OPTIMIZING ESTABLISHMENT OF HIGH NEED PEDIATRIC ACUTE MYELOID LEUKEMIA PATIENT DERIVED XENOGRAFT MODELS

CHILDREN'S ONCOLOGY GROUP

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BACKGROUND

- Pediatric acute myeloid leukemia (pAML) is an aggressive hematologic malignancy with an overall survival rate that has plateaued around 60%.[1]
- pAML is associated with high rates of relapse, chemotherapy resistance, and therapy-associated death; thus, novel therapeutics with favorable side effect profiles are urgently needed.[1]
- pAML patient-derived xenograft (PDX) models are vital for pre-clinical evaluation of new therapeutic agents; however, the lack of pAML PDX models poses a notable barrier to pre-clinical testing in pAML drug development.[2] Additionally, existing pAML PDX models represent only a few common molecular subtypes, leaving many high-risk variants unrepresented.
- In collaboration with the Children's Oncology Group (COG), we have generated the largest known collection of serially passaging pAML PDX models in the US (currently 43 pAML models, https://pdxportal.research.bcm.edu).[2])
- The addition of immuno-deficient mouse (IDM) strains, including NSGS, NRGS, NBSGW, and MISTRG/MISTRG6, may address low myeloid engraftment rates and broaden the diversity of available models.[2]
- An additional method that has been tested with hopes of increasing human AML cell engraftment in IDM mice is pre-conditioning the mice with irradiation or busulfan.[3]

MOUSE STRAINS

Abbreviation	Full Name	Background Strain	Cause of Immunodeficiency
NSGS	• NOD-scid IL2Rgnull-IL3/CSF2/KITLG	• NOD scid gamma (NSG)	• Severe combined immunodeficiency mutation (Prkdc^{scid}) • Complete knockout of IL2RG gene
NRGS	• NOD-Rag1$^{-/-}$ IL2Rgnull-IL3/CSF2/KITLG	• NOD rag gamma (NRG)	• Targeted knockout mutation in RAG1 • Targeted knockout mutation in IL2RG gene
NBSGW	• NOD-scid IL2Rgnull-KIT^{W-41J}	• Kit^{W-41J} mice were intercrossed with NSG mice • Kit^{W-41J} is a missense mutation that results in impaired hematopoiesis	• Severe combined immunodeficiency mutation (Prkdc^{scid}) • Targeted knockout mutation in IL2RG gene
MISTRG	• M-CSF$^{h/h}$ IL-3/GM-CSF$^{h/h}$ SIRPa$^{h/h}$ TPO$^{h/h}$ RAG2$^{-/-}$ IL2Rg$^{-/-}$	• MITRG mice – RAG2$^{-/-}$ IL2Rg$^{-/-}$M-CSF$^{h/h}$ IL-3/GM-CSF$^{h/h}$ TPO$^{h/h}$ • MITRG does not have humanized SIRPa	• Targeted knockout mutation in RAG2 • Targeted knockout mutation in IL2RG gene
MISTRG6	• M-CSF$^{h/h}$ IL-3/GM-CSF$^{h/h}$ SIRPa$^{h/h}$ TPO$^{h/h}$ RAG2$^{-/-}$ IL2Rg$^{-/-}$IL-6$^{h/h}$	• MISTRG mice	• Targeted knockout mutation in RAG2 • Targeted knockout mutation in IL2RG gene

AIM

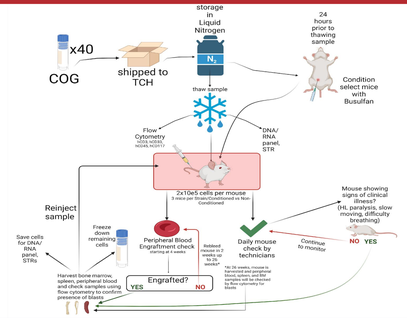
- To evaluate different IDM strains of mice and pre-conditioning with busulfan to optimize engraftment of pAML samples.
- We aim to characterize engraftment rates across various strains and experimental conditions as well as establish additional serially passaging high-need pAML PDX models.

