Highlights from IMS 20th meeting 2023

Daniele Derudas

La spettrometria di massa

30-31 gennaio 2024
BOLOGNA, Royal Hotel Carlton

DISCLOSURES

NO RELEVANT DISCOSURES

International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

Shaji Kumar, Bruno Paiva, Kenneth C Anderson, Brian Durie, Ola Landgren, Philippe Moreau, Nikhil Munshi, Sagar Lonial, Joan Bladé, Maria-Victoria Mateos, Meletios Dimopoulos, Efstathios Kastritis, Mario Boccadoro, Robert Orlowski, Hartmut Goldschmidt, Andrew Spencer, Jian Hou, Wee Joo Chng, Saad Z Usmani, Elena Zamagni, Kazuyuki Shimizu, Sundar Jagannath, Hans E Johnsen, Evangelos Terpos, Anthony Reiman, Robert A Kyle, Pieter Sonneveld, Paul G Richardson, Philip McCarthy, Heinz Ludwig, Wenming Chen, Michele Cavo, Jean-Luc Harousseau, Suzanne Lentzsch, Jens Hillengass, Antonio Palumbo, Alberto Orfao, S Vincent Rajkumar, Jesus San Miguel, Herve Avet-Loiseau

Standard IMWG response criteria‖	
Stringent complete response	Complete response as defined below plus normal FLC ratio** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)††
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease ¶¶,‖‖	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: Serum M-protein (absolute increase must be ≥0·5 g/dL); Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; Urine M-protein (absolute increase must be ≥200 mg/24 h); In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); Appearance of a new lesion(s), ≥50% increase from nadir in SPD§§ of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease

International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

Shaji Kumar, Bruno Paiva, Kenneth C Anderson, Brian Durie, Ola Landgren, Philippe Moreau, Nikhil Munshi, Sagar Lonial, Joan Bladé, Maria-Victoria Mateos, Meletios Dimopoulos, Efstathios Kastritis, Mario Boccadoro, Robert Orlowski, Hartmut Goldschmidt, Andrew Spencer, Jian Hou, Wee Joo Chng, Saad Z Usmani, Harea Zamagni, Kazuyuki Shimizu, Sundar Jagannath, Hans E Johnsen, Evangelos Terpos, Anthony Reiman, Robert A Kyle, Pieter Sonneveld, Paul G Richardson, Philip McCarthy, Heinz Ludwig, Wenming Chen, Michele Cavo, Jean-Luc Harousseau, Susanne Lentzsch, Jens Hillengass, Antonio Palumbo, Alberto Orfao, S Vincent Rajkumar, Jesus San Miguel, Herve Avet-Loiseau

(Continued from previous page)

Clinical relapse	Clinical relapse requires one or more of the following criteria: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features) related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice; Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD§§ of the measurable lesion; Hypercalcaemia (>11 mg/dL); Decrease in haemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions; Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma; Hyperviscosity related to serum paraprotein
Relapse from complete response (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% plasma cells in the bone marrow; Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcaemia see above)
Relapse from MRD negative (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% clonal plasma cells in the bone marrow; Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcaemia)

cancers

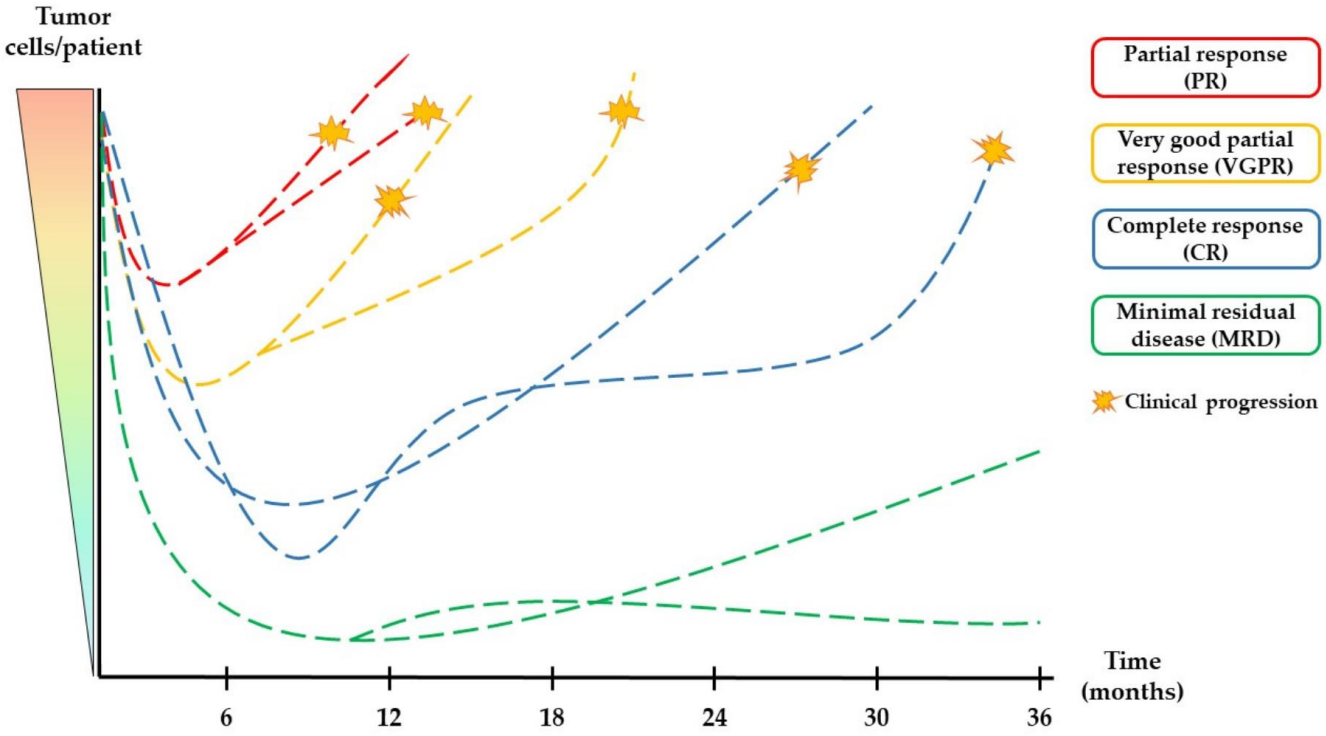
MDPI

Review

Minimal Residual Disease in Multiple Myeloma: Past, Present, and Future

Alejandro Medina-Herrera, María Eugenia Sarasquete *, Cristina Jiménez, Noemí Puig and Ramón García-Sanz

Department of Hematology, University Hospital of Salamanca (HUSA/IBSAL), CIBERONC, CIC-IBMCC (USAL-CSIC), 37007 Salamanca, Spain; amedinah@saludcastillayleon.es (A.M.-H.); jscris@usal.es (C.J.); npuig@saludcastillayleon.es (N.P.); rgarcias@usal.es (R.G.-S.)
* Correspondence: mesolonso@saludcastillayleon.es



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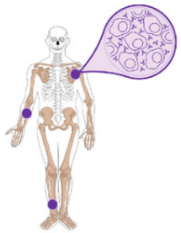
	Standard MFC	NGF	ASOqPCR	NGS	ddPCR
Applicability	90–100%	90–100%	40–75%	~90%	Comparable to qPCR
Sensitivity	10^{-4}–10^{-5}	10^{-5}–10^{-6}	10^{-4}–10^{-5}	10^{-5}–10^{-6}	At least 10^{-5}
Standardization	No	EuroFlow	EuroMRD	ClonoSEQ *	Ongoing
Turnaround time	1 day	1 day	≥1 week	4 days–1 week	≥1 week
Specific primers/probes	Not applicable	Not applicable	Yes	No	Yes
Standard curve	Not applicable	Not applicable	Yes	No	No
Influenced by SHM	No	No	Yes	Yes	Yes
Baseline BM	No	No	Yes	Yes	Yes
Fresh sample (processing time)	Yes (24–48 h)	Yes (24 h)	No	No	No

Technique	Source of specimen	Method	Level of detection	Reference	Limitation
MFC	Bone marrow aspirate	Uses multiple surface and cytoplasmic markers (colors) to identify phenotypically aberrant clonal plasma cells	Depends on the number of markers tested	Rawstron et al[2]	Cannot be done on stored sample
(ASO)-qPCR	Bone marrow aspirate	Identify clonal MM plasma cell–specific *IGH* gene rearrangements	10^{-5}	Bakkus et al[3]	Requires patient-specific primers
NGF	Bone marrow aspirate	Standardized MFC with automate readouts	$> 10^{-5}$	Flores-Montero et al[4]	Cannot be done on stored sample
NGS	Bone marrow aspirate	DNA is amplified using primers designed for IGH-VDJH, IGH-DJH, or IGK and sequenced to determine the presence and quantity of clonal DNA sequence	$> 10^{-5}$	Ladetto et al[5]	Dominant sequence might not be identified in < 10% of cases

Baseline PET/CT and BM sampling for MRD (if needed by technique; e.g., NGS)



a

Baseline presentation

Patients with only **BM disease (no FL at PET/CT)**

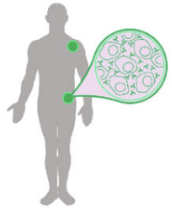
Initial MRD monitoring

PB MRD (MS or NGF/NGS)

If PB MRD neg: perform BM MRD

If BM MRD neg: need for sustained evaluation?

