P020

Comparing Minimal Residual Disease Detection in Multiple Myeloma using NGS-Based LymphoTrack® Assays and Flow Cytometry

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Introduction

Multiple myeloma (MM), which can be identified by the presence of >10% clonal plasma cells in bone morrow (BM), is the second most common hematological malignancy. Multiparameter flow cytometry (MFC) is a standard tool used to detect and monitor MM patients. Next generation sequencing (NGS) based methods have demonstrated advantages with improved sensitivity, and international organizations (NCCN, IMWG and ESMO) have recently included NGS for minimal residual disease (MRD) assessment in MM. Here we report results of a pilot study comparing the detection of MRD using NGS-based LymphoTrack® Assays and MFC by testing 101 specimens from MM subjects.

Materials and Methods

The LymphoTrack® *IGH* and *IGK* Assays for the MiSeq were manufactured under cGMP standards per ISO 13485. Master Mix with 24 different indices to allow the simultaneous testing of multiple samples and targets on the same MiSeq® flow cell.

The workflow for the LymphoTrack *IGH* FR1, *IGH* FR2, *IGH* FR3, and *IGK* Assays is depicted below:

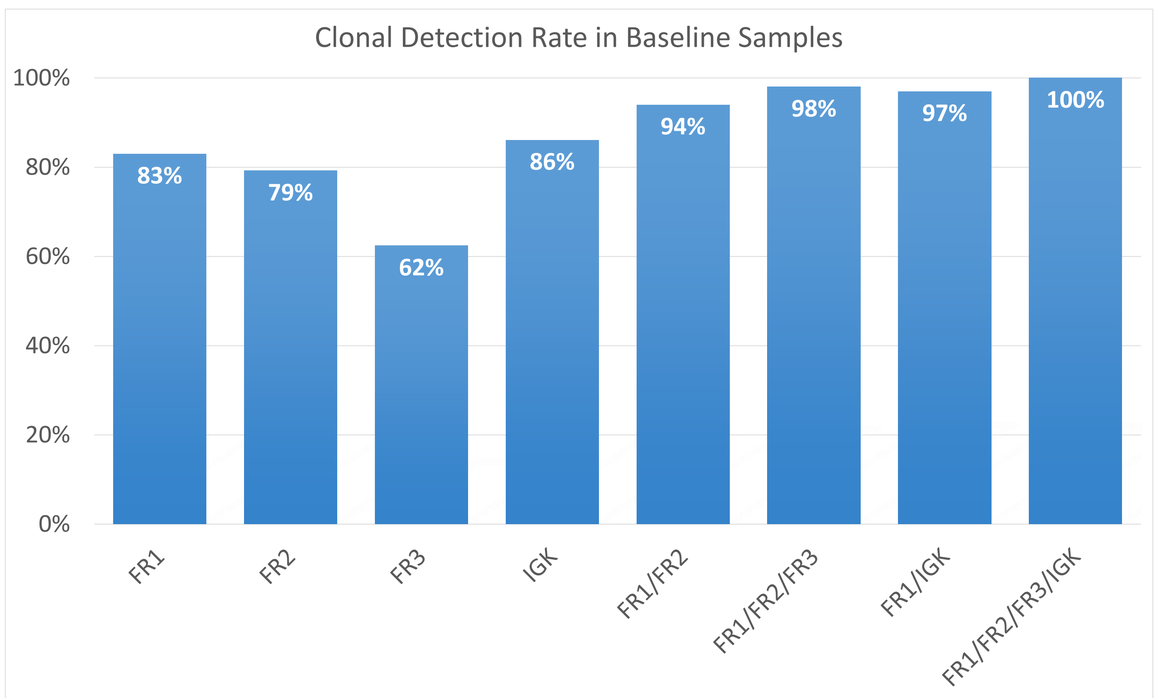
PCR → Purify Amplicons → Quantify Amplicons → Pool Library → Run MiSeq® → Analyze Data

