H44

Minimal Residual Disease Detection of Lymphoid and Plasma Cell Neoplasms Using a Next-Generation Sequencing (NGS)-Based Assay

Caleb Ho, MD; Juan Gomez-Gelvez, MD; Mustafa H Syed, MS; Kseniya Petrova-Drus, MD, PhD; Ahmet Zehir, PhD; Wayne Yu, BS; Tessara Baldi, BS; Marc Ladanyi, MD; Ahmet Dogan, MD, PhD; Jinjuan Yao, MD, PhD; Khedoudja Nafa, PharmD, PhD; Maria E. Arcila, MD

Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY

Introduction

Lymphoid and plasma cell neoplasms are characterized by clonally-restricted T-cell receptor (TCR) or immunoglobulin (Ig) rearrangements. Across clinical laboratories, this is generally demonstrated with standardized multiplex polymerase chain reaction (PCR) assays, in which V-J or D-J products are separated by fragment sizes on capillary electrophoresis (CE). However, this approach has relatively low sensitivity and does not provide the specific clonal sequence information required for tracking a clone at low level or in minimal residual disease (MRD) setting. In this study, we assessed the performance of a NGS-based assay, LymphoTrack®(LT) (Invivoscribe, San Diego, CA), for detection of low level and MRD among various lymphoid and plasma cell neoplasms in comparison to CE and flow cytometry (FC) assays.

Material and Methods

DNA was extracted from bone marrow, blood, and formalin-fixed paraffin-embedded tissue from 48 patients with diagnostic and post-therapy (PT) samples. For clonal Ig rearrangement, PCR primers flanking the *IGH* conserved framework region 1 (FR1) in VH and conserved JH region were used. For clonal TCR rearrangement, primers flanking the *TRG* conserved Vγ and Jγ regions were used. The amplified products were sequenced on the Illumina MiSeq platform, and analyzed with the proprietary LymphoTrack® analysis software, which provided the quantitation and V-J gene family usages of all unique sequences. With the aid of an in-house developed software, MSK-LymphoClone, the patient-specific diagnostic clonal sequences were used to detect residual disease involvement in subsequent samples, and compared to concurrent CE and 10-color FC results available at MSKCC.

Results

	Diagnostic Samples	Post-Therapy Samples
Acute Lymphoblastic Leukemias	11	14
Mature B-cell Neoplasm	16	20
Mature T-cell Neoplasm	9	13
Plasma Cell Neoplasm	12	15
Total	48	62

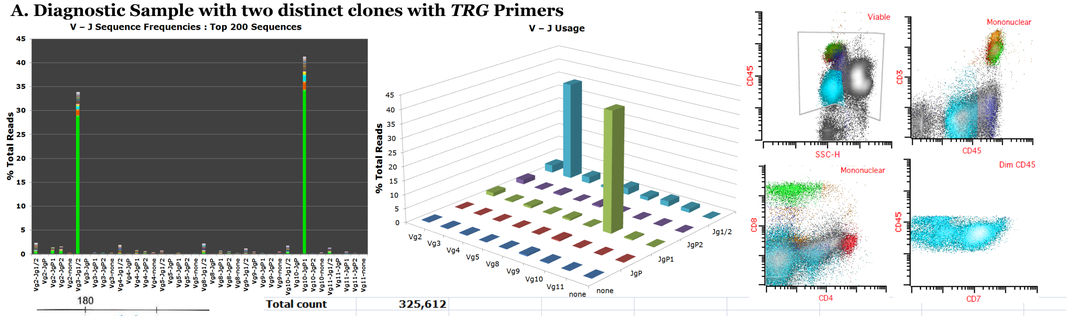
Table 1: Lymphoid and Plasma Cell Neoplasm cases used for Residual Disease Detection by LymphoTrack®

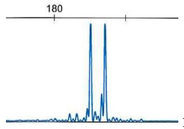
Table 2: Summary of Total Sequencing Reads and Percentage of Reads supporting Residual Disease

