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# POSTER ABSTRACTS

## 602.MYELOID ONCOGENESIS: BASIC

### Rapid and Accurate Remethylation of *Dnmt3a* Deficient Hematopoietic Cells with Restoration of DNMT3A Activity

*Yang Li*[1], *Haley Abel, PhD*[2], *Michelle Cai, MD*[3], *Taylor Lavalle*[4], *Tiankai Yin, BA*[5], *Nichole Helton*[6], *Amanda Smith*[2], *Christopher A Miller, PhD*[6], *Timothy J Ley*[6]

[1]Division of Oncology, Department of Internal Medicine, Washington University in St. Louis School of Medicine, Saint Louis, MO
[2]Washington University in St. Louis, St. Louis, MO
[3]Division of Oncology, Department of Internal Medicine, Washington University in St. Louis, St. Louis, MO
[4]University of Missouri School of Medicine, Columbia, MO
[5]Washington University in St. Louis, Saint Louis, MO
[6]Division of Oncology, Department of Internal Medicine, Washington University in St. Louis School of Medicine, St. Louis, MO

Heterozygous loss-of-function mutations in the *DNMT3A* gene are the most common cause of clonal hematopoiesis, and among the most common initiating events for Acute Myeloid Leukemia (AML). A reduction of DNMT3A activity causes a canonical, focal hypomethylation phenotype in hematopoietic cells, which is associated with immortalization of hematopoietic stem cells, and blockage of myeloid differentiation. The methylation defect can be partially reversed with restoration of physiologic levels of *DNMT3A* expression over a period of several months (Ketkar et al., *PNAS*, 2020; PMID 35313694).To identify a more efficient remethylation strategy, we first characterized the DNA methylation phenotypes of bone marrow cells from mice with hematopoietic cell deficiency of *Dnmt3a*, *Dnmt3b* (or both enzymes), or expressing the dominant negative *Dnmt3a* $^{R878H}$ mutation (equivalent to R882H in humans; the most common mutation found in AML patients). Using these different mouse models as substrates, we then defined the patterns and completeness of DNA remethylation after "adding back" supraphysiologic levels of wild type DNMT3A1, DNMT3B1, DNMT3B3 (an inactive splice isoform of DNMT3B), or DNMT3L (a catalytically inactive "chaperone" that augments the activity of DNMT3A and DNMT3B in early embryogenesis), with MSCV-based retroviruses transduced into primary mouse hematopoietic stem/progenitor cell *s*. Overexpression of WT DNMT3A for 2 weeks *in vitro* (˜3 fold) can accurately reverse the differentially methylated regions (DMRs, which are >99% hypomethylated) of *Dnmt3a* deficient hematopoietic cells, or cells expressing the R878H mutation. The DMRs of *Dnmt3b* deficient mouse bone marrow cells can be corrected by overexpression of DNMT3A, DNMT3B1, or DNMT3B3; however, DNMT3B3 failed to remethylate the DMRs in *Dnmt3a/Dnmt3b* double knockout mouse bone marrow cells. Since DNMT3B3 is an inactive enzyme that does not cause remethylation in the absence of DNMT3A, these data suggest that DNMT3B3 functions in a DNMT3A-dependent manner in hematopoietic cells. Importantly, both DNMT3B3 and DNMT3L have been shown to facilitate DNA methylation by acting as a chaperone for DNMT3A (Duymich et al., *Nat. Communication*, 2016; PMID 27121154). *In vitro*, DNMT3L copurified with DNMT3A leads to an increase in DNMT3A methyltransferase activity that is ˜5 times greater than DNMT3B3 copurified with DNMT3A. Remarkably, overexpression of DNMT3L (which is not expressed in adult hematopoietic cells, or AML cells) can completely correct the hypomethylation phenotype of *Dnmt3a* $^{R878H/+}$ bone marrow cells within 2 weeks of *in vitro* overexpression, or 4 weeks of *in vivo* overexpression ( **Figure 1A and 1B**), probably by augmenting the activity of WT DNMT3A encoded by the residual WT allele in these heterozygous mutant cells. Two weeks of overexpression of DNMT3A or DNMT3L in *Dnmt3a* $^{R878H/+}$ bone marrow cells (cultured *in vitro* with IL-3, SCF, FLT3L, and TPO to maintain HSPC populations) can induce a differentiation response, increasing the fraction of mature myeloid cells in the cultures by ˜4-fold. This finding was recapitulated *in vivo* where one month of overexpression of *DNMT3A* or *DNMT3L* in transplanted *Dnmt3a* $^{R878H/+}$ mouse bone marrow cells increased the fraction of mature myeloid cells by 2-fold by DNMT3L and 4-fold by DNMT3A. Together,these data show that the focal, canonical DNA hypomethylation phenotype of *Dnmt3a* $^{R878H/+}$ hematopoietic cells can be accurately and efficiently corrected by overexpressing WT DNMT3A or DNMT3L for several weeks. We have also shown that the restoration of methylation in *Dnmt3a* $^{R878H/+}$ mutant cells alters the developmental fate of progenitors, increasing the proportion of mature myeloid cells. These data suggest that *DNMT3L* expression may represent a novel approach for restoring DNMT3A activity in AMLs initiated by *DNMT3A* mutations, and could possibly alter AML cell

fate. Additional studies are underway to determine whether the correction of DNMT3A activity in *Dnmt3a* $^{R878H/+}$ -initiated AML cells with retroviral addback of DNMT3A or DNMT3L will cause a differentiation response that alters AML cell fate.

