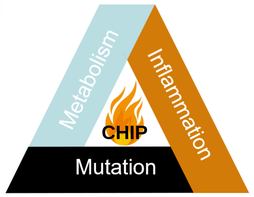
NLRP3 inflammasome activity drives clonal hematopoiesis of indeterminate potential

Rachel L. Gessner[1], Zhonghe Ke[1], Rachel Culp-Hill[2], Wolfgang Schleicher[1], Katia E. Niño[1], James S. Chavez[1], Atif Shaikh[3], Angelo D'Alessandro[2], and Eric M. Pietras[1]

[1]Division of Hematology; [3]Department of Biochemistry and Molecular Genetics; [3]Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

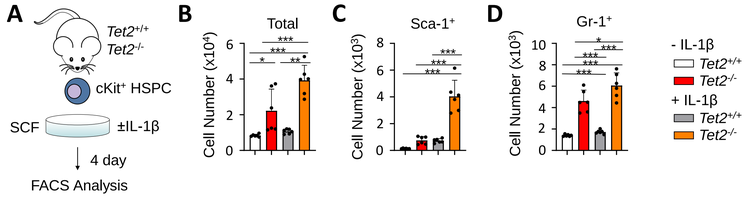
Introduction

- **Clonal hematopoiesis of indeterminate potential (CHIP) is a condition associated with the development of severe hematologic malignancies and cardiovascular disease.**
- **CHIP is distinguishable by the preferential expansion of hematopoietic stem and progenitor cells (HSPC) often harboring mutations in genes such as TET2, an epigenetic regulator.**
- **CHIP has been found more often in patients possessing proinflammatory tissue environments.**
- **In *Tet2*-deficient HSPC, IL-1β has been found to be overexpressed.**
- **The NLRP3 inflammasome is responsible for the maturation and release of IL-1β and IL-18.**



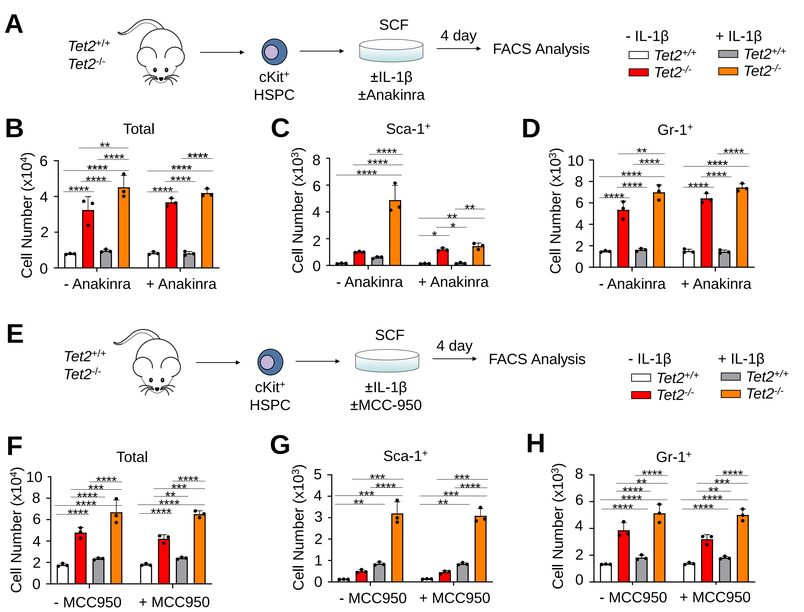
Results

1 Tet2$^{-/-}$ HSPC undergo selective expansion *in vitro*.



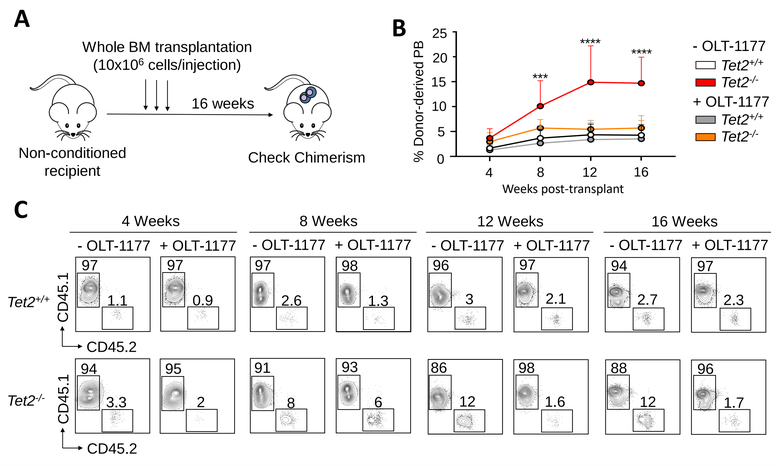
A, Experimental scheme for *Tet2*$^{-/-}$ cKit^{+} liquid culture in the presence of IL-1β; **B**, Number of live cells; **C**, Sca-1^{+} cells and; **D**, Gr-1^{+} cells from 4-day culture (n=6). One-way ANOVA was used for statistical analysis *p<0.05 **p<0.01 ***p<0.001.

