Novel analysis strategy of T-cell receptor NGS data to develop patient-specific clonotype panels and detect minimal residual disease (MRD) in T-cell acute lymphoblastic leukemia (T-ALL)

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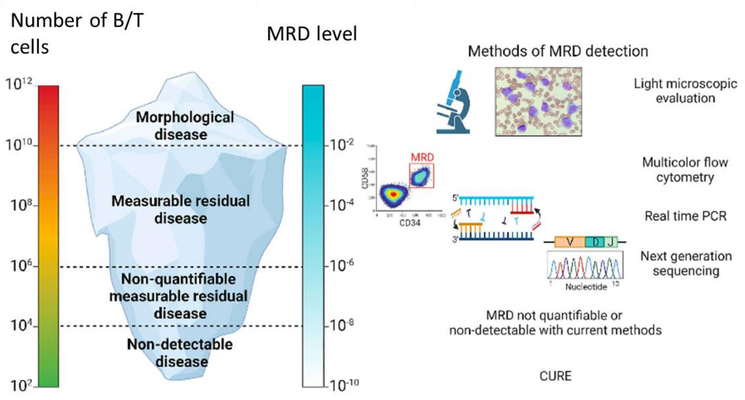
Background

MRD testing in Hematological Malignancies



clonoSEQ® By Adaptive

Uses NGS to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK and IgL receptor gene sequences in B-cell derived malignancies.

DLBCL CLIA-validated in peripheral blood and bone marrow
Diffuse large B-cell lymphoma (DLBCL)

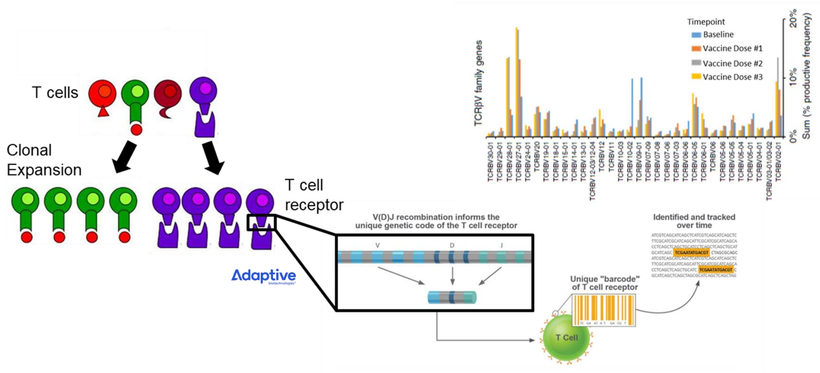
B-ALL FDA-cleared in bone marrow, CLIA-validated in peripheral blood
B-cell acute lymphocytic leukemia (B-ALL)

CLL FDA-cleared and CLIA-validated in bone marrow and peripheral blood
Chronic lymphocytic leukemia (CLL)

MULTIPLE MYELOMA FDA-cleared in bone marrow, CLIA-validated in peripheral blood

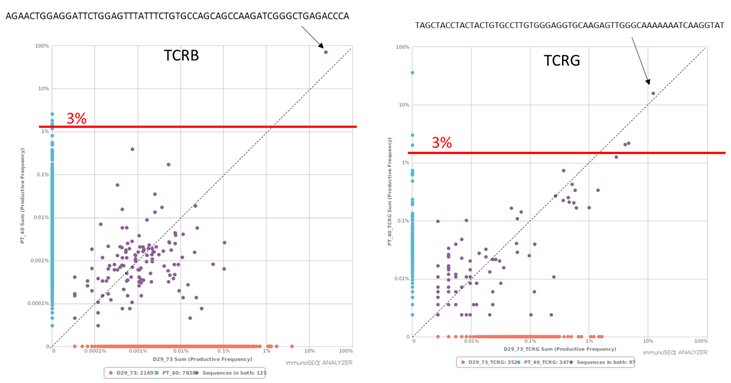
T Cell Receptor (TCR) Profiling

Clonotype expansion of T cell receptors is measured longitudinally by sequencing of samples

