3049 - STRESS AND DISTRESS HEMATOPOIETIC STEM CELLS BY IMMUNE STIMULI
Roi Gazit
Ben-Gurion University of the Negev, Beer-Sheva, Israel

Hematopoietic Stem Cells (HSCs) are generating all types of blood and immune cells. They regenerate along healthy life, and even more so following immunological stimuli. We can prospectively isolate naïve-HSCs; however, there is little understanding of immune-activated HSCs. Part of the difficulties come from usage of multiple surface-markers, some of which are changing drastically after stimuli. The Fgd5-mCherry reporter mouse allows better identification of HSCs. Importantly, we observed no significant changes in frequencies, and total-numbers, of the primitive HSCs (lineage-cKIT+ Sca1 +CD150+Fgd5mCherry+) within the bone-marrow upon acute or extended immune-stimulation by pIpC. Furthermore, we found that these Fgd5+ HSCs retained robust long-term multipotent activity, while adjacent LSKCd350+Fgd5mCherry- do not. RNA-Seq analysis revealed differently expressed surface markers, including CD317 and CD69, being the first HSC-activation markers. CD317 reveal that all HSCs are rapidly responding to stimuli, while CD69 suggest differences of further activation. Furthermore, chronic long-term immune stimulation exerts deleterious effects on HSCs, and may predispose toward malignancy. We are also interested in various types of immune stimuli that broaden understanding of HSCs as part of the immune system. Understanding HSC's activation is suggesting the opportunity to enhance a needed response, or to blunt an excessive activation that may lead to exhaustion.

3050 - NOVEL ROLE OF PRL-3 PHOSPHATASE IN HEMATOLOGICAL MALIGNANCIES
Phyllis Chong1, Wee Joo Chng2, Julia Lim2, jianbiao Zhou2
1Cancer Science Institute of Singapore, Singapore, Singapore; 2CSI Singapore, Singapore, Singapore

Overexpression of PRL-3, an oncogenic phosphatase, was identified as a novel cluster in newly diagnosed multiple myeloma (MM) patients. However, the regulation and oncogenic activities of PRL-3 in MM warrants further investigation. Here, we report that IL-6 activates STAT3, which acts as a direct transcriptional regulator of PRL-3. Upregulation of PRL-3 increased myeloma cell viability and re-phosphorylates STAT3 in a biphasic manner through direct interaction and deactivation of SHP2, thus blocking the ggP130 (Y759)-mediated repression of STAT3 activity. Abrogation of PRL-3 reduced myeloma cell survival, clonogenicity and tumorigenesis, and detailed mechanistic studies revealed a “de-activation” of effector proteins such as Akt, Erk1/2, Src, STAT1 and STAT3. Furthermore, loss of PRL-3 efficiently abolished nuclear localization of STAT3 and reduced its occupancy on the promoter of target genes c-myc and mcl-1, and anti-apoptotic genes bcl-2 and bcl-xl. We also demonstrated a role of PRL-3 in the acquired resistance of myeloma cells towards bortezomib, which can be overcome by PRL-3 silencing. Of clinical relevance, STAT3 and PRL-3 expression is positively correlated in five independent cohorts, and STAT3 activation signature was significantly enriched in patients with high PRL-3 expression. Furthermore, PTP4A3 is a biomarker to identify high-risk MM patients that exhibited poor prognosis and inferior outcome even when treated with novel combinational therapeutics (PI and IMiD). Conclusively, our results supported a feedforward mechanism between STAT3 and PRL-3 that prolongs pro-survival signaling in MM, and proposed the targeting of PRL-3 as a validated therapeutic opportunity in MM.

3052 - ENHANCED ENRICHMENT OF SMALL CD31+ BLAST-LIKE HUMAN CORD BLOOD CELLS
Gary Gilmore, Darlene DePasquale, John Lister, Santosh Sadashive
Allegheny Health Network, Pittsburgh, United States

We previously reported the enrichment and sorting of small CD31+ cells from human umbilical cord blood by magnetic bead depletion of lineage+ and CD34+ cells. We also reported different lineage/CD34+ depletion strategies [combining Lineage depletion and CD34+ UltraPure kits vs Diamond CD34+ kit] results in different input populations for sorting; CD31+ cells could be sorted from either. Here we report adding the missing component from the alternate lineage-depletion cocktail [anti-CD61 microbeads to Lineage depletion kit/anti-CD123 microbeads to Diamond kit] results in identical input populations, with the CD31+ target cells comprising >20% of input population, permitting rapid, high-purity sorting. We have performed sorts with Influx [B-D] and Tyto [Milenyi] sorters, yielding 125,000 to 750,000 cells [92-98% pure] in 15 minutes. We show purified small CD31+ blast-like cells express the ESC marker alkaline phosphatase, as well as CD4, CD90 and CscR4.

We have been able to successfully isolate our CD31+ cell population from frozen cord blood samples stored in DMSO/FBS after red cell depletion. Frozen storage results in decreased MNC recovery, but CD31+ cell yields appear unaffected, further improving the enrichment of the CD31+ cell population. This is similar to what we have seen with frozen samples for CD34+ HSC isolation. The ability to use frozen samples is greatly improved by the using of Miltenyi’s Tyto Running Buffer, a cGMP-compliant wash and transport solution which contains Tytonase to prevent cell clumping. Tyto Running Buffer can be used in the magnetic bead depletion steps without affecting overall yields. These findings permit rapid sorting of highly purified CD31+ cells, which can be used for gene expression profiling and cell culture.

3054 - SMALL-MOLECULE TELOMERE INHIBITOR ATTENUATES THE LEUKEMIA-INITIATION POTENTIAL IN DRUG-RESISTANT LEUKEMIA CELLS
Jiali Gu1, Yingdai Gao2, Yinghui Li3, Yahui Ding5, Huier Gao2, Yafang Li3, Yue Sun2
1Institute of Hematology & Blood Diseases Hospital, tianjin, China (People’s Republic); 2Institute of Hematology & Blood Diseases Hospital Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China (People’s Republic); 3Nankai University, Tianjin, China (People’s Republic)

The drug resistance is the most often obstacle in leukemia treatment and failing to eradicate the quiescent leukemia stem cell (LSCs) is believed as the main reason of relapse. Numerous studies have indicated that abnormal maintenance of telomere is related to the sensitivity of cancer cells to therapy. Based on these facts, we designed a lot of compounds and conducted large scale screening. In this study, we identified a small molecule compound IX, an imatinib derivative, as a potential therapeutic candidate of drug-resistant leukemia treatment. IX can eradicate drug-resistant leukemia cells or LSCs, while has little effect on the normal progenitor cells in vitro. We also showed that IX can relieve the tumor burden of CML xenograft mice model and prolong the life span of them. Meanwhile, it can inhibit the abnormal elongation of telomere not only by effectively decreases the telomerase activity of drug-resistant leukemia cells, but also by affecting ALT mechanism. Besides, the suppression of Wnt signaling pathway by IX may plays an important role in eradicating drug-resistant cells. Taken together, our findings suggest that IX could be a novel therapeutic agent for the treatment of refractory leukemia.