	# of Total Sequencing Reads	% of Reads supporting Residual Disease
Lowest	50,634	0.0020
Highest	7,026,781	76.2467
Median	1,106,490	1.0119

Results

A. Diagnostic Sample with two distinct clones with *TRG* Primers



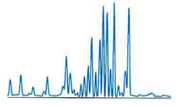


Total count 325,612

Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Cumulative %	
1	TGGGTAAGACAAC	148	111498	Vg10	JgP1	34.2425955	34.2425955	DS1
2	AGAATCAGTAGAC	144	94089	Vg3	Jg1/2	28.8960481	63.1386435	DS2
3	TGGGTAAGACAAC	148	5600	Vg10	JgP1	1.7198383	64.8584819	
4	TGGGTAAGACAAC	148	4604	Vg10	JgP1	1.4139528	66.2724347	
5	AGAATCAGTAGAC	144	4188	Vg3	Jg1/2	1.2861934	67.5586281	

Flow: 8% Abnormal T-Lymphoblasts; 12% Blasts on Aspirate Smears

B. 1 Month Post 2nd Allogeneic Stem Cell Transplant

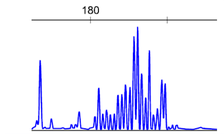


Total count 435,837 DS2 not found

Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Column1	
359	GGAATCAGCCCAC	138	81	Vg4	JgP1	0.0185849		
360	GGAGTCAGTCCAC	151	81	Vg2	JgP2	0.0185849		
361	TGGGTAAGACAAC	148	81	Vg10	JgP1	0.0185849		DS1
362	TGGGTAAGACAAC	156	81	Vg10	Jg1/2	0.0185849		
363	GGATTCAGTCCAC	147	81	Vg2	JgP2	0.0185849		
364	GGAGTCAGTCCAC	142	81	Vg2	Jg1/2	0.0185849		

Flow: No Abnormal T cells; Morphologic Remission

C. 6 Months Post 2nd Allogeneic Stem Cell Transplant

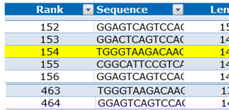


Total count 914,912 DS1 not found

Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Column1	
282	GGAGTCAGTCCAC	145	215	Vg2	Jg1/2	0.0234995		
283	GGAGTCAGTCCAC	150	215	Vg2	JgP2	0.0234995		
284	AGAATCAGTAGAC	144	214	Vg3	Jg1/2	0.0233902		DS2
285	GGAATCAGCCCAC	139	214	Vg4	Jg1/2	0.0233902		
286	GGAGTCAGTCCAC	152	213	Vg2	JgP2	0.0232809		
287	TGGGTAAGACAAC	152	212	Vg10	JgP1	0.0231716		

Flow: 0.0026% Abnormal T-Lymphoblasts; 1% Blasts on Aspirate Smears

D. 8 Months Post 2nd Allogeneic Stem Cell Transplant



Total count 688,110

Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Column1
152	GGAGTCAGTCCAC	155	357	Vg2	JgP2	0.0518812	
153	GGACTCAGTCCAC	148	354	Vg3	Jg1/2	0.0514453	
154	TGGGTAAGACAAC	148	351	Vg10	JgP1	0.0510093	DS1
155	CGGCATTCCGTCA	145	347	Vg9	JgP	0.0504280	
156	GGAGTCAGTCCAC	147	344	Vg2	JgP1	0.0499920	
463	TGGGTAAGACAAC	138	93	Vg10	JgP1	0.0135153	
464	GGAGTCAGTCCAC	147	93	Vg2	JgP2	0.0135153	
465	AGAATCAGTAGAC	144	92	Vg3	Jg1/2	0.0133700	DS2
466	GGACTCAGTCCAC	148	92	Vg3	Jg1/2	0.0133700	
467	GGAGTCAGTCCAC	150	92	Vg2	JgP2	0.0133700	
468	GGAATCAGCCCAC	135	92	Vg4	none	0.0133700	

Flow: No Abnormal T cells; Morphologic Remission

Fig. 1: Residual Disease Detection in a Patient with Relapsed T-Lymphoblastic Leukemia/Lymphoma, using *TRG* Primers on LymphoTrack®. The patient shows evidence of subsequent relapse by PET scan, and is currently on palliative therapy.

