Single-cell NGS methods to track tumor precursor cells in HTLV-1 and BLV leukemia models

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Background:
Increasing evidence indicates that cancer cells sequentially accumulate changes that confer deregulated growth. Developments in single-cell NGS mean that it is now feasible to trace tumors' life histories and unravel key questions in cancer evolution. However, one of the greatest obstacles to investigating tumor evolution is finding and isolating the tumor precursor cell. In HTLV-1-associated ATL the transformed cell and its early precursor share a unique taggable proviral integration site (IS). Here we leverage the closely-related BLV leukemia model in sheep to develop single-cell genomic approaches to trace the malignant cell's history and identify drivers of abrupt clonal expansion observed at tumor onset. To this end, we exploit the unique advantage of being able to retrospectively trace the rare tumor ancestor clone at multiple time-points prior to overt cancer.

Methods and Results:
We developed a customized plate-based Target-seq approach in which full-length transcriptome and proviral/host genomic regions of interest are simultaneously sequenced at single-cell level. By targeting provirus and tumor-specific IS, we were able to identify (i) random infected cells, (ii) infected cells belonging to the tumor clone, allowing retrospective tracing of the tumor ancestor at multiple time-points. We also exploited the transcript full-length properties to reconstruct the leukemic B-cell receptor (BCR) consensus sequence. The possibility to screen/identify precursors according to tumor BCR transcriptomic signatures generated an additional layer of selection. Altogether, this IS/BCR dual screening approach ensures robust identification of rare tumor ancestors and their transcriptomic profiles. In parallel, we are developing a custom microfluidics workflow to screen thousands of primary cells in a high-throughput and cost-effective manner. This is required for exploring time-points with extremely low frequencies of precursor cells.

Conclusions
We have developed single-cell NGS approaches that have the potential to track pre-leukemic clones and unveil key drivers of the oncogenic switch governing unrestrained clonal expansion.

Disclosure of Interest Statement:
None.