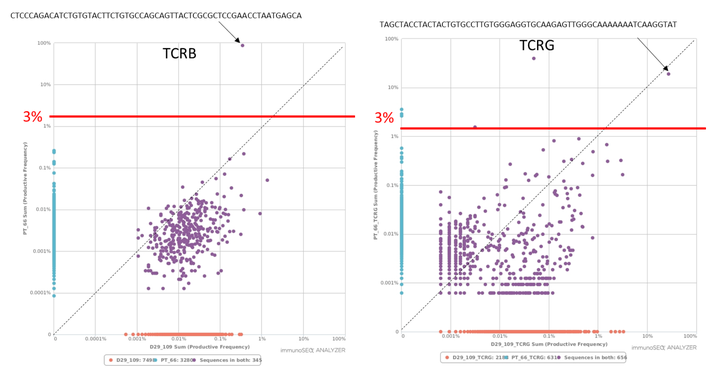


MRD Detection Post-Treatment

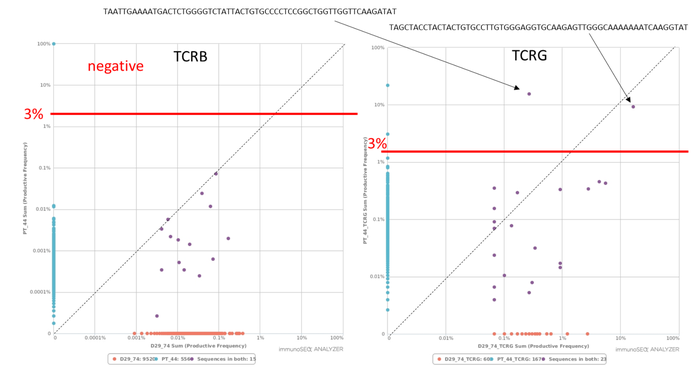
Detection of the target TCRβ and TCRγ sequences in a patient with confirmed MRD by multiparametric flow cytometry (mpFC)



Detection of the target TCRβ and TCRγ sequences in a patient with negative MRD by multiparametric flow cytometry (mpFC)



Detection of target TCRγ sequences in a patient with negative MRD both by multiparametric flow cytometry (mpFC) and NGS-MRD



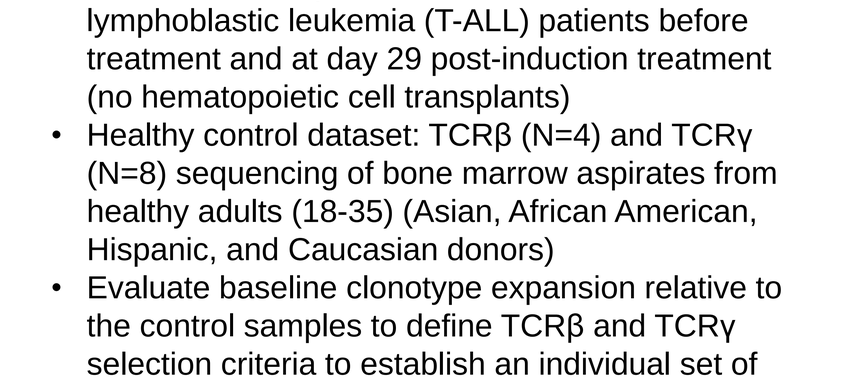
Experimental Objectives

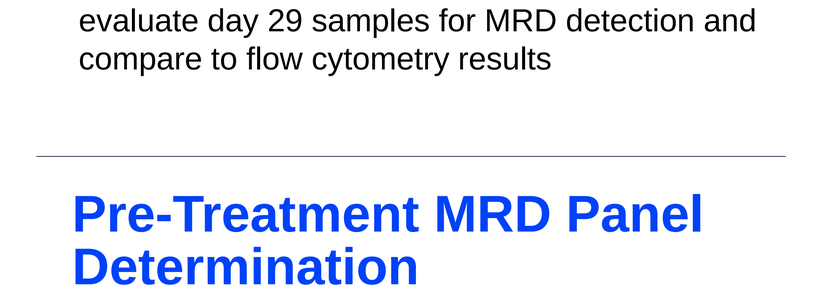
- Goal: Use a training set of retrospective patient data to propose a putative clinical test to use for MRD detection in T-cell derived hematological malignancies
  - Data source: Adaptive Biotechnologies immuneACCESS® database (world's largest collection of TCR and BCR sequences) and the immunoSEQ Analyzer® platform

