AN ULTRA-SENSITIVE METHOD FOR SEQUENCING AND MONITORING M-PROTEIN IN PERIPHERAL BLOOD

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Introduction

With the improvement of therapy, Minimal Residual Disease (MRD) tracking is of high importance to manage Multiple Myeloma disease (MM). Most sensitive MRD assays to date are based on quantification of clonal plasmocytes by next generation sequencing or flow cytometry in bone marrow aspirates. However, such methods have shown limitations such as being invasive, sample heterogeneity and lacking the possibility for frequent sampling. Frequent MM monitoring on blood at equivalent sensitivity than achieved in bone marrow could provide actionable information on disease activity and detect early signs of progression.

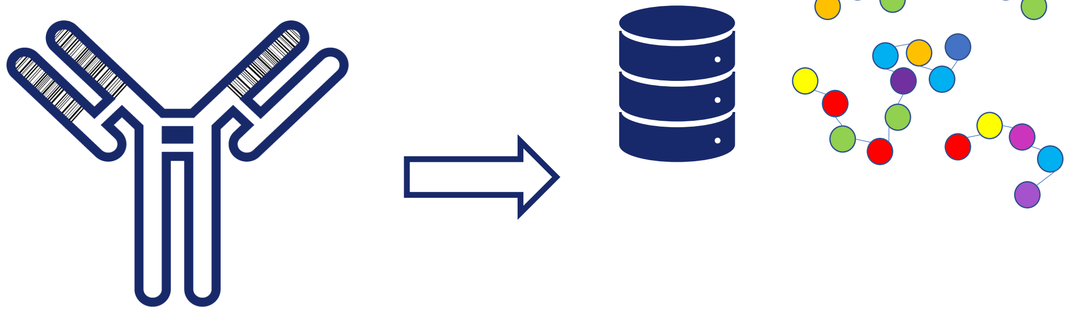
M-protein is a well-established biomarker used for MM diagnostic and monitoring. Mass Spectrometry (MS) has been introduced as a possibility to monitor M-protein [1]. Intact protein measurement by MS has the drawback of lacking in sensitivity with high interference from the polyclonal background, in contrast clonotypic peptides originating from the variable region of the M-protein are unique for each patient relieving this interference.

The aim of this study is to demonstrate the feasibility of sequencing and monitoring M-protein in serum samples with M-InSight.

Method

Therapy response of 41 Multiple Myeloma patients from the IFM-2009 clinical trial (NCT01191060) was used to evaluate the assay.

M-InSight assay:



M-InSight uses a novel de novo approach using Peaks Ab[2] software to sequence the M-protein with MS from serum that is further assembled into full length HC (Heavy Chain) and LC (Light chain) sequences

Several clonotypic peptides were further chosen with the use of an in-house bioinformatics algorithm to select the best candidate. Each peptide is chosen to be specific to each patient (CDR region) from both chains.

Clonotypic peptides are used to quantitate M-protein in patients' serum during each phase of the treatment.

All 41 M-proteins were sequenced by MS, and then compared to RNA sequencing data based on total cDNA from all expressed genes. Trust4[3] was used to construct clonotypes and to identify clonal molecular fingerprints and finally their clonotypic peptides based on transcriptomic datasets.

M-protein concentrations were determined by calibration on a sample with a known M-protein concentration quantified by an agarose gel electrophoresis system (Hydrasys 2, Sebia).

Figure 1 shows the clinical trial design of IFM 2009.

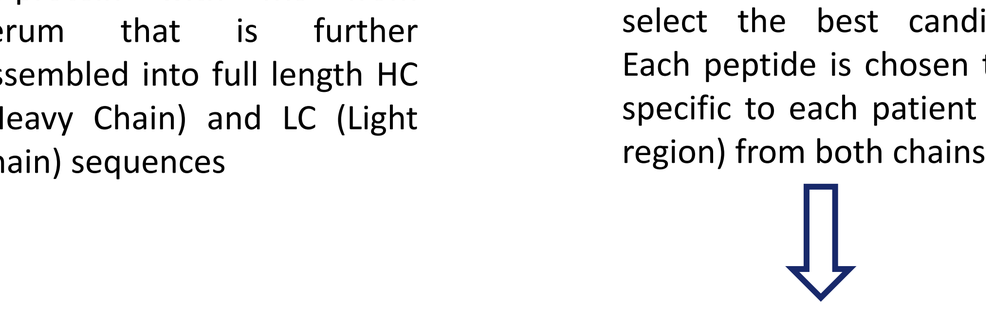


Figure 1: Design of IFM 2009 clinical trial

Results

De novo sequencing using MS showed a very high concordance with the RNA sequences obtained from total cDNA assay alignment (Figure 2). The 41 patients used in the study showed a minimum coverage of 92% and median of 97% regardless the isotype. However, a strong alignment requires a sample with M-protein concentration >2 g/L; a lowest coverage was observed for a FLC-K patient with a non measurable amount of M-protein with SPEP.