If BM MRD pos: repeat at later timepoint (after a clinical phase? After a fixed duration of time?)



b

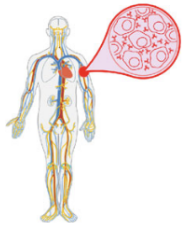
Patients with **EMD/focal lesions at PET/CT**

PB MRD (MS or NGF/NGS)

If PB MRD neg: perform PET/CT and BM MRD

If BM MRD and PET/CT neg: need for sustained MRD

If BM MRD pos: repeat at later time point (after a clinical phase? After a fixed duration of time?)



c

Patients with **circulating plasma cells**

PB MRD NGF (or MS/NGS)

If PB MRD neg: perform BM MRD and PET/CT

If BM MRD and PET/CT neg: need for sustained MRD? Only PB monitoring?

If BM MRD pos: repeat at later time point (after a clinical phase? After a fixed duration of time?)

For **high-risk patients**: high-risk CA, LDH, ISS II/III → consider multiple methods (imaging + BM)

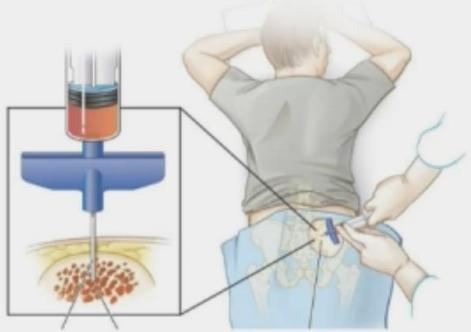
IMWG MRD criteria

IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Imaging-positive MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue

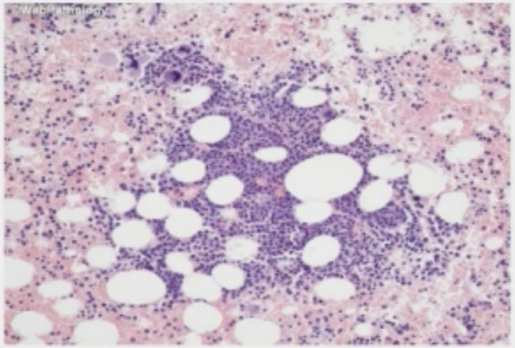
Kumar S, et al. Lancet Oncol. 2016 Aug;17(8):e328-e346

Limitations of MRD analysis in the bone marrow of patients with MM

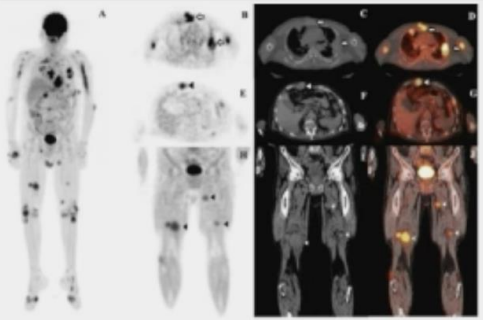
Invasive procedure



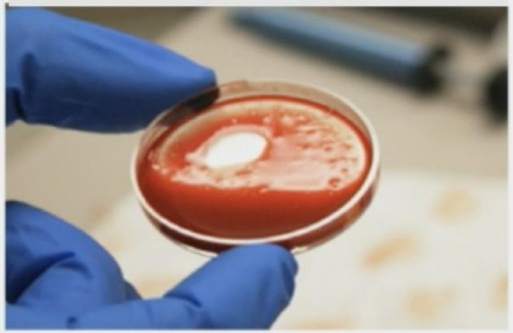
Patchy infiltration



Extramedullary disease



Hemodilution



There is a need for development of less invasive methods

Bone Marrow Biopsies Are No Walk in the Park

By Yolanda Brunson-Sarrabo • November 19, 2019 • 3 min read

Last updated: April 2023

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Unsettling and stressful

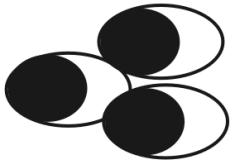
During my first unexpected experience with this unknown procedure, I could be heard screaming all the way in the reception area. The experience was/is unsettling and stressful. I always say that with technology and the advancement of medicine, it still blows me away how some procedures still seem so very barbaric. Though I understand the need to extract these specific marrow plasma cells mostly from the back hip bone (iliac crest)... well, let's just say it's a hard pill to swallow during the process!

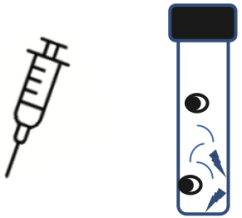
Polish Myeloma Consortium

AT THE FOREFRONT UChicago Medicine

https://blood-cancer.com/living/bone-marrow-biopsy

Bone marrow biopsy		Liquid biopsy
Less convenient and more invasive	**Accessibility**	More convenient and less invasive
Limited to marrow clones	**Risk stratification**	Can identify disseminated disease and hidden lesions (extramedullary)
Less comprehensive	**Genetic information**	More comprehensive
Possible false negativity for patchy infiltration	**Diagnosis information**	More comprehensive






ONCOLOGY RESEARCH
2023 31(3): 271-274

Tech Science Press

Liquid biopsy and blood-based minimal residual disease evaluation in multiple myeloma

Alessandro GOZZETTI*, Monica BOCCHIA

Division of Hematology, University of Siena, Azienda Ospedaliera Universitaria, Siena, 53100, Italy


STEVEN SPIELBERG PRESENTS
BACK TO THE FUTURE
PG
A ROBERT ZEMECKIS FILM

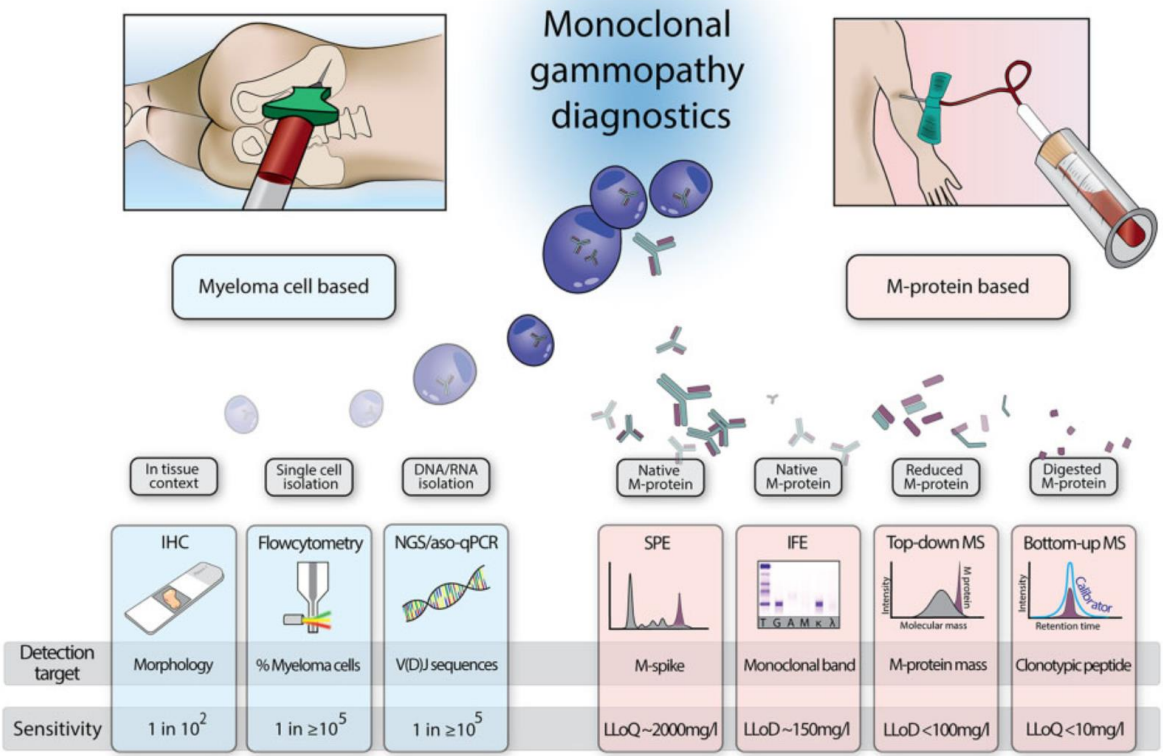
Technique	Source of specimen	Method	Level of detection	Reference	Limitation
LC MALDI-TOF or mass-fix mass spectrometry	Serum	M-protein detection by scanning the overall mass distribution of denatured intact immunoglobulin LCs	< 0.01 g/dL	Mills et al[6]	Variable resolution can affect level of detection
Clonotypic mass spectrometry	Serum	Ig trypsin digestion and detection of peptides specific to the M-protein antigen-binding region, also called the complementarity-determining region	0.001 g/L	Bergen et al[7]	Identifying unique clonotypic peptide depends on sequencing and might be difficult in some cases
BloodFlow	Peripheral blood	Immunomagnetic enrichment of circulating plasma cells followed by NGF	10^{-8}	Notarfranchi et al[8]	Requires 50 mL peripheral blood sample

Clinical Chemistry 66:3
421-433 (2020)

Review

Mass Spectrometry for Identification, Monitoring, and Minimal Residual Disease Detection of M-Proteins

M. Zajec,[a,b,†] P. Langerhorst,[c,†] M.M. VanDuijn,[b] J. Gloerich,[c] H. Russcher,[a] A.J. van Gool,[c] T.M. Luider,[b] I. Joosten,[c] Y.B. de Rijke,[a,‡] and J.F.M. Jacobs[c,*,‡]



IHC, immunohistochemistry; LLoD, lower limit of detection; LLoQ, lower limit of quantification.