Results

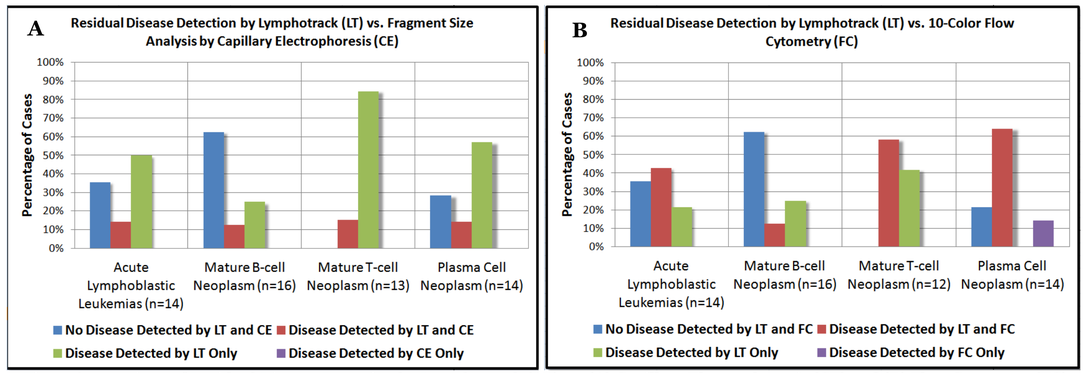


Fig. 2: Comparison of Residual Disease Detection by Lymphotrack (LT) vs. (A) Capillary Electrophoresis (CE) and (B) Flow Cytometry (FC)

- In 2/14 Plasma Cell Neoplasm PT samples, FC detected suspicious atypical plasma cells (0.0007% and 0.0028% of total WBC), but no clonal sequence detected by LT.
  - In one sample, total sequencing reads was suboptimal for MRD detection (65,960 total reads).
  - In the other sample, two subsequent samples from the same patient showed no evidence of disease by all detection methods.
- In 12 PT samples from 10 patients, LT detected residual disease, while neither FC nor CE detected disease.
  - 2/10 patients showed subsequent overt evidence of persistent/recurrent diseases, with median follow-up time of 3 months.
- In 18 PT samples from 17 patients, there is no evidence of residual disease by all detection methods.
  - 16/17 patients showed no subsequent evidence of disease, with median follow-up time of 2.7 months.

Conclusion

Compared to capillary electrophoresis and flow cytometry, LymphoTrack® provides comparable or better MRD detection sensitivity of lymphoid neoplasms, and with increased diagnostic certainty by utilizing patient-specific clonal sequences for MRD detection.

Acknowledgements

The authors would like to thank the Diagnostic Molecular Pathology Laboratory members and Invivoscribe for their technical support.

References

1. Emerson RO, et al. Defining the alloreactive T cell repertoire using high-throughput sequencing of mixed lymphocyte reaction culture. *PLoS One* 2014;9(11):e111943
2. Logan AC, Et al. Immunoglobulin and T cell receptor gene high-throughput sequencing quantifies minimal residual disease in acute lymphoblastic leukemia and predicts post-transplantation relapse and survival. *Biol Blood Marrow Transplant* 2014;20:1307-13.
3. Miller, J.E. (2013). Principle of Immunoglobulin and T Cell Receptor Gene Rearrangement. In Cheng L, Zhang D, Eble J.N. (Eds), *Molecular Genetic Pathology* (2nd Ed). New York, USA: Springer Science & Business Media.
4. Schumacher JA, et al. A comparison of deep sequencing of TCRG rearrangements vs traditional capillary electrophoresis for assessment of clonality in T-Cell lymphoproliferative disorders. *Am J Clin Pathol.* 2014;141(3):348-59
5. Wu D, et al. Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IGH. *Clin Cancer Res.* 2014;20(17):4540-8

AMP Annual Meeting, November 10-12 2016, Charlotte, NC