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# POSTER ABSTRACTS

## 605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

### Therapeutic Effects and Metabolic Rewiring upon Glutaminase Loss in T-ALL

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive lymphoid neoplasm that requires intensified chemotherapy for treatment. Despite significant improvements in clinical outcomes with these chemotherapy regimens, still 20-25% of pediatric cases relapse, and the prognosis of these refractory T-ALLs remains extremely poor, underscoring the urgent need to explore novel targets for more effective approaches. The identification of highly prevalent (~60% patients) activating mutations in NOTCH1 has brought considerable interest in developing targeted anti-NOTCH1 therapies, offering promising alternatives to chemotherapy. However, early efforts in the clinic with $\Upsilon$-secretase inhibitors (which block a proteolytical cleavage critical for the activation of NOTCH1) have been hampered by limited and heterogenous therapeutic responses. Therefore, further advances in treatment of T-ALL require a deeper understanding of the basic mechanisms driving this disease.

We have previously discovered a NOTCH1-controlled metabolic pathway that determines response to anti-NOTCH1 therapies. Specifically, glutaminolysis, which replenishes TCA cycle intermediates to maintain metabolism, is a critical pathway for leukemia cell growth downstream of NOTCH1. As such, inhibition of glutaminolysis pharmacologically or genetically (via deletion of glutaminase, *Gls*) is highly synergistic with anti-NOTCH1 therapies. However, Gls-deficient T-ALLs eventually progress, highlighting the need for improved therapeutic strategies. Moreover, the glutamine fate and the compensatory mechanisms to overcome Gls loss in leukemic cells *in vivo* are still poorly understood.

To investigate the underlying mechanisms mediating the resistance to Gls loss, we systematically traced the nutrient utilization in isogenic Gls-positive and Gls-negative T-ALLs by intravenously infusing $^{13}$C-lactate, $^{13}$C-glucose and $^{13}$C-glutamine in leukemic mice. Quantitative analysis indicates that Gls-positive T-ALL cells use primarily glutamine as a carbon source to feed the TCA cycle. However, TCA labeling from glutamine decreases in Gls-deficient T-ALL cells, while lactate contribution to label TCA intermediates significantly increases, suggesting a potential compensatory metabolic rewiring mechanism to overcome Gls loss uncoupling TCA from glycolysis. In addition, we observed a reduction of the levels of palmitate, palmitoleate and some polyunsaturated fatty acids, together with a significant downregulation of the expression of *Fasn*, a central regulator of fatty acid anabolism, in Gls-deficient T-ALL cells. These results suggest a switch to lipid catabolic processes to support leukemic cell growth in absence of Gls *in vivo*.

Gene expression profiling and proteomics analyses of these leukemias with isogenic loss of Gls revealed a strong enrichment in heme/porphyrin-biosynthesis signatures as heme-biosynthetic genes were systematically upregulated in Gls-deficient T-ALL cells as compared to T-ALL control cells. Indeed, using a loss-of function CRISPR-based genetic screening in combination with BPTES, a potent glutaminase inhibitor, in a human T-ALL cell line *in vitro*, we identified heme-biosynthesis pathway as a potential modulator, whose disruption could sensitize T-ALL cells to glutaminase inhibition.

Overall, our findings formally show that while glutaminase plays a key role in T-ALL progression, still cells rapidly rewire their metabolism to compensate for Gls loss demonstrating a marked metabolic flexibility. Moreover, our results have unveiled previously unknown metabolic vulnerabilities that could be exploited in combination with glutaminase inhibitors in the clinical setting with the potential to improve the efficacy of T-ALL therapies and lead to better outcomes for patients.

**Disclosures** No relevant conflicts of interest to declare.

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