Profiling the TCR beta repertoire in liquid biopsies from NSCLC donors

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Abstract# B54

Introduction

During infection, and in cancer, the immune system's response to antigen leads to changes in the T-cell repertoire. T-cell clonal expansions can be measured by sequencing the antigen-specific loci in the T-Cell Receptor beta gene (TCRβ). In oncology research TCRβ sequencing is being explored as a predictor for response to immunotherapy as well as immune related adverse events (IRAE) post-immunotherapy. Recent studies have focused on two metrics, T-cell clonality and TCR convergence as potential biomarkers. Non-invasive testing for these markers can be achieved using peripheral blood lymphocytes (PBL). In this study PBL specimens from donors previously diagnosed with NSCLC were evaluated using TCRβ sequencing. Additionally, to model T-cell repertoire changes due to antigen stimulation, primary PBL were challenged *in vitro* with cytomegalovirus (CMV) antigen.

Methods

Part 1: PBL from Non-Small Cell Lung Cancer (NSCLC) donors

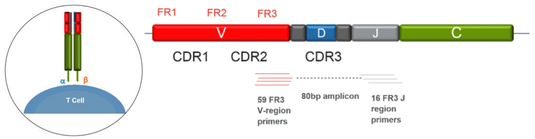
- Blood was collected from N=5 NSCLC donors into Streck RNA BCT®. RNA was extracted from buffy coat cells using the Qiagen RNeasy® FFPE Kit.
- Qubit™ RNA HS assay was used to quantify RNA, and Agilent RNA 6000 Nano Assay was used to determine the RNA integrity scores (RIN).
- cDNA was generated using SuperScript™ IV VILO™ Kit (Thermo Fisher Scientific).

Part 2: CMV model antigen study (Astarte Biologics)

- Specimens:
  - Primary PBMC from 4 different normal healthy donors
  - Donors were seropositive for CMV antigen and HLA-A02-positive
- Treatment conditions:
  1. Baseline: Unstimulated cells
  2. Lysate: Whole-cell Lysate from CMV-infected cells
  3. Peptide: CMVpp65$_{495\text{-}503}$ peptide (NLVPMVATV), HLA-A201-restricted
- After 6 days of culture in the presence of IL-2 cells were harvested for flow cytometry to measure pp65-responsive CD8+ T-cells and for RNA
- RNA for TCRβ Sequencing was extracted using Qiagen RNeasy® Mini Kit

TCRβ Sequencing

- Oncomine TCR Beta-SR Assay (Thermo Fisher Scientific)



- cDNA input: determined based on CD3 qualification assay (measures the CD3 fraction of mixed cell populations and DNA amplifiability)
- Sequencing:
  - Instrument: Ion GeneStudio™ S5 Plus
  - Templating: Ion Chef™, Ion 530™ Chips
  - 25pM pooled libraries (12 specimens/chip)

Results

Table 1. Sample and TCR metrics for specimens collected from NSCLC donors

Donor	RIN Score	cDNA Input (ng)	Library Conc (pM)	Raw Reads (million)	Productive and Rescued Reads (%)	# Clones	Clonality	TCR Convergence
1	2.5	400	473	1.55	74%	49914	0.09	0.016
2	3.9	180	457	1.99	73%	44299	0.24	0.021
3	6.8	72	1101	2.13	79%	58357	0.15	0.026
4	4	324	1004	1.99	83%	61936	0.14	0.033
5	2.3	34	45	1.17	63%	10731	0.48	0.005

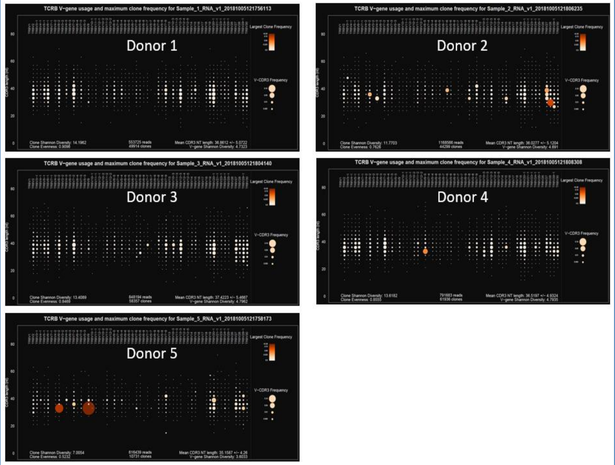
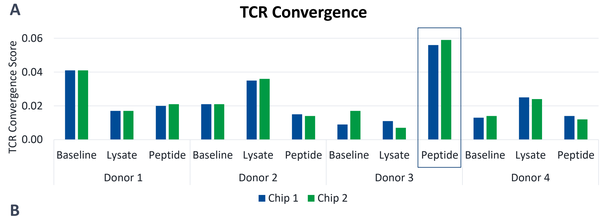