Proteomic technologies for protein profiling

Technique	Method Description
2-dimensional polyacrylamide gel electrophoresis (2D-PAGE)	The protein content of a sample is resolved on a gel in two dimensions according to mass and charge; the gels are stained, and the spot intensities in the samples are analyzed among the multiple gels.
2D-DIGE	Each protein sample of interest is labelled with a different fluorophore (Cy3, Cy5, or Cy2) that binds covalently to the epsilon amino group of lysine residues.
Protein microarrays	Direct labelling or labelled secondary antibodies are used to identify bound proteins once targeted proteins in one sample bind to probes on a "forward" microarray, and vice versa for "reverse" microarrays.
Surface-enhanced laser desorption/ ionization time-of-flight mass spectrometry (SELDI-TOF MS)	Retention chromatography and mass spectrometry principles are combined, offering a fast, high-throughput, and relatively sensitive screening approach for complicated protein samples. Proteins can also be separated, detected, and analyzed at the femtomole level straight from biological materials. This allows for the discovery of many analytes and the analysis of many diverse samples while studying multiple biological variables at the same time.
Matrix-assisted laser desorption/ ionization-time of flight mass spectrometry (MALDI-TOF MS)	Application of a protein mixture onto a gold plate, desorption of proteins from the plate using laser energy, and determination of the protein masses, with comparison of peak intensities between several different samples.
Liquid Chromatography with tandem mass spectrometry (LC-MS-MS)	Separation of a mixture of peptides (derived from trypsin-catalyzed protein digestion) through one-, two-, or three-dimensional LC and determination of peptide masses through MS-MS.
Isotope-coded affinity tag (ICAT)	Chemical tagging of proteins on cysteine residues with a heavy or light stable isotope; after labelling samples are combined, proteins are digested with trypsin, and tagged peptides are extracted via affinity chromatography; both samples are then concomitantly analyzed using LC-MS-MS.

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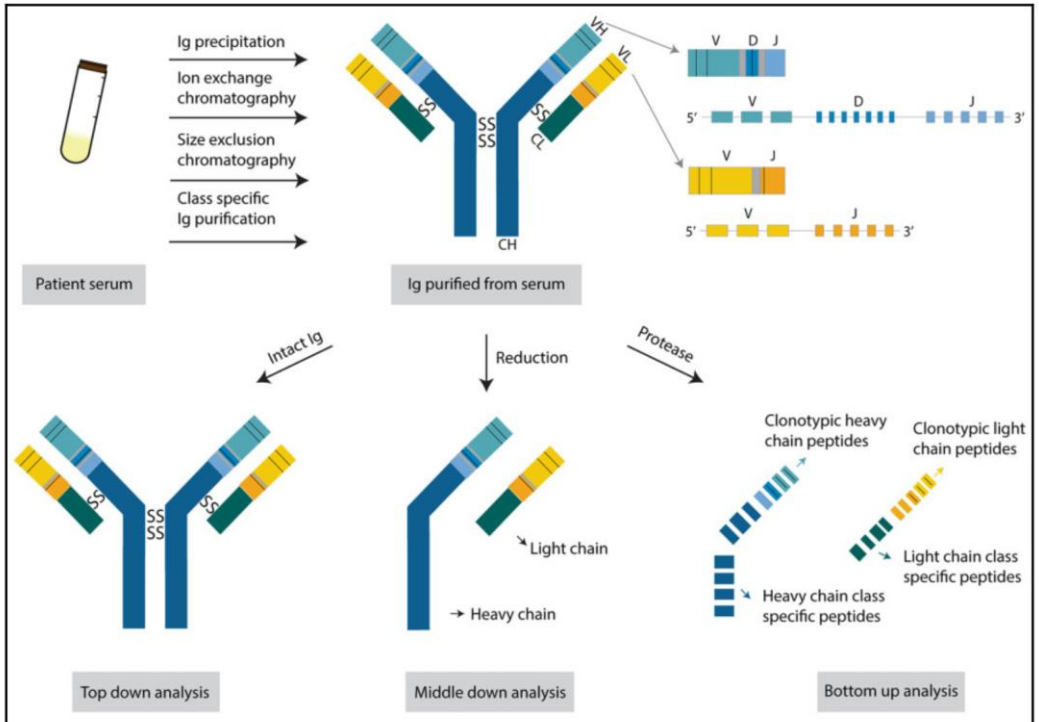

Ig characterization flowchart using MS methodology. After purification, Ig can be characterized as an intact protein (top-down), reduced into Ig fragments (middle-down), or digested into peptides (bottom-up). The complementarity-determining regions, indicated with black lines in the rearranged V(D)J regions, constitute the most variable parts of the Ig and thus are ideal for selection of clonotypic peptides. CH, constant part of heavy chain; CL, constant part of light chain; SS, disulfide bond; VH, variable part of heavy chain; VL, variable part of light chain.

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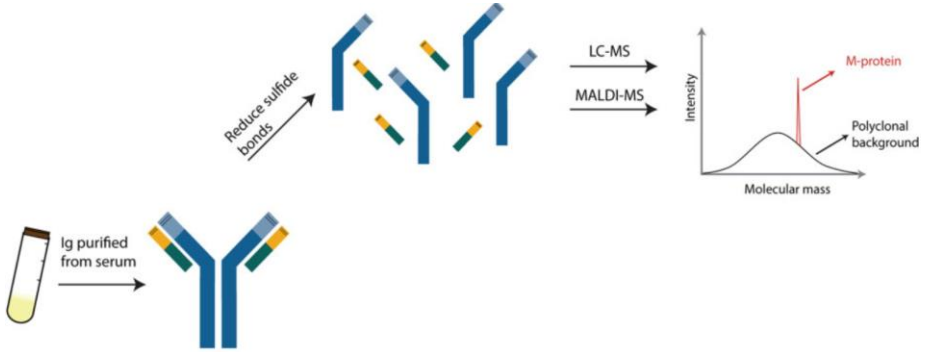
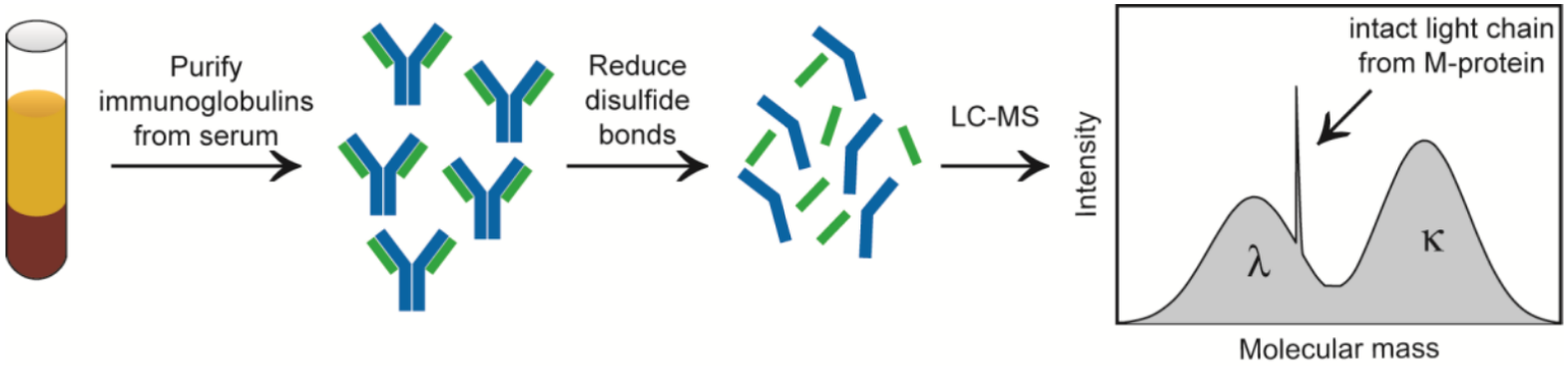
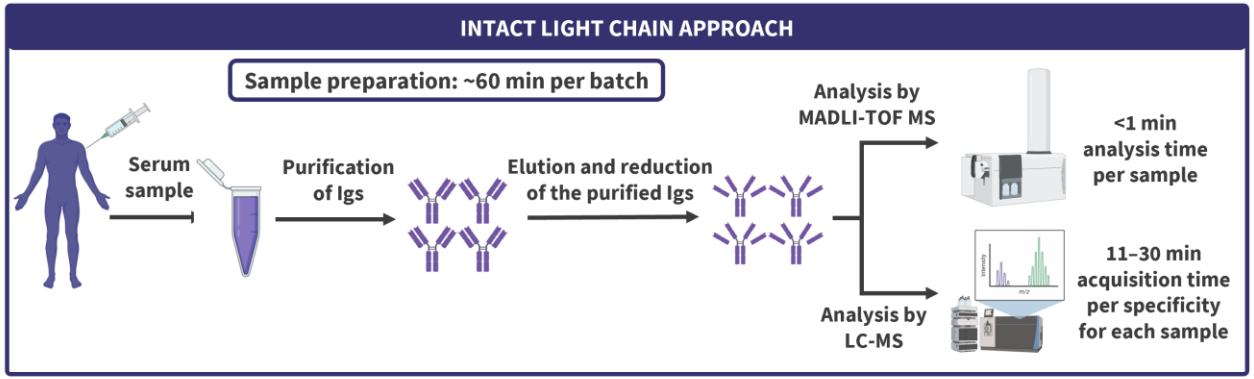
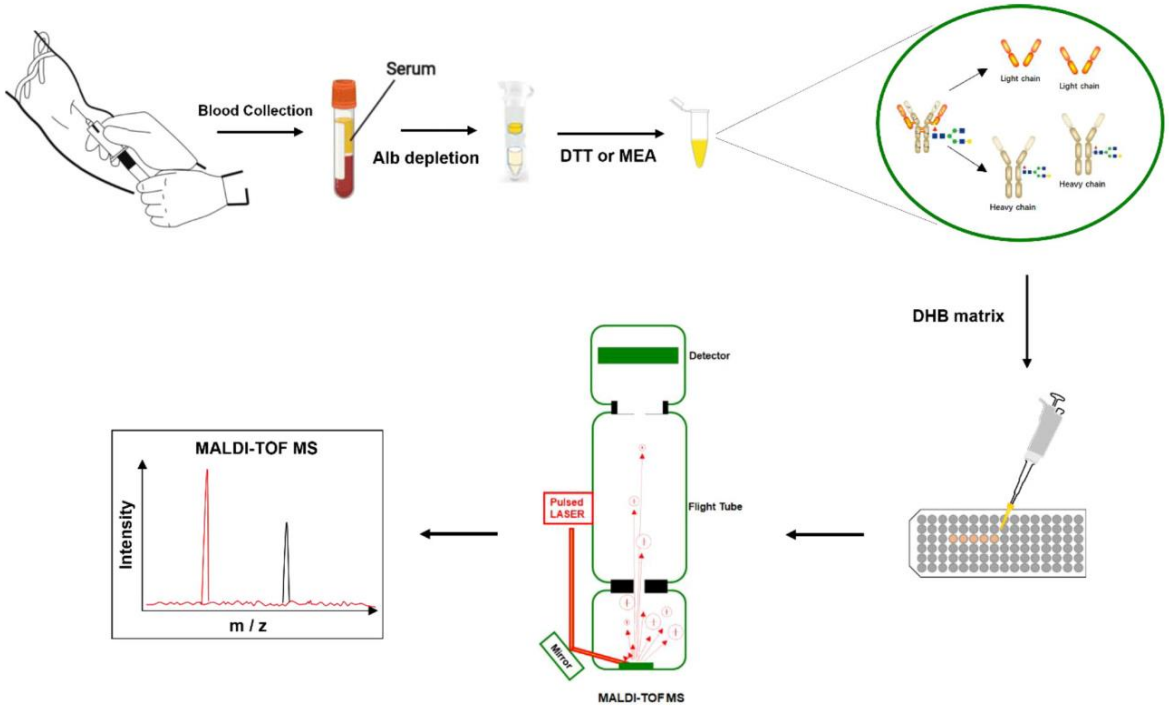


