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# POSTER ABSTRACTS

## 621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

### An Integrated Multimodal Framework for Noninvasive TCL Disease Detection and Monitoring

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Background: Early noninvasive identification of therapeutic responses could help accelerate the development of more effective therapies for T-cell lymphoma (TCL), and to improve patient outcomes. However, standardized noninvasive disease-monitoring strategies remain elusive for the most common mature T-cell tumors. Therefore, the development of highly sensitive and accurate disease monitoring methods for TCL represents a strong unmet clinical need. We tackled this challenge using an integrated liquid biopsy strategy leveraging both cell-free DNA (cfDNA) and cell-free RNA (cfRNA), by simultaneously interrogating tumor-specific aberrations, including somatic mutations, clonotypic TCR rearrangements, and TCL gene expression signatures.
Methods: We studied 83 serial blood samples from 36 patients diagnosed with TCL and treated in the US or Japan, including 63 longitudinal samples from 30 cases from Stanford University receiving standard TCL therapies (NCT00398177), and 20 blood samples from 6 subjects with relapsed/refractory TCL treated in Kyushu University hospital. Cell-free DNA samples for all cases were profiled to simultaneously detect somatic mutations by CAPP-Seq (Newman et al 2016 Nature Biotech) as well as clonotypic VDJ rearrangements by SABER (Shukla et al 2020 ASH, Sworder et al 2022 Cancer Cell). Therefore, an integrated CAPP-Seq targeted panel was designed for hybrid capture to target recurrently mutated genes in diverse T-cell neoplasms along with TCR rearrangements at 4 loci. Captured libraries were sequenced to a median deduplicated depth of ~3000x. For CAPP-Seq, single nucleotide variants (SNVs) were identified by the analysis of tumor tissue or pre-treatment cfDNA; constitutional germline variants and clonal hematopoiesis (CHIP) variants were censored using matched DNA from either CD4+ T-cell depleted PBMCs or buccal swabs. For SABER, tumor clonotypic VDJ sequences were defined as most prevalent clone in tumor tissue or pretreatment plasma cfDNA. Plasma cfRNA was profiled for a subset of patients (20 serial samples from 13 patients) using RARE-Seq (Nesselbush et al, in preparation). We then measured correlations between radiographic disease status and circulating disease levels, as measured using mutant ctDNA and clonotypic TCR molecules, and cfRNA gene expression signatures.
Results: Among the 36 TCL subjects, all cases (100%) could be noninvasively detected in plasma cfDNA using either somatic mutations (mutant ctDNA only: 86%) or using clonotypic cell-free TCR rearrangements (cfTCR only: 88%). When considering the concentrations of these distinct tumor reporters, mutant ctDNA levels (hGE/mL plasma) and cfTCR levels (# of clones/mL plasma) were significantly correlated (Pearson $R = 0.65$, $p<0.01$, Fig. 1A). When considering longitudinal measurements during and after therapy, circulating levels of both ctDNA and clonotypic cfTCR were significantly lower in patients experiencing objective responses (CR/PR) than in patients experiencing SD/PD ($p<0.01$). Moreover, when comparing acellular plasma versus cellular (PBMC) blood fractions, circulating disease levels were significantly higher in plasma and often became undetectable

in PBMCs (Fig. 1B). When considering TCR repertoires, CDR3 diversity was significantly lower at timepoints of measurable disease (pre-treatment, SD, PD). Furthermore, clonotypic tumor cell-free TCR (tumor cfTCR) fragments were significantly shorter than non-tumor cfTCR molecules ($p<0.01$). When considering cfRNA, a gene expression signature of activated CD4 memory T-cells was strongly correlated with both mutant ctDNA and tumor cfTCR levels (R=0.85, 0.64), and was higher at timepoints of active TCL disease.

Conclusions: Noninvasive TCL detection and monitoring appears feasible through liquid biopsies interrogating cfDNA and cfRNA, with advantages over PBMCs. The complementarity for disease detection using tumor-specific somatic mutations, clonotypic TCR rearrangements, and gene expression levels, strongly suggests synergies to enable an integrated multimodal liquid biopsy framework for TCL. This novel platform should allow more accurate assessment of disease status and MRD, and to inform risk-adapted treatment strategies in TCL.

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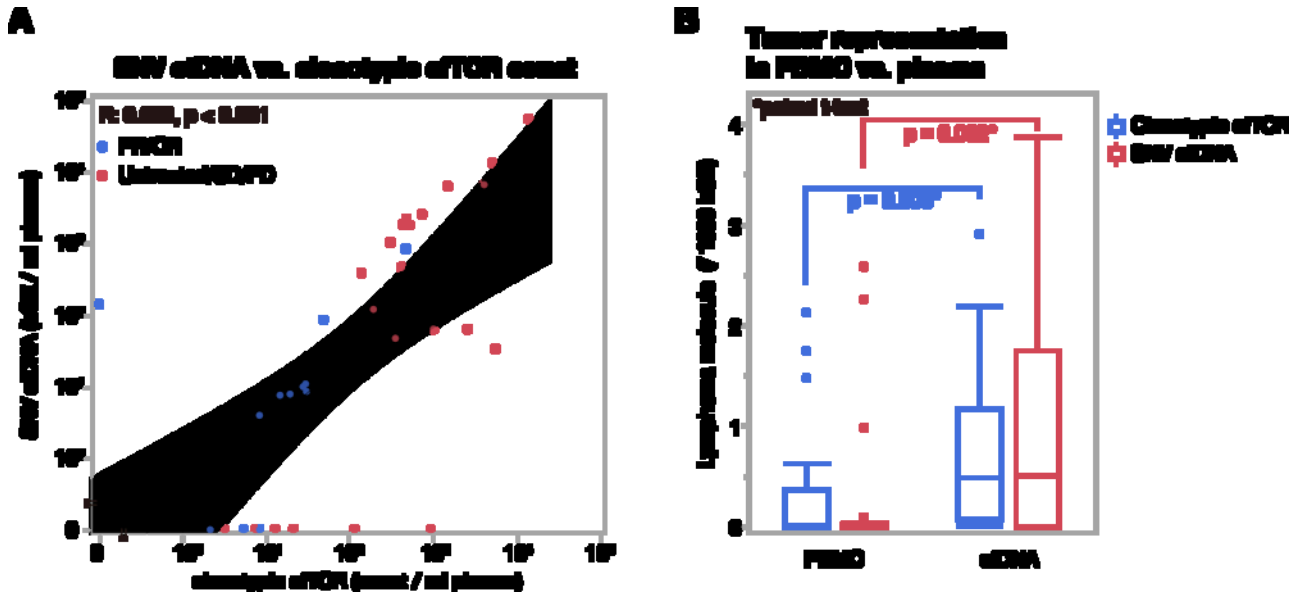


**Figure 1**

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