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ORAL ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Impaired Efficacy and Durability of Hematopoietic Stem Cell Engraftment Caused By Samd9l-Deficiency within Bone Marrow Niches

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Germline mutations in the *SAMD9* and *SAMD9L (SAMD9/L)* genes on chromosome 7q drive both childhood myelodysplastic syndrome (MDS)/secondary acute myeloid leukemia (sAML) associated with monosomy 7, and adult-onset MDS/sAML associated with distinct somatic genetic alterations. The ortholog for *SAMD9/L* in mice is *Samd9l*. Gain of Function (GoF) and Loss of Function (LoF) *Samd9l* alleles predispose to MDS at distinct ages. LoF models show that *Samd9l*-deficient mice, like humans, are more likely to develop MDS during adulthood, while GoF models result in early onset bone marrow (BM) dysfunction and MDS/sAML.

Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for bone marrow failure (BMF), MDS, and AML arising from germline genetic syndromes. Our previous research demonstrated that deficits in host BM microenvironment (ME) niches contribute to hematopoietic failure and poor HSC engraftment in murine models of many MDS predisposition syndromes associated with BMF. As we have observed clinical cases where poor engraftment impaired outcomes in patients with *SAMD9/L* mutations, we recently hypothesized, BM niche functions critical to facilitating HSC engraftment may be impaired due to niche-intrinsic deficits caused by *SAMD9/L* mutations. Herein, we present data testing this hypothesis, demonstrating *Samd9l* deficiency alters BM ME composition, extracellular matrix (ECM) regulation, and proinflammatory cytokine signaling resulting in impaired donor HSC engraftment after HSCT.

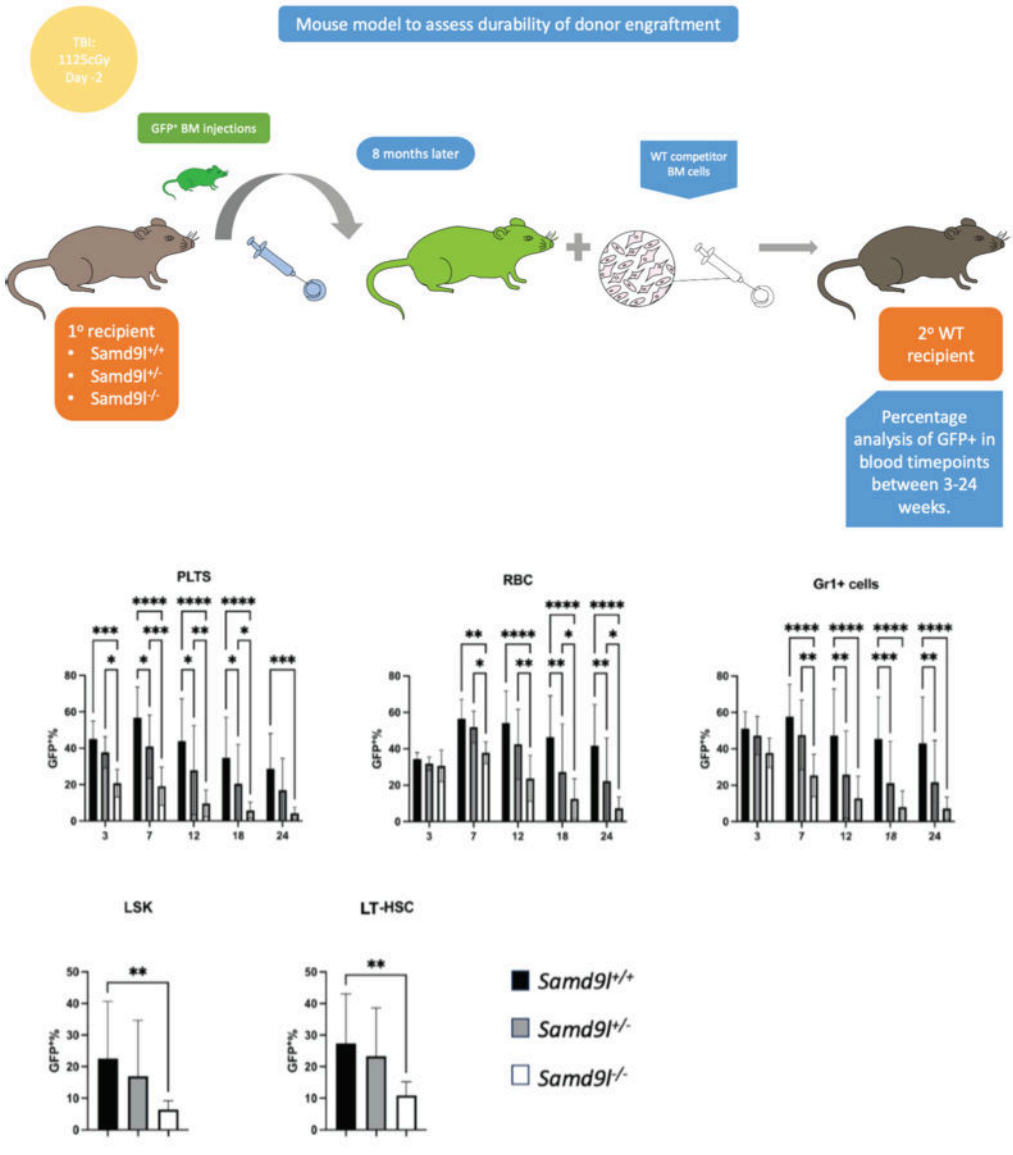
We performed primary (1 ^{ry}) and secondary (2 ^{ry}) HSCT to model impacts of *SAMD9/L* deficiency on BM niche function during HSCT using WT (*Samd9l* $^{+/+}$), *Samd9l* $^{+/-}$, and *Samd9l* $^{-/-}$ mice as HSCT recipients of healthy WT GFP $^{+}$ donor BM to measure the capacity of Samd9l LoF niches to engraft donor HSCs. To assess changes in niche composition and gene expression during HSCT, flow cytometry, multiplex ELISA, and RNASeq analysis were performed on WT, *Samd9* $^{+/-}$ and *Samd9* $^{-/-}$ mice. Compared to WT control niches, *Samd9* $^{+/-}$ and *Samd9* $^{-/-}$ niches exhibited impaired efficiency of donor HSC engraftment at 3 weeks post-1 ^{ry} HSCT, as measured by competitive 2 ^{ry}-HSCT performed with 1 ^{ry} recipient BM harvested at this timepoint. Beyond 30 weeks post-2 ^{ry}-HSCT in this model, GFP $^{+}$ reconstitution of GR1 $^{+}$ myeloid, RBC's, and platelets was reduced by 25%, 18%, and 35% in 2 ^{ry} recipients of *Samd9* $^{+/-}$1 ^{ry} recipient BM and by 26%, 28%, and 32% in 2 ^{ry} recipients of *Samd9* $^{-/-}$ 1 ^{ry} recipient BM. Durability of HSC engraftment by 8 months was even more significantly impaired in *Samd9* $^{+/-}$ and *Samd9* $^{-/-}$ niches, with *Samd9* $^{-/-}$ mice exhibiting low survival by this timepoint. Competitive 2 ^{ry} HSCT performed 8 months post-1 ^{ry} HSCT showed reduction in trilineage GFP $^{+}$ blood cells out to 24 weeks post 2 ^{ry}-HSCT, including reductions in GFP $^{+}$ Gr1 $^{+}$ myeloid cells by 50% and 83% in 2 ^{ry} recipients of *Samd9l* $^{+/-}$and *Samd9l* $^{-/-}$1 ^{ry} recipient BM, respectively, compared to control 1 ^{ry} recipient BM. Similar reductions in BM long-term HSC and Lin $^{-}$Sca-1 $^{+}$cKit $^{+}$ HSC/progenitor cells in 2 ^{ry} recipients of *Samd9l* $^{-/-}$compared to *Samd9l* $^{+/+}$ 1 ^{ry}recipient BM 8 months post 1 ^{ry}-HSCT.

