aspects of heterogeneity and heatmaps of the first principle component of each aspect constructed to visualise pathway level transcriptional variability and transcriptionally distinct subsets in this refined HSC population. **Results:** Hierarchical clustering of cells from normal bone marrow and CML at diagnosis revealed the transcriptional profile distinguishing normal HSCs, BCR-ABL+ leukemic HSCs and BCR-ABL- leukemic HSCs. This also defined subsets of cells with a divergent transcriptional profile, including reduced expression of G2/M phase mitotic pathways, which may be consistent with a quiescent phenotype. Adjusting for cell cycle eliminated the distinction between BCR-ABL+ and BCR-ABL- cells without preserving the dichotomy between cells from normal and leukemic mouse, suggesting an important role for extrinsic cell signaling in regulating transcription in this HSC population. Inclusion of TKI-treated cells in the PAGODA analysis demonstrated persistence of this proposed quiescent subset following treatment. **Summary/Conclusion:** Aggregating pathway level information into "aspects" of heterogeneity using PAGODA, and applying hierarchical clustering has given new insights into the differing transcriptional profiles of leukemic HSCs, and revealed a transcriptionally distinct subset of cells that may represent a reversibly quiescent, apoptosis-resistant cell population consistent with the disease-maintaining leukemic stem cell. This corroborates previous evidence and provides further support for an important role of extrinsic cell signaling in modifying the transcriptional state of HSCs.

**S126 COMBINATION OF ASCIMINIB (ABL001) WITH ATP-COMPETITIVE TYROSINE KINASE INHIBITORS TARGETS EARLY CML PROGENITOR CELLS.**

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**Background:** The BCR-ABL1 chimeric oncoprotein drives chronic myeloid leukemia (CML) pathogenesis. The kinase activity of BCR-ABL1 (K252A) has important implications for drug discovery, as BCR-ABL1 inhibitors must be selective against other kinases (TKIs) that cause side effects, and against the normal healthy tissue, thereby sparing normal, healthy cells from the toxic effects of the TKI. Preclinical studies have shown that asciminib selectively inhibits the growth of BCR-ABL1 positive (+) cells regardless of the presence of BCR-ABL1 point mutations. Clinical trials for patients with CML or Ph+ acute lymphoblastic leukaemia utilizing asciminib alone and in combination with TKIs are currently underway and preliminary results are promising. **Aims:** To assess if dual inhibition of BCR-ABL1 leads to improved treatment outcomes in preclinical studies.

**Methods:** Here, we assess the effects of asciminib, alone and in combination with ATP-competitive TKIs (IM, nilotinib (NIL) and ponatinib (PON)) in CML cell lines and primary CD34+ chronic phase (CP)-CML stem/progenitor cells (HSPC). We assessed synergy using resazurin read-outs using Compusyn Software. We performed cell counts, apoptosis, cell-cycle and proliferation assays to determine the effect of drug combinations in CML cell lines and primary samples; and confirmed effects on primitive cells using colony-forming cell (CFC) and long-term culture initiating cell (LTC-IC) assays in vitro.

**Results:** Dose-response studies using the resazurin assays in CML cell lines (Bv173, K562, KCL22) indicate that asciminib is potent at low nanomolar concentration, even in cells that express the BCR-ABL1 T315I point mutation (KCL22). Apoptosis and cell cycle assays’ assessed by FACS showed that the inhibitory effects of asciminib were maintained in KCL22 and expressing cells when asciminib was used in combination with PON. Washout studies with asciminib in KCL22 expressing cells demonstrated a prolonged phenotypic response using low-nanomolar doses of asciminib as the cells failed to regrow and had irreversible cell-cycle damage. Primary CD34+ CML HSPCs demonstrated proliferation arrest and increased apoptosis (70-100% increase relative to control; p < 0.001) when treated for up to 72 hours with asciminib, alone and in combination with IM or NIL LTC-IC and CFC assays, determining the functional activity of primitive CML HSPCs in vitro, demonstrated that the combination of asciminib with IM or NIL reduced colony outputs (60-90% decrease relative to control, p < 0.001), beyond that achieved with each drug alone (40-80% relative to control, p < 0.001), and in separate experiments, with minimal effect on normal HSPC.

**Summary/Conclusion:** These results suggest that asciminib represents a novel therapeutic approach with effects on primitive CP-CML HSPCs both as a single agent and in combination with TKI and has efficacy in cells expressing the multi-TKI resistant T315I mutation. We are now investigating the mechanism of action for asciminib, alone and in combination with NIL, by RNA-sequencing.

**S127 CHRONIC MYELOID LEUKEMIA IS ASSOCIATED WITH INCREASED NETRUPHIL EXTRACELLULAR TRAP FORMATION THAT IS AUGMENTED BY PONATINIB – A POTENTIAL CONTRIBUTOR TO VASCULAR TOXICITY?**

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**Background:** Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) driven by the occurrence of the bcr-abl1 fusion gene. Cardiovascular (CVS) and thrombotic complications are increasingly reported with the use of certain tyrosine kinase inhibitors (TKIs) used to treat CML such as ponatinib. The mechanism underlying these CVS adversities is not fully understood but may involve off-target effects that alter the function of vascular endothelial cells as well as other cells involved in the pathogenesis of thrombosis.

More than a decade ago Brinkmann and colleagues (Science, 2004) described the occurrence of neutrophil extracellular traps (NETs). In response to various stimuli, neutrophils can expell extracellular strands of decondensed DNA in complex with histones and other neutrophil granular proteins. These structures have the ability to ensure and kill microbes but are also implicated in the pathogenesis of autoimmunity and thrombosis. Previous studies demonstrated that NETs have a role in the increased thrombotic tendency associated with Philadelphia-negative MPNs (Wolch et al, STM 2018). **Aims:** To study NET formation in CML and evaluate the possible effect of the different TKIs on NET formation.

**Methods:** Neutrophils were isolated from patients with CML (n = 7) and from age and gender matched controls (n = 7) by Ficol gradient. NET formation was analyzed in-vitro following neutrophil stimulation with phorbol 12-myristate 13-acetate (PMA), ionomycin and lipopolysaccharides (LPS) and assessed by the NETosis Assay Kit (Cayman chemical) as well as by immunofluorescence (IF) studies. PAD4 and citrullinated H3 (H3cit) expression was determined by Western blot. Membrane expression of CD11b and reactive oxygen species (ROS) production were analyzed by flow cytometry. NET formation and ROS production were assessed in resting and stimulated conditions and after ex-vivo pre-treatment of neutrophils with clinically relevant concentrations of various TKIs.

**Results:** Neutrophils from patients with CML had increased NET formation up to 72% relative to control (p = 0.03) and after stimulation with PMA (p = 0.002). Pretreatment of neutrophils with ponatinib significantly augmented NET formation as compared to DMSO, imatinib...