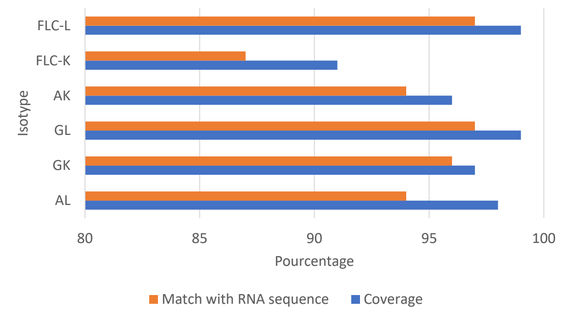
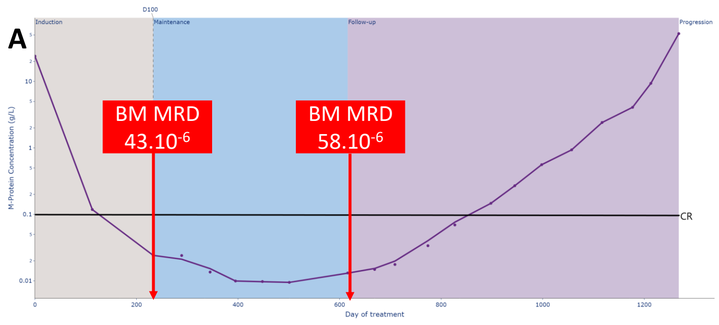


Figure 2: Bood-based De novo sequencing by MS of M-protein, pourcentage of coverage and match with the RNA seq obtained from bone marrow cDNA extract

Figure 3A shows the M-protein concentration of a patient from Arm A who has a relapse starting during the maintenance, while figure 3B shows the concentration of M-protein of a patient from Arm B that has a long stable disease at MRD neg level for over 2 years. Results show a very high sensitivity, with M-protein still detectable by M-InSight despite MRD negativity determined by next generation sequencing on bone marrow aspirate.



Isotype	AL
Treatment Arm	A
M Spike	24
FLC Kappa (mg/L)	8,15
FLC Lambda (mg/L)	126
Best assessment	sCR
PFS (months)	40
Lowest concentration (mg/L)	9,1
Relapse alert	660 days
Progression	1264 days

Isotype	GL
Treatment Arm	B
M Spike	64,1
FLC Kappa (mg/L)	1,07
FLC Lambda (mg/L)	255
Best assessment	sCR
PFS (months)	69
Lowest concentration (mg/L)	0,3
Relapse alert	1510 days
Progression	NA

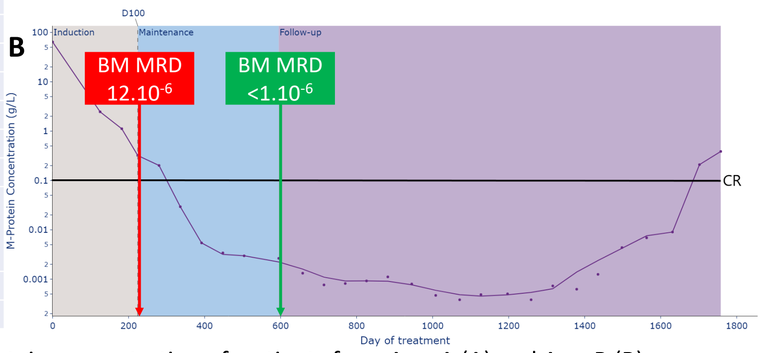


Figure 3: M-protein concentration of patients from Arm A (A) and Arm B (B)

Conclusions

The newly developed and validated M-InSight assay is presented as an ultra-sensitive fully blood-based assay to sequence and monitor M-protein with the possibility for frequent non-invasive MRD monitoring. De novo sequencing using MS on serum sample showed a high concordance versus sequences obtained from RNA seq.

The best sensitivity achieved by M-InSight, detecting 0.2 mg/L of M-protein, was 1000- and 100-fold more sensitive compared to SPE and intact protein MS method, respectively[4]. High dynamic range of the assay allows the monitoring of the M-protein at each stage of the disease.

References

1. Noori S. Blood Cancer J. 24;13(1) (2023)
2. Zhang J, Mol Cell Proteomics. (2012)
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4. Kohlhagen, M., CCLM, 59 (1) (2021)