Intact protein method




INTACT LIGHT CHAIN APPROACH
Sample preparation: ~60 min per batch
Serum sample
Purification of Igs
Elution and reduction of the purified Igs
Analysis by MADLI-TOF MS
Analysis by LC-MS
<1 min analysis time per sample
11–30 min acquisition time per specificity for each sample

Workflow diagram of the MALDI-TOF MS method for the screening test of M- protein in serum



MALDI-TOF-MS for rapid screening analysis of M-protein in serum

Jie Li[1*], Anping Xu[1], Weijie Xie[1], Bowen Li[1], Cunliang Yan[1], Yong Xia[1], Chao Liang[2,3,4*] and Ling Ji[1*]

[1]Department of Laboratory Medicine, Peking University Shenzhen Hospital, Shenzhen, China, [2]Department of Biology, School of Life Sciences, Southern University of Science and Technology, Shenzhen, China, [3]Institute of Integrated Bioinfomedicine and Translational Science (IBTS), School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, Hong Kong SAR, China, [4]State Key Laboratory of Proteomics, National Center for Protein Science (Beijing), Beijing Institute of Lifeomics, Beijing, China

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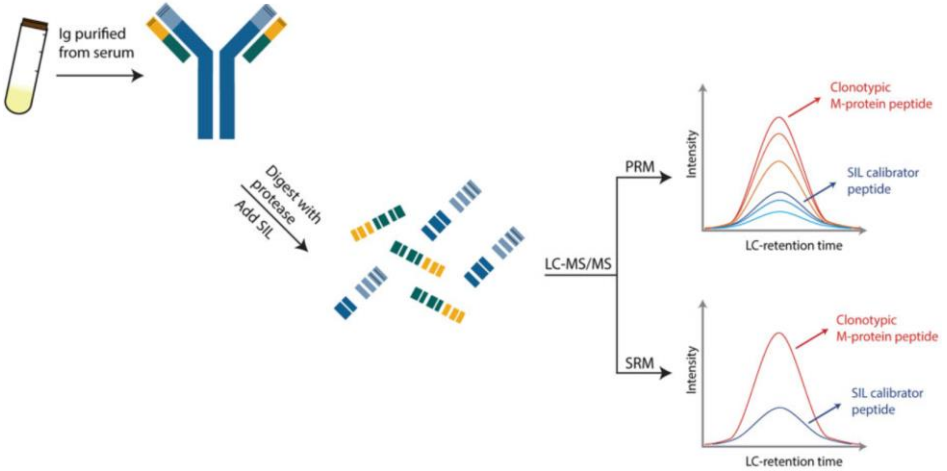
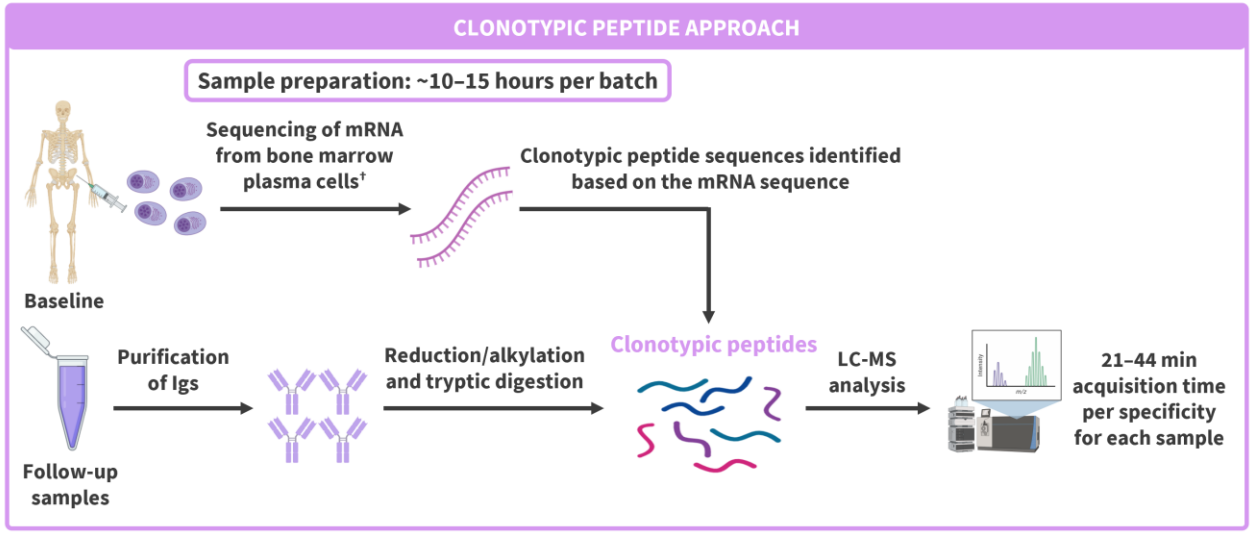
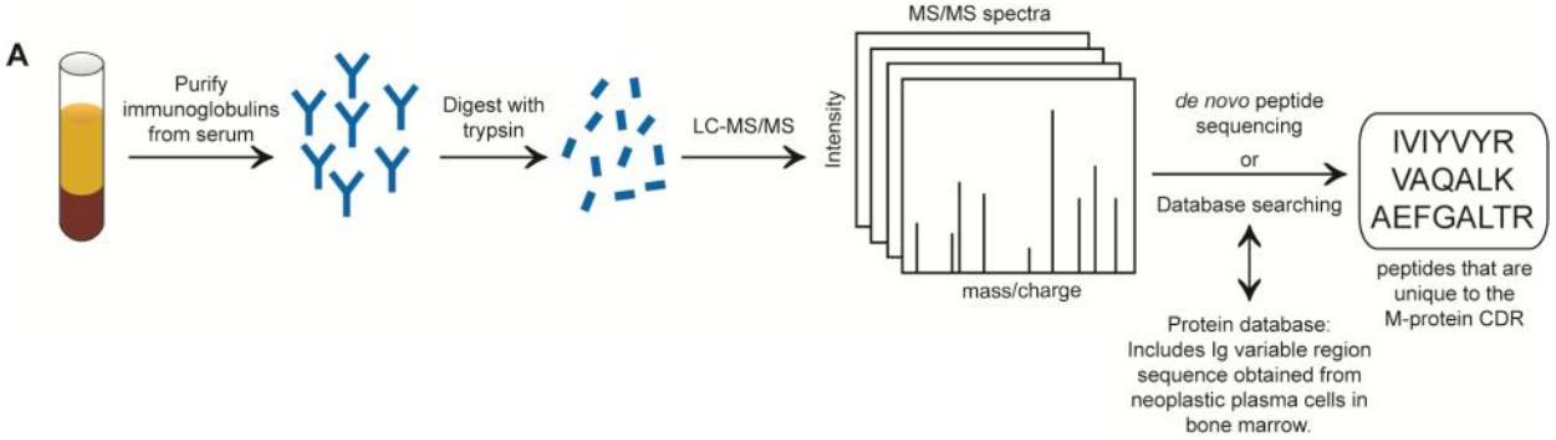
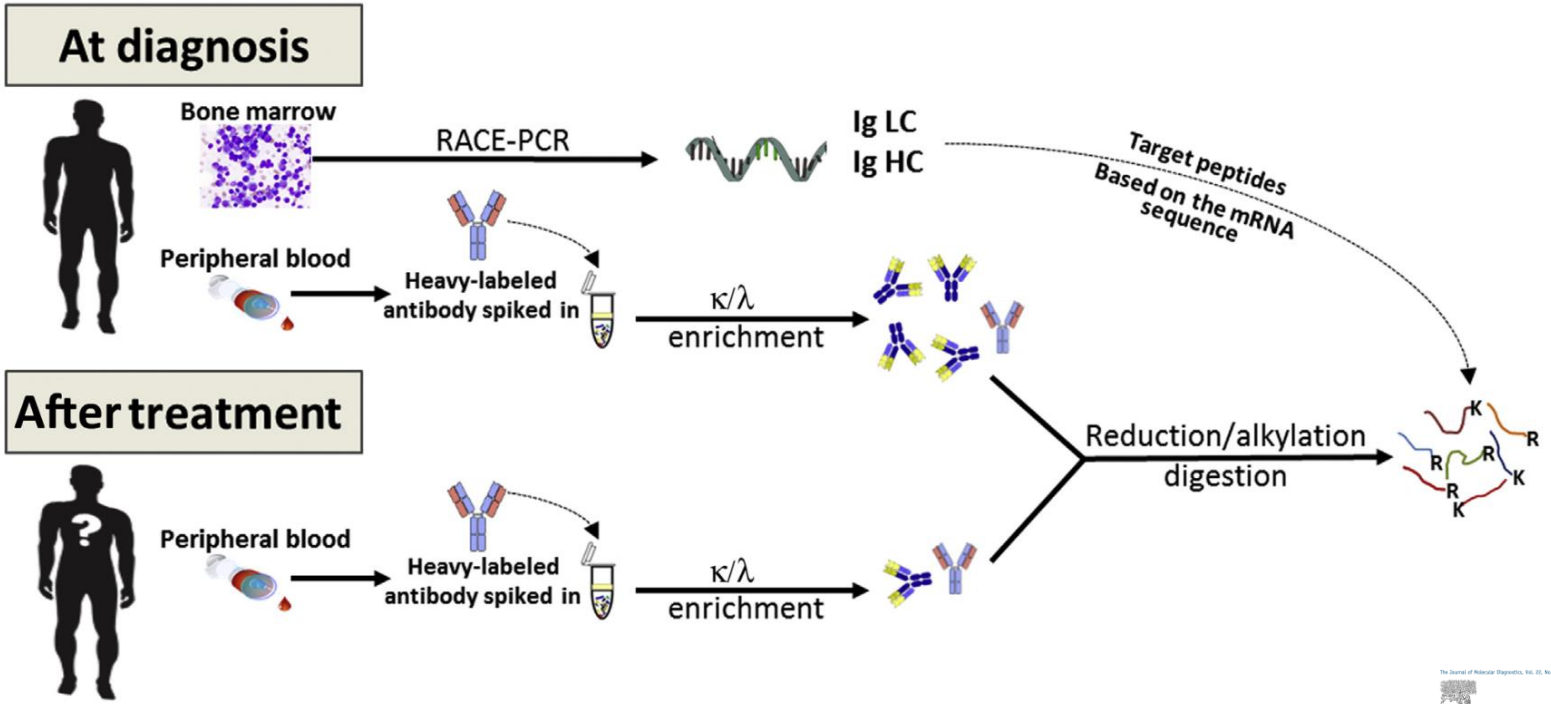



