Development of a Mouse Myeloid Progenitor Cell Therapeutic for Treatment of HS-ARS in BALB/c mice

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Abstract: Exposure of the hematopoietic system to lethal doses of radiation results in severe neutropenia, which increases susceptibility to opportunistic infections. We have developed a method to generate mouse myeloid progenitor cells (mMPC) *ex vivo* from purified mouse hematopoietic stem cells (HSC) to treat severe neutropenia. We have previously shown that cryopreserved mMPC pooled from several major histocompatibility complex disparate donors engraft across allogeneic barriers and provide cell-dose dependent radioprotection in mice exposed to supralethal radiation doses (9-15 Gy) (Singh *et al.*).

The aim of this study was to elucidate the potential of mMPC to prevent death from lower radiation doses where death is caused by the hematopoietic sub-syndrome of acute radiation syndrome (HS-ARS) in BALB/c mice (6-7 Gy). We demonstrate that mMPC protect BALB/c mice from radiation lethality in a radiation dose and mMPC dose dependent manner. In this model mMPC transiently engraft and generate myeloid progeny during the critical period of hematopoietic failure, enabling autologous hematopoietic recovery.

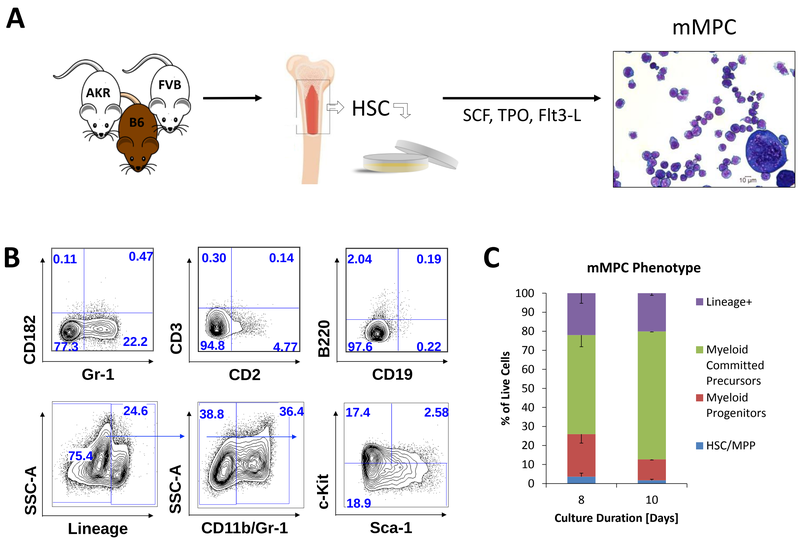

Figure 1. ***Ex vivo*****-derived mMPC are composed of myeloid committed progenitor cells. A.** Schematic of *ex vivo* mMPC generation from bone marrow HSC of AKR$^{H\text{-}2k}$, B6.PI-Thy1.1$^{H\text{-}2b}$ and FVB$^{H\text{-}2q}$ mouse strains. HSC are isolated individually from each donor strain as c-Kit^{+}Thy1.1^{mid}Lineage$^{-/low}$Sca-1^{+} (KTLS) cells by FACS and cryopreserved. KTLS from all three donor strains are thawed and pooled in equal parts for culture in X-vivo 15 media supplemented with 50 ng/mL rmSCF, 5 ng/mL rmTPO, 30 ng/mL rmFlt3L, Primocin, and L-Glutamine. mMPC are harvested after 6 to 10 days of culture and cryopreserved for future use. A picture of a representative cytospin of mMPC harvested after 8 days of culture (stained with May-Grünwald/Giemsa) is shown at 40x (Olympus BX40 microscope). **B.** Representative post-thaw flow cytometry plots of mMPC harvested after 8 days of culture. Top: Markers of mature neutrophils (Gr-1^{+}CD182^{+}), T cells (CD2^{+}CD3^{+}), and B cells (B220^{+}CD19^{+}). Bottom: Markers of committed myeloid precursors (Lin^{-}CD11b/Gr-1^{+}, Lin^{-}CD11b/Gr-1^{-}c-Kit^{-}), myeloid progenitors (Lin^{-}CD11b/Gr-1^{-}c-Kit^{+}Sca-1^{-}), and HSC and multipotent progenitors (HSC/MPP) (Lin^{-}CD11b/Gr-1^{-}c-Kit^{+}Sca-1^{+}). **C.** Phenotyping of mMPC post thaw demonstrates increasing myeloid commitment with increasing culture duration. Mean and standard deviations are shown for 8 day (n=6) and 10 day cultures (n=2). Lineage markers = B220, CD3, CD4, CD5, CD8, CD11c, CD19, CD115, CD182, FcεR1α, IL-7Rα, TER-119.

