

## MRD TRACKING IN BLOOD USING A DE NOVO SEQUENCING FROM SPEP GEL OF M-PROTEIN IN MULTIPLE MYELOMA PATIENTS

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### INTRODUCTION

**Multiple Myeloma (MM)** is considered as a rare blood disease, characterized by the uncontrolled growth of malignant plasma cells in the bone marrow, leading to the secretion of monoclonal immunoglobulin (M-protein).

Current routine techniques like serum protein electrophoresis (SPEP) and immunofixation (IF) offer reliable detection, but their sensitivity is limited for complete response assessment.

**Sebia's M-inSight® assay** emerged as a novel, ultra-sensitive, and non-invasive liquid biopsy tool for monitoring **Minimal Residual Disease (MRD)** in MM patients.

**M-inSight®** utilizes cutting-edge mass spectrometry (MS) to accurately identify and quantify unique clonotypic peptides. Unlike traditional MS techniques analyzing intact M-proteins, **M-inSight®** bypasses interference from the polyclonal background by targeting these unique clonotypic peptides.

Identifying the best clonotypic peptides, typically done with a baseline serum sample, is required for patient signature. This study proposes a combined approach utilizing the high sensitivity of **M-inSight®** with the specificity of HYDRAGEL SPE for M-protein sequencing.

This combination aims to provide an addition to the **M-inSight®** workflow proving a powerful tool for longitudinal monitoring of **MRD** in MM patients.

### METHOD

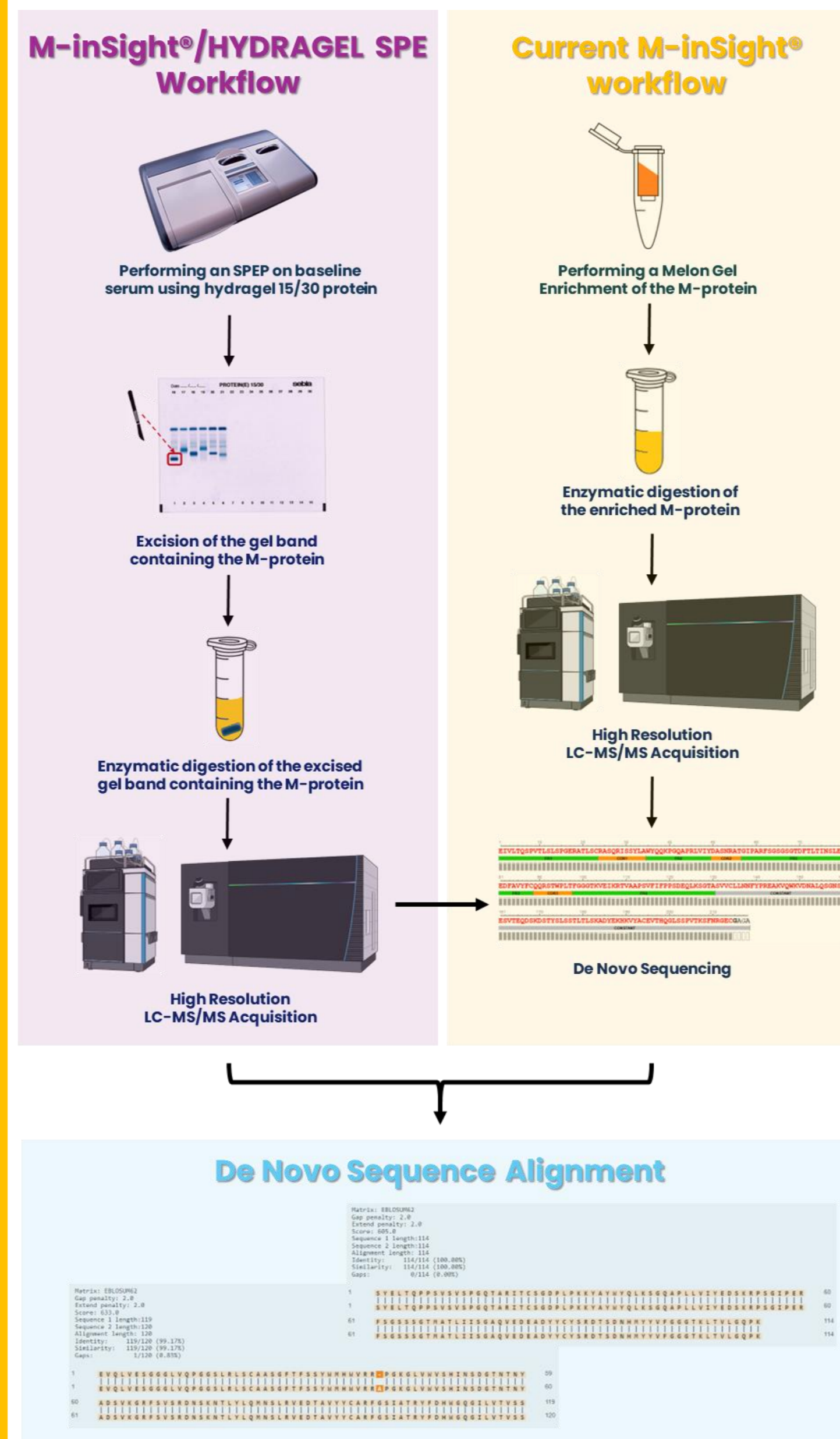


Figure 1: Workflow used for this study.

