Uroporphyrinogen Decarboxylase (*UROD*) is a Therapeutically Targetable Dependency in Acute Erythroid Leukemia

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Background

- Although great advances in profiling and understanding the genomic landscape of acute myeloid leukemia (AML) and acute erythroid leukemia (AEL), therapies have remained mostly unchanged.
- We developed mouse models of AEL harboring mutations in recurrently mutated genes in AEL through CRISPR-Cas9 genome editing of hematopoietic stem and progenitor cells (HSPCs) and transplant assays.
- Here, **to identify druggable dependencies in AEL,** we performed a genome wide CRISPR/Cas9 knockout screen using the CRISPR Knockout Pooled Library Brie in two mouse AEL cell lines and validated results in human AEL cell lines.

Brie Library CRISPR Knockout Screen

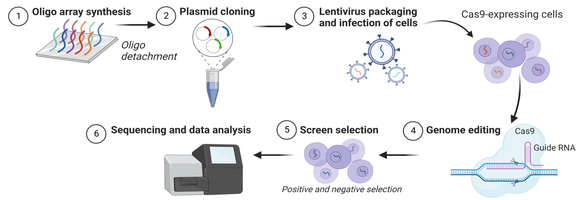


Figure 1: Screen Overview
Previously established mouse cell lines were harvested from moribund mice and cultured according to *Iacobucci et al. Blood 2021*. Virus was added to cells at a 17.5 MOI and cultured for 72 hours. Infected cells were selected using puromycin at a concentration of 10 ng/mL for 72 hours. Cells were cultured for 21 days.

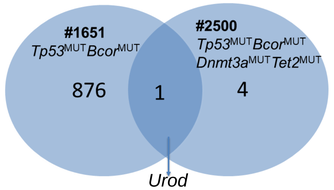


Figure 2: Screen Results Comparison
DNA from passages one and nine from each screen were extracted and sequenced for gRNA enrichment. Data were analyzed by the Model-based Analysis of Genome-wide CRISPR-Cas9 Knockout (MAGeCK) method: 877 and 5 significantly depleted genes were identified in #1651 and #2500 AEL cell lines, respectively. Only one gene was conserved between the two cell lines, Uroporphyrinogen decarboxylase (*Urod*).

UROD is an enzyme in the heme biosynthesis pathway that is responsible for catalyzing the conversion of uroporphyrinogen III to coproporphyrinogen.

Heme Pathway in AEL

UROD and other genes from the heme pathways have significantly higher expression and activity in AEL patients compared to other leukemia subtypes.

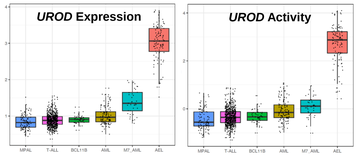


Figure 3: *UROD* is highly expressed and active in AEL
Patient gene expression and activity data was obtained and analyzed by NetBID.

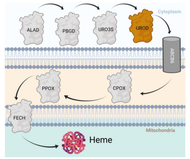


Figure 4: Heme Biosynthesis

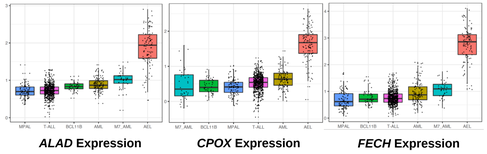


Figure 5: Heme Pathway Expression
Additional members of the heme biosynthesis pathway are overexpressed in AEL.

UROD Knockdown in Cell Lines

UROD Knockdown in Mouse AEL cell lines

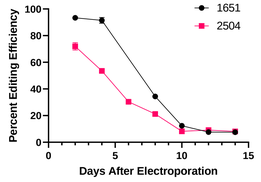


Figure 6: Editing efficiency of UROD Knockdown
Cells were electroporated with a sgRNA/Cas9 ribonucleoprotein complex with an sgRNA targeting *Urod* at a different locus to that used in the Brie screen. Editing efficiency was monitored over time using the Tracking of Indels by DEcomposition (TIDE) tool.
#1651: mutations in *Tp53, Bcor;* #2504: mutations in *Tp53, Bcor, Dnmt3a, Tet2*

Significant loss of proliferation and survival of *Urod*-deficient cells compared to *Urod* wild-type cells.

UROD Knockdown in Human AEL Cell Lines

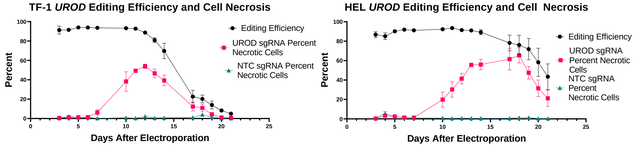


Figure 7: Genome editing and apoptosis assay in human AEL cells (TF-1 and HEL)

- Editing efficiency for *UROD* declined overtime: *UROD*-deficient cells are at a detriment compared to their wild-type counter parts.
- Necrotic cells increased in the *UROD*-knockdown group compared to the non-targeting control (NTC) group as assessed by apoptosis assay: *UROD* knockdown leads to necrotic cell death.
- There is a significant loss in editing efficiency after the peak of necrotic cells, showing that the *UROD*-knockout cells are at a remarkable growth disadvantage compared to the wild-type tumor cells.