Following TBI, *Samd9l* $^{+/-}$and *Samd9l* $^{-/-}$ BM niches contained similar percentages of osteoprogenitors, mesenchymal stem cells, and endothelial cells compared to WT controls. Multiplex ELISA on BM plasma collected 48hrs post-TBI revealed that *Samd9l* $^{+/-}$and *Samd9l* $^{-/-}$deletion in the BM ME led to markedly reduced expression of IFN-γ (43% and 55% reduction in *Samd9l* $^{+/-}$and *Samd9l* $^{-/-}$), Eotaxin (33%/36%), GM-CSF (47%/47%), and IL-1a (48%/65%). In contrast, expression of niche cell

extracts showed markedly increased levels of IL-17 (4 fold) and CXCL11 (1.4 fold) in *Samd9l* $^{+/-}$and of IL-17 (5.3 fold) IL-12p70 (1.3 fold), and CXCL11 (1.5 fold) in *Samd9l* $^{-/-}$, suggesting that aberrantly active Th17 responses caused by niche *Samd9l* deficiency may be responsible for impairing HSC function and maintenance post-HSCT. RNASeq analysis showed downregulation of ECM genes in both *Samd9l* $^{-/-}$and *Samd9l* $^{+/-}$models.

Our data demonstrates BM niche function is impaired by Samd9l deficiency in a gene dosage-dependent manner. These deficits are associated with increased expression of a specific inflammatory cytokine pathway, suggesting that aberrant growth factor receptor recycling due to *Samd9l* deficiency may drive stress hematopoiesis and alter HSC cell fate decisions leading to failure of durable donor HSC maintenance post-HSCT.

Disclosures No relevant conflicts of interest to declare.



Samd9l$^{+/+}$, *Samd9l*$^{+/-}$ and *Samd9l*$^{-/-}$ mice received transplantations of 10^6 whole bone marrow (BM) cells from healthy wild-type GFP$^+$ donors after 1100cGy total body irradiation (TBI). The durability of donor engraftment in the primary recipients was assessed at 8 months after BM transplantation (BMT) by competitive secondary transplantation assays. Durable donor hematopoietic stem cell (HSC) engraftment was impaired in *Samd9l*-deficient recipients, as indicated by reduced GFP$^+$ reconstitution in platelets (PLT), red blood cells (RBC), and Gr1$^+$ myeloid cells in the peripheral blood of secondary recipients out to 24 weeks after secondary competitive BMT, and reduced GFP$^+$ percentages in the Lin-Sca1+cKit+ (LSK) progenitors and Lin-Sca1+cKit+CD48-CD150+ long-term(LT)-HSCs in the BM of secondary recipients at 29 weeks after secondary competitive BMT. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, one-way ANOVA or unpaired t-test.

Figure 1

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