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# POSTER ABSTRACTS

## 602.MYELOID ONCOGENESIS: BASIC

### Pyrimidine Starvation Is a Targetable Cancer Vulnerability: Mechanisms of Nucleotide Homeostasis

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**Introduction:** Acute myeloid leukemia (AML) is a heterogeneous disease, though all AML exhibit a characteristic block in differentiation. Hence, much effort has been made to develop small molecules that promote leukemic cell differentiation in the treatment of AML. In 2016 we identified the enzyme dihydroorotate dehydrogenase (DHODH) as a therapeutic target in AML. DHODH is a mitochondrial enzyme that converts dihydroorotate to orotate in the 4th step of *de novo* pyrimidine synthesis. Without DHODH enzymatic activity, a cell is forced to rely on autophagy or extracellular salvage to maintain its intracellular pyrimidine pool. DHODH inhibitor therapy has shown broad preclinical efficacy across multiple cancer types including, glioma, neuroblastoma, breast, small cell lung and pancreatic cancer suggesting that malignant cells are specifically impaired in their ability to maintain nucleotide homeostasis during periods of limited availability. Despite these very encouraging pre-clinical results, the translation into human clinical trials has been met with limited anti-cancer activity and therapeutic success. This discordance between pre-clinical and clinical benefit likely speaks to our limited understanding of how normal and malignant cells respond to pyrimidine starvation.

**Methods:** Using in vitro and in vivo assays to compare normal hematopoietic progenitor and leukemic cells we interrogated key nodes in pyrimidine synthesis to identify the mechanisms behind this selective, anti-leukemic activity in the context of nucleotide starvation: [1] Nucleotide measurement by HPLC, [2] Flux experiments by isotopic tracing, [3] Polysome profiling to compare the transcriptome and translatome, [4] Quantification of ribosome recycling by examining the lysosomes following ultracentrifugation and [5] Direct visualization of lysosomes using new techniques in cryogenic electron microscopy.

**Results:** Treatment of leukemia cells with brequinar blocked *de novo* synthesis and led to the rapid depletion of intracellular pyrimidines. In comparison, normal hematopoietic CD34 positive progenitor cells had less depletion, suggesting a selective vulnerability of leukemic cells over normal cells. We used isotopically labeled glutamine (15N) and uridine (13C) to quantify the contribution of *de novo* synthesis and extracellular salvage under control and starvation conditions. The leukemia cell line THP1 showed a higher reliance on *de novo* synthesis than normal CD34 positive hematopoietic progenitors ( **Figure 1A, left graphs**). Furthermore, following DHODH inhibition, THP1 cells were less capable of extracellular salvage compared to normal cells ( **Figure 1A, right graphs**). Polysome profiling analysis in THP1 cells showed a dramatic decrease in translating ribosomes as well as dramatic decrease in ribosomal RNA. We identified a short-list of 22 genes whose translation is prioritized during nucleotide deprivation. Following DHODH inhibition and nucleotide starvation, cells upregulate their number of lysosomes, as quantified by flow cytometry. In addition, the contents of the lysosome include ribosomal proteins, whose abundance increased during nucleotide starvation, suggesting an ongoing recycling of ribosomes to meet the nucleotide demand of leukemic cells during starvation. Finally, for the first time we have been able to directly visualize (by cryogenic electron microscopy) ribosomes within lysosomes during periods of nucleotide starvation, a finding that was completely absent in leukemia cells cultured under normal conditions ( **Figure 1B**).

**Conclusion:** We hypothesized that the transformation from normal to malignant cell is accompanied by changes in nucleotide metabolism that render the transformed cells more reliant on *de novo* synthesis, establishing a metabolic vulnerability (and inherent therapeutic window) in the use of DHODH inhibitors. Our work confirms that leukemic blasts differ in their dependence on *de novo* nucleotide synthesis and its ability to garner nucleotides via salvage or autophagy pathways. This balance

appears to be disturbed in the setting of malignancy such that leukemic cells are empirically more sensitive to treatment targeting pyrimidine synthesis. Understanding the differences in how leukemic and normal cells handle their nucleotide pools at baseline, and under starvation conditions, will guide the clinical utility of this class of inhibitors.