Samples from 16 newly diagnosed non eligible for transplantation MM patients from a Sanofi clinical trial (NCT02513186) were chosen to assess the de novo sequencing in gel of the M-protein.

All patients were selected from VRDI Part B cohort (bortezomib, lenalidomide, dexamethasone) consisting of evaluating the preliminary efficacy (complete response [CR] rate) of isatuximab administered at the selected dose in combination with bortezomib based regimen.

Serum samples were separated through electrophoresis gel on Hydrasys 2 (Sebia). The M-protein bands were excised and directly digested with specific enzymes for the de novo sequencing using mass spectrometry.

Some serum samples were sequenced using Melon gel enrichment and variable domain sequences were aligned using a bioinformatics online tool (<https://en.vectorbuilder.com/tool/sequence-alignment.html>) to allow a direct comparison of the 2 sequences obtained with both preparations.

Clonotypic peptides have been selected from the hypervariable regions of variable domain from both chain sequences. Each peptides were then monitored by M-inSight for all patients at different time point.

### RESULTS

The **M-inSight®/HYDRAGEL SPE** tandem successfully achieved a sequence coverage of at least 95% with 98% confidence for both chains. This workflow demonstrates a significantly higher confidence level for patient L's sequence compared to the current **M-inSight®** method. The synergy between **M-inSight®** technology and HYDRAGEL SPE ensures high specificity and reliability in de novo sequencing.

Patient	M-protein concentration (g/L)	Sample at	% of sequence coverage				% similarity of Variable Domain		Peptides concordances
			Current M-inSight® Workflow		M-inSight®/HYDRAGEL SPE Workflow		Heavy	Light	
Patient A	33.8	Diagnosis	98%	100%	95%	100%	90.65%	98.23%	✓
Patient B	30.6	Diagnosis	100%	100%	100%	100%	99.26%	100%	✓
Patient C	13.7	Diagnosis	98%	95%	100%	100%	97.52%	85.96%	✓(2/4)
Patient D	55.9	Diagnosis	99%	100%	99%	100%	96.03%	98.15%	✓
Patient E	25.6	Diagnosis	97%	98%	100%	100%	89.47%	98.26%	✓(4/5)
Patient F	10.1	Diagnosis	92%	100%	99%	100%	78.26%	99.11%	✓(2/3)
Patient G	14.8	Diagnosis	97%	100%	100%	100%	87.14%	100%	✓
Patient H	25.9	Diagnosis	100%	100%	100%	100%	100%	81.51%	✓
Patient I	38	Diagnosis	100%	97%	100%	100%	95.16%	97.27%	✓
Patient J	44.5	Diagnosis	100%	100%	100%	100%	98.39%	100%	✓
Patient K	52.2	Diagnosis	100%	100%	100%	100%	98.33%	96.33%	✓
Patient L	n/a	ClD1	93%	81%	96%	99%	81.54%	80.95%	✓
Patient M	37.8	Diagnosis	99%	100%	100%	100%	95.38%	100%	✓
Patient O	n/a	Diagnosis	98%	100%	99%	99%	90%	100%	✓
Patient P	3.26	Diagnosis	98%	100%	100%	100%	91.67%	100%	✓

% of sequence coverage		% similarity of VD		
≥95%	<95%	≥90%	≥80%	80%<

Table 1: Comparative Evaluation of M-inSight® Workflows for M-Protein De Novo Sequencing and MRD Monitoring.

Sequence alignment of variable domains revealed a high degree of similarity between patients, with 13 out of 16 patients exhibiting 90-100% similarity for the light chain and 12 out of 16 for the heavy chain. **Patient F** showed a lower similarity of less than 80% for the heavy chain, potentially indicating a free light chain condition.

Comparison of sequences from both preparations revealed 100% concordance for clonotypic peptides in 13 out of 16 patients, validating the new combined approach for MRD monitoring of a total of 302 serum samples. In 3 out of 16 patients, peptide concordance was at least 50%.

