Relapse due to inability of available therapies to eradicate leukemia stem cells is central to the pathogenesis of *T-cell acute lymphoblastic leukemia (T-ALL).* In addition, all T-ALL patients today generally receive the same type of treatment despite evidence of different underlying abnormalities in individual patients. The specific objective of this proposal is to define and validate a new tumor suppressor CD25 (IL2 receptor α, IL2Rα), which could serve as a biomarker for patient stratification in T-ALL. The central hypothesis is that CDK6-mediated suppression of CD25 is required for initiation of T-ALL by activated Notch1, and induction of CD25 mediates the therapeutic response to CDK6 inhibition in established T-ALL. We postulate that the CD25 expression in human T-ALL patients will affect formation and progression of human T-ALL. Guided by extensive preliminary data, the central hypothesis will be tested in one specific aim in this proposal: To determine the LIC frequency from $CD25^+$ and $CD25^-$ human T-ALL leukemic cells. To accomplish this, $CD25^+$ and $CD25^–$ primary T-ALL cells will be sorted and LSCs frequency determined by injecting serial dilutions of sorted $CD25^+$ and $CD25^–$ leukemic cells (10^5, 10^4, 10^3, 10^2 and 10) into NSG mice. With 6 mice per dilution, we sort CD25+/- populations from 3 patient samples. Mice will be monitored and bled weekly to determine the percentage of circulating human CD45+ cells in the peripheral blood, and sacrificed when determined to be moribund. Kaplan-Meier survival curves and statistical analyses were performed using GraphPad Prism software, $p < 0.05$ was considered statistically significant. LIC frequency will be calcualted using ELDA software as we have done previously. Primary pediatric T-ALL patients that express wild type or mutant NOTCH1 and/or wild type or mutant PTEN will be analyzed. Overall, the outcome of the proposed studies will suggest that the baseline level of expression of CD25 in human T-ALL patients and if CD25 could serve as a novel tumor suppressor and response to inhibition of CDK6 kinase activity, which could serve as a valuable biomarker for predicting the clinical response of human T-ALLs to a CDK4/6 inhibitor. Moreover, the outcome of the proposed studies will be both scientifically and clinically impactful and will generate adequate preclinical data leading to a more comprehensive application such as RO1.