A murine model for human early/immature T-cell precursor acute lymphoblastic leukemia (EITP ALL)

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ABSTRACT

Early/immature T cell precursor acute lymphoblastic leukemia (EITP ALL) represents a subset of human leukemias distinct from other T-ALL, and associated with poor prognosis. Clinical studies have identified chromosomal translocations involving the NUP98 gene and point mutations of IDH genes as recurrent mutations in patients with EITP-ALL. In a recent study using genetically engineered mice, we demonstrated that cooperation of an Idh2R140Q mutation with a NUP98-HOXD13 (NHD13) fusion gene resulted in EITP-ALL. Highlights of this double transgenic mouse model included the similarity of the immunophenotypic, mutational and gene expression landscape with human EITP-ALL. Additional studies showed that the Idh2R140Q/NHD13 EITP-ALL are sensitive to selective mutant IDH2 inhibitors in vitro, leading to the possibility that these mice can serve as a useful model for the study of EITP ALL development and therapy.

Early T-cell precursor (ETP) leukemia and early immature T-ALLs represent a group of human leukemia that have an immunophenotype and gene expression profile that is distinct from other T-ALLs, and has historically been associated with poor prognosis [1, 2]. Because ETP leukemias and immature T-ALLs have similar immunophenotype and gene expression profile we and others elected to group them as early/immature T cell precursor (EITP) ALL [2, 3]. The characteristic immunophenotype is negative for markers of mature thymocytes (CD4 and CD8) but positive for markers of myeloid or hematopoietic stem cells.

Highly specific gain of function mutations in isocitrate dehydrogenase 1 or 2 (IDH1/2) were identified in patients with acute myeloid leukemia (AML) over a decade ago [4, 5], and have more recently been associated with T-cell leukemias [2, 3, 6, 7], especially EITP ALL [2, 3]. The characteristic immunophenotype is negative for markers of mature thymocytes (CD4 and CD8) but positive for markers of myeloid or hematopoietic stem cells.

Coincidentally, whole exome sequencing of AML which developed in mice that expressed a NUP98-HOXD13 (NHD13) transgene revealed that 21% of these samples had acquired an Idh1p.R132H mutation, suggesting that an NHD13 transgene might collaborate with an Idh1/2 mutation to generate AML [11]. Since IDH2p.R140Q is the most common IDH mutation seen in patients with AML [4], we predicted that the combination of these two mutations would lead to AML, and generated Idh2R140Q transgenic mice that were crossed with NHD13 transgenic mice to test this hypothesis. As anticipated, we found that the onset of leukemia was significantly accelerated in Idh2R140Q/NHD13 EITP-ALL mice compared to single transgenic NHD13 or Idh2R140Q mice [3]. Surprisingly, the vast majority of leukemias that developed in Idh2R140Q/NHD13 double transgenic mice were not AML, but rather an immature T-cell leukemia that displayed an immunophenotype which was consistent with either DN1 thymocytes (CD4−CD8−CD44+CD25−CD90+Kit+), DN2 thymocytes, (CD4−CD8−CD44+CD25−CD90−Kit+), or an immunophenotype intermediate between DN1 and DN2. Additionally, lack of surface CD3 (but presence of cytoplasmic CD3), and the presence of clonal Tcrb DJ (but not complete VDJ) gene rearrangements suggested that...
the murine \textit{Idh2}^{R140Q}/NHD13 DN1/DN2 leukemias were similar to human EITP-ALL.

