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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Overexpression of the Signaling Integrator *Gab2* Accelerates AML Development in Mice with *Dnmt3a* ^{R878H} and *Npm1* ^{cA} Mutations

Michael H. Kramer, MDPhD[1], *Stephanie Richardson*[2], *Yang Li*[2], *Nichole Helton*[2], *Daniel George*[2], *Michelle Cai, MD*[3], *Sai Mukund Ramakrishnan, MS*[2], *Christopher A Miller, PhD*[2], *Timothy J Ley*[2]

[1]Division of Oncology, Department of Medicine, Washington University School of Medicine, St Louis, MO
[2]Division of Oncology, Department of Internal Medicine, Washington University in St. Louis School of Medicine, St. Louis, MO
[3]Division of Oncology, Department of Internal Medicine, Washington University in St. Louis, St. Louis, MO

NPM1 and *DNMT3A* are among the most commonly mutated genes in Acute Myeloid Leukemia (AML), affecting 25-30% of patients. Importantly, these mutations co-occur much more frequently than expected by chance, with ~15% of AML samples containing mutations in both genes.This synergy is recapitulated in mouse models, where mice with either *Dnmt3a* ^{R878H} or *Npm1* ^{cA} mutations develop leukemia with a long latency and low penetrance, whereas mice with both *Dnmt3a* ^{R878H} and *Npm1* ^{cA} mutations develop leukemia in 8-12 months with nearly 100% penetrance (Dovey et al., *Blood*, 2017; Loberg et al., *Leukemia*, 2019).To better understand the mechanisms underlying this synergy, and the process of leukemic transformation, we have characterized pre-leukemic bone marrow and 9 independent spontaneous AMLs that have arisen in these mice. These spontaneous AMLs rapidly cause fatal leukemias in secondary recipients; whole genome sequencing of all tumors revealed one or more human AML-like cooperating mutations in each, including canonical mutations in *Ptpn11* (E69K, A72V and S506L), an activating *Kit* ^{D818Y} mutation, a *Cbl* exon 9 deletion, a *Cbl* ^{Y369H} mutation,and an amplification of *Flt3* associated with >20 fold overexpression of WT *Flt3*. Characterization of the pre-leukemic transcriptomes and methylomes demonstrated little change in the pre-leukemic state for several months, followed by dramatic and canonical shifts in transcription and methylation after transformation. Remarkably, 8 of the 9 AMLs contained an amplification of the entirety of murine chromosome 7 as the sole structural variant in each genome. We analyzed panel sequencing data from a set of published AMLs arising in this model (Loberg et al., *Leukemia*, 2019), and also exome sequencing data from a different *Npm1* ^{cA} driven tumor model (Dovey et al., *Blood*, 2017); these AMLs also developed complete or partial amplification of chromosome 7 in 7 of 8 and 10 of 15 cases, respectively. Through integration of these datasets, we identified a minimally amplified 8.9 Mbp region on chromosome 7, containing 209 genes, including two candidate genes (*Gab2, Pak1*) that show overexpression in both murine and human AMLs with *DNMT3A* and *NPM1* mutations. Retroviral constructs expressing each cDNA (with an IRES-GFP tag) were transduced into lineage depleted bone marrow cells from preleukemic WT, *Dnmt3a* ^{R878H} , *Npm1* ^{cA} or *Dnmt3a* ^{R878H} x *Npm1* ^{cA} mice, and transplanted into recipient mice. Overexpression of *GAB2* (but not "Empty Vector" or *PAK1*) induced significant expansion of hematopoietic cells from the *Dnmt3a* ^{R878H}x *Npm1* ^{cA}mice (and to a lesser extent, from *Npm1* ^{cA} mice),but not *Dnmt3a* ^{R878H} mice or WT mice (**Figure 1A**), suggesting that the mutations in *Npm1* and *Dnmt3a* synergize to account for this phenotype. Strikingly, overexpression of *GAB2* in *Dnmt3a* ^{R878H}x *Npm1* ^{cA}bone marrow led to development of AML in 5 of 5 engrafted mice in less than 5 months ($p<10^{-6}$ compared to untransduced marrow; **Figure 1B**). 0 of 3 engrafted mice with "Empty Vector" (GFP only) in *Dnmt3a* ^{R878H}x *Npm1* ^{cA}bone marrow and 0 of 3 engrafted mice with overexpression of *PAK1* in *Dnmt3a* ^{R878H}x *Npm1* ^{cA}bone marrow developed leukemia during this 5 month period. Taken together, these data suggest that AML-associated mutations in *DNMT3A* and *NPM1* allow for the selective expansion of cells overexpressing *GAB2* as an important step towards leukemic transformation. In human myeloid neoplasms, amplification of chromosome 11q (containing human *GAB2*) is a recurrent cytogenetic event, observed in 1.5% of cases (Papaemmanuil, *NEJM*, 2016), and expression of *GAB2* across AML samples containing *NPM1* mutations is increased ~50% compared to healthy CD34+ cells or promyelocytes. GAB2 is known to bind to GRB2 and SHP2/PTPN11 to facilitate signaling from receptor tyrosine kinases (including FLT3) to downstream pathways (including RAS/RAF/MEK/ERK, PI3K and AKT, in some contexts). These data nominate *GAB2* overexpression as a factor that may be relevant for the progression of founding clones with *DNMT3A* and *NPM1* mutations to overt AML. Additional studies to define its protein interactome in preleukemic cells, its mechanism of action, and how leukemia-causing mutations in *DNMT3A* and *NPM1* shape the fitness landscape to select for *GAB2* overexpression are underway.

