Development of an HLA-matched humanized immune system mouse supporting primary AML PDX engraftment for drug efficacy studies

Mara Gilardi[1], Paolo Schiavini[1], Laura Griffin[2], Brandon Walling[1], Veronica Zanon[1], Nektaria Papadopoulou[1], Paul Heverly[1], Stefano Cairo[1], Philip Dube[2], Monika Buczek[2], Marianna Zipeto[1], Michael Ritchie[1]

[1] Champions Oncology, Inc., [2] Taconic Biosciences, Inc.

ABSTRACT

Acute Myeloid Leukemia (AML) is a devastating hematological malignancy with a global incidence of approximately 120,000 new cases annually and a five-year survival rate below 30%. Despite advances in therapeutic strategies, treatment outcomes remain poor. The complexity of AML, characterized by genetic heterogeneity and clonal evolution, presents significant challenges for the development and testing of novel therapeutics in clinically relevant settings.

AML research relies heavily on cell lines and syngeneic models, or immunodeficient models without an added human immune system component. While these systems provide valuable insights, they fail to fully recapitulate the genetic and phenotypic diversity of primary human AML tumors. Traditional models often lack the tumor microenvironment and immune interactions critical for therapeutic evaluation. Furthermore, existing models generally rely on extensively passaged samples, which lose the heterogeneity and clonal diversity intrinsic to AML, undermining their clinical relevance.

To address these limitations, we developed an in vivo AML platform using human CD34+ engrafted NOG-EXL mice. NOG-EXL was selected as the preferred in vivo model as it has been successfully engrafted with primary AML PDXs, albeit not also with a humanized immune system component. This novel platform was built on a bank of over 50 patient-derived AML models that have been passaged, preserving their native tumor characteristics. Humanized NOG-EXL mice, transgenic for human IL-3 and GM-CSF, were used to promote primary AML engraftment as well as CD34+ stem cell differentiation into a wider repertoire of human myeloid and lymphoid lineages. The models are fully characterized with RNA sequencing, proteomics, diagnostic data, and detailed clinical histories. By retaining the complexity and clonal architecture of primary AML tumors, these models provide a truly translational system for preclinical drug testing.

AML donors were evaluated in humanized NOG-EXL. CD34+ engraftment was confirmed in humanized NOG-EXL mice followed by engraftment with AML primary cells. Human immune cell engraftment and AML cancer progression were assessed at different timepoints through flow cytometry on peripheral blood and bone marrow. A custom flow cytometry panel was used to identify the human immune cells present as well as the main AML sub-populations including the AML progenitors, AML blasts and monoblasts.

This novel approach bridges a critical gap in AML research by offering a robust, clinically relevant platform that reflects real-world disease biology by demonstrating an in vivo model with primary never passaged AML PDX in the context of a human immune system. The. Champions AML humanized model sets a new standard for preclinical evaluation, promising to accelerate the development of personalized therapies and improve outcomes for AML patients worldwide.

MATERIALS & METHODS

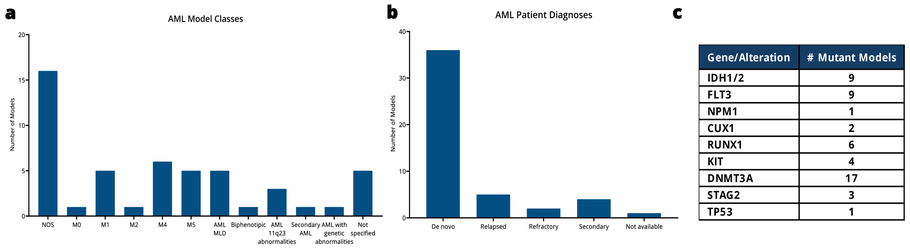
Primary AML samples: Sourced from Champions Oncology's AML tumor bank, featuring over 50 fully characterized patient-derived models maintaining in a never-passaged state to preserve heterogeneity and clonal evolution these models truly represent the patients in the clinic. Target expression, mutational profile, proteomics, and clinical histories have been used for model selection.

