Lymphatic exudate is a novel source of tumor-associated immune cells

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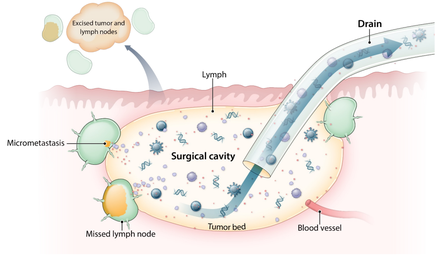
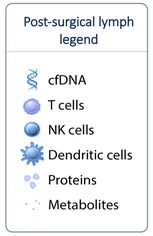
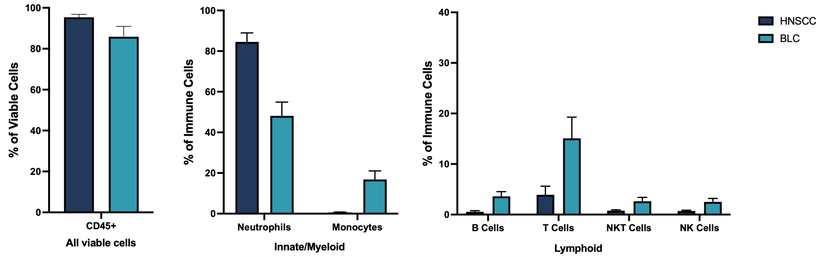
Introduction

Autologous tumor-infiltrating lymphocyte (TIL) therapy has demonstrated efficacy in multiple solid tumor types, but therapy requires surgery and extensive manufacturing. Lymphatic exudate ("lymph") is routinely collected from surgical drains following initial tumor resection. Although usually discarded, we have shown that this novel proximal biofluid is rich in ctDNA and prognostic of recurrence in head and neck squamous carcinoma (HNSCC) patients. Here, we investigate lymph as a novel, accessible source of tumor-associated lymphocytes.





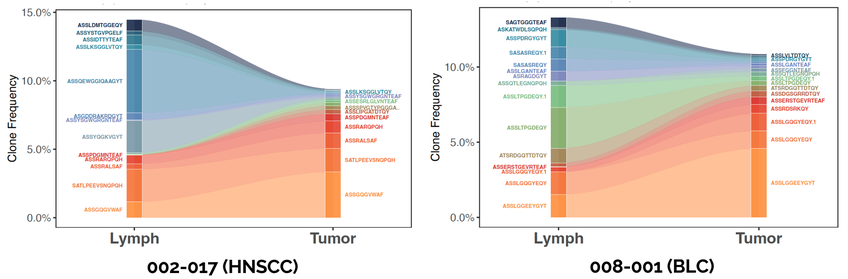
Post-surgical Lymph is rich in immune cells



	HNSCC	BLC
CD45+	95.36%	85.85%
T cells	3.92%	15.08%
NKT cells	0.78%	2.63%
B cells	0.55%	3.60%
Monocytes	0.37%	7.90%

Lymph cells were primarily CD45+. Neutrophils were the most abundant cell type, consistent with reported post-surgical increase. Within the lymphoid compartment, T cells were the dominant cell population, with B, NK and NKT Cells also detected.

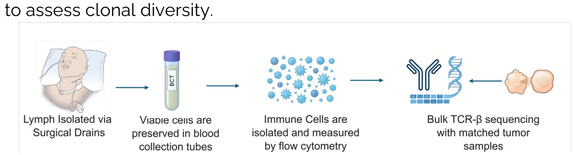
Shared TCR Landscape Across Lymph and Tumor



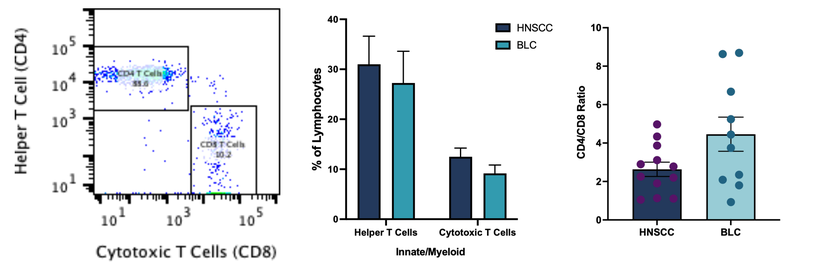
In patients with high tumor overlap, the top 20 shared clones between tumor and lymph were visualized. In patient 002-017, 9 of the top 20 clones showed preferential expansion at the tumor site (>1.5x higher in tumor than lymph). Similarly 8 out of the top 20 clones showed preferential expansion at the tumor site in patient 008-001. This is consistent with antigen-driven expansion of tumor-reactive T cells. Other shared clones were enriched in lymph, suggesting recent systemic activation, mobilization or potential exhaustion.