Disclosures No relevant conflicts of interest to declare.

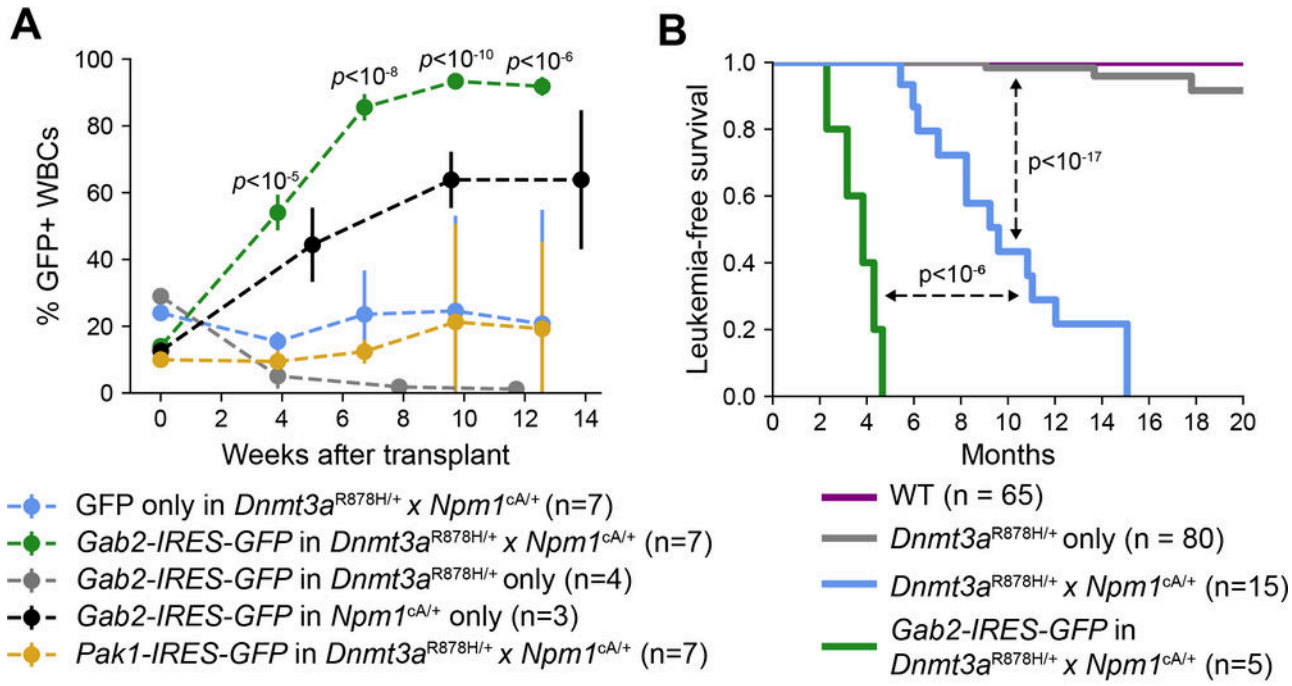

Figure. A) Overexpression of *Gab2* cDNA in *Dnmt3a*$^{R878H/+}$ x *Npm1*$^{cA/+}$ marrow causes highly significant expansion of the expressing cells in vivo over 12 weeks, compared to an Empty Vector control ("GFP only", light blue). *Pak1* overexpression (yellow) does not cause expansion. Overexpression of *Gab2* in *Dnmt3a*$^{R878H/+}$ marrow cells (grey) or WT cells (not shown) did not cause expansion, while overexpression of *Gab2* in *Npm1*$^{cA/+}$ marrow (black) causes partial expansion. Retroviral vectors were used to overexpress the indicated cDNA in lineage-depleted, CD45.2+ hematopoietic stem and progenitor cells that were then transplanted into CD45.1+ recipient mice. GFP positivity of CD45.2+ peripheral white blood cells (WBCs) was measured over time via flow cytometry. **B)** Mice of the indicated genotypes were monitored for the development of leukemia. Retroviral overexpression of *Gab2* in the *Dnmt3a*$^{R878H/+}$ x *Npm1*$^{cA/+}$ background significantly accelerates the development of AML, compared to mice with the *Dnmt3a*$^{R878H/+}$ x *Npm1*$^{cA/+}$ genotype alone.

Figure 1

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