COMPREHENSIVE IMMUNOPHENOTYPING AND GENOMICS FROM SINGLE BLOOD TUBE FOR T-CELL DYNAMICS IN CLINICAL TRIALS

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Background: Analysis of peripheral whole blood is a cornerstone of investigational therapeutic development. Phase I/II trials request multiple blood draws from patients to understand pharmacokinetics and pharmacodynamics; in cases where they are experiencing severe disease, reducing draw volumes is desirable. Long distances between collecting hospitals and bioanalytical labs risks sample degradation. To conserve patient whole blood samples, we coupled deep immunophenotyping and transcriptomic workflows using a single, cryopreserved tube as input (figure 1). This method allowed characterization of T-cell immune state by flow cytometry, TCR diversity and transcriptomics in a global clinical trial of Non-Hodgkin Lymphoma (NHL).

Methods: Peripheral blood mononuclear cells (PBMC) were collected from NHL patients (n = 165; NCT03625037) and age matched normal healthy volunteer donors (n = 24). PBMC extraction was performed in three central labs located in Europe, Asia, and North America. A T-cell focused, 16-parameter flow cytometry panel was performed on PBMCs and then RNA was purified from the remaining cells in the tube. T-cell receptor β sequencing (TCRseq) using a multiplex PCR (Illumina) and transcriptomics using EdgeSeq (HTG Molecular) was performed. In parallel, patient whole blood samples were assessed by flow cytometry in the central labs. Data from all three platforms was integrated and analyzed with linear models in R.

Results: We compared immunophenotyping from patient whole blood (overnight shipped) to cryopreserved PBMCs from the same blood draw; the viable cell percentage of PBMCs after thawing averaged 55%. T-cell populations in high viability PBMC samples (>70%) were correlated to whole blood estimates (average Spearman’s ρ of 0.7). Samples with viability >30% retained correlation across the immune subsets of interest (T naïve, effector, central memory and regulatory). Our flow cytometry workflow consumed 1M cells, 70% of patients profiled had sufficient sample remaining for TCR and transcriptomic assays (500k+ cells; average RNA yield of 350ng).

Combining TCR and transcriptomics with flow cytometry enables a composite picture of T-cell activity. Statistical analysis revealed CD4+ naïve proportions were the primary driver of TCR diversity. Top genes associated with diversity were CD69, a well-known activation marker, DUSP10/MKP5, an immune regulator1 and GPR183/EBI2 an oxysterol receptor with a role in lymphoid development.2,3

Conclusions: This streamlined workflow is amenable to global clinical trials with long shipping distances and reduces blood draw requirements. Integration of flow cytometry, TCR diversity and the transcriptome allows a deeper understanding of T-cell activity that can support mechanism of action analyses.

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REFERENCES


Ethics Approval: The protocol and informed consent were approved by an independent ethics committee or institutional review board before initiation. The trial was done in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines, Declaration of Helsinki, and all applicable regulatory requirements. All patients gave written informed consent.

Consent: Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Trial Registration: GEN3013 Trial in Patients With Relapsed, Progressive or Refractory B-Cell Lymphoma (NCT03625037)