CLONOTYPIC PEPTIDE APPROACH
Sample preparation: ~10–15 hours per batch
Sequencing of mRNA from bone marrow plasma cells†
Clonotypic peptide sequences identified based on the mRNA sequence
Baseline
Purification of Igs
Reduction/alkylation and tryptic digestion
Clonotypic peptides
LC-MS analysis
21–44 min acquisition time per specificity for each sample
Follow-up samples

Clonotypic peptide method





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The Journal of Molecular Diagnostics

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Mass Spectrometry—Based Method Targeting Ig Variable Regions for Assessment of Minimal Residual Disease in Multiple Myeloma

Carla D. Martins,* Sarah Huet,* San S. Yi,* Maria S. Ritorto,* Ola Landgren,† Ahmet Dogan,* and Jessica R. Chapman*

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Advantages and disadvantages of methodologies approved by the IMWG*

Methodology	Cost, $	Advantages	Disadvantages
SPE	7–67	Widely available Relatively low cost	Interference from tmAbs Unsuitable for monitoring patients with non-secretory and oligosecretory MM Cannot accurately quantify IgA MPs that migrate in the beta-region
sIFE	22–200	Widely available Relatively low cost	Interference from tmAbs
Serum FLC	34–120	Widely available Greater sensitivity for detection of low-level FLC MPs than sIFE	Does not specifically measure the monoclonal FLC and relies on the FLC ratio to detect monoclonal FLC, making it more difficult to interpret in the context of renal impairment due to the reduced clearance rate

Advantages and disadvantages of methodologies approved by the IMWG*

Intact light chain MALDI-TOF MS	145	Greater sensitivity Can detect additional risk factors for PCD Can differentiate MPs from therapeutic antibodies	Only available in the USA
NGF	450	High sensitivity Standardized methodology	Requires BM biopsy to obtain a suitable sample Samples need to be processed within 24-48 hours of collection
NGS	1,950	High sensitivity Fast turnaround time for large samples Clone evolution BM reconstitution[7]	Requires BM biopsy to obtain a suitable sample
PET-CT	870	Can monitor intra-and extra-medullary disease	False-negative results were observed in patients with hexokinase deficiency Exposure to high doses of ionizing radiation Patients may need to travel significant distances to access appropriate scanners

FLC, free light chain; IMWG, International Myeloma Working Group; MALDI-TOF MS, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; MM, multiple myeloma; MP, monoclonal protein; MRD, minimal residual disease; NGF, next-generation flow; NGS, next-generation sequencing; PCD, plasma cell disorders; PET-CT, positron-emission tomography-computed tomography; sIFE, serum immunofixation electrophoresis; SPE, serum protein electrophoresis; tmAb, therapeutic monoclonal antibody.

*Adapted from Giles, *et al.*[1]

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Review

Mass Spectrometry for Identification, Monitoring, and Minimal Residual Disease Detection of M-Proteins

M. Zajec,[a,b,†] P. Langerhorst,[a,†] M.M. VanDuijn,[b] J. Gloerich,[c] H. Russcher,[a] A.J. van Gool,[c] T.M. Luider,[b] I. Joosten,[c] Y.B. de Rijke,[a,‡] and J.F.M. Jacobs[a,*,‡]

Characteristics of techniques to monitor multiple myeloma disease activity in bone marrow and serum.

	Bone marrow sampling			Serum sampling			
	MFC	ASO-qPCR	NGS	SPE/CE	IFE/IS-CE	Top-down MS	Bottom-up MS
Applicability, %	~100	~65	~90	~100	~100	~100	~100
Quantitative	Yes (clonal PCs)	Yes [V(D)J seq.]	Yes [V(D)J seq.]	Yes (M-spike)	No (visual M-peak)	No (arbitrary units)	Yes (internal standard)
Analytical sensitivity	1 in $\geq 10^5$	1 in $\geq 10^5$	1 in $\geq 10^5$	LLoQ ~2 g/L	LLoD ~150 mg/L	LLoD ≤100 mg/L	LLoQ ≤10 mg/L
Baseline sample	Important but not mandatory	Mandatory	Mandatory	Not needed	Not needed	Not needed	Important but not mandatory
Fresh sample	Needed (<36 h)	Not needed[a]	Not needed[a]	Not needed	Not needed	Not needed	Not needed
Sample volume	$\geq 5 \times 10^6$ cells[b]	$\geq 1 \times 10^6$ cells[b]	$\geq 1 \times 10^6$ cells[b]	500 µL	500 µL	≤ 100 µL	≤ 100 µL
Nonrepresentative sampling	Extramedullary and patchy disease			Non-secretory multiple myeloma (no M-protein biomarker)			
Turnaround time	2–3 h	BS: 3–4 weeks FU: ≤ 5 days	≤10 days	≤3 days	≤5 days	≤1 days	BS: 4–5 weeks[c] FU: ~5 days
Global availability	Intermediate	Limited	Limited	High	High	Few sites RUO[d]	Few sites RUO

BS, baseline sample (target identification); CE, capillary electrophoresis; FU, follow-up sample; IS, immunosubtraction; LLoD, lower limit of detection; LLoQ, lower limit of quantification; PC, plasma cell; RUO, research use only; M-spike, spike of the M-protein.

