Abstract: S129

Title: PREEMPTIVE TARGETING OF PRELEUKEMIC CELLS IN DOWN SYNDROME WITH PATHWAY-DIRECTED THERAPIES: A NOVEL APPROACH TO PREVENT MYELOID LEUKEMIA

Abstract Type: Oral Presentation

Session Title: Acute myeloid leukemia - Biology & translational research 2

Background:

The challenge of preventing myeloid malignancies at a preleukemic stage remains significant, primarily due to the low frequency of malignant transformations and a scarcity of effective models for developing preemptive therapies. In this context, myeloid leukemia in Down syndrome (ML-DS), evolving from transient abnormal myelopoiesis (TAM) in over 20% of cases, offers a unique model for research. TAM, occurring in up to 30% of newborns with Down syndrome, is marked by prenatal origins, trisomy 21, and mutations in GATA1. Hence, ML-DS and the precedent TAM present genetically simple models for studying leukemia progression.

Aims:

Our research aims to establish preemptive treatments for myeloid malignancies, focusing on eradicating preleukemic cells in children with Down syndrome to prevent the progression to overt leukemia, which would obviate the need for intensive chemotherapy, thereby sparing patients from its significant side effects.

Methods:

We employed CRISPR/Cas9-based dropout and differentiation screens on a recently developed murine fetal hematopoietic stem/progenitor cell model that simulates human TAM/ML-DS, incorporating GATA1s mutations and the overexpression of chromosome 21 oncogenes. Our approach utilized a custom sgRNA library targeting genes associated with FDA-approved drugs, integrating screening outcomes with gene expression data from TAM/ML-DS patients, essentiality scores, and pathway enrichment analyses. This was followed by evaluating candidate genes and their corresponding drugs in the murine TAM model, human cell lines, primary patient cells, and xenografts.

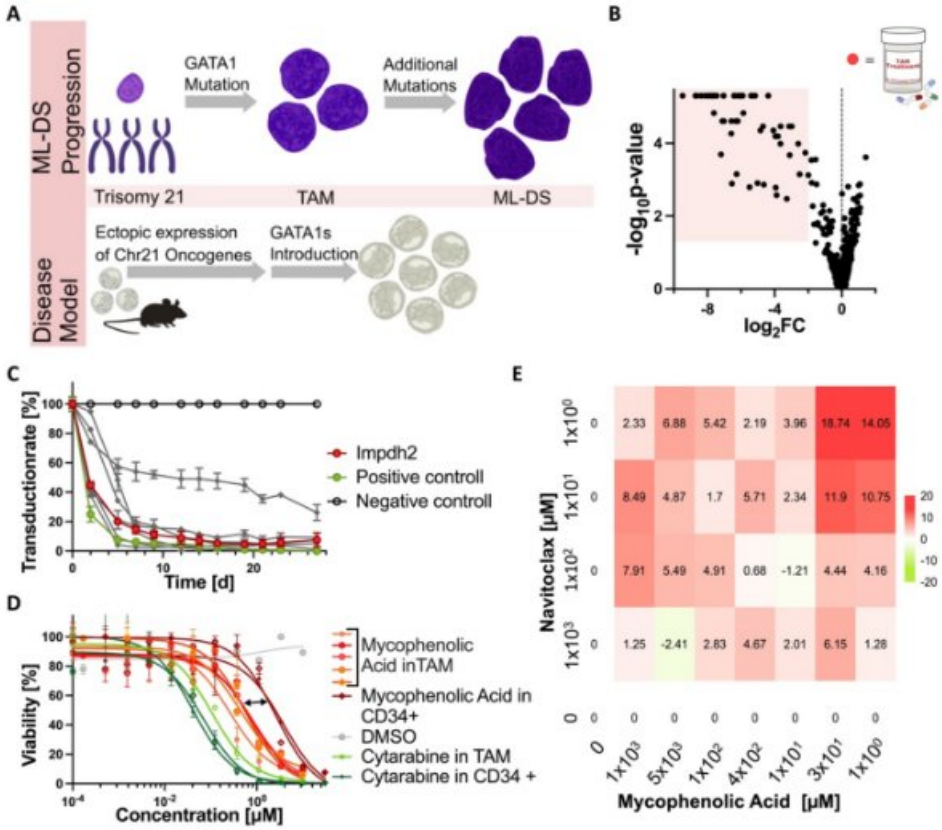
Results:

Moving beyond conventional drug screening methods, we adopted a novel strategy using CRISPR/Cas9-based dropout and differentiation screens, integrated with bioinformatics for pathway analysis. This approach was based on the premise that gene knockouts might mimic the therapeutic effects of drugs targeting the same proteins. Our objective was to identify drugs critical for the proliferation and halted differentiation in our recently developed murine TAM model. The investigation underscored a significant reliance on the purine biosynthesis pathway, a finding consistent in both our model and human trisomy 21 cell lines. Through a comprehensive analysis, IMPDH2 emerged as a key gene whose disruption led to increased megakaryocytic differentiation and apoptosis. This discovery guided us to Mycophenolic Acid (MPA) and its prodrug, mycophenolate mofetil (MMF), known for their selective action in preventing organ transplant rejection with minimal side effects in children. Remarkably, our data showed high specificity of these drugs in targeting the identified pathway, validated by the effective treatment of patient-derived TAM blasts, leading to cell cycle arrest and apoptosis. Prolonged exposure to lower doses further promoted megakaryocytic differentiation, aligning with predictions from IMPDH2 knockout. Additionally, our search for combinatory treatments revealed the BCL2/BCL-XL inhibitor navitoclax as a synergistic partner, expanding the repertoire of potential therapeutic strategies.

Summary/Conclusion:

Our comprehensive screening approach has underscored the reliance of TAM on the purine biosynthesis and

BCL2/BCL-XL pathways, proposing MMF and navitoclax as novel, effective treatments to eliminate preleukemia and prevent the transition to overt leukemia in patients. This study not only offers immediate therapeutic avenues for children with Down syndrome but also sets the stage for developing preemptive treatments for other malignancies.



(A) Disease Progression and TAM Model. **(B)** Example for CRISPR/Cas Screen outcome. **(C)** Pathway dependency validation. **(D)** Drug Validation in patient derived TAM Blasts. **(E)** Synergy Score between Mycophenolic Acid and Navitoclax in exemplary ML-DS PDX Cells.

Keywords: Myeloid leukemia, Down Syndrome