101 paired BM samples from MM patients were tested in this study. The MFC methods utilized an 8-color direct immunofluorescence technique and tested 1-5 million cells. Our NGS based MRD assessment tested less than ~1/10 as many cell equivalents of genomic DNA (0.7µg, ~107,000 cell equivalents) from the collected specimens which were blinded prior to testing with four LymphoTrack Assays (*IGH* FR1, FR2, FR3 and *IGK*) on a MiSeq®. LymphoTrack Software was used, which automatically sorted data by target and index. The primary clonal rearrangement (clonotype) identified by LymphoTrack FR1 or FR2 Assay was then used to track disease in the 91 subsequent samples. LymphoQuant® Internal Control was added to each PCR reaction at 100 cell equivalents to estimate the cell equivalents within each sample. LymphoTrack Software and LymphoTrack MRD software were used to analyze the sequencing results from baseline and follow up samples, respectively.

Results: Baseline Samples by LymphoTrack Assays – MiSeq®

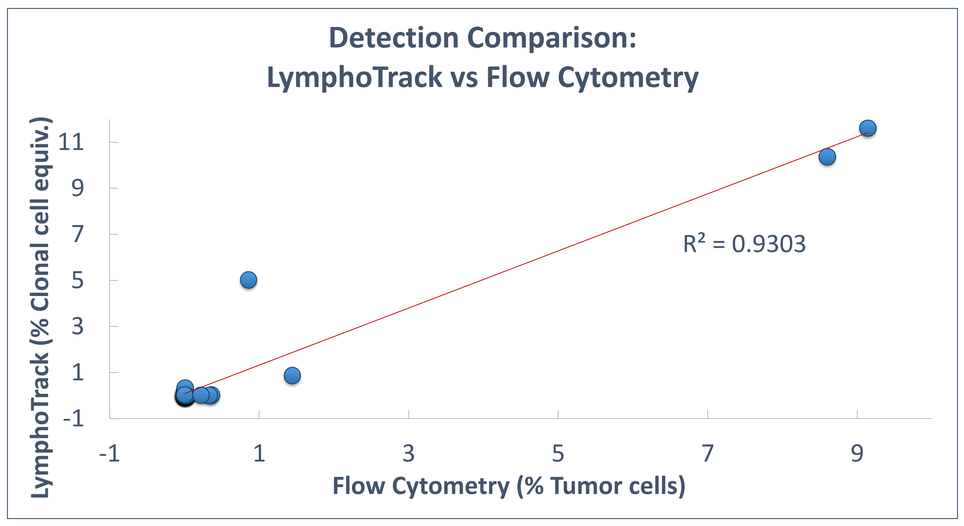
Individual Assays	FR1	FR2	FR3	*IGK*
Clonal (C)	84/101 (83.2%)	80/101 (79.2%)	63/101 (62.4%)	87/101 (86.1%)
Non-Clonal (NC)	15/101 (14.9%)	21/101 (20.8%)	38/101 (37.6%)	14/101 (13.9%)
Invalid (I)	2/101 (2.0%)	0/101 (0%)	0/101 (0%)	0/101 (0%)

Combined Assay Results	FR1/FR2	FR1/FR2/FR3	FR1/*IGK*	FR1/FR2/FR3/*IGK*
Clonal (C)	95/101 (94%)	99/101 (98%)	98/101 (97%)	101/101 (100%)
Non-Clonal (NC)	6/101 (6%)	2/101 (2%)	2/101 (2%)	0/101 (0%)
Invalid (I)	0/101 (0%)	0/101 (0%)	1/101 (1%)	0/101 (0%)

