LOW DOSE SPACE RADIATION AFFECTS LONG-TERM SURVIVAL OF BONE MARROW PROGENITOR CELL POPULATIONS

S. P. Sasi[1], D. Park[1], K.X. Walsh[2], J. Carrozza[3,4], X. Yan[1,4], and D. A. Goukassian[1,2,4]
[1]Genesys Research Institute, 736 Cambridge Street, CBR-342, Brighton, MA 02135, sharath.sasi@steward.org,
[2]Boston University School of Medicine, 700 Albany Street, W611, Boston, MA 02118, kxwalsh@bu.edu,
[3]Steward St. Elizabeth's Medical Center, 736 Cambridge Street, Brighton, MA 02135, joseph.carrozza@steward.org
[4]Tufts University School of Medicine, 736 Cambridge Street, Brighton, MA 02135, david.goukassian@tufts.edu

BACKGROUND: Radiation (IR)-induced chromosomal instability was demonstrated in the bone marrow (BM) for up to 24 months after full body IR with either X-rays or neutrons, indicating that chromosomal instability can be initiated and maintained in vivo. However, there is a significant gap in studies to date assessing full body space IR-induced survival of BM progenitor cell populations. It was shown for myeloid and lymphoid BM-derived stem and progenitor cells that after space flights the numbers of these cells are reduced to just one-half of their normal levels. Decrease in the total number of BM-derived endothelial progenitor cell (EPCs) and their lineage precursors Early- and Late-Multi-Potent Progenitor cells (E-MPPs and L-MPPs) could contribute to the pathogenesis of ischemic and/or peripheral vascular diseases, as well as for maintenance of normal vascular homeostasis in the heart and other organs. No data on BM E-MPP, L-MPP or EPC survival and proliferation during and after space flights, are currently available.

METHODOLOGY: Astronauts will be exposed to IR composed of a spectrum of low-fluence protons (^{1}H) and HZE nuclei (i.e., iron - ^{56}Fe). Therefore, we evaluated the effect of low-dose full body single dose of ^{1}H at 0.5 Gy, 1 GeV/n and 0.15 Gy, 1GeV/n of ^{56}Fe-IR on survival (FACS analysis of double positive Annexin V/propidium iodide staining - less than 2n DNA) and proliferation (CyQUAT cell proliferation assay kit) of BM EPCs before, 2, 5, 24 hours, 1, 2 and 4 weeks after ^{1}H and ^{56}Fe-IR. BM EPCs were isolated and maintained ex-vivo in corresponding selective EBM2 medium for 48-72 hours. To determine longitudinally the survival of E-MMPs and L-MMPs in the bone marrow after a full body single 50 cGy ^{1}H- and 15 cGy ^{56}Fe-IR (both ions at 1 GeV/n) C57BL/6 mice were euthanized at 1, 2, 4, 8, 12, 28 and 40 weeks post-IRs. BM cells were subjected to density gradient centrifugation to isolate mononuclear cells (MNCs). MNCs were triple-stained with FITC-labeled RAM34 (consists of CD34, c-kit, and Sca1), PE-Cy7-labeled AC133, and PE-labeled hematopoietic lineage negative (Lin^{min}) cocktail (CD3/B220/CD11b/TER-119/Ly-6G) then sorted for E-MPP and L-MMP.

RESULTS: *BM EPCs ex-vivo* - Our results showed that after full-body ^{1}H- and ^{56}Fe-IR there was 2.5-3.5-fold increase in BM EPC apoptosis ex-vivo, with the peak 3.5-fold increases in apoptosis for ^{56}Fe at 5hrs ($p<0.001$) and for ^{1}H at 24hrs ($p<0.001$). By day 7 the apoptosis was decreased to near control levels in both ion-IR EPCs. However, there was a gradual increase in EPC apoptosis for ^{1}H and ^{56}Fe between days 7-28, with maximum 3-fold ($p<0.003$) increase in BM EPC apoptosis on day 28 for both ion-IR mice. These findings indicate that there is a cyclical (early 5 hrs and delayed 28 days) increase in BM EPC apoptosis after a single low dose ^{1}H or ^{56}Fe IR. There was no significant change in ex-vivo expanded BM EPCs proliferation in ^{1}H- and ^{56}Fe-IR up to 7 days post-IR. Compared to 24 hrs (both ions), there was a ~20% (^{1}H, $p<0.05$) and ~45% (^{56}Fe, $p<0.02$) increase in the rate of EPC proliferation on day 14, but the rate of EPC proliferation for both ions had dropped significantly ($p<0.001$) below or near control levels on day 28. *BM E-MPP and L-MPP fluorescent cell sorting up to 10 month post-IR* - Compared to control mice, ^{1}H-IR increased the number of both E-MPPs (665%) and L-MPPs (203%) by 1 week, whereas ^{56}Fe-IR decreased E-MPP (74%) and L-MPPs (65%) at 1 week post-IR, suggesting an initial stimulation by ^{1}H and "a hefty" damage by ^{56}Fe in the BM milieu. In ^{56}Fe-IR mice, E-MPPs were somewhat recovered between 4 and 12 weeks but declined again below ~55-70% of control levels between 28-40 weeks. In ^{1}H-IR mice, E-MPPs were close to control levels up to 4 weeks, but declined >50% at 8 and 28 weeks. These long-lasting cyclical effects in the number of BM-derived E-MPPs and L-MPPs after a single ^{1}H or ^{56}Fe IR dose strongly suggest the presence of prolonged non-targeted effects in BM milieu, among other factors, perhaps, due to inflammation, oxidative stress, etc.

SUMMARY. Our ex-vivo BM EPC data may suggest that early increase in BM-derived EPC apoptosis may be a direct effect of single low dose ^{1}H- or ^{56}Fe-IR, whereas later increase in apoptosis and decrease in proliferation could be a result of delayed non-targeted effects. Our longitudinal E-MPP and L-MPP studies showed that despite an initial ^{1}H-IR-induced increase in the number of BM E-MPPs and L-MPPs cell populations, both ^{1}H-IR and ^{56}Fe-IR have profound and long-lasting (28-40 months) *NEGATIVE* effects, >50% decreases in the number of E- and L-MPPs. The function of the surviving fraction of E-MPPs, L-MPPs and EPCs and implications for the cardiac homeostasis, as well as cardiac repair and regeneration, remains unknown. These findings warrant inquiry into the mechanistic studies of functional capacity of the surviving fraction of BM progenitor cell populations.