGHT FOR PATHOGEN REDUCTION OF RED BLOOD CELL CONCENTRATES

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Transfusion-related sepsis is the most frequent infectious complication of blood transfusion. With millions of units transfused per year, product safety is key to public health but currently relies upon donor deferral and product testing. Pathogen reduction technologies can also be used, with several systems CE marked or FDA approved for platelet and plasma use, but only one CE marked for whole blood. 405 nm violet-blue light has recently demonstrated potential for in situ bacterial reduction of ex vivo stored plasma and platelet products, along with preliminary antiviral potential in plasma. This study assesses the potential compatibility of 405 nm antimicrobial light with red blood cell (RBC) components.

Sheep RBC were analysed, showing light transmissibility and absorption characteristics over wavelengths of 220-1100 nm using neat–1000X dilutions. Irradiances of 10-100 mW/cm^2 405 nm light were then applied to red cell samples with ~1.3 & 7.8 mm depths over 10-60 minutes, giving doses of 9-360 J/cm^2. Treatment effects on cell integrity were demonstrated by microscopy and photometric detection of leaked haemoglobin using the Harboe method.

RBC transmission analysis highlighted the high opacity of RBC suspension at 18% haematocrit with 1 cm depths, with peak absorption found to be at 417 nm. Results suggested the requirement of sample depths <1 cm to provide potential for penetration. Photometric analysis demonstrated RBC damage was influenced by the irradiance levels and exposure times used. Haemolysis increased by 0.37 & 1.26% over 15-60 minutes with 20 & 50 mW/cm^2 irradiances respectively. A greater increase of 5.06% was observed by 100 mW/cm^2 exposures over 15-30 minutes. Haemolysis rose sharply during 100 mW/cm^2 exposures and was supported by a visual decrease in stained viable cells. Low treatment doses ≤90 J/cm^2 with irradiances ≤50 mW/cm^2, showed potential compatibility, producing haemolysis below the 0.8% European limit.

Future work will build on these findings and investigate the potential for antimicrobial efficacy of a 405 nm light treatment system at levels compatible with RBC physiology.

3063 – DISTINCT POPULATIONS OF MULTIPOTENT PROGENITORS AND HEMATOPOIETIC STEM CELLS EMERGE FROM HEMOGENIC ENDOTHELIUM IN THE MURINE EMBRYO

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During embryonic development, blood cells emerge from hemogenic endothelium (HE), producing waves of hematopoietic progenitors prior to the emergence of rare hematopoietic stem cells (HSC), which have the unique ability to self-renew and generate all cell types of the adult hematopoietic system. Prior studies have demonstrated that yolk sac-derived erythromyeloid progenitors and HSC originate from distinct populations of HE. However, it is not known whether the earliest lymphoid-competent and multipotent progenitors originate from the same population of HE that gives rise to HSC. To investigate this, we combined index sorting of single hemogenic precursors with stromal co-culture that enables simultaneous detection of HSC and multilineage hematopoietic potential, to functionally validate surface markers that may distinguish hemogenic precursors with different hematopoietic fates. We found that expression of