Expression patterns in pediatric AML

**Research Objective:**
Unsupervised heirarchical clustering of patients based on expression (n = 283)

E-selectin is a cell adhesion glycoprotein that is expressed on endothelial cells and has been implicated in therapeutic resistance. E-selectin recognizes numerous glycan epitopes, expressed by human leukocytes, that contain α(1,3)- or α(1,4)-fucose and α(2,3)-sialic acid on a lacto/neolactosamine backbone.

- In most myeloid leukemias, leukemic blasts express ligands of E-selectin (ex. CD44, ESL-1).
- These ligands contain the epitope of the carbohydrate sidechain Lewis X (α3L), which is important for cell-to-cell recognition and adhesion, and mediates leukocyte rolling along the vascular endothelium.

- Cell surface expression of α3L:
  - Increases adhesion of leukemic blasts to vascular endothelial cells
  - Facilitates sequestration of blasts to the bone marrow vascular niche, where they are prevented from entering normal circulation
  - Prevents leukemic blasts from being readily accessed by chemotherapy compounds

**This process can, in turn, lead to cell adhesion-mediated drug resistance, and resultant poor clinical outcome**

- Uprosalen, an E-selectin antagonist drug, prevents leukemic cell homing to the vascular niche and was shown to be a potent adjunct to therapeutics. The combination of chemotherapy and uproleselan produced a reduction in tumor burden and improved survival rates in multiple myeloma (MM) and adult acute myeloid leukemia (AML), as compared to chemotherapy treatment alone.

- Previous studies established that leukemic cell surface levels of E-selectin ligands are correlated with response to uproleselan.

**Research Objective:** We questioned whether transcriptome profiling of E-selectin ligand-forming glycosylation genes can be used to identify elevated E-selectin ligand expression in patients with pediatric acute myeloid leukemia (AML), and subsequently which AML patients might be best treated to (and benefit from) treatment with uproleselan.

**Patient Cohort and Methods**

**Patient Population:** Ribo-depleted RNA-seq data was obtained from patients treated in COG AAML1031 (N = 1,074)
- Patients ranged from <1 to 29.6 years of age
- Median patient age was 9.94 years
- Samples were obtained at diagnosis from bone marrow

**Analysis:** All analyses were performed in R (v 3.5.2)
- Overall survival (OS) was used to correlate gene expression levels (in log2 TPM) with outcome via Cox proportional hazards regression models
- Transcriptome profiling of glycosylation genes identifies correlation with clinical outcome in AML

**Expression Patterns in Pediatric AML**

**Figure 1.** Expression of 24 genes from 5 functional groups of interest, each containing a selection of genes involved in the synthesis (glycosyltransferases) or degradation (glycosidases) of the ligands of E-selectin.

**Figure 2.** Unsupervised hierarchical clustering of patients based on expression of the E-selectin ligand glycosylation genes of interest. 7 of the genes were excluded from further analysis due to low expression levels. Expression of the remaining 17 genes varies by patient cytogenetics.

**Figure 3.** Univariate Cox regression models were used to identify genes associated with OS. A multivariate model of the remaining 15 glycosylation genes identified ST3GAL4 & FUT7 as independent prognostic indicators of OS.

**Figure 4.** Synthesis of α3L(E-selectin) ligands is a cell adhesion glycoprotein that is expressed on endothelial cells and has been implicated in therapeutic resistance. E-selectin recognizes numerous glycan epitopes, expressed by human leukocytes, that contain α(1,3)- or α(1,4)-fucose and α(2,3)-sialic acid on a lacto/neolactosamine backbone.

**Figure 5.** Patients highly expressing FUT7 (i.e. the highest quartile of expression) had significantly worse outcome than low expressors (i.e. lowest 3 quartiles of expression). Similarly, patients in the highest quartile of ST3GAL4 expression had a 5-year OS of 53.9% vs. 68.3% (p < 0.001) (B). Patients highly expressing FUT7 (i.e. the highest quartile of expression) had a 5-year OS of 53.9% vs. 68.3% (p < 0.001). Patients highly expressing FUT7 (i.e. the highest quartile of expression) had a 5-year OS of 53.9% vs. 68.3% (p < 0.001).

**Figure 6.** A subset of patients highly expressed both genes (ST3GAL4/FUT7high) (A). These individuals had particularly adverse survival (45.8% OS vs 71.0% OS, p < 0.001). Patients highly expressing either ST3GAL4 or FUT7 (but not both, aka ST3GAL4/FUT7mix) had a 5-year OS of 55.5%, illustrating what may be a compounding unfavorable impact conferred on survival (B).

**Future Studies:**
To validate ST3GAL4 & FUT7 as potential predictive biomarkers, we will evaluate the in vivo response of bone marrow mononuclear cells from primary patient samples to the combined treatment of E-selectin inhibitors (uproleselan) and chemotherapy in the presence of endothelial cells.

We anticipate patients with high levels of ST3GAL4 & FUT7 will exhibit sensitivity to E-selectin blockade compared to those with lower expression.