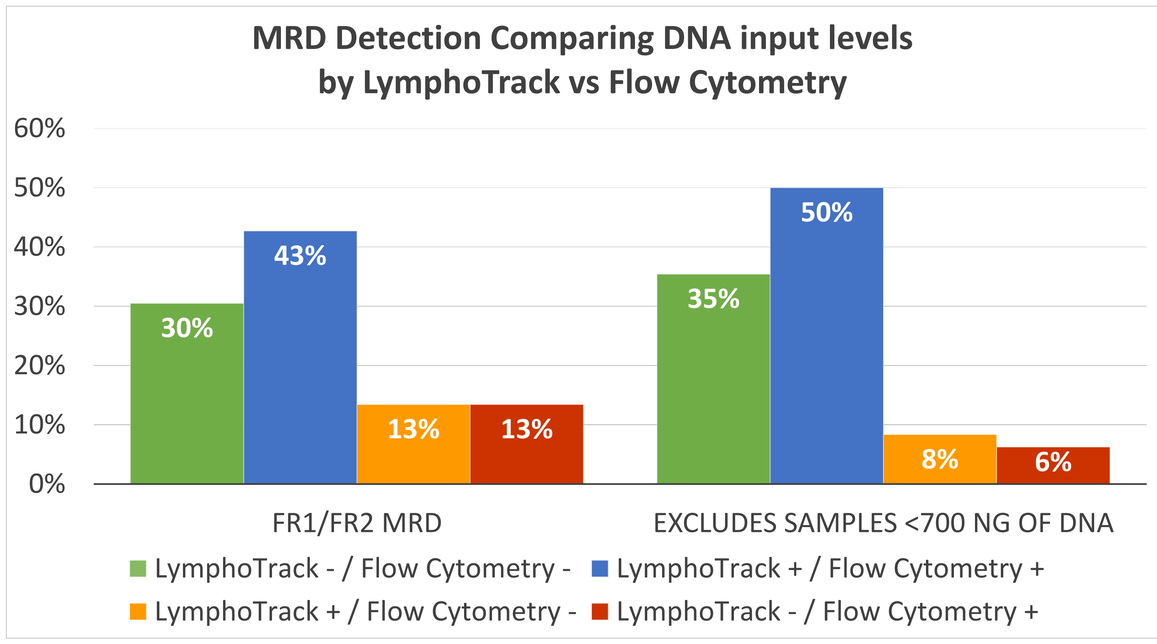


Results: MRD Samples by LymphoTrack Assays – MiSeq®

MRD Samples for FR1/FR2		MFC	
LymphoTrack® FR1/FR2 MiSeq® Assay	N=82	Detected	Not Detected
	Detected	35	11
	Not Detected	11	25
	Concordance	73.2%	



Samples (N=11) are LymphoTrack - / MFC+					
Sample	LymphoTrack DNA input (Cell Equiv.)	%Tumor Cells by MFC	Sample	LymphoTrack DNA input (Cell Equiv.)	%Tumor Cells by MFC
9	700ng (~1.1x10^5)	0.00051	59	289ng (~4.4x10^4)	0.001
41	700ng (~1.1x10^5)	0.06288	80	225ng (~3.5x10^4)	0.03204
44	700ng (~1.1x10^5)	0.003336	83	138ng (~2.1x10^4)	0.086148
47	486ng (~7.5x10^4)	0.036504	90	243ng (~3.7x10^4)	0.789004
49	430ng (~6.6x10^4)	0.04552	101	237ng (~3.6x10^4)	0.19221
51	263ng (~4.0x10^4)	0.0008			



MRD Samples (Excluding <700 ng DNA)		MFC	
LymphoTrack *IGH* FR1 MiSeq® Assay Alone	N=48	Detected	Not Detected
	Detected	23	4
	Not Detected	3	18
	Concordance	85.4%	

Conclusions

- LymphoTrack assays were able to detect clonotype sequences in 100% of baseline samples from MM subjects.
- LymphoTrack *IGH* FR1 Assay achieved 85.4% agreement with MFC in detecting MRD - despite testing only 1/10th cell equivalents used in MFC assessment.
- LymphoTrack assays when spiked with LymphoQuant Internal Control were able to report clonal cell percentage in MRD samples and have a good correlation (R^2= 0.93) with MFC measured % Tumor cells.
- Unlike MFC assays, the LymphoTrack Assays and accompanying bioinformatics software can be validated for submission to regulatory authorities worldwide.

AMP Global May 16-18 2019 Hong Kong