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+ leukaemic HSCs and BCR-ABL- leukaemic 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 whilst preserving the dichotomy between cells from normal and leukemic marrow, 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 leukaemic HSCs, and revealed a transcriptionally distinct subset of cells that may represent a reversibly quiescent, apoptosis-resistant cell population consistent with the disease-maintaining leukaemic 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.

**S128 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 the BCR-ABL1 complex with histones and other neutrophil granular proteins. These structures have the ability to ensnare 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 Ficoll gradient. NET formation was analyzed *ex-vivo* following neutrophil stimulation with phorbol 12-myristate 13-acetate (PMA), ionomycin and lipopolysaccharides (LPS) and assessed by flow cytometry (Hematology Institute and Blood Bank, Meir Medical Center, Kfar Saba, Israel).

**CONTRIBUTOR TO VASCULAR TOXICITY? THAT IS AUGMENTED BY PONATINIB – A POTENTIAL CONTRIBUTOR TO VASCULAR TOXICITY?**

A. Teleman1,2, G. Granot3, A. Shacham-Abulafia3,4, M. H. Ellis1,4, O. Yarchovsky-Dolber1,4, P. Raanan1,2, O. Wolch1,2
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**Background:** Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) driven by the occurrence of the bcr-abl fusion gene. Cardiovascular (CVS) and thrombotic complications are increasingly reported with the use of certain tyrosine kinase inhibitors (TKI’s) used to treat CML such as ponatinib. The mechanism underlying these CVS adverseities 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.

Methods: Neutrophil extracellular traps (NETs) formation was analyzed by flow cytometry. NET formation and ROS production were assessed in CML neutrophils treated with clinically relevant concentrations of various TKI’s. NET formation was confirmed by immunofluorescence microscopy. Membrane expression of neutrophil granule proteins was determined by Western blot. NET formation was assessed using CompuSyn Software. We assessed the ability of asciminib alone and in combination with TKIs to reduce NET formation and ROS production.

**Results:** Treatment with asciminib at sub-therapeutic concentrations (40–80% relative to control) and in combination 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 LEUKAEMIA IS ASSOCIATED WITH INCREASED NEUTROPHEL EXTRACELLULAR TRAP FORMATION THAT IS AUGMENTED BY PONATINIB – A POTENTIAL CONTRIBUTOR TO VASCULAR TOXICITY?**

A. Teleman1,2, G. Granot3, A. Shacham-Abulafia3,4, M. H. Ellis1,4, O. Yarchovsky-Dolber1,4, P. Raanan1,2, O. Wolch1,2
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**Background:** Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) driven by the occurrence of the bcr-abl fusion gene. Cardiovascular (CVS) and thrombotic complications are increasingly reported with the use of certain tyrosine kinase inhibitors (TKI’s) used to treat CML such as ponatinib. The mechanism underlying these CVS adverseities 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 expel extracellular strands of decondensed DNA in complex with histones and other neutrophil granular proteins. These structures have the ability to ensnare 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.
and dasatinib treated neutrophils (p = 0.03 for all comparisons). ROS generation was significantly enhanced in CML patient samples compared to controls (p < 0.01). Ponatinib treatment also led to an increase of ROS levels in neutrophils from patients with CML. PAD4 and H3K31 expression was higher in neutrophil lysates form patients with CML (n = 3) as compared to controls (n = 3), (p = 0.05, p = 0.01, respectively).

Summary/Conclusion: CML is associated with an increase in NET formation which is further augmented by ponatinib. Further studies are needed to assess the possible role of increased NET formation in CML on the CVS and thrombotic complications associated with ponatinib exposure.