Figure 1. Spectratyping plots for NSCLC specimens. Circles are bins containing all clones with a particular V gene-CDR3 nucleotide (nt) length combination. Circle size indicates the frequency of all clones contained in the bin, and color indicates the frequency of the most prevalent clone present in the bin (darker orange with higher frequency). Expanded clones are visible in Donor 5, which has the highest clonality score of the 5 donors (Table 1).

Table 2. Sample and TCR metrics for CMV model antigen study

Donor	Treatment	RIN Score	cDNA Input (ng)	Library Conc (pM)	Raw Reads (million)	Productive and Rescued Reads (%)	# Clones
1	Baseline	10	46	593	1.6	83%	31804
	Lysate	10	25	788	1.99	85%	12504
	Peptide	10	29	385	1.52	84%	26901
2	Baseline	10	43	708	1.54	82%	25459
	Lysate	9.9	46	1093	1.99	83%	11250
	Peptide	10	107	687	2.16	83%	31367
3	Baseline	10	38	290	1.45	81%	29964
	Lysate	10	48	630	1.77	84%	27647
	Peptide	10	63	401	1.43	83%	26131
4	Baseline	10	40	563	1.56	83%	31672
	Lysate	10	30	835	1.7	83%	6683
	peptide	10	29	608	1.78	84%	36051

Results



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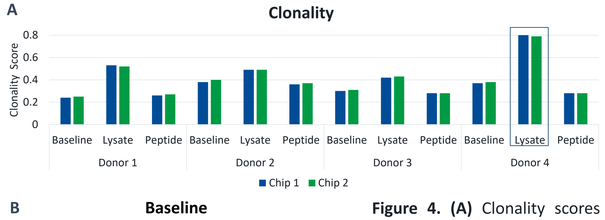
Variable	Joining	CDR3 AA	CDR3 NT	Total Counts	Frequency	Rank
TRBV12-3	TRBJ1-2	ASSSAYYGYT	GCCAGCAGCTCAGCGTACTATGGCTACACC	19545	0.0356	3
TRBV12-3	TRBJ1-2	ASSSAYYGYT	GCCAGCAGTTCGGCTTACTATGGCTACACC	2931	0.0053	23
TRBV20-1	TRBJ1-1	SARQGDTEAF	AGTGCTAGGCAGGGAGACACTGAAGCTTTC	1725	0.0031	31
TRBV20-1	TRBJ1-1	SARQGDTEAF	AGTGCTAGACAGGGGGACACTGAAGCTTTC	652	0.0012	62

Figure 3. (A) TCRβ convergence scores for the CMV model antigen study. Results are shown for replicate 530 chips. **(B)** Two examples of convergent T-cell clones (clones with identical amino acid sequence but variable nucleotide sequence, which can be driven by chronic antigen exposure) are shown for Donor 3 cells stimulated with pp65 peptide.



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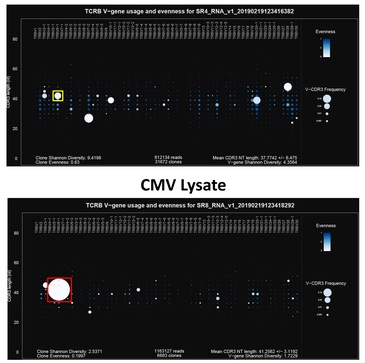
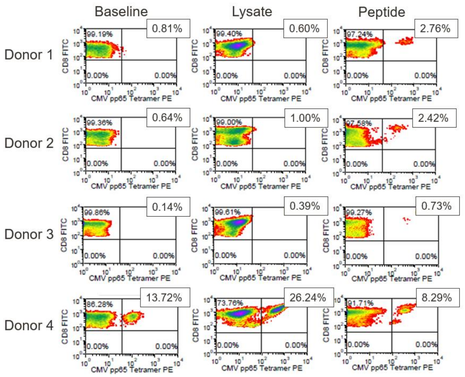


Figure 4. (A) Clonality scores for the CMV model antigen study. **(B)** Spectratyping plots for Donor 4 showing clonal expansion in the Lysate condition relative to baseline. Darker blue circles indicates increasing evenness and lower clonality (clonality = 1 - evenness). The large circle (red box) contains 57 clones. The most prevalent clone and driver of the high clonality score for this sample was observed at 76% frequency. This clone was present in the baseline sample at 6.5% frequency (yellow box).