[a]DNA must be extracted within 36 h, analysis performed on archived DNA.

[b]More cells increase sensitivity.

[c]Without internal standard, the turnaround-time is equal to FU.

[d]Implemented in routine diagnostics at the Mayo Clinic in 2018.

ARTICLE Open Access

Mass spectrometry for the evaluation of monoclonal proteins in multiple myeloma and related disorders: an International Myeloma Working Group Mass Spectrometry Committee Report

David L. Murray[1], Noemi Puig[2], Sigurdur Kristinsson[3], Saad Z. Usmani[4], Angela Dispenzieri[1,5], Giada Bianchi[6], Shaji Kumar[7], Wee Joo Chng[7,8,9], Roman Hajek[10], Bruno Paiva[11], Anders Waage[12,13], S. Vincent Rajkumar[7] and Brian Durie[14]

List of IMWG recommendations regarding mass spectrometry.

Intact LC MALDI-TOF can be used in lieu of immunofixation in the clinically assessment of patients and the assessment of patients on clinical trials.

We endorse the use of mass spectrometry to aid in distinguishing therapeutic antibodies from endogenous M-proteins.

We recognize that using mass spectrometric methods in lieu of conventional IFE may lead to lower rates of CR, and therefore cross-comparisons of CR rates in trials done in different time periods is not recommended.

We endorse the collection of further data from mass spectrometry (MALDI-TOF, miRAMM, or clonotypic peptide approach) to document the ability to test for MRD negativity in the peripheral blood, and to guide timing of BM tests for next-generation flow cytometry and NGS studies.

We encourage further investigations to further clarify the relationship and implications of N-linked glycosylation in MGUS progression to myeloma and AL amyloidosis.

Citation/s (assay name)	Analyte	Type of MS analysis	LOD, mg/L	LOQ, mg/L
Mills, *et al.* 2016 (MALDI-TOF MS)	Intact light chain	MALDI-TOF MS	<100	450
Ashby, *et al.* 2018 Sakrikar, *et al.* 2021 (QIP-MS/EXENT)	Intact light chain	MALDI-TOF MS	8–15†	15†
Mills, *et al.* 2015 (MiRAMM)	Intact light chain	LC-MS	5	NR
Puig, *et al.* 2021 (QIP-MS/EXENT)	Intact light chain	LC-MS	NR	NR
Langerhorst, *et al.* 2021	Clonotypic peptide	LC-MS	NR	1
Liyasova, *et al.* 2021 (EasyM)	Clonotypic peptide	LC-MS	0.5–1	2
Zajec, *et al.* 2018	Clonotypic peptide	LC-MS	0.1–0.3	0.4–0.9
Martins, *et al.* 2020	Clonotypic peptide	LC-MS	0.1–1.5	NR

LC-MS, liquid chromatography-mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; NR, not reported; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MS, mass spectrometry; QIP-MS, quantitative immunoprecipitation mass spectrometry.

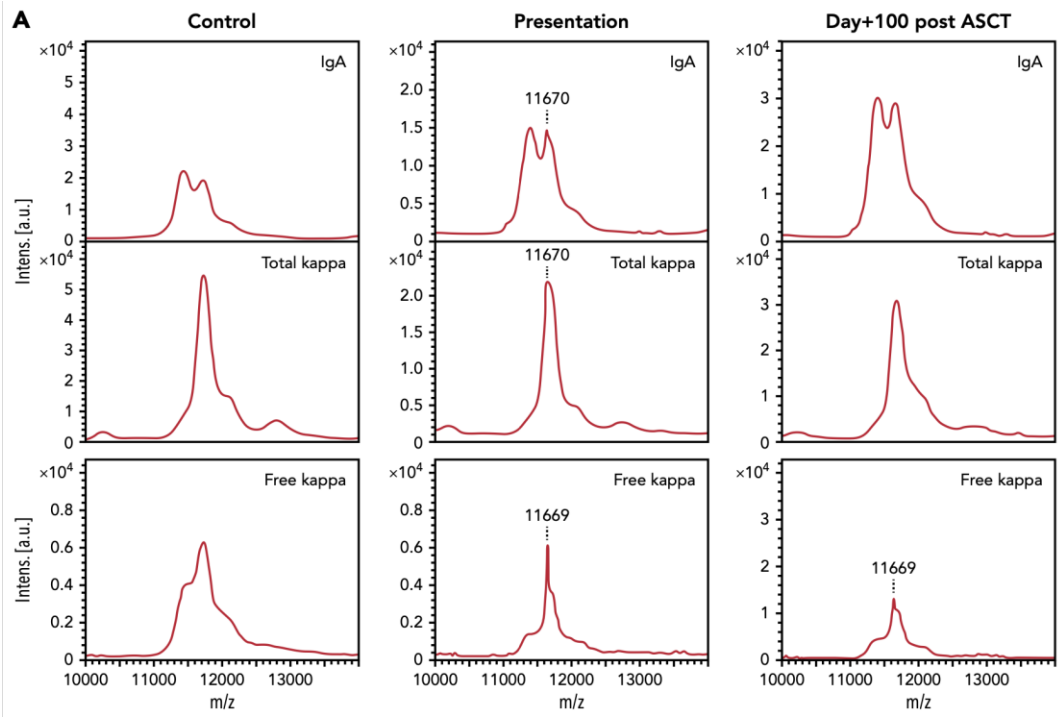
*Adapted from Giles, *et al.*[1]

†Reported for intact immunoglobulin monoclonal peptides and not monoclonal free light chain.

Redefining nonmeasurable multiple myeloma using mass spectrometry

Hannah V. Giles,[1,2] Mark A. Cook,[2,3] Mark Trehane Drayson,[4] Gordon Cook,[5,6] Nicola Jane Wright,[7] Simon John North,[7] Stephen Harding,[7] David A. Cairns,[6] Anna Hockaday,[6] Martin F. Kaiser,[8] Paul Moss,[1,2] Faith E. Davies,[9] Gareth J. Morgan,[10] Graham Jackson,[11,12] and Guy Pratt[1,2]

[1]University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; [2]University of Birmingham, Birmingham, United Kingdom; [3]Bristol Myers Squibb, Boudry, Switzerland; [4]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom; [5]St James's Institute of Oncology, Leeds, United Kingdom; [6]Cancer Research UK Institute of Clinical Trials Research, University of Leeds, Leeds, United Kingdom; [7]The Binding Site Ltd, Birmingham, United Kingdom; [8]The Institute of Cancer Research, Royal Marsden Hospital, London, United Kingdom; [9]Perlmutter Cancer Center and [10]Department of Hematology, NYU Langone Health Care, New York, NY; [11]Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom; and [12]Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom



(A) A 0.086 g/L IgA k paraprotein that was detectable in the presentation sample of a patient with NSMM (immunofixation negative, IgG 7.31 g/L, IgA 4.1 g/L, IgM 0.8 g/L, serum free k 79.4 mg/L, serum free l 22.7 mg/L, sFLCr 3.50) at m/z 11 670 and monoclonal k FLC were detectable at m/z 11 669 for the doubly charged light chains, respectively. At day1100 after ASCT, the sFLCr had normalized (serum free k 22.7 mg/L, serum free l 14.12 mg/L, sFLCr 1.60) but MS detected residual monoclonal k FLC with the same m/z as detected in the baseline sample

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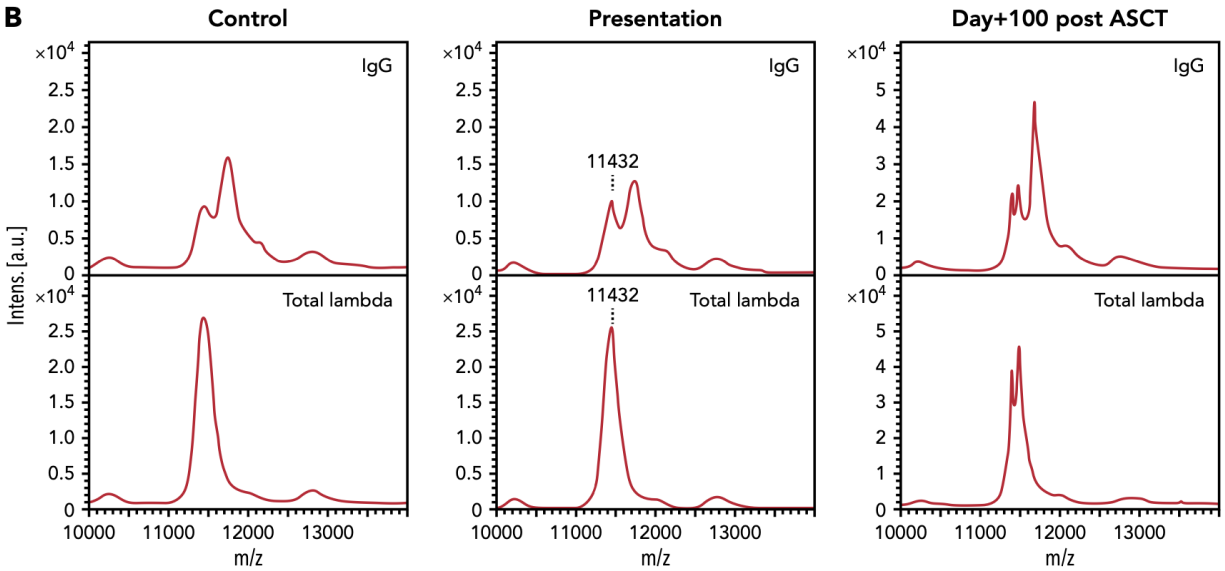

(B) A 0.14 g/L IgG l monoclonal protein detectable by MS at m/z 11 432 for the doubly charged light chain in the presentation sample from a patient with NSMM (immunofixation negative, IgG 5.40 g/L, IgA 0.5 g/L, IgM 0.39 g/L, serum free k 5.46 mg/L, serum free l 6.08 mg/L, sFLCr 0.90). At day1100 after ASCT oligoclonal peaks are present in the IgG and l spectra but no residual monoclonal protein is detectable at m/z 11 432.