Early immature thymic progenitors that retain both lymphoid and myeloid lineage potential have been identified as the target cell population for EITP-ALL transformation \cite{1, 12}. Examination of young, clinically healthy (i.e, no evidence of leukemia) \textit{Idh2}^{R140Q}/NHD13 double transgenic mice showed a severe block in thymocyte maturation, and an expansion of DN thymocytes with oligoclonal Tcrb DJ rearrangement. Although NHD13 transgene alone impairs thymocyte differentiation \cite{3, 13}, this is potentiated by the addition of the \textit{Idh2}^{R140Q} transgene, resulting in a severe differentiation block at the DN2 to DN3 transition. The differentiation

**Figure 1:** \textit{Idh2}^{R140Q}/NHD13 double transgenic mice develop EITP ALL resembling the human disease. Top, Generation of \textit{Idh2}^{R140Q}/NHD13 mice. Middle, Leukemic cells display blast morphology, DN1 immunophenotype, and clonal Tcrb DJ rearrangement. GL, germline (non-rearranged) Tcrb. Bottom, Acquired mutations identified by whole exome sequencing (WES) in \textit{Idh2}^{R140Q}/NHD13 leukemia compared to those in NHD13-only leukemia. Mutations in orange are common in human EITP. RNA-seq from murine EITP ALL (\textit{Idh2}^{R140Q}/NHD13) compared to murine non-EITP ALL. GSEA shows similarity between genes enriched in human and mouse EITP.
block characterized at an immunophenotype level was also evident at a transcriptional level as Idh2R140Q/NHD13 DN1/DN2 leukemias were enriched for genes expressed in DN1 thymocytes as compared to DN3 thymocytes. Taken together, these findings support the hypothesis that Idh2R140Q/NHD13 leukemias originated from early T cell precursors in the thymus, similar to human EITP-ALL [1].

The genomic landscape of human EITP-ALL has been recently characterized [8, 12], and mutations that are more prevalent in EITP-ALL as compared to non-EITP T-ALL have been identified. Using whole exome sequencing we were able to show that acquired mutations in human EITP-ALL (such as KRAS, NRAS, PTPN11, JAK3, SH2B3, SETD2, and EZH2) were enriched in Idh2R140Q/NHD13 DN1/DN2 T-ALL, and NOTCH1 mutations, which are less common in EITP T-ALL were also less common in the Idh2R140Q/NHD13 DN1/DN2 T-ALL. Finally, gene set enrichment analysis (GSEA) showed that the gene expression profile of Idh2R140Q/NHD13 DN1/DN2 T-ALL was similar to that of human EITP-ALL, further reinforcing the potential of this mouse model in elucidating transformation pathways relevant for understanding human EITP-ALL (Figure 1).

Enasidenib (AG-221) is a potent selective inhibitor of the mutant IDH2 enzyme which has recently been approved for treatment of relapsed or refractory AML patients with IDH2 mutations [14]. Using an OP9-DL1 co-culture system we established an immortalized Idh2R140Q/NHD13 DN cell line and found that treatment of these immortalized cells with AG-221 led to marked decrease in cell proliferation suggesting targeting Idh2 mutations may be an effective treatment for EITP-ALL as well as AML.

The study by Goldberg and colleagues [3] demonstrated that collaboration of an IDH2 mutation with a NUP98-HOXD13 translocation leads to a highly penetrant EITP-ALL by targeting early thymic progenitor cells. In the context of human disease, it is important to note that both NUP98 translocations and IDH1/2 mutation have been reported as recurrent events in EITP-ALL [6–8, 15, 16]. Additionally, the report [3] demonstrated that the Idh2R140Q/NHD13 DN1/DN2 T-ALL recapitulates human EITP-ALL in terms of immunophenotype, Tcrb gene rearrangements, gene expression profile, and landscape of acquired mutations. We predict that the Idh2R140Q/NHD13 mouse model will serve as an excellent tool to study EITP biology and identify therapies for patients with EITP leukemia.

Author contributions

Conceptualization: Vijay Negi and Peter D. Aplan. Writing original draft: Vijay Negi and Peter D. Aplan. Writing review and editing: Vijay Negi and Peter D. Aplan. Supervision: Peter D. Aplan.

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CONFLICTS OF INTEREST

Peter D. Aplan receives royalties from the NIH Technology Transfer Office for the invention of NUP98-HOXD13 mice.
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