Detection of Clonal Rearrangements in Multiple Myeloma Samples using LymphoTrack® Assays

H045

Ying Huang[1], Austin Jacobsen[1], Jeff Panganiban[1], Edgar Vigil[1], Kasey Hutt [1], Joshua Heilman[1], Martin Blankfard[2], Even H Rustad[2], Malin Hultcrantz[2], Ola Landgren[2] and Jeffrey E. Miller[1]

[1]Invivoscribe, Inc., San Diego, USA, [2]Memorial Sloan Kettering Cancer Center, New York, USA

Introduction

Multiple myeloma (MM) is a malignancy of plasma cells. Currently, multiparameter flow cytometry (MFC) is the tool most often used to detect and monitor MM in patients. However, MFC requires fresh specimens and is difficult to standardize between test centers. Since genomic DNA is stable and the assays, platforms, and the accompanying software can be easily standardized between test centers, we developed next generation sequencing (NGS)-based assays with bioinformatics software to detect the clonal Ig rearrangements associated with MM and track Minimal Residual Disease (MRD). Here we report the results of a pilot study of clonal rearrangement detection and tracking by testing 193 anonymized/blinded MM specimens using LymphoTrack Assays. These assays identify B-cell populations by targeting the *IGHV* Leader, *IGH* FR1, FR2, FR3, and *IGK*. Thirty (30) follow-up samples were also tested to demonstrate MRD utility.

Materials and Methods

- Five (5) LymphoTrack® Assays (*IGHV* Leader, *IGH* FR1, *IGH* FR2, *IGH* FR3 and *IGK*) for the MiSeq®, each with available with 24 indices, were designed, developed, and manufactured per cGMP, then QC tested and validated under a QSR-compliant regulatory system.
- 50 ng of genomic DNA (only ~7,700 cell equivalents) from 193 MM bone marrow (BM) baseline specimens were procured, anonymized and blinded prior to testing with the four LymphoTrack Assays (*IGH* FR1, FR2, FR3 and IGK) using the MiSeq® platform.
- Libraries generated from all 4 assays were purified, harmonized, pooled, and sequenced in a single MiSeq run. Fourteen (14) samples that tested negative by the above 4 assays were reflex tested with the 5th LymphoTrack Assay, IGHV Leader.
- The most prominent clonal rearrangement sequence identified by one of the LymphoTrack Assays was then tracked using the single assay.
- 200 ng DNA (~ 30,000 cells), typically from "last pull" aspirates with low tumor cell content were tested from 30 subsequent residual follow up specimens.
- In order to generate an estimation of lymphoid cell equivalents within each specimen LymphoQuant® Internal control (IC; 100 cell equivalents) was added to each PCR reaction in follow up specimens.
- LymphoTrack® Software (MiSeq®) and LymphoTrack MRD software were used to analyze the sequencing results from baseline and follow up samples, respectively.

Conclusions

• Despite testing baseline specimens which were generally from "last pull" aspirates with low tumor cell content (50ng ~= 7,700 cell equivalents), LymphoTrack Assays were shown to detect clonality in about 80% of MM baseline research specimens.

• Despite testing only 200 ng DNA (~ 30,000 cells), typically from "last pull" aspirates with low tumor cell content, the same LymphoTrack Assays and testing procedures were able to track the clonal sequences in 87% of MM follow up specimens.

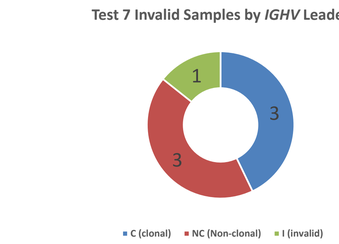
• The presence of LymphoQuant IC within each PCR reaction allowed the % clonal cells within each specimen to be estimated.

• LymphoTrack Assays can be potentially useful tools to identify and monitor disease status in MM samples at both diagnosis and subsequent time points throughout the course of treatment.

• Unlike MFC, the LymphoTrack Assays and accompanying bioinformatics software can be easily standardized between laboratories and submitted for approval to regulatory authorities worldwide.