Santoclyte et al. Blood Cancer Journal (2021)11:78
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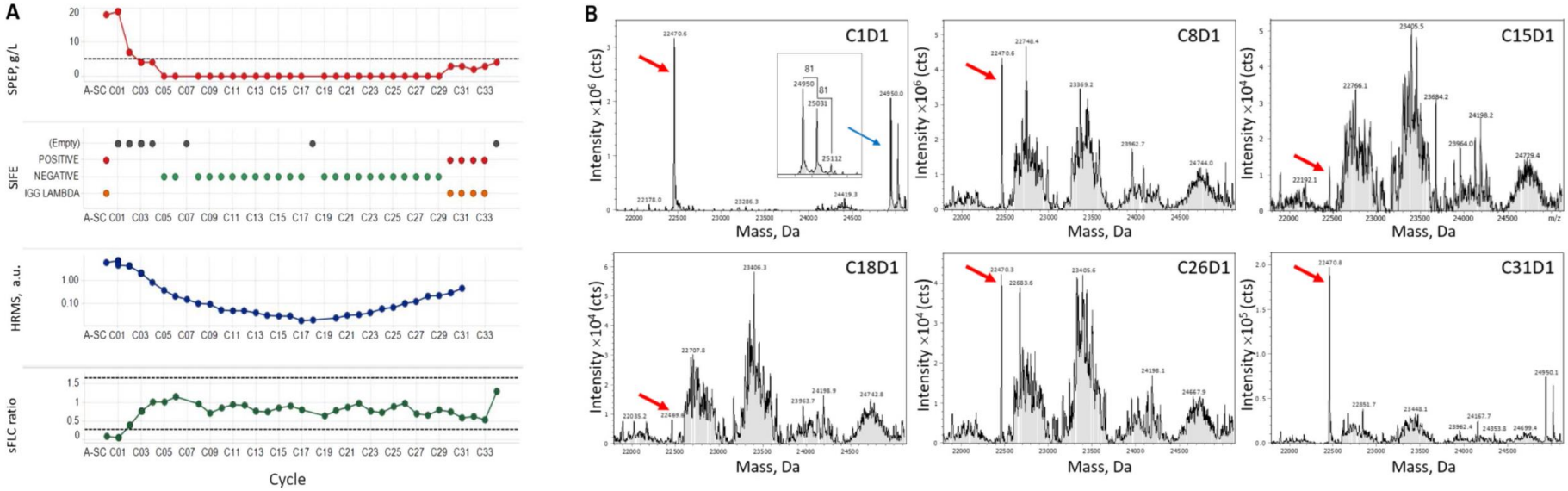
Blood Cancer Journal

CORRESPONDENCE Open Access

Sensitive multiple myeloma disease monitoring by mass spectrometry

Rasa Santoclyte[@], Chelsea Jin[2], James Pratt[2], Ron Ammar[2], Keyur Desai[2], Mohan Bolisetty[1], Prianka Das[1], Mihaela Popa-McKiver[2] and Oscar Puig[@3,4]



A SPEP, SIFE, HRMS, and sFLC results for subject 122. **B** HRMS profiles for subject 122 showing detectable monoclonal light chain peak at 22,470.6 ± 1.5 Da in all the time points. The additional peaks in 25,000 mass region represent glycosylated heavy chains at 1⁄2 mass based on the difference between the adjacent peaks, which is 81 Da or 1⁄2 mass of hexose residue (162 Da).

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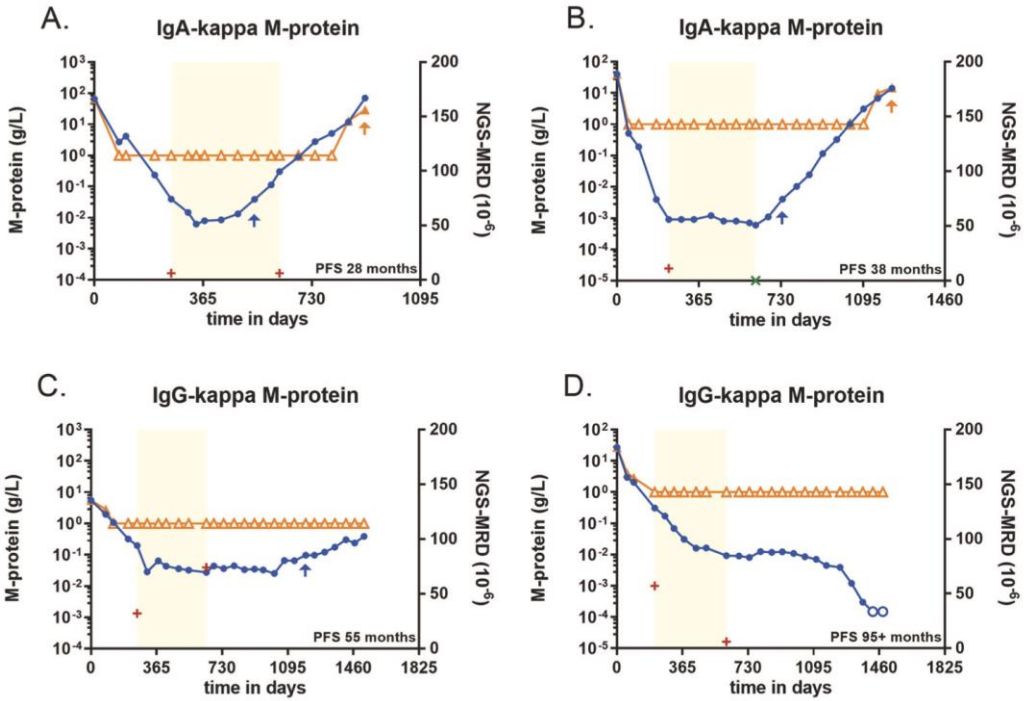
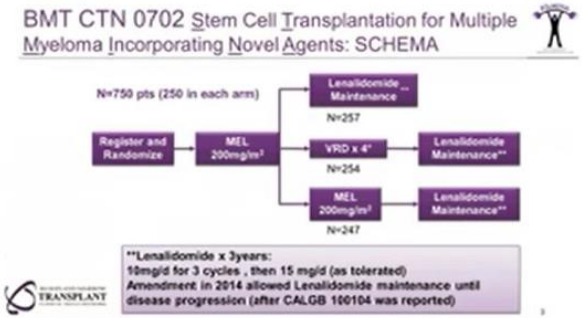
Dynamic monitoring of myeloma minimal residual disease with targeted mass spectrometry



Dynamic monitoring profiles in the IFM 2009 cohort. Open symbols indicate the analyte could not be detected. Orange triangles show SPEP data for the M-protein; blue dots show MS-MRD data. The yellow area indicates the period of maintenance treatment, with NGS- MRD assessments at the start and end. A positive NGS-MRD result is shown as a red plus; a negative NGS-MRD result as a green X. The blue arrow shows early evidence of progression based on MS-MRD data; the orange arrow shows where progression was seen in the data from the IFM 2009 study. **A** Patients with early progression during maintenance treatment. Patient 020001, IgA-kappa M-protein. B Patients with progression within one year after end of maintenance treatment. Patient 052003, IgA-kappa M-protein. **C** Patients with progression more than one year after end of maintenance treatment. Patient 044012, IgG-kappa M-protein. **D** Patients with no progression observed, this particular patient reached MS-MRD negativity after 1400 days of follow-up. Patient 025007, IgG-kappa M-protein.