HIGH NEED GENOMIC SUBTYPES

Primary Fusion	Cytogenetics	Key Characteristics	Frequency Among pAML Patients	# of Samples
CBFA2T3::GLIS2	inv(16)(p13.3;q24.3)	Infants, AMKL, Fibrotic Bone Marrow, Low PB disease burden	1.9% (n=41)	3
DEK::NUP214	t(6;9)(p22.3;q34.1)	Often seen with FLT3-ITD	1.8% (n=39)	5
ETV6 Fusions (CCND3, LMBR1, MNNX1, NIPBL)	t(7;12) 12p13.2 rearrangement	Only seen in children <2 years old	0.7% (n=15)	5
FUS::ERG	t(16;21)(p11.2;q22.2)	Median age 8.5 years old	0.6% (n=12)	5
KAT6A::CREBBP	t(8;16)(p11.2;p13.3)	Poor prognosis if >90 days at dx, High spontaneous remissions ff <90 days at dx	0.6% (n=12)	2
MECOM	t(3;21)(26.2;q22) for RUNX1::MECOM	While fusion is rare, overexpression is more common and associated with poor prognosis	0.3% (n=7)	3
NUP98::KDM5A	t(11;12)(p15;p13)	Often seen in children <4 years old	1.4% (n=31)	3
NUP98::NSD1	t(5;11)(q35;p15.5)	Highly associated with FLT3-ITD, evenly distributed by age	4.9% (n=106)	3
NUP98::HOXA9	t(7;11)(p15;p15)	Very rare	0.2% (n=4)	3
RBM15::MKL1	t(1;22)(p13;q13)	Infants and M7	0.8% (n=17)	3
RUNX1::CBFA2T2 or RUNX1::CBFA2T3	ins(21;20)(q22;q11q11) t(6;21)(q24;q22)	For RUNX1::CBFA2T3, 76% M1/M2 FAB classification	0.5% (n=10)	5

COG provided 40 patient samples from 11 rare "high-need" genomic subtypes of pAML. "High-need" based on possible availability of targeted agents, lack of available PDX models, and/or dismal current event free survival. Frequency data per 2173 patients from AAML1031 (n=1173), AAML0531 (n=827), AAML03P1 (n=104), or CCG-2961 (n=69) with a primary fusion classification.

PDX WORKFLOW



PDX MODEL ESTABLISHMENT

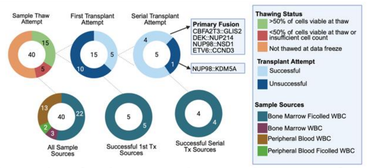


Figure 1. Schematic of primary and secondary engraftment rates for all samples trialed for the high need PDX model establishment. To date, 20 of 40 patient samples have been thawed with 15 samples suitable for use (>50% cells viable at thaw). Five achieved primary engraftment and four were successfully serially transplanted.

SERIALLY TRANSPLANTED SAMPLES SURVIVAL CURVES

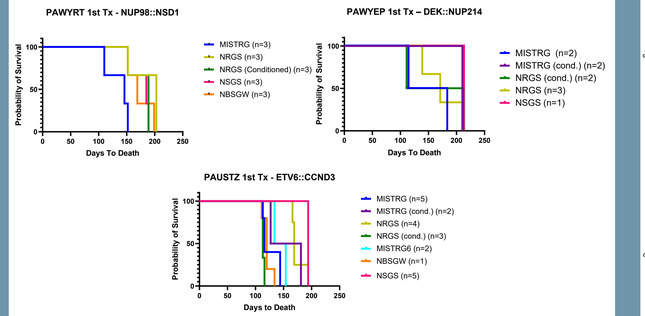


Figure 2. Survival of serially transplanted samples indicates possible strain-specific effects. PAWYRT (NUP98::NSD1), PAWYEP (DEK::NUP214) and PAUSTZ (ETV6::CCND3) 1st transplant (tx) were injected into mice of various strains; survival rates varied by IDM strain. Strain-specific effect on survival could not be assessed with certainty for PAVPKA (CBFA2T3::GLIS2) and PAVWPW (NUP98::KDM5A) due to limited numbers of mice injected and resultant harvesting of all mice on similar days to avoid loss of sample.