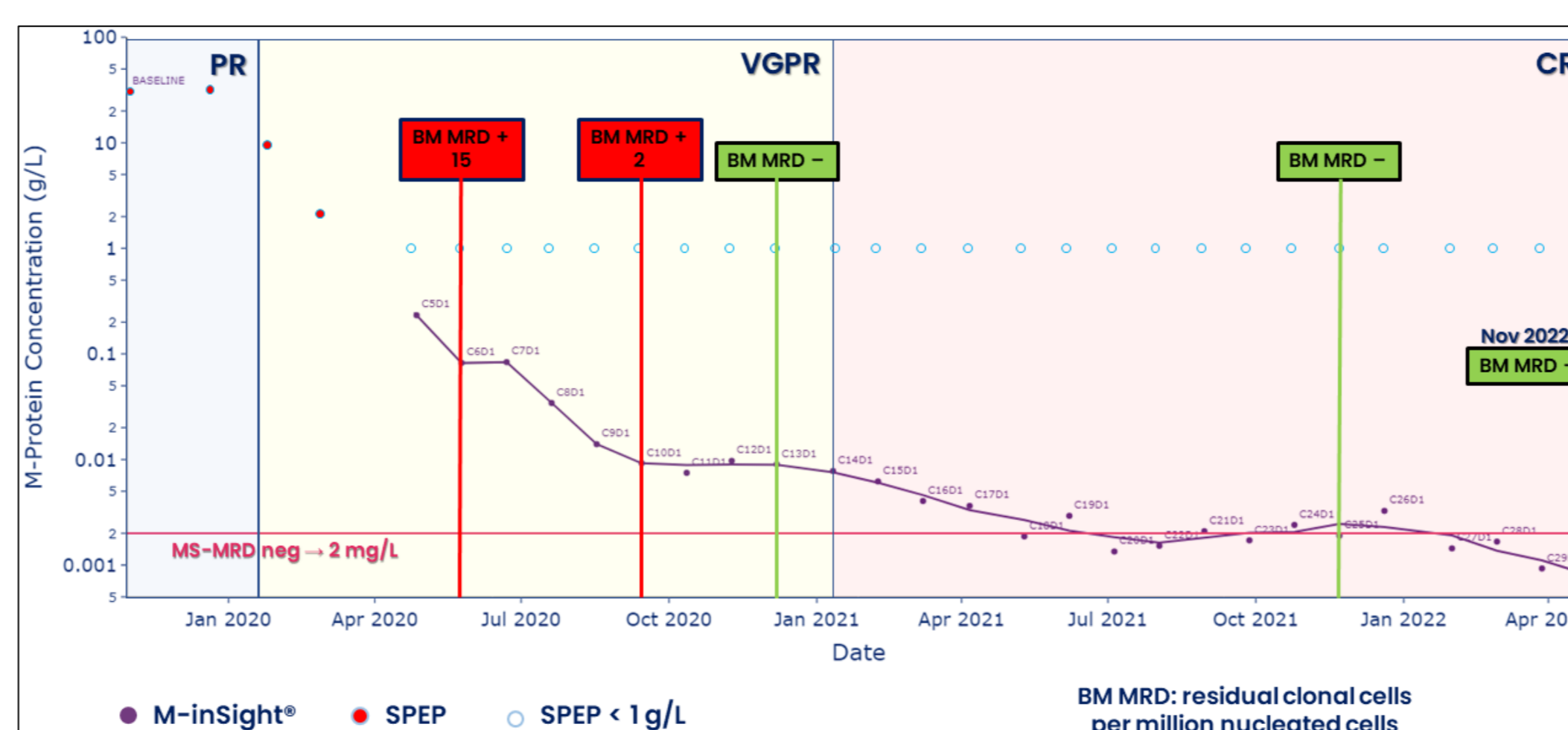


Figure 2: Evaluation of M-Protein Concentration and MRD by M-inSight® for patient B.

The **M-inSight®/HYDRAGEL SPE** tandem successfully monitored the disease in 5 patients with stable disease and low levels of M-protein. All 4 patients who developed progression of disease (PD) had a 2-fold or greater increase in M-protein at 2 consecutive timepoints.

The combined approach also identified new confident clonotypic peptides in two patients with sustained MRD-negative status, suggesting its enhanced sensitivity and ability to provide additional information.