Humanized NOG-EXL Mice: NOG mice transgenic for human IL-3 and GM-CSF (NOG-EXL) were used as these support not only high levels of CD34+ engraftment with resulting myeloid and lymphoid differentiation but also a high success rate in primary AML engraftment and growth. NOG-EXL mice engrafted with human CD34+ hematopoietic stem cells (HSCs) were provided by Taconic Biosciences. HSC donors were selected based on HLA matching to specific AML tumors and HSC engraftment was confirmed by flow cytometry by Taconic Biosciences.

AML Progression & Immune Profiling: Custom flow cytometry panel used to track AML blasts, monoblasts, and AML progenitors and human immune cell subpopulations have been developed ensuring proper engraftment in peripheral blood and bone marrow at different time points. CD34+ Donors and AML Donors were specifically mismatched on one HLA criteria in order to differentiate healthy donor and diseased AML myeloid cells by flow cytometry.

RESULTS

AML primary sample collection and multi-omic characterization:



c

Gene/Alteration	# Mutant Models
IDH1/2	9
FLT3	9
NPM1	1
CUX1	2
RUNX1	6
KIT	4
DNMT3A	17
STAG2	3
TP53	1

Fig 1: A snapshot of our AML bank: a living and diverse bank of primary AML patient-derived models established from tumor biopsies from patients pretreated with the latest generation therapies, including models pretreated with advanced therapies like Gilteritinib, Mylotarg, and CSF1R inhibitors, as well as those with del(7q). a) Shows the AML model classes. Our AML bank is comprised of patient-derived specimens across a range of subtypes which includes M1, M2, M4, M5, NOS and others. b) Shows the patient diagnosis. Every model in the AML bank is fully characterized with clinical data, NGS analysis, proteomics, phospho-proteomics, and kinase activity, clinical annotations coupled with molecular datasets and in vivo responses. c) Shows genomic alteration data. The bank includes models with common mutations in FLT3 (ITD), IDH1/IDH2 and NPM.

Model information: AML primary CTG-2239 model:

a

- *FAB classification/Subtype: NOS*
- *Tumor type: acute myeloid leukemia (AML)*
- *Diagnosis: de Novo*
- *Treatment history: naïve*
- *RNAseq WES Proteomics: available*
- *Patient history: available*
- *In vivo and ex-vivo data: available*

b

Agent	Response
Venetoclax	Responder
Tipifarnib	Partial Responder
Gilteritinib	Resistant
5-Aza+Veneto	Responder

c **HLA information**

HLA A	25:01, 34:02
HLA B	35:01, 39:01
HLA C	06:02, 12:03

Fig.2: a) The list reports specific model information. b) In vivo efficacy data performed with CTG-2239. c) HLA information (sequencing data).

Schematic representation of the experimental workflow and flow cytometry gating strategy:

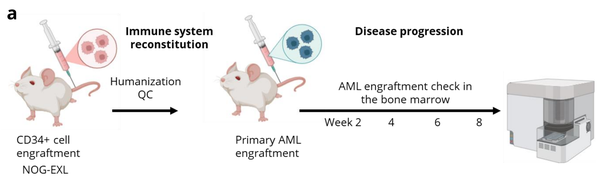


Fig.3: a) Experimental Workflow. b) Gating strategy assessing immune and AML cells engraftment.

Kinetics of engraftment of human primary AML cells in humanized NOG-EXL:

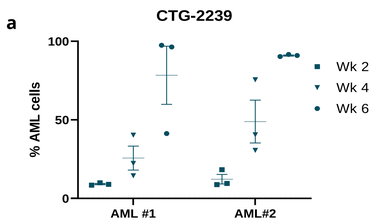


Fig.4: a) AML disease progression over time in the humanized AML models in NOG-EXL. 2 different CD34+ donors were used to perform the humanization (#1 and #2). The model was characterized by analyzing the bone marrow by flow cytometry at different timepoints. In the graph we report the % of AML cells (each data point represents one mouse).

RESULTS

The engraftment of a fully functional immune system to test different therapeutic strategies:

T cell numbers in AML are often lower than in healthy individuals, and their functionality is impaired due to immune suppression by leukemic blasts, myeloid cells and alterations in the bone marrow microenvironment. By leveraging NOG-EXL ability to support the reconstitution of a complete immune system, this model replicates the immunosuppressive microenvironment of diseased bone marrow. The model displayed physiologically relevant levels of T cells and myeloid cells, including M2 immunosuppressive macrophages.