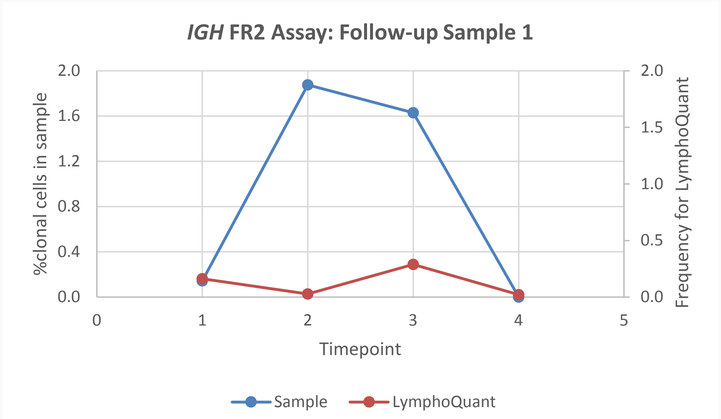
Results: Using LymphoTrack® Assays - MiSeq® to Detect Clonality in Baseline MM Samples

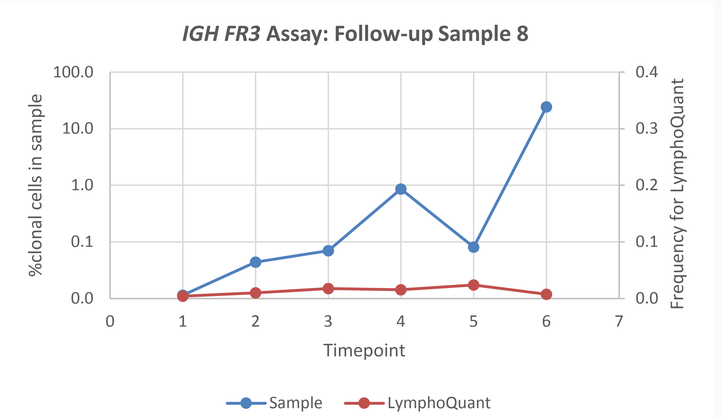
Total N=193	*IGH* FR1	*IGH* FR2	*IGH* FR3	*IGK*	Overall (*IGH* FR1/FR2/FR3 + *IGK*)
C (Clonal)	88/193 (45.6%)	81/193 (42.0%)	90/193 (46.6%)	106/193 (54.9%)	152/193 (78.8%)
NC (Non-clonal)	83/193 (43.0%)	109/193 (56.5%)	101/193 (52.3%)	78/193 (40.4%)	34/193 (17.6%)
I (Invalid)	22/193 (11.4%)	3/193 (1.6%)	2/193 (1.0%)	9/193 (4.7%)	**7**/193 (3.6%)



Results: Using LymphoTrack® Assays - MiSeq ® to Track Sequences in Follow up MM Samples

Follow-up Sample	Assay	MRD Results	% Clonal cells in sample	LymphoQuant Frequency
Sample1_TP1	FR2	Detected	0.144	1.63E-01
Sample1_TP2	FR2	Detected	1.876	2.83E-02
Sample1_TP3	FR2	Detected	1.628	2.89E-01
Sample1_TP4	FR2	Detected	0.001	2.16E-02
Smaple2_TP1	FR2	Detected	0.004	1.22E-01
Smaple2_TP2	FR2	Detected	0.003	1.16E-01
Sample3_TP1	IGK	Detected	0.002	2.63E-01
Sample3_TP2	IGK	Detected	0.001	2.71E-02
Sample4_TP1	FR3	Not Detected	0.000	7.12E-02
Sample5_TP1	IGK	Detected	1.717	1.17E-01
Sample5_TP2	IGK	Detected	0.093	1.44E-01
Sample6_TP1	IGK	Detected	0.001	2.15E-01
Sample6_TP2	IGK	Detected	0.003	7.35E-02
Sample6_TP3	IGK	Detected	0.004	9.63E-02
Sample6_TP4	IGK	Detected	0.019	1.05E-01
Sample6_TP5	IGK	Detected	0.002	1.25E-01
Sample7_TP1	FR3	Detected	2.773	5.38E-02
Sample7_TP2	FR3	Detected	0.346	1.55E-02
Sample8_TP1	FR3	Detected	0.011	4.14E-03
Sample8_TP2	FR3	Detected	0.044	9.97E-03
Sample8_TP3	FR3	Detected	0.070	1.77E-02
Sample8_TP4	FR3	Detected	0.862	1.55E-02
Sample8_TP5	FR3	Detected	0.081	2.38E-02
Sample8_TP6	FR3	Detected	24.223	7.46E-03
Sample9_TP1	FR2	Detected	1.180	1.19E-01
Sample9_TP2	FR2	Detected	0.035	2.57E-01
Sample10_TP1	FR3	Not Detected	0.000	2.93E-01
Sample10_TP2	FR3	Detected	0.002	1.51E-02
Sample11_TP1	FR1	Not Detected	0.000	8.15E-01
Sample12_TP1	FR3	Not Detected	0.000	2.46E-01
Overall detecting samples: 26/30 (87%)				





AMP, November 1-3 2018, San Antonio, TX, USA