Comparison of LymphoTrack® Assays - MiSeq® and Flow Cytometry for Clonality and Minimum Residual Disease Assessment in Multiple Myeloma

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Background

Multiple myeloma (MM), characterized by presence of excess plasma cells (PCs) in bone marrow (BM), accounts for about 10% of all hematological malignancies. Multiparameter flow cytometry (MFC) is a standard tool used to detect and monitor MM in patients. PCR-based NGS methods have been developed to identify patient-specific gene rearrangements (clonotypes) within the immunoglobulin (*Ig*) loci, and recently international organizations such as NCCN, IMWG, and ESMO have included NGS as a recommended tool for MRD assessment in MM. We developed NGS-based LymphoTrack Assays and bioinformatics software to detect and track clonal rearrangements in B-cell malignancies using the Illumina MiSeq platform. Here we compare the ability of the LymphoTrack *IGH* FR1, FR2, FR3, and *IGK* assays to identify and track patient-specific clonotypes with detection and monitoring by MFC on 101 anonymized, paired diagnostic and MRD MM specimens.

Methods

- Schematic Illustration of the *IGH* gene

- Schematic Illustration of the *IGK* gene

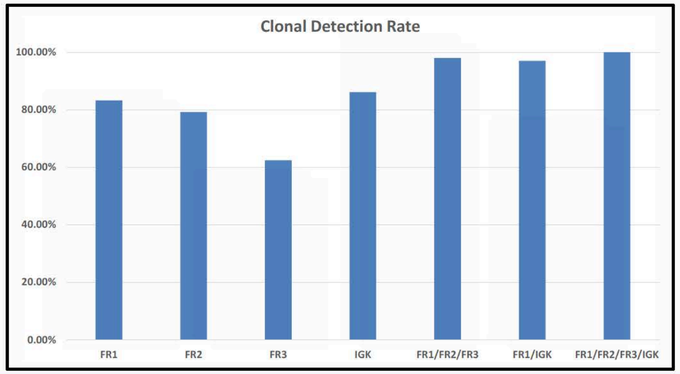
- The workflow for the LymphoTrack *IGH* FR1, *IGH* FR2, *IGH* FR3, and *IGK* Assays - MiSeq

- The LymphoTrack *IGH* FR1 , *IGH* FR2, *IGH* FR3, and *IGK* Assays for the MiSeq were manufactured under cGMP standards per an ISO 13485 certified QMS. Each assay consists of a one step PCR Master Mix with 24 different indices to allow the simultaneous testing of multiple samples and targets on the same MiSeq flow cell.
- A complimentary bioinformatics software package: LymphoTrack Dx Software - MiSeq & LymphoTrack MRD Software (RUO), were developed and validated under ISO13485 design control.
- 101 paired (diagnostic and MRD) BM samples from MM subjects were tested by MFC utilizing a 8-color direct immunofluorescence technique.
- Genomic DNA was extracted from the same BM samples, anonymized, and blinded for testing with the LymphoTrack *IGH* FR1, *IGH* FR2, *IGH* FR3 and *IGK* Assays - MiSeq.
- DNA from diagnostic samples were tested using 50 ng of DNA for each 4 LymphoTrack assays (*IGH* FR1, *IGH* FR2, *IGH* FR3 and *IGK*) to identify sample-specific clonotype, which was then tracked using a single assay in MRD samples. Libraries from diagnostic samples were sequenced with all targets combined together.
- DNA from 84 MRD samples, whose paired diagnostic samples were *IGH* FR1 positive, were tested using 700 ng of DNA (or highest amount available) for the LymphoTrack *IGH* FR1 assay.
- LymphoQuant® control DNA from clonal cells was added to each PCR reaction at 1,000 cell equivalency when testing these MRD samples to allow the estimation of cell equivalents within each MRD sample tested.
- MiSeq FASTQ files from diagnostic and MRD samples were analyzed by the LymphoTrack Dx Software – MiSeq and LymphoTrack MRD Software (RUO), respectively.

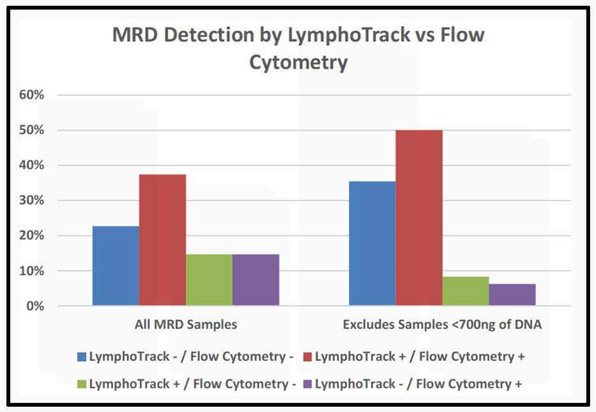
Results: Diagnostic Samples by LymphoTrack Assays - MiSeq

Individual Assays	FR1	FR2	FR3	IGK
Clonal (C)	84/101 (83.2%)	80/101 (79.2%)	63/101 (62.4%)	87/101 (86.1%)
Non-Clonal (NC)	15/101 (14.9%)	21/101 (20.8%)	38/101 (37.6%)	14/101 (13.9%)
Invalid (I)	2/101 (2.0%)	0/101 (0%)	0/101 (0%)	0/101 (0%)

Individual Assays	FR1/FR2/FR3	FR1/IGK	FR1/FR2/FR3/IGK
Clonal (C)	99/101 (98.0%)	98/101 (97%)	101/101 (100%)
Non-Clonal (NC)	2/101 (2%)	2/101 (2.0%)	0/101 (0%)
Invalid (I)	0/101 (0%)	1/101 (1%)	0/101 (0%)



Results: MRD Samples by LymphoTrack *IGH* FR1 Assay - MiSeq



Results: MRD Samples by LymphoTrack *IGH* FR1 Assay - MiSeq

MRD Samples Excluding <700ng DNA		Flow Cytometry	
LymphoTrack FR1 MiSeq Assay	N=48	Detected	Not Detected
	Detected	24	4
	Not Detected	3	17

Concordance	85.4%
Sensitivity	88.9%
Specificity	81.0%

Discordant MRD Sample	LymphoTrack NGS FR1 MiSeq with LymphoQuant		Multiparameter Flow Cytometry	
	Total reads	MRD Status (Cell Equivalent)	%Tumor Plasma cells/sample	MRD Status
9	829,841	Not detected	5.10E-04	Detected
41	687,943	Not detected	6.29E-02	Detected
44	746,321	Not detected	3.34E-03	Detected
17	705,001	Detected (1.40E-03)	0.00E+00	Not detected
24	778,407	Detected (7.49E-03)	0.00E+00	Not detected
27	862,395	Detected (1.08E-02)	0.00E+00	Not detected
60	895,466	Detected (6.56E-01)	0.00E+00	Not detected

Conclusions

- The combination of LymphoTrack *IGH* FR1, *IGH* FR2, *IGH* FR3 and *IGK* Assays - MiSeq was able to detect clonotype sequences in 100% of diagnostic samples from MM subjects.
- The LymphoTrack *IGH* FR1 Assay – MiSeq, by itself, achieved 85.4% agreement with MFC in detecting paired MRD samples from MM subjects.
- With the LymphoTrack Dx Assays, the same reagents and workflow were utilized for both initial clonality testing and for tracking of clonal populations in MM samples.
- Unlike MFC assays, the LymphoTrack Assays and accompanying bioinformatics software can be submitted for approval to regulatory authorities worldwide.

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