2 NLRP3 inhibition does not prevent *Tet2*$^{-/-}$ HSPC expansion *in vitro*.



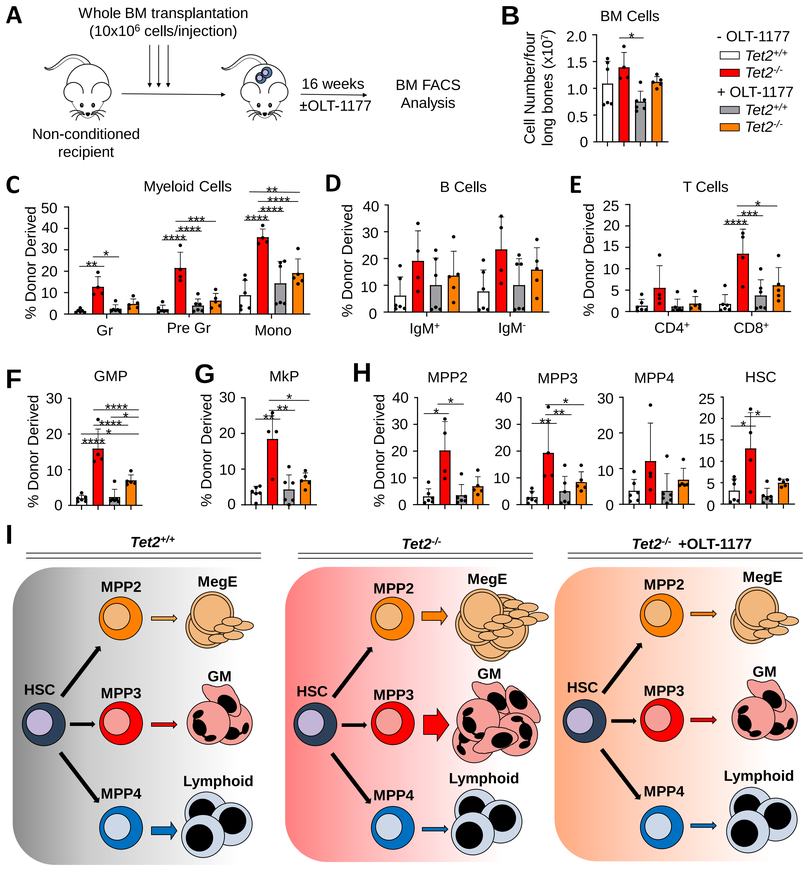
A, Experimental scheme for 4-day liquid culture of *Tet2*$^{-/-}$ cKit^{+} HSPC with IL-1β and Anakinra – an inhibitor of IL-1 receptor; **B**, Total number of live; **C**, Sca-1^{+} and; **D**, Gr-1^{+} cells from culture (n=3); **E**, Experimental scheme for 4-day liquid culture of *Tet2*$^{-/-}$ cKit^{+} HSPC with IL-1β and MCC950 – an inhibitor of NLRP3 inflammasome; **F**, Total number of live; **G**, Sca-1^{+} and; **H**, Gr-1^{+} cells from culture (n=3). One-way ANOVA was used for statistical analysis *p<0.05 **p<0.01 ***p<0.001.

3 NLRP3 inhibition selectively suppresses *Tet2*$^{-/-}$ donor-derived hematopoiesis in peripheral blood.



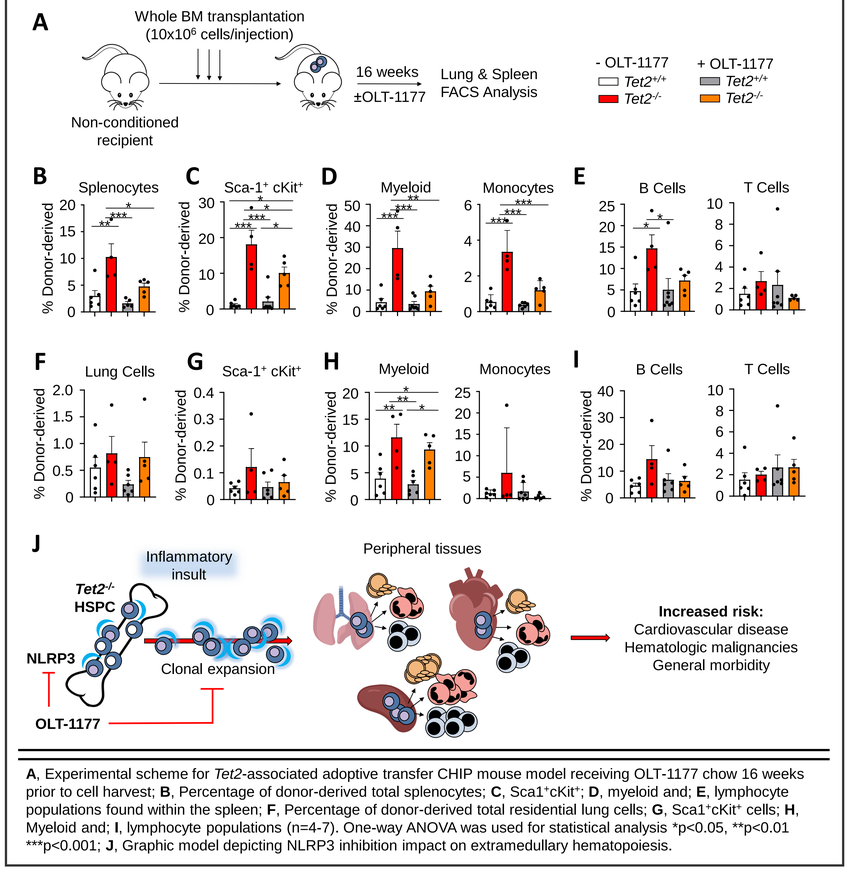
A, Experimental scheme for *Tet2*-associated CHIP mouse model adoptive transfer receiving 7.5 g/kg OLT-1177, an NLRP3 inflammasome inhibitor, chow; **B**, Percent of donor-derived cells present in the peripheral blood over 16-week treatment post-transplantation; **C**, FACs plots of donor and host populations. Two-way ANOVA was used for statistical analysis *p<0.05 **p<0.01 ***p<0.001.