Experimental Set-up

- Patient data set: TCRβ and TCRγ sequencing of bone marrow aspirated from N=43 T-cell acute lymphoblastic leukemia (T-ALL) patients before treatment and at day 29 post-induction treatment (no hematopoietic cell transplants)
- Healthy control dataset: TCRβ (N=4) and TCRγ (N=8) sequencing of bone marrow aspirates from healthy adults (18-35) (Asian, African American, Hispanic, and Caucasian donors)
- Evaluate baseline clonotype expansion relative to the control samples to define TCRβ and TCRγ selection criteria to establish an individual set of clones that will be used for MRD evaluation at day 29 (individualized patient panels)
- Using each patient's individual TCRβ/TCRγ panel, evaluate day 29 samples for MRD detection and compare to flow cytometry results

Pre-Treatment MRD Panel Determination





Conclusions and Next Steps

Results Summary

- Pre-treatment analysis of TCRβ and TCRγ clonotypes revealed expansion of multiple clones for an individualized MRD panel for each patient
- These candidate TCRβ and TCRγ clones were not expressed at a frequency greater than 0.08% in the healthy control samples
  - Represent the neoplastic T lymphoblasts
- MRD panels of all productive rearrangements that were expressed more than 3% of the total TCRβ and TCRγ sequences detected in the pretreatment sample allowed 43/43 (100%) for longitudinal MRD testing
- A positive detection of MRD was defined as identification of at least one of the clones in the top 50 TCRβ and TCRγ rearrangements
- This strategy successfully identified MRD in day 29 samples that were both mpFC positive and negative
  - Suggests enhanced sensitivity of MRD detection

Next Steps

- Evaluate a prospective cohort of bone marrow samples and compare to current diagnostic MRD
- Correlate results with patient clinical status to determine sensitivity
- Repeat this retrospective training in whole blood samples
- Evaluate retrospective and prospective patient cohorts of bone marrow and blood in other T cell derived leukemias
- Develop a test to predict GVHD with allogeneic stem cell transplant using a similar strategy

Abstract

MRD detection in leukemias is characterized by identification of pathologic biomarkers despite lack of morphological detection of leukemia cells by light microscopy. The diagnostic armamentarium to assess MRD in T and B cell-derived hematological malignancies includes multiparametric flow cytometry (mpFC), real-time quantitative PCR (qPCR), and NGS of hypervariable receptor junctions to indiscriminately assess clonal frequencies. Of these diagnostic tools, NGS-MRD is 10-100-fold more sensitive and provides distinct clinical value. Specifically, MRD levels in ALL are associated with significantly improved overall survival and event free survival after induction treatment. While there are clinically approved NGS-MRD tests for B-cell derived malignancies, a standardized biostatistical analysis in T-cell derived leukemias has yet to be approved. The objective of this work was to use a training set of retrospective data from T-ALL patients to propose a putative clinical test for MRD detection. NGS of T-cell receptor (TCR) β and γ clonotypes was obtained via Adaptive Biotechnologies immuneACCESS® database and analyzed on the immunoSEQ Analyzer® platform. T-ALL samples included bone marrow aspirates before treatment and after induction therapy; control samples were from healthy adults aged 18-35 (Asian, African American, Hispanic, and Caucasian). Pre-treatment analysis of TCRβ and TCRγ clonotypes revealed expansion of multiple clones that could be used as putative biomarkers in an individualized panel. Since these candidate TCRβ and TCRγ clones were not expressed at a frequency greater than 0.08% in the healthy control cohort, they were presumed to represent the neoplastic T lymphoblasts. Using all productive rearrangements that were expressed more than 3% of the total TCRβ or TCRγ sequences in the treatment-naive sample, 43/43 samples qualified for longitudinal MRD testing. These panels were used to evaluate the corresponding post-induction treatment samples and positive MRD detection was defined as identification of at least one of the clones in the top 50 TCRβ or TCRγ rearrangements. This strategy identified MRD in post-induction treatment samples that were both mpFC positive and negative, which suggests enhanced sensitivity of MRD detection. Collectively, these data establish a novel MRD analysis strategy for T-ALL using NGS of TCRβ and TCRγ clonotypes that is potentially translatable to all T-cell derived hematological malignancies. More sensitive MRD assessments could impact therapeutic decisions for maintenance therapy or candidacy for hematopoietic cell transplant in patients with a higher risk of relapse. Future efforts will evaluate a longitudinal patient cohort of T-cell derived leukemias prospectively for comparison to matched whole blood samples to facilitate less invasive testing.