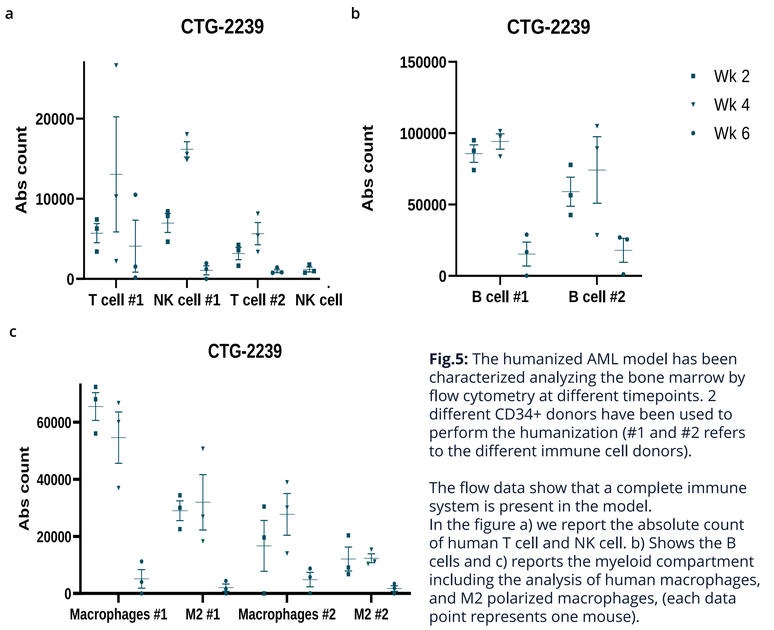


Fig.5: The humanized AML model has been characterized analyzing the bone marrow by flow cytometry at different timepoints. 2 different CD34+ donors have been used to perform the humanization (#1 and #2 refers to the different immune cell donors).

The flow data show that a complete immune system is present in the model.
In the figure a) we report the absolute count of human T cell and NK cell. b) Shows the B cells and c) reports the myeloid compartment including the analysis of human macrophages, and M2 polarized macrophages, (each data point represents one mouse).

SUMMARY

For the first time, we developed a humanized in vivo AML model using NOG-EXL mice engrafted with primary AML patient-derived xenografts (PDXs) and human CD34+ cells.
This innovative mouse model allows for the study of AML progression, immune interactions, and therapeutic responses by flow cytometry, in clinically relevant models.
This breakthrough model bridges the gap between preclinical and clinical research, setting a new standard for testing immune-based therapies, such as checkpoint inhibitors, CAR-T cells, and novel combination treatments, in a difficult-to-model disease.

A novel humanized immunocompetent AML model: Combines primary never-passaged AML cells with a functional human immune system in NOG-EXL mice including T cells, B cells, macrophages, M2 macrophages and NK cells.

Clinically relevant AML diversity: AML primary never passaged models maintains genetic and clonal heterogeneity, preserving real-world tumor biology offering clinical translatable data.

Improved immunotherapy evaluation: The immune and AML cells engraftment mimics the numbers present in the bone marrow of patients in the clinic. This model enables, for the first time, robust testing of AML immunotherapies and combination treatments in clinically and biologically relevant settings.

REFERENCES

1) Pharmaco-Pheno-Multiomic Integration Reveals Biomarker Profiles and Therapeutic Response Prediction Models in Leukemia and Ovarian Cancer. Gilad Silberberg, Brandon Walling, Amy Wesa, Alessandra Audia, Ido Sloma, Yi Zeng, Guanghui Han, Jia Tang, Paige Pammer, A'ishah Bakayoko, Xuan Ren, Daniel Ciznadija, Bandana Vishwakarma, Yaron Mosesson, Marianna Zipeto, Michael Ritchie. bioRxiv 2022.06.14.495846; doi: https://doi.org/10.1101/2022.06.14.495846

2) https://www.taconic.com/products/mouse-rat/nog-portfolio/humanized-immune-system-mice/hunog-exl scan for more info:

Scan to Download

2025 All Rights Reserved