Methods and Materials

Lymph was collected 24 hours post-surgery from 11 HPV- HNSCC and 14 bladder cancer (BLC) patients in K_2EDTA blood collection tubes. Cells were isolated from lymph and immune populations were characterized by flow cytometry using the Miltenyi 8-color immunophenotyping kit (human). Bulk T-cell receptor β (TCR-β) sequencing was performed using the OmniSeq® TCRβ repertoire assay on 17 patient sets with matched tumor (11 HNSCC, 6 BLC). Two BLC patients failed sequencing QC. TCR repertoires were compared between tumor and lymph and Simpsons clonality was used to assess clonal diversity.

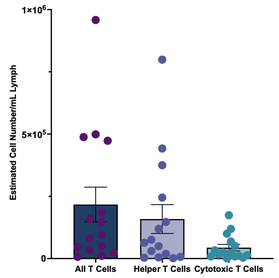


Lymph contains both Cytotoxic and Helper T Cells



Both CD4+ and CD8+ T Cells were detected in all patient lymph samples at variable frequencies. CD4/CD8 ratios ranged from 1-4.97 in HNSCC and 0.11-8.69 in BLC, compared to the typical 1-3 range observed in healthy individuals.

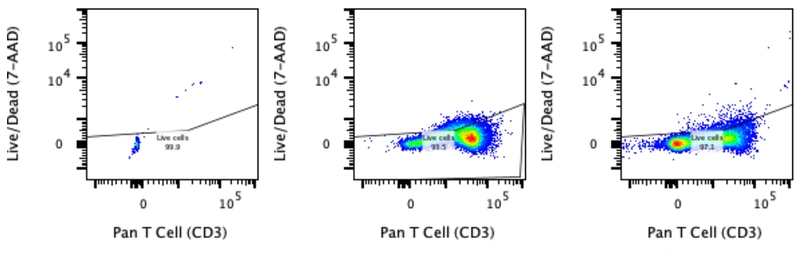
Lymph as a Rich Source of Tumor-Associated T Cells



T cells / mL	216,989 (6,740 – 958,000)
Routine drain volume (24 hours post-op)	30 mL
T cells / drain	**6.5 million (0.2 – 28.7 million)**

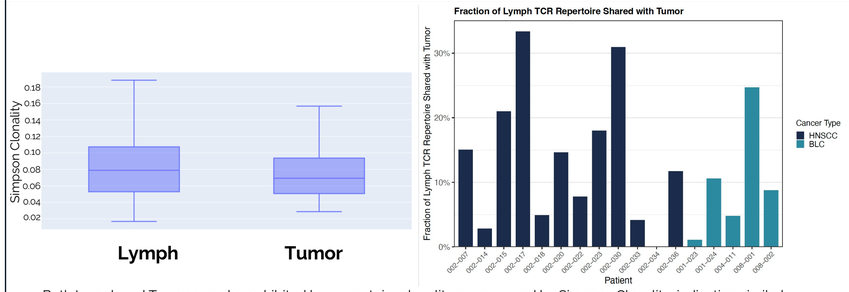
T-cell numbers were calculated from flow-cytometry frequencies and total viable cells counts (Trypan Blue) per mL of lymph. Based on a routine 30 mL drain volume at 24 hours post-operation, an average of 6.5 million T cells can be isolated from lymph per day.

Lymph cells remain viable post-thaw



Cryopreserved lymph cells or peripheral blood mononuclear cell (PBMC) control were thawed and stained with MACS 8-color immunophenotyping panel. Lymph cells showed high viability (7-AAD-negative population) post-thaw in both indications.

Lymph and Tumor share High T-Cell Clonal Overlap



Both Lymph and Tumor samples exhibited low repertoire clonality as measured by Simpson Clonality, indicating similarly polyclonal repertoires across both compartments. CDR3 sequence analysis revealed high overlap of T-Cell clones between tumor and lymph. Approximately 5-35% of lymph T cells were tumor-associated, considerably higher than what is typically observed in peripheral blood. Taken together, lymph T Cell repertoires indicate a high proportion of tumor-associated lymphocytes.

Conclusions and Future Work

Post-surgical lymph contains viable immune populations with substantial TCR overlap with tumor infiltrating lymphocytes. Lymph could enable the isolation of tumor-associated T cells without the challenges around metastasectomy. Post-surgical lymph T cells may represent a novel, abundant, low cost, and easily accessible source for adoptive cell therapy.

Future work characterizes the activation, exhaustion and cell state of lymph T Cells with single-cell RNA-seq and in vitro functional tests.

More information

Please contact Wendy Winckler: wwinckler@dropletbiosci.com

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