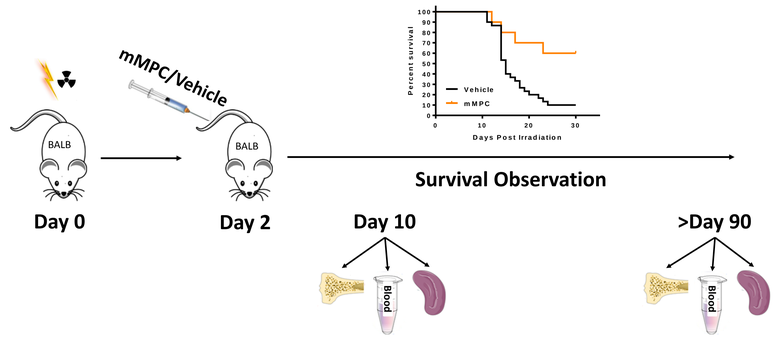

Figure 2. HS-ARS study design. 8-10 week old male BALB/c mice (H-2^{d}) from Jackson Laboratory are lethally irradiated between the hours of 8 and 11 a.m. in a pie-shaped restrainer on a rotating platform at shelf-position 7 using a Faxitron CP-160 X-ray system set at 160 kV and 6.3 mA (Dose Rate ~0.71 Gy/min). Two days post irradiation mMPC are thawed and diluted in cryomedia (CryoStor Base, 8.5% Dimethyl Sulfoxide, 5% Human Serum Albumin) to the desired concentration and infused in a total volume of 0.1 mL by retro-orbital i.v. injection. The vehicle group is infused with the same volume of cryomedia only. Mice are observed at least once daily to determine 30-day survival under basic veterinary care. Significance of survival curves is determined by pair-wise Log-rank analysis using GraphPad Prism 6.0. Cohorts of surviving mice are selected randomly for scheduled sacrifice and analysis of blood, bone marrow, and spleen to assess cellularity using a Hemavet hematology analyzer and a NucleoCounter. Chimerism and immune cell composition is assessed in all tissues using a Beckman Coulter Gallios flow cytometer. Flow cytometry is used to distinguish host (H-2^{d}) and recipient (H-2^{b}, H-2^{k}, H-2^{q}) major histocompatibility class I molecules which are expressed on the majority of cells. Differences between vehicle and mMPC infused mice are tested for significance by unpaired two-tailed T-test using GraphPad Prism 6.0.

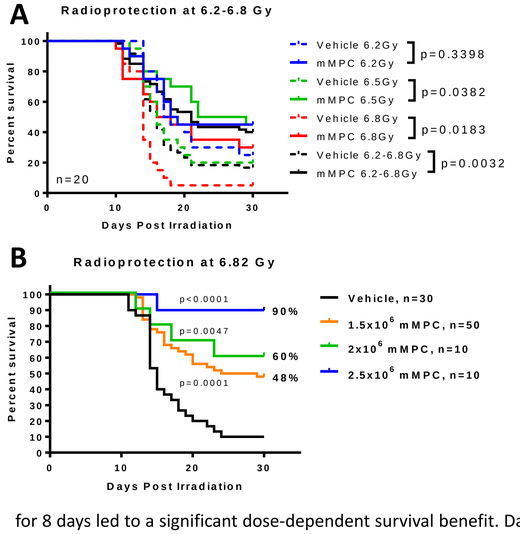

Figure 3. mMPC protect BALB/c mice from radiation doses targeting 30-day lethalities between 50-99%. A. Infusion of lethally irradiated mice with 3x10^{6} mMPC cultured for 10 days led to significantly improved survival compared to mice that received vehicle at 6.5 Gy from 20% to 45% and at 6.8 Gy from 5% to 30%. Pooled across all radiation doses, infusion of mMPC significantly impoved survival (40%) compared to vehicle infusion (16.7%). **B.** Infusion of mice exposed to 6.8 Gy with 1.5x10^{6}, 2.0x10^{6}, or 2.5x10^{6} pooled allogeneic mMPC cultured for 8 days led to a significant dose-dependent survival benefit. Data pooled from 5 mMPC lots and 3 independent experiments. In line with phenotyping results, this data demonstrates that mMPC cultured for 8 days (**B**) are more potent radioprotectors than mMPC cultured for 10 days (**A**).

References:

Singh VK, Christensen J, Fatanmi OO, Gille D, Ducey EJ, Wise SY, Karsunky H, Sedello AK 2012. Myeloid Progenitors: A Radiation Countermeasure that is Effective when Initiated Days after Irradiation. Radiation Research 177(6), pp. 781-91.