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## Figure 1

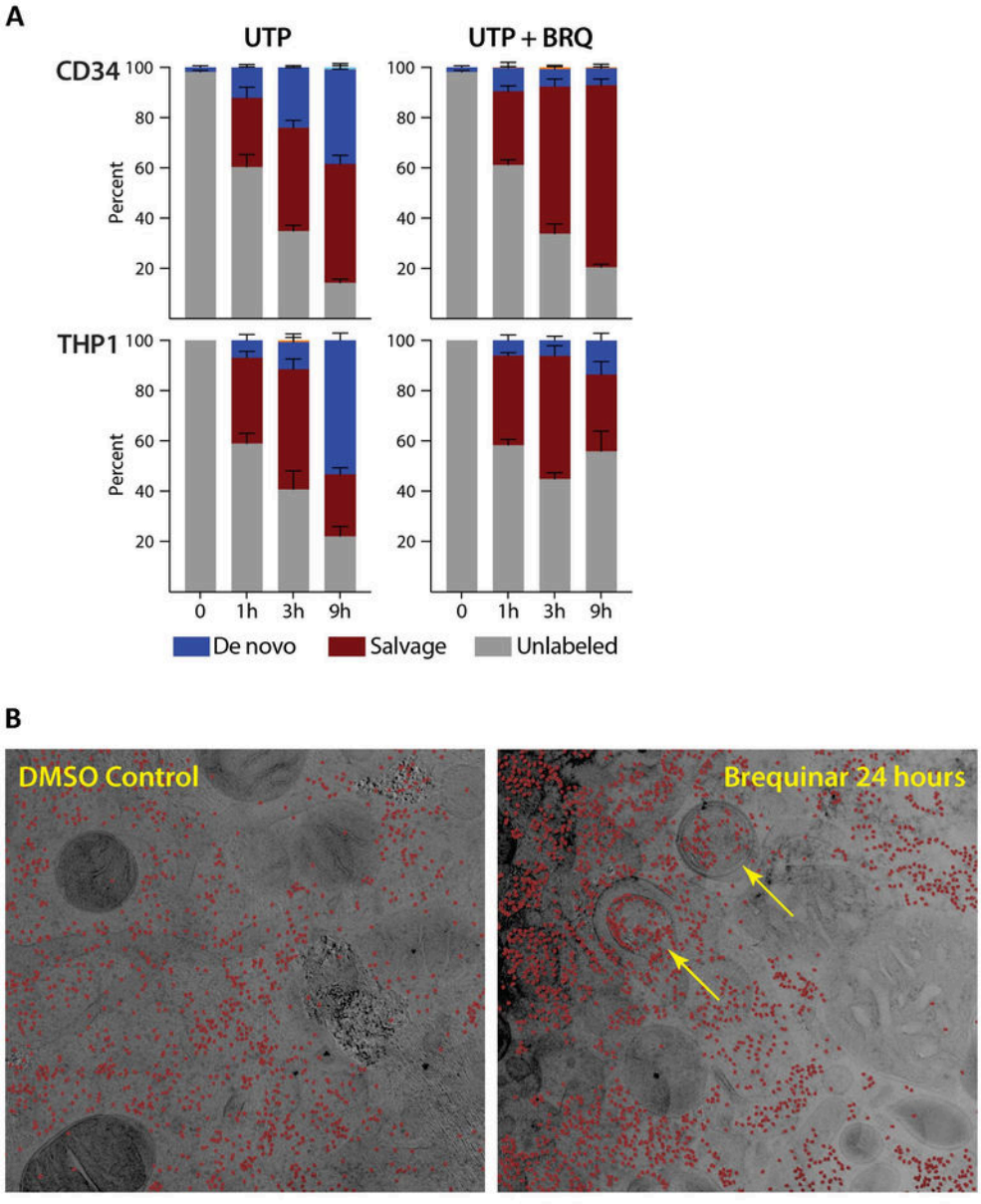


**Figure 1. Pyrimidine starvation is a targetable cancer vulnerability. A.** Cells maintain their intracellular nucleotide pools by *de novo* synthesis (blue) or extracellular salvage (red). Inhibition of DHODH by brequinar (BRQ) halts *de novo* synthesis and forces a cell to rely on extracellular salvage. THP1 cells are more reliant on *de novo* synthesis (bottom left) and less capable of salvage (bottom right) following DHODH inhibition by BRQ compared to normal CD34$^{+}$ cells (top right). **B.** Ribosome 60S subunit detection in THP1 cells using cryo-EM HRTM suggests that after BRQ treatment (1 uM, 48-hours) ribosomes can be found in membrane-enclosed compartments such as the lysosomes (yellow arrows). Representative images of THP1 slices from cells treated with DMSO (left) or BRQ (right) for 48h. Ribosomes detected by HRTM are shown in red. Scalebar represents 1 μm.

**Figure 1**

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