**Modeling and Therapeutic Targeting in Acute Lymphoblastic Leukemia I**

**S128** BCP- AND T-ALL CELLS HIDE IN DISTINCT NICHES OF THE BONE MARROW

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**Background:** Persistence of minimal residual disease (MRD) is a strong indicator for drug resistance in acute lymphoblastic leukemia (ALL). The contribution of the bone marrow microenvironment to resistance remains incompletely understood. In-vivo imaging in a murine model of T-cell ALL (T-ALL) infirms the existence of distinct predilection sites for leukemia cells in the bone marrow. A recent report based on experiments with patient-derived- xenografts (PDX) has suggested a role for a dormant subpopulation in B-cell precursor ALL (BCP-ALL), possibly in close contact to the extravascular side of endothelial cells of bone marrow (after induction chemotherapy), we show that BCP-ALL cells are located in close contact to the extravascular side of endothelial cells of bone marrow sinusoids. BCP-ALL cells were enriched within a distance below 5 mm from endothelial cells and reproducibly more distant from arterioles and transition zones. In contrast, T-ALL PDX at early engraftment and after induction chemotherapy were spread over the bone marrow with a marked tendency to cluster close to the endothelium after induction chemotherapy (~30 % of all T-ALL cells). We next attempted to identify subsets with slow cell cycle rates using Carboxyfluorescin-succinimidyl ester (CFSE) labelled ALL PDX. In our model of induction chemotherapy, proliferation of BCP- as well as T-ALL cells was transiently decelerated. However, we could not detect any persistence of a reproducible dormant ALL subpopulation in BCP-ALL, possibly in close contact to the extravascular side of endothelial cells of bone marrow sinusoids. BCP-ALL cells were enriched within a distance below 5 mm from endothelial cells and reproducibly more distant from arterioles and transition zones. In contrast, T-ALL PDX at early engraftment and after induction chemotherapy were spread over the bone marrow with a marked tendency to cluster close to the endothelium after induction chemotherapy (~30 % of all T-ALL cells). We next attempted to identify subsets with slow cell cycle rates using Carboxyfluorescin-succinimidyl ester (CFSE) labelled ALL PDX. In our model of induction chemotherapy, proliferation of BCP- as well as T-ALL cells was transiently decelerated. However, we could not detect any persistence of a reproducible dormant ALL subpopulation in BCP-ALL, possibly in close contact to the extravascular side of endothelial cells of bone marrow sinusoids. BCP-ALL cells were enriched within a distance below 5 mm from endothelial cells and reproducibly more distant from arterioles and transition zones. In contrast, T-ALL PDX at early engraftment and after induction chemotherapy were spread over the bone marrow with a marked tendency to cluster close to the endothelium after induction chemotherapy (~30 % of all T-ALL cells).

**Summary/Conclusion:** Our data reconcile previous reports by revealing robust differences between BCP- and T-ALL with respect to the topology of the leukemia niche in MRD. BCP-ALL MRD was detected mostly in perivascular space, while T-ALL MRD was less specifically distributed with some clusters at the endothelium. This provides the basis to investigate molecular determinants of BCP-ALL interactions with the vascular compartment, which may leverage new candidate targets for therapeutic intervention.

**S129** TP53 SOMATIC MUTATIONS AS PRE-LEUKEMIC EVENTS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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**Background:** Alterations of the tumor suppressor gene TP53 are observed in 15–20 % of patients with acute lymphoblastic leukemia (ALL) and are associated with resistance to standard treatment regimens and inferior survival. TP53 mutations (TP53mut) have been reported as an early leukemogenic event in patients with acute myeloid leukemia. In ALL, however, only limited data are available.

**Aims:** Study the pre-leukemic mutation pattern of TP53 gene in ALL patients. Therefore, we analyzed the kinetics of the TP53mut in bone marrow and peripheral blood in the group of 43 adult ALL patients harboring TP53mut at the diagnosis (Dx) and compared it to the kinetics of standard EuroMRD IG/TR minimal residual disease (MRD). Moreover, TP53mut were assessed in the distinct hematopoietic populations.

**Methods:** Patients received induction/consolidation treatment according to the GMALL 07/03 and GMALL register protocol. For each patient, TP53-mutations were analyzed in at least one Dx/MRD-positive (n = 54) and one MRD-negative sample, if available (n = 41). The presence of TP53mut was quantified using UMI-based next generation amplicon sequencing (sensitivity: 5x10^-3). In 5 patients, selected based on TP53mut-status and on the availability of