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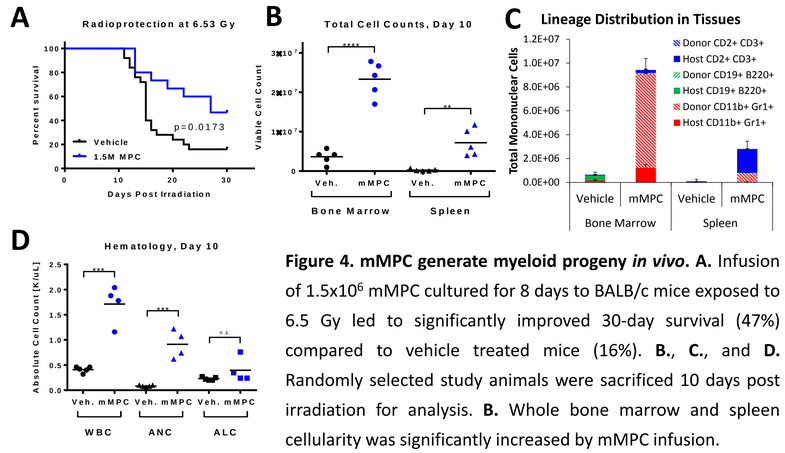

Figure 4. mMPC generate myeloid progeny *in vivo*. A. Infusion of 1.5x10^{6} mMPC cultured for 8 days to BALB/c mice exposed to 6.5 Gy led to significantly improved 30-day survival (47%) compared to vehicle treated mice (16%). **B.**, **C.**, and **D.** Randomly selected study animals were sacrificed 10 days post irradiation for analysis. **B.** Whole bone marrow and spleen cellularity was significantly increased by mMPC infusion. **C.** Flow cytometry analysis shows that mMPC generate CD11b^{+}Gr-1^{+} myeloid cell progeny in the bone marrow and spleen. An increase in host CD2^{+}CD3^{+} T cell numbers in the spleen indicate a possible allogeneic response to mMPC by residual host T cells. **D**. Whole blood cell (WBC) and absolute neutrophil counts (ANC) but not absolute lymphocyte count (ALC) were significantly improved in mMPC compared to vehicle infused mice.

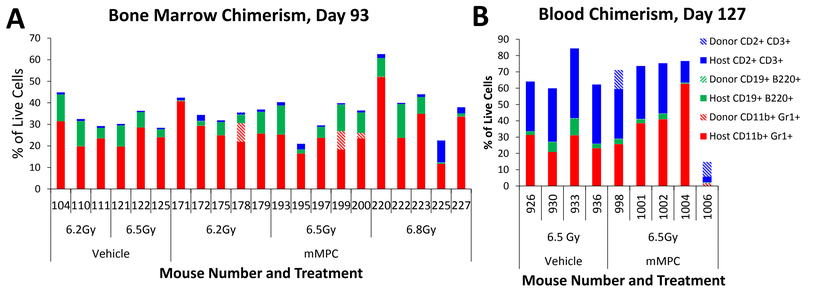

Figure 5. mMPC Engraftment is transient. A. Mice were irradiated with 6.2, 6.5, or 6.8 Gy and infused with vehicle solution or with 3x10^{6} mMPC cultured for 10 days. Data from the flow cytometry analysis of host (H-2^{d}) and donor (H-2^{b,k,q}) lineage distribution in the bone marrow of each surviving mouse from each treatment group is shown on study Day 93. Bone marrow cells are predominantly host cells with only 3 mice showing a small fraction of donor CD11b^{+}Gr-1^{+} myeloid cells. **B.** Mice were irradiated with 6.5 Gy and infused with vehicle solution or with 1.5x10^{6} mMPC cultured for 8 days. Flow cytometry analysis of host (H-2^{d}) and donor (H-2^{b,k,q}) lineage distribution in the blood of surviving mice on study Day 127 shows that blood cells are predominantly host derived with 2 mMPC infused mice showing a small fraction of donor CD2^{+}CD3^{+} T cells.

Discussion: These results show proof-of-concept in a mouse model that pooled, allogeneic myeloid progenitor cells generated *ex vivo* are a potent therapeutic treatment for HS-ARS. mMPC rapidly generate functional myeloid cells during the critical period of hematopoietic failure following radiation exposures targeting 50-99% lethality by Day 30, and increase survival in a dose dependent manner. Engraftment by mMPC is transient and enables autologous hematopoietic recovery. Cellerant Therapeutics is currently developing a human myeloid progenitor cell product, CLT-008, as an effective bridging therapy for severe neutropenia associated with chemotherapy, cord blood transplant, and HS-ARS.