DYNAMICS OF HSPC REPOPULATION IN NON-HUMAN PRIMATES REVEALED BY A DECADE-LONG CLONAL TRACKING

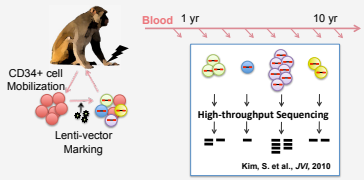
Sanggu Kim[1,5], Namshin Kim[8], Angela Presson[2], Samson Chow[3,5], Gay Crooks[4], Cynthia Dunbar[7], Dong Sung An[5,6], Robert Donahue[7] and Irvin Chen[1,5]

Dept. of Microbiology, Immunology & Molecular Genetics[1], Biostatistics[2] and Molecular and Medical Pharmacology[3] , Pathology & Laboratory Medicine[4], UCLA AIDS Institute[5], school of nursing[6], UCLA, California, National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland [7], USA. Korean Bioinformation Center, Daejeon, South Korea [8].

BACKGROUND: Hematopoietic stem and progenitor cells (HSPC) have tremendous therapeutic potentials. To date, however, our understanding of HSPCs in the primate system lags far behind the level desired for current and future therapies using these cells, due primarily to the lack of proper assay systems to examine the full regenerative potential of primate HSPC.

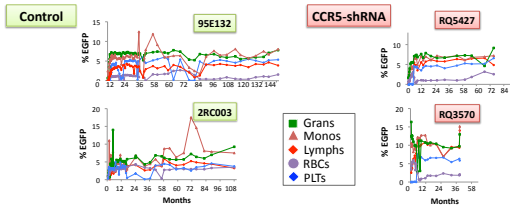
METHODOLOGY: Four rhesus macaques were followed over 4 – 12 years after transplantation of autologous CD34+ HSPCs marked with lentiviral vectors expressing either anti-HIV shRNA against the HIV-1 co-receptor, C-C chemokine receptor type 5 (CCR5-shRNA) or expressing only EGFP (Control). Vector marked clones in serially collected blood were analyzed by large-scale vector integration site (VIS) sequencing and bioinformatics analysis of genomic VIS sequences.

Sequencing-based Clonal Tracking

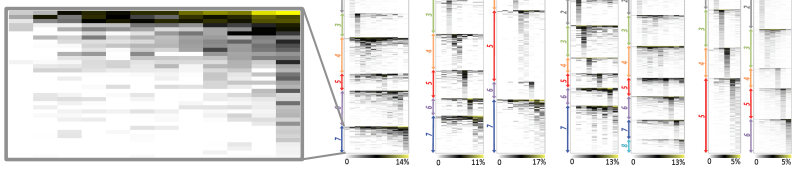


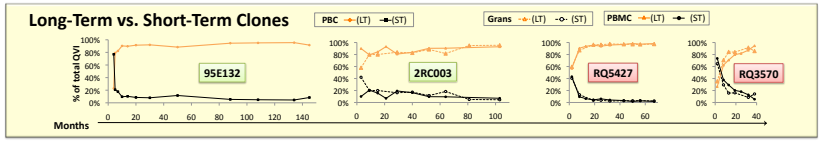
RESULTS:

Long-term, Stable Hematopoietic Reconstitution

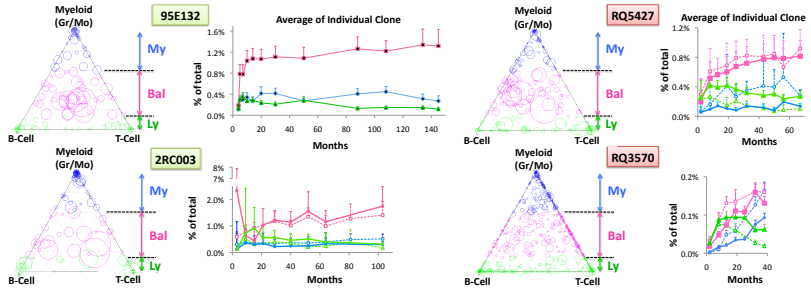


Stable Reconstitution Maintained By Sequential Clonal Repopulation. Thousands of HSPC clones were identified, repopulating sequentially over time, clustered into groups with different kinetics of repopulation.

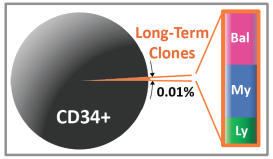


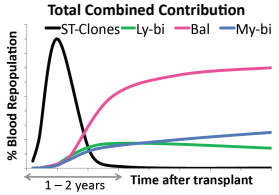


Functional Subtypes within the Long-Term Clones.



Functional Subtypes Within the Long-Term Clones.
Consistent with recent discoveries in murine HSC studies, the long-term repopulating clones could be distinctively grouped into "myeloid-biased (My)", "lymphoid-biased (Ly)", and "balanced (Bal)" subtypes based on their unique lineage output potentials. Interestingly, a small number of Bal type long-term clones predominated from 1-2 years post-transplant, contributing up to 25 – 71 % of total repopulating cells in test animals.





CONCLUSIONS: The first system level description of the detailed *in vivo* behavior patterns of primate HSPCs, providing new insights into primate repopulation and a potential frame of reference for future HSPC-based gene-therapies. No notable effect of CCR5-shRNA expression upon clonal repopulation was observed.

Accepted for ***Cell Stem Cell***.