Flow cytometric assessment of T cell clonality for early detection of ATL: comparison with RNA-Seq

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Background:
To identify HTLV-1 carriers at high risk of developing ATL, we established a flow cytometric assay that tests for oligoclonal expansions within HTLV-1-infected CCR4+CD26- T-cells (OCI-flow assay). To evaluate the sensitivity by which our assay can detect expanded T-cell clones in carriers without ATL, we sequenced TCR-α and -β chain complementarity determining region 3 (CDR3) regions in flow-sorted peripheral blood mononuclear cell (PBMC) samples from donors with a range of OCI-flow scores.

Methods:
Two groups of donors were studied: HTLV-1-carriers with an OCI-flow>0.770 (high risk of ATL, n=11, nine with one expanded clone and two with two expanded clones), and OCI-flow≤0.770 (low risk of ATL, n=10). PBMCs were flow-sorted to obtain: CD4+CCR4+CD26- cells expressing the dominant TCRVβ subunit (OCI-flow>0.770 samples only), other CD4+CCR4+CD26- cells and other CD4+ T-cells, resulting in 13 sorted fractions predicted to contain expanded clones, and 40 fractions predicted not to contain expanded clones. The proviral burden of each fraction was quantified by qPCR. RNA was extracted, subjected to RNA-Seq, and CDR3 sequences were analysed using MiXCR. Unique TCR-α/-β CDR3 sequences which comprised >20% of TCR-α/-β CDR3 sequences were called as expanded clones.

Results:
RNA-Seq/CDR3 sequence analysis detected expanded clones in 11/13 fractions predicted to contain clones, and 1/40 fractions predicted not to contain a clone ($p<0.00001$, Fisher's exact test). Clone-containing fractions had a median of 0.99 proviral copies per cell. Half of expanded clones expressed multiple TCR-α species, (n=4, 1TCR-α/1TCR-β; n=5, 2TCR-α/1TCR-β; n=1, 3TCR-α/1TCR-β; n=2, 1TCR-α/0TCR-β), indicative of expression of nonproductively rearranged TCR-α chains. Of the two remaining fractions predicted to contain clones, one had a dominant CDR3 just below the threshold for calling a clone (19%), and one had no obviously dominant CDR3 sequence.

Conclusion:
The sensitivity of the OCI-flow assay is equivalent to RNA-Seq/CDR3 sequence analysis for the detection of expanded HTLV-1-infected clones.

Disclosure of Interest Statement:
Nothing to disclose.