**Disclosures Smith:** *Incite Corporation:* Current Employment, Current equity holder in publicly-traded company.

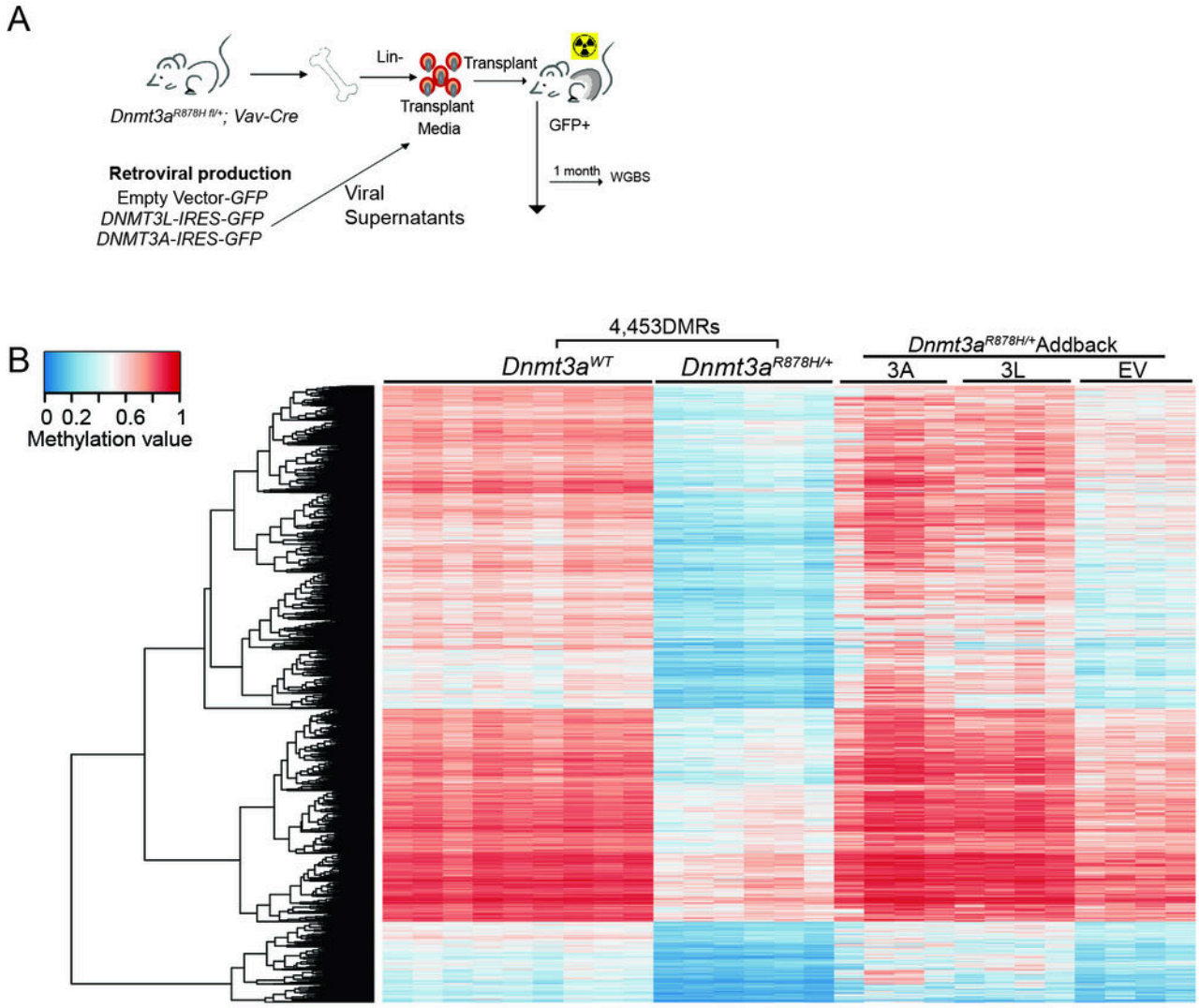


**Figure1. Effects of retroviral addback in *Dnmt3a*$^{R878H/+}$ bone marrow cells *in vivo*.** **A.** Schematic workflow for an in vivo addback experiment using *Dnmt3a*$^{R878H/+}$ mouse bone marrow cells. **B.** Heatmap showing the methylation values for individual samples of 4,453 DMRs defined by comparing whole genome bisulfite sequencing (WGBS) data from WT (n=9) vs. *Dnmt3a*$^{R878H/+}$ (n=6) fresh bone marrow samples. Values for the same DMRs were plotted passively for *Dnmt3a*$^{R878H/+}$ samples that were retrovirally transduced with a *DNMT3A1* expressing vector ("3A", n=4), a *DNMT3L* expressing vector ("3L", n=4) or an empty vector ("EV", n=4), transplanted into recipient mice, and harvested 1 month later. WGBS was performed on purified GFP+ cells (since all vectors express an IRES GFP tag) from each mouse. Red represents high methylation values, and blue represents low methylation values. Note the remethylation associated with DNMT3A1 or DNMT3L addback in each independent mouse sample. Some remethylation is also seen with the empty vector, because the stress of transplantation increases the expression of endogenous *Dnmt3a* in the expanded pool of mouse hematopoietic progenitor cells (data not shown).

**Figure 1**

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