Results

Figure 5 (right). pp65 tetramer assay results for CMV model antigen study. Positive FITC and PE staining indicates the presence of pp65 responsive CD8+ T-cells. The % positive cells are shown.

Table 3 (below). A family of clones (TRBV12-3 TRBJ1-2 ASSSAxYxYT) expanded in all samples in response to CMV challenge, and the expansion observed by sequencing correlates with pp65 tetramer staining as shown in Figure 5.



Donor	Condition	Variable	Joining	CDR3 AA	CDR3 NT	Total Counts	Frequency	Rank
1	Baseline	12-3	1-2	ASSSANYGYT	GCCAGCAGTTCGGCGAACTATGGCTACACC	31	0.007%	1300
	Lysate	12-3	1-2	ASSSANYGYT	GCCAGCAGTTCGGCTAACTATGGCTACACC	123	0.010%	470
	Peptide	12-3	1-2	ASSSANYGYT	GCCAGCAGTTCGGCTAACTATGGCTACACC	4003	0.85%	8
2	Baseline	12-3	1-2	ASSSAHYGYT	GCCAGCAGTTCCGCTCACTATGGCTACACC	25	0.004%	2379
	Lysate	12-3	1-2	ASSSAHYGYT	GCCAGCAGTTCCGCTCACTATGGCTACACC	151	0.012%	426
	Peptide	12-3	1-2	ASSSAHYGYT	GCCAGCAGTTCCGCTCACTATGGCTACACC	30031	3.0%	3
2	Baseline	12-3	1-2	ASSSANYRYT	GCCAGCAGTTCGGCTAACTATCGCTACACC	74	0.012%	639
	Lysate	12-3	1-2	ASSSANYRYT	GCCAGCAGTTCGGCTAACTATCGCTACACC	1528	0.12%	80
	Peptide	12-3	1-2	ASSSANYRYT	GCCAGCAGTTCGGCTAACTATCGCTACACC	150381	15.1%	1
3	Baseline	12-3	1-2	ASSSYYGYT	GCCAGCAGTTCATACTATTATGGCTACACC	5	0.001%	13121
3	Lysate	12-3	1-2	ASSSAYYGYT	GCCAGCAGTTCGGCTTACTATGGCTACACC	15	0.002%	3817
3	Lysate	12-3	1-2	ASSSAYYGYT	GCCAGCAGCTCCGCCTACTATGGCTACACC	4	0.000%	13941
3	Lysate	12-3	1-2	ASSSAYYGYT	GCCAGCAGCTCAGCGTACTATGGCTACACC	96	0.012%	455
	Peptide	12-3	1-2	ASSSAYYGYT	GCCAGCAGCTCAGCGTACTATGGCTACACC	19545	3.6%	3
4	Baseline	12-3	1-2	ASSSANYGYT	GCCAGCAGTTCAGCTAACTATGGCTACACC	489	0.080%	54
	Lysate	12-3	1-2	ASSSANYGYT	GCCAGCAGTTCAGCTAACTATGGCTACACC	6061	0.52%	8
	Peptide	12-3	1-2	ASSSANYGYT	GCCAGCAGTTCAGCTAACTATGGCTACACC	485	0.075%	85
4	Baseline	12-3	1-2	ASSSANYGYT	GCCAGCAGCTCCGCTAACTATGGCTACACC	50	0.008%	646
	Lysate	12-3	1-2	ASSSANYGYT	GCCAGCAGCTCCGCTAACTATGGCTACACC	69	0.006%	257
	Peptide	12-3	1-2	ASSSANYGYT	GCCAGCAGCTCCGCTAACTATGGCTACACC	32	0.005%	1950

Conclusions

- The Oncomine TCRβ assay can detect repertoire features with high resolution using PBL isolated from liquid biopsies.
- Profiling of the TCRβ repertoire using the Ion Torrent platform represents a valuable new solution due to the technology's low substitution error rate and given that substitution errors mimic TCR convergence.
- We are currently pursuing studies to evaluate the clinical utility of sequencing the immune repertoire in NSCLC patients receiving immunotherapy.

Acknowledgments

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