4 NLRP3 inhibition suppresses expansion of *Tet2*$^{-/-}$ HSPC and myeloid cells in the bone marrow



A, Experimental scheme for *Tet2*-deficient adoptive transfer CHIP mouse model receiving OLT-1177 chow treatment for 16 weeks prior to bone marrow harvest; **B**, Absolute number of cells present in the lineage negative compartment of four long bones; **C**, Percent of donor-derived myeloid cells; **D**, B cells; **E**, T cells; **F**, GMP; **G**, MkP, **H**, MPP2, MPP3, MPP4, and HSC found in the bone marrow (n=4-7). Two-way ANOVA was used for statistical analysis *p<0.05 **p<0.01 ***p<0.001; **I**, Graphic model of *Tet2* cell populations plus the addition of OLT-1177.

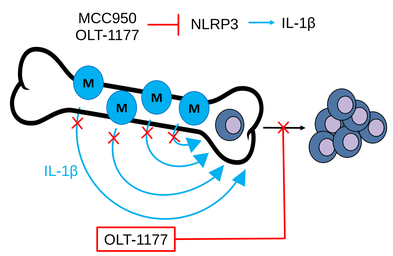
5 NLRP3 inhibition reduces burden of Tet2$^{-/-}$ myeloid cells in peripheral tissues



A, Experimental scheme for *Tet2*-associated adoptive transfer CHIP mouse model receiving OLT-1177 chow 16 weeks prior to cell harvest; **B**, Percentage of donor-derived total splenocytes; **C**, Sca1^{+}cKit^{+}; **D**, myeloid and; **E**, lymphocyte populations found within the spleen; **F**, Percentage of donor-derived total residential lung cells; **G**, Sca1^{+}cKit^{+} cells; **H**, Myeloid and; **I**, lymphocyte populations (n=4-7). One-way ANOVA was used for statistical analysis *p<0.05, **p<0.01 ***p<0.001; **J**, Graphic model depicting NLRP3 inhibition impact on extramedullary hematopoiesis.

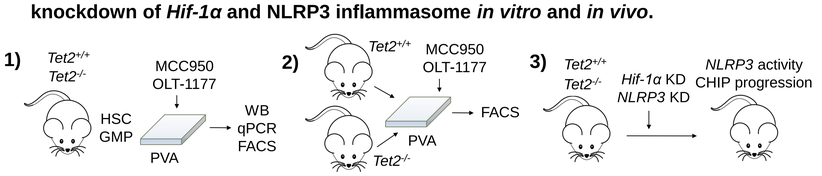
Conclusions

- ***Tet2*$^{-/-}$ HSPC aberrantly expand *in vitro*; IL-1β further exacerbates this phenotype.**
- **Short-term exposure to MCC950 or Anakinra in liquid culture have no significant effect on HSPC proliferation. Other cells within the bone marrow niche, such as monocytes, may play an unknown role.**
- **Inhibition of NLRP3 inflammasome via OLT-1177 *in* vivo reduces *Tet2*$^{-/-}$ expansion particularly within the myeloid lineage of the peripheral blood, bone marrow.**
- ***Tet2*$^{-/-}$ extramedullary hematopoiesis in the spleen is significantly reduced with OLT-1177 treatment.**



Future Directions

1) **Isolate cell populations of interest – HSC and GMP – and maintain on PVA coated liquid culture plate to identify cell type with most NLRP3 activity and/or sensitivity.**
2) **Conduct *Tet2*$^{+/+}$ *Tet2*$^{-/-}$ competitive liquid culture assay in the presence of MCC950 or OLT-1177 to test for *in vitro* preferential expansion of *Tet2*$^{-/-}$.**
3) **Understand the mechanism of action of OLT-1177 in the mouse model by shRNA knockdown of *Hif-1α* and NLRP3 inflammasome *in vitro* and *in vivo*.**



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