Almost 3,800 children each year develop leukemias, making them the most common pediatric cancer in the US. Seventy-five to eighty percent of these pediatric leukemias are of the acute lymphocytic variety (ALL), and they predominantly involve precursor B cells. Our laboratory is the sole CLIA and College of American Pathologists (CAP)-certified laboratory in our institution, a Children’s Oncology Group Phase I research center, that offers clinically validated multiparameter flow cytometric assessment of patient peripheral blood (PB), bone marrow (BM), and tissue samples to support the diagnostic services of our Center for Cancer and Blood Disorders. We receive and analyze at least 15 pediatric leukemia/lymphoma samples each month, and over 90% of them are precursor B-cell ALL samples.

The overarching goal of this proposal is to test the hypothesis that a unique cellular and tumor-cell-associated phosphoprotein signature identifies patients who are insensitive to standard front line chemotherapy used in pediatric precursor B-cell ALL. This hypothesis posits that this signature measured at baseline (prior to induction chemotherapy) predicts failure of front line chemotherapy and disease relapse. This study will help identify, right at the time of diagnosis, patients who will benefit from stem cell transplantation versus conventional chemotherapy, rather than delay this decision, which currently is made by evaluating the persistence of leukemic blasts (minimal residual disease) 4–6 weeks post initiation of induction chemotherapy.

Specific Aim 1: To identify a cellular-phosphoprotein signature in the bone marrow (BM) and/or peripheral blood (PB) of pediatric precursor B-cell ALL samples that stratifies patient risk of failure to respond to chemotherapy. Although a 90% cure rate is observed for pediatric B-cell ALL associated with favorable prognostic factors, almost one out of every four patients experiences front line therapy failure. Accordingly, there is a need to identify novel actionable biomarkers that can help predict response to therapy and potentially identify new therapeutic targets. There is increasing evidence from “deep-profiling” studies of adult AML and follicular lymphomas that phosphoprotein signatures identify unique phenotypes of tumor cell subsets that predict responses to therapy. Hence there is a critical need to identify similar actionable biomarkers specifically for the pediatric population affected by ALL. We will utilize the BD Phosflow™ Violet Fluorescent Cell Barcoding Kit and established and published protocols to address Specific Aim 1. Fresh BM/PB samples will be treated with monoclonal antibodies (mAbs) to identify precursor B-ALL cells [CD19+CD10+CD34+ surface immunoglobulin (sIg)] and their associated phosphoprotein signature will be evaluated in the absence or presence of exogenous signaling inputs such as PMA/ ionomycin, IFN-gamma, CpG, IL4, soluble CD40-ligand (CD40L) trimer, and mAbs targeting class I and class II CD34 epitopes. The choice of some of these signaling inputs is based on the fact that infections can be a common presenting feature in these patients and that human bone
marrow CD34+ cells express functional Toll-like receptors. CpG treatment can enhance expression of CD40 and cytokine receptors on B-ALL cells. Hence, in some experiments, the phosphoprotein signature will be evaluated after combined treatment with CpG+IL4+IFN-gamma and CD40L. Based on the choice of our signaling inputs, the phosphoprotein signature will include evaluation of the following intracellular molecules: p-ERK, p-Stat1, p38, NF-kappa B p65, pStat6, and Syk. mAbs directed against phosphorylated versions of these molecules are available from BD. Due to the lack of commercially available CD19-specific antibodies with proven agonistic activity, this signaling input will not be utilized in this protocol. The acquired data will be analyzed using Cytobank software. It is possible that a phosphoprotein signature that informs clinical decision making might lie in a cellular subset other than the primary tumor cells, as observed in adult follicular B-cell lymphoma, in which the IL7-mediated activation of Stat5 in tumor infiltrating T cells helped stratify risk of disease relapse. Follow-up studies and future grant applications will address these issues.

Specific Aim 2: To define a threshold value of "CD19-ligand" (CD19L) expressing cells in the patient BM samples at baseline that predict disease relapse. CD19 is a B-cell-specific marker that is expressed throughout the entire spectrum of B-cell development. Normal and ALL-associated precursor B cells lack the expression of slg, CD21, and CD81. It has been recently reported that signaling through CD19 alone following engagement with CD19L expressed on T cells and T-cell precursors in the BM might regulate B-cell responses by inducing apoptosis of potentially hyperactive or autoreactive B cells in the BM. We will perform flow cytometric analyses of mature T cells (surface CD3+) and precursor T cells (cytoplasmic CD3+, nuclear TdT+, CD7+, CD34+) in BM samples to detect the expression of CD19L. CD19L protein is highly homologous to the TOX family of HMG-box proteins and can be detected using the commercially available eFluor® 660-conjugated rat anti-human/mouse TOX mAb. A purified mouse anti-human CD19L-specific mAb also exists, but is currently not available through commercial vendors. Five hundred thousand total events will be collected from each sample, and the percentage of CD19L-expressing cells will be determined in the index/diagnostic BM sample.

One hundred index samples will be analyzed, and results from both specific aims will be correlated with subsequently observed disease relapse or sustained control to define a cellular signature that predicts the likelihood of disease recurrence and chemotherapy failure.

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