Figure 8: Immunoblotting Validation
- Samples with high editing efficiency did not express UROD at detectable levels.
- At the later time points when the editing efficiency decreases, UROD expression recovers, supporting that UROD is a dependency in these cells and the only cells that survive in the assay are those that express UROD.
- We assessed heme production indirectly by immunoblotting for ALAS1, which is negatively regulated by cellular heme, thus we observed that *UROD* knockdown cells had ALAS1 at higher levels compared to NTC. The highest concentrations of ALAS1 was at day 14 of the TF-1 cells, which coincides with the highest number of necrotic cells.

Metabolic effects of UROD knockdown

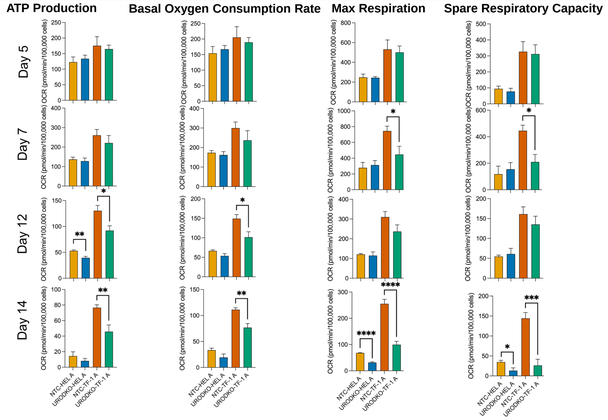


Figure 9: Metabolic Profiling upon UROD Knockdown in human AEL cells
To investigate the effect of UROD knockout on the metabolic function of the human AEL cell lines, we performed a Seahorse assay to analyze mitochondrial respiration and ATP production in a longitudinal fashion. UROD loss led to a significant decrease in all measured parameters in the TF-1 cells, most notably in maximal respiration and spare respiratory capacity.

In Vivo UROD Knockdown

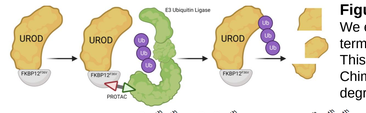


Figure 10: dTAG System Overview
We engineered an FKBP12^{F36V} fusion to the N-terminus of the UROD protein in AEL cell lines. This allows targeting by a Proteolysis Targeting Chimera (PROTAC) that favors protein degradation by an E3 ubiquitin ligase.

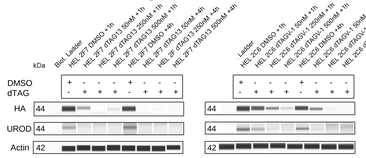


Figure 11: dTAG13 treatment
To optimize the degradation conditions upon dTAG13 treatment we performed a serial dilution (50nM, 250nM, and 500nM) and time course (1 or 4 hours) in two engineered clones.
- Complete degradation of UROD at 500nM after four hours in both clones and the same at 1 hour in the 2C6 clone.
- Significant degradation at all other time points and concentrations.

Figure 12: UROD dTAG13 treatment in long term culture
To further validate our dTAG system, we performed a longitudinal study to ensure complete suppression of UROD expression.
- We are able to completely remove UROD expression with dTAG treatment.
- We continue to see increased ALAS1 expression in the UROD-degraded samples, showing a decrease in cellular heme, further indicating that we have disrupted UROD functionality and the cell's ability to synthesize heme.

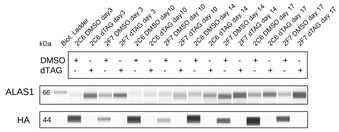
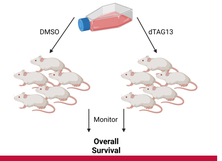


Figure 13: In Vivo UROD Targeting
We plan on performing an *in vivo* targeting of UROD as a therapeutic vulnerability using the dTAG system in mice. We will have a dTAG13 treated group and a DMSO treated group as a negative control. We will monitor the mice for symptoms of disease and compare outcomes between the two groups.



Conclusions

- We performed two genome-wide CRISPR-Cas9 knockout screens in genetically distinct mouse AEL cell lines.
- Uroporphyrinogen Decarboxylase (*Urod*) is a conserved dependency in mouse and human cell lines.
- Seahorse assays show a decrease in mitochondrial output and flow cytometry staining for Annexin V and PI show an increase in necrotic cell death following loss of UROD expression.
- *In vivo* studies are currently being conducted to further validate UROD as a targetable therapeutic vulnerability in AEL.

References: Iacobucci I, Wen J, Meggendorfer M, et al. Genomic subtyping and therapeutic targeting of acute erythroleukemia. Nat Genet. 2019;51(4):694-704; Iacobucci I, Qu C, Varotto E, et al. Modeling and targeting of erythroleukemia by hematopoietic genome editing. Blood. 2021;137(12):1628-1640.

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