SERIALLY TRANSPLANTED SAMPLES DISEASE BURDEN

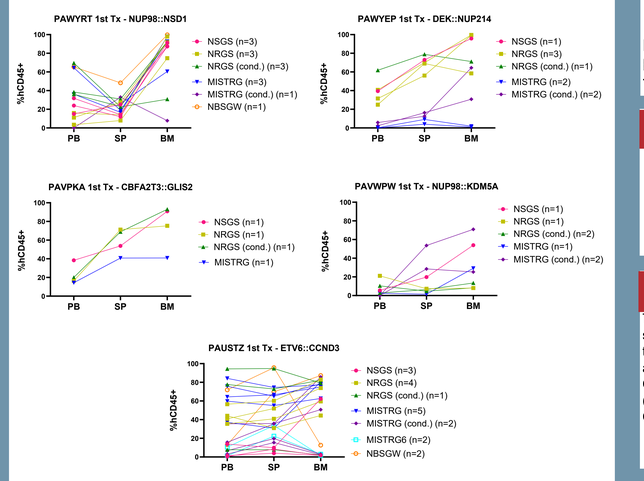


Figure 3. Disease burden varies by both model and IDM strain. Across all strains and high-need models, peripheral blood (**PB**) often demonstrated the lowest level of disease burden. Bone marrow (BM) typically had the highest disease burden, though with some strains/samples (e.g. DEK::NUP214, CBFA2T3::GLIS2 and ETV6::CCND3) spleen (SP) approached or exceeded BM levels. Only 1st tx data is shown, but serial tx data is available.

SAMPLE EXPANSION

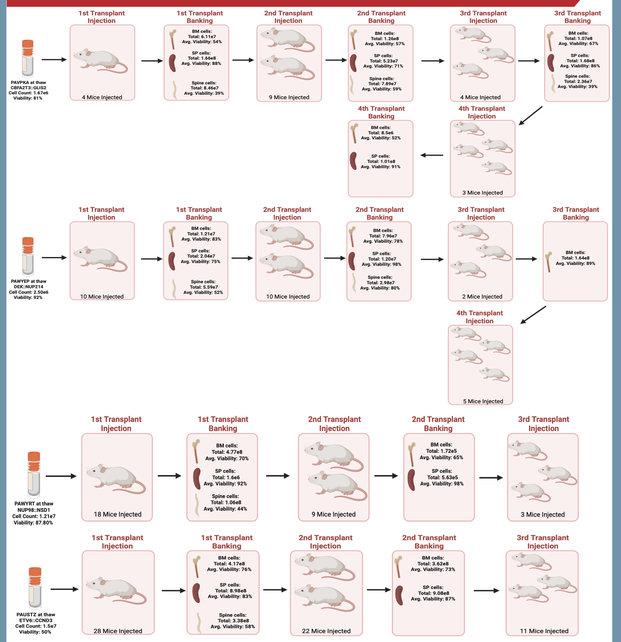


Figure 4. High-need sample expansion. Four high-need pAML samples have been successfully serially transplanted (PAVPKA, PAWYEP, PAWYRT & PAUSTZ). The figure illustrates the extent to which each sample has expanded and banked.

FUTURE DIRECTIONS

- Firefly luciferase transduction of models to evaluate disease burden using non-invasive imaging.
- Distribution of models to the larger research community for collaborative scientific advancement.
- Utilization of PDX models to further knowledge of pAML and test novel agents

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Bald Is Beautiful

Blood Cancer United

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