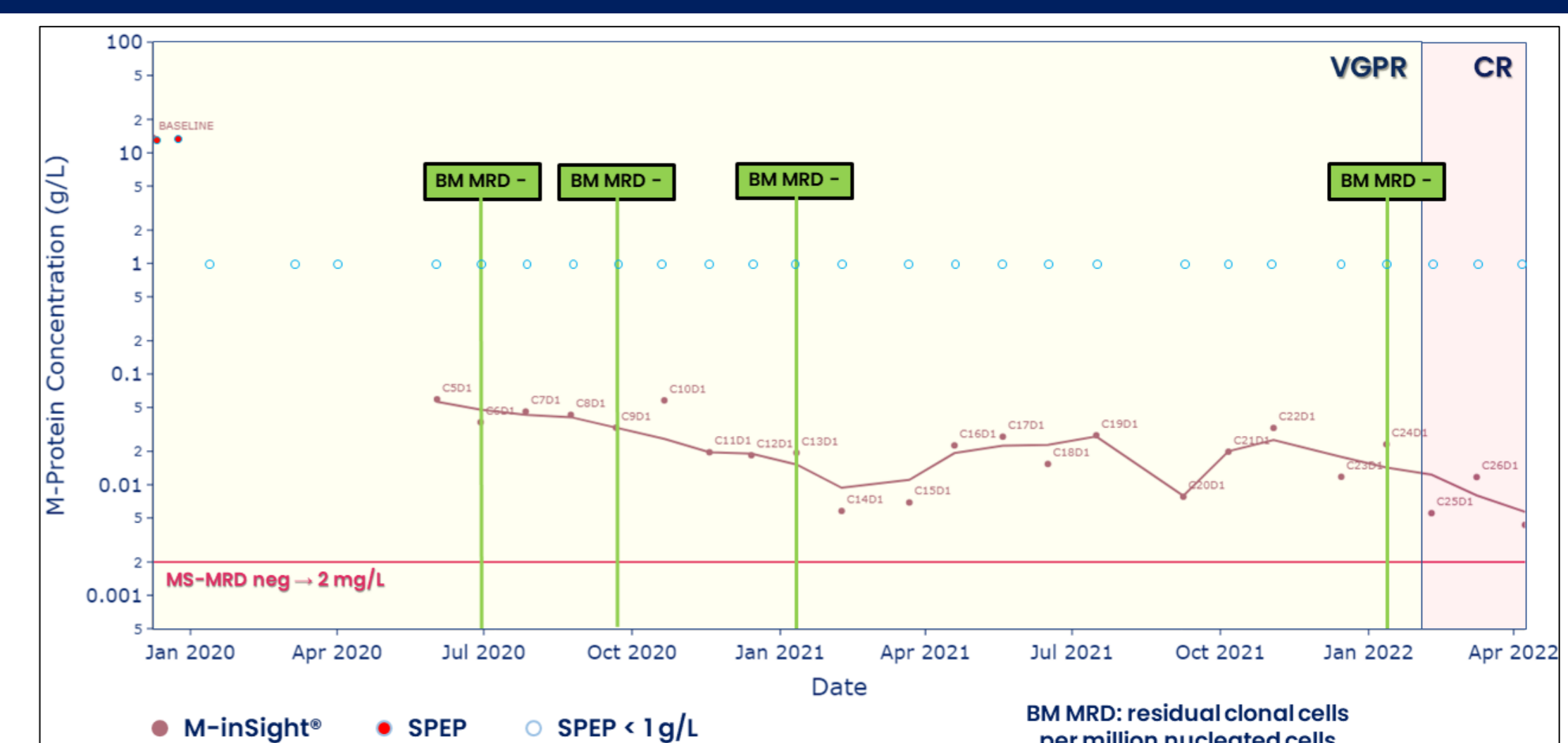


Figure 3: Evaluation of M-Protein Concentration and MRD by M-inSight® for patient C with a sustained MRD-negative status.

Concordance of 26 paired samples		M-inSight®	
		+	-
BM MRD	+	16	0
	-	6	4

Table 2: Concordance analysis of M-InSight® and BM MRD.

**M-inSight®** showed a high-level concordance with Bone Marrow (BM) MRD. All patients with NGS-detected MRD positivity at a level of  $10^{-6}$  were also MS-MRD positive and their M-protein levels could be quantified using this method. Additionally, 60% of BM MRD negative patients were identified as MS-MRD positive by M-InSight®, suggesting its enhanced sensitivity and potential as reliable alternative to BM MRD assessment. The lowest detectable M-protein concentration using M-inSight® was 0.01 mg/dL (10µg/dL).

### CONCLUSIONS

- ✓ High degree of similarity of M-protein sequences obtained from the gel and directly from serum demonstrates the specificity and robustness of **M-inSight®** in serum.
- ✓ The combined **M-inSight®/HYDRAGEL SPE** approach appears to be an alternative sample preparation method for highly sensitive and specific M-protein sequencing.

- ✓ Results showed that **M-inSight®** identified MS-MRD positivity in a significant number of patients who were BM MRD negative, highlighting its increased sensitivity.
- ✓ **M-InSight®** showed strong concordance with Bone Marrow MRD, suggesting its potential as a reliable alternative for MRD assessment.

### REFERENCES

- <sup>3</sup>Noori S. *Blood Cancer Journal*, 2023
- <sup>4</sup>Zajec M. *Clinical Chemistry*, 2020

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