

Title: Synergistic Drug Combinations targeting Nelarabine Resistance in T-cell Acute Lymphoblastic Leukemia**Authors:**

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy and is classified into B-cell ALL and T-cell ALL (T-ALL), with T-ALL comprising approximately 15 % of cases. While survival rates for T-ALL have improved, outcomes for relapsed patients remain poor, with an overall survival at 35 %. Nelarabine, a nucleoside analogue targeting T-cells, remains the only specific agent for relapsed T-ALL. However, clinical response rates have been modest (~50 %), highlighting the urgent need to understand resistance mechanisms and develop more effective treatment combinations.

We investigated mechanisms of Nelarabine resistance in T-ALL. RNA-sequencing analysis of T-ALL patient samples (N=39) did not reveal significant differences in the expression of genes involved in Nelarabine transport or metabolism. Screening of 14 T-ALL cell lines revealed a wide range of sensitivity to Nelarabine. In two resistant lines we performed a combination screen with 527 drugs to identify synergistic partners. This screen highlighted three distinct drug classes with potential synergistic efficacy, namely DNA repair, cell growth, and proliferation, that are undergoing validation in targeted analyses. To further elucidate resistance mechanisms, we will conduct a genome-wide CRISPR-Cas9 knockout screen in resistant T-ALL lines, where depleted gRNAs under drug treatment will pinpoint genes, whose loss sensitizes cells to Nelarabine. Additionally, sensitive lines will be exposed to gradually increasing doses of Nelarabine to generate resistant derivatives, providing insights into pathways underlying acquired resistance.

This project seeks to increase the efficacy of Nelarabine and to complete preclinical studies required to advance promising drug combinations into early-phase clinical trials.