Disposition of patients, samples, and testing

Patients with available samples were tested with Mass-Fix



Objective: To test the hypothesis that Mass-Fix is superior to existing methodologies to predict for survival outcomes in a prospective clinical trial

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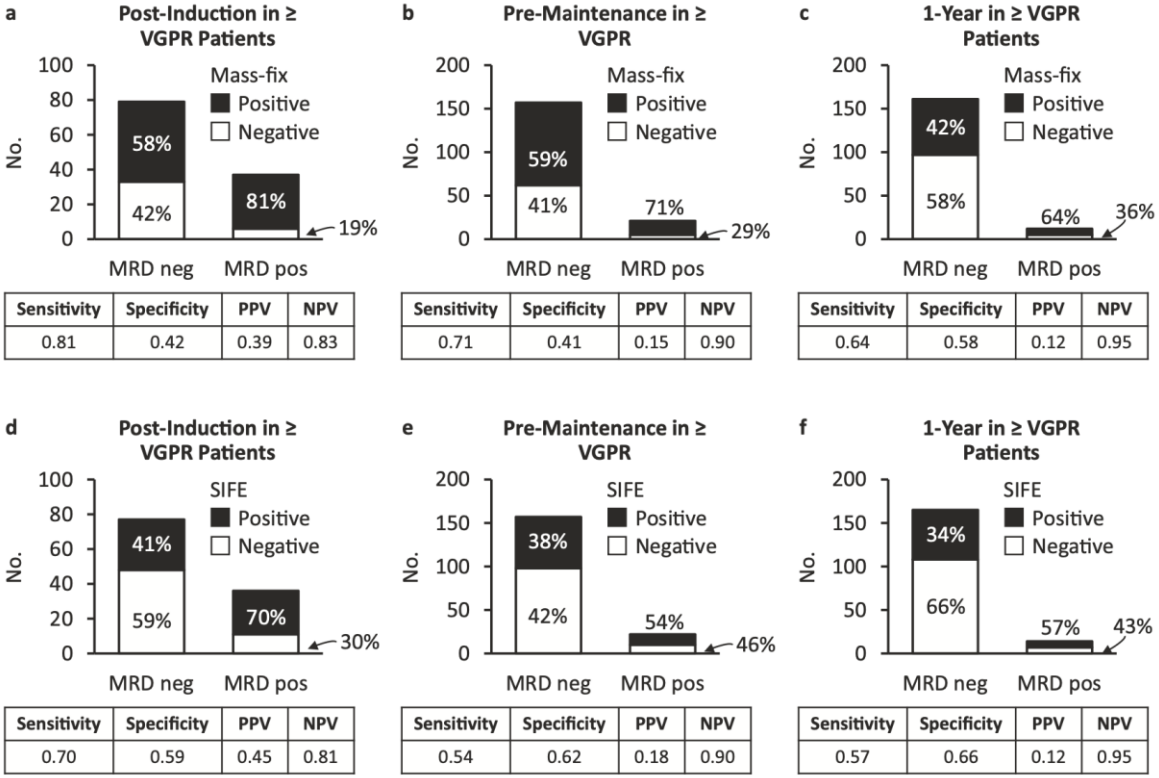
Mass-Fix better predicts for PFS and OS than standard methods among multiple myeloma patients participating on the STAMINA trial (BMT CTN 0702 /07LT)

Angela Dispenzieri[1,2✉], Amrita Krishnan[3], Bonnie Arendt[3], Beth Blackwell[4], Paul K. Wallace[5], Surendra Dasari[5], Dan T. Vogl[6], Yvonne Efebera[7], Mingwei Fei[8], Nancy Geller[9], Sergio Giralt[10], Theresa Hahn[3], Alan Howard[11], Mindy Kohlhagen[2], Heather Landau[10], Parameswaran Hari[4], Marcelo C. Pasquini[4], Muzaffar H. Qazilbash[12], Philip McCarthy[3], Nina Shah[13], David H. Vesole[14], Edward Stadtmauer[9] and David Murray[2]



Sensitivity	Specificity	PPV	NPV
0.81	0.42	0.39	0.83

Sensitivity	Specificity	PPV	NPV
0.71	0.41	0.15	0.90

Sensitivity	Specificity	PPV	NPV
0.64	0.58	0.12	0.95

Sensitivity	Specificity	PPV	NPV
0.70	0.59	0.45	0.81

Sensitivity	Specificity	PPV	NPV
0.54	0.62	0.18	0.90

Sensitivity	Specificity	PPV	NPV
0.57	0.66	0.12	0.95

Performance of serum Mass-Fix as compared to bone marrow MRD. a–c performance of Mass-Fix among patients in CR or better at 3 time points; **d–f** performance of SIFE among patients in VGPR or better at 3 time points.

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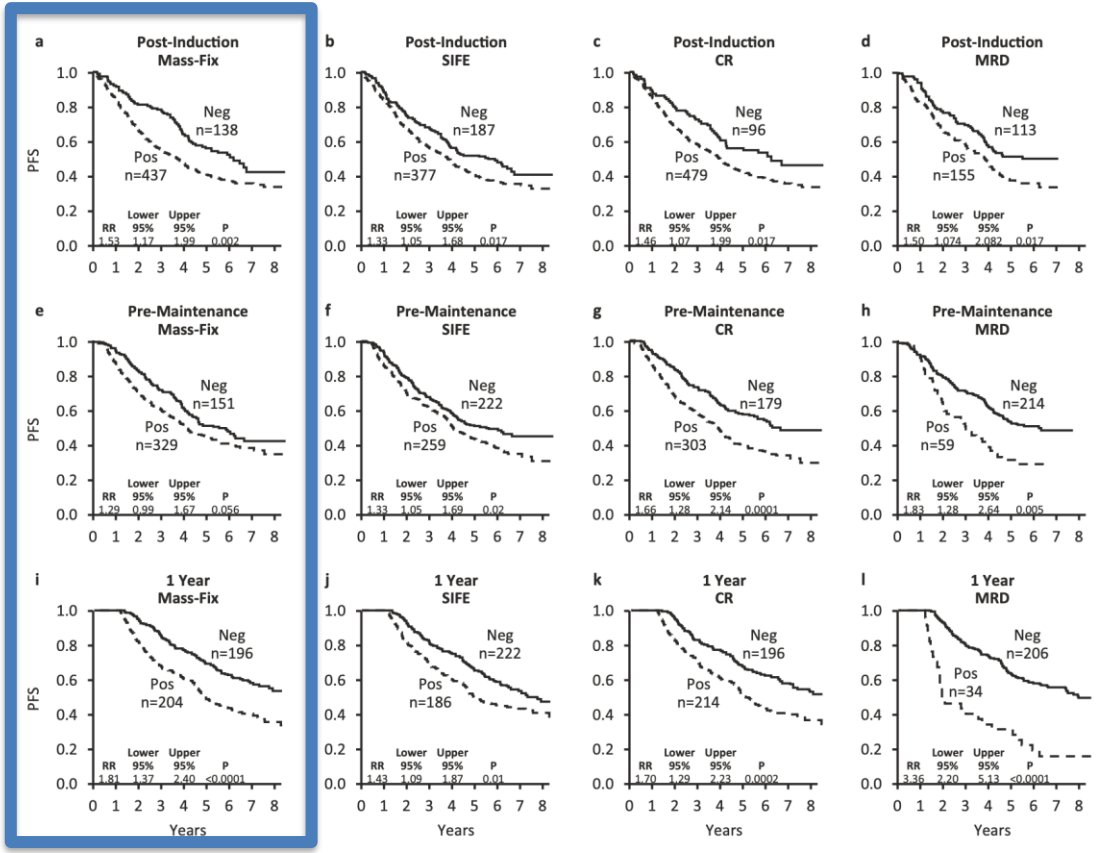

Progression free survival based on response measurement at the time points. a–d post-induction sample; **d–h** pre-maintenance sample; **i–l** 1 year post enrollment sample.

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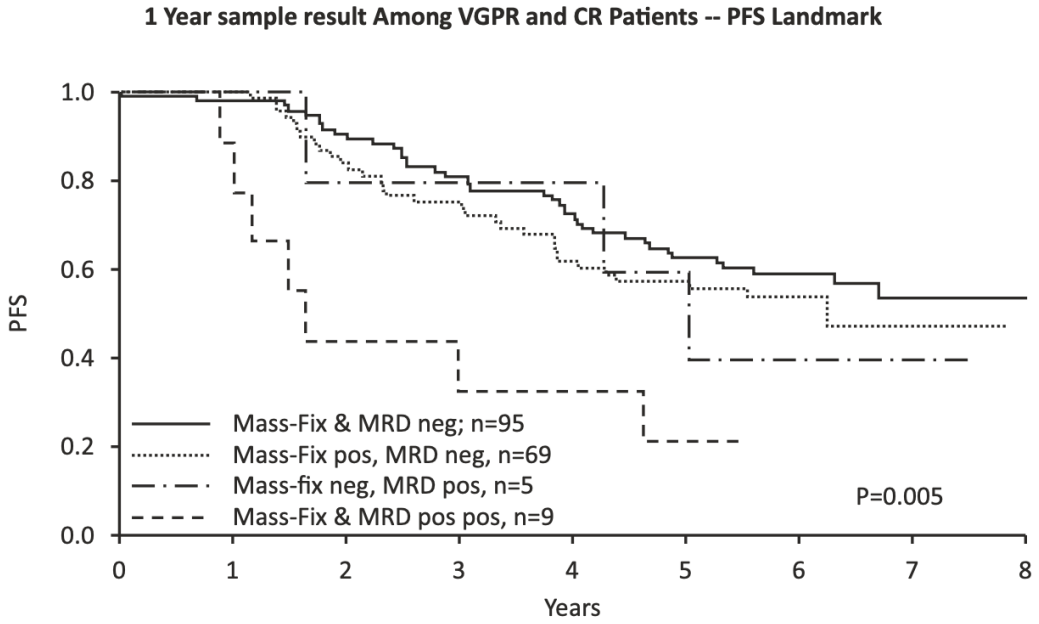

Interaction between Mass-Fix and MRD status and PFS using 1-year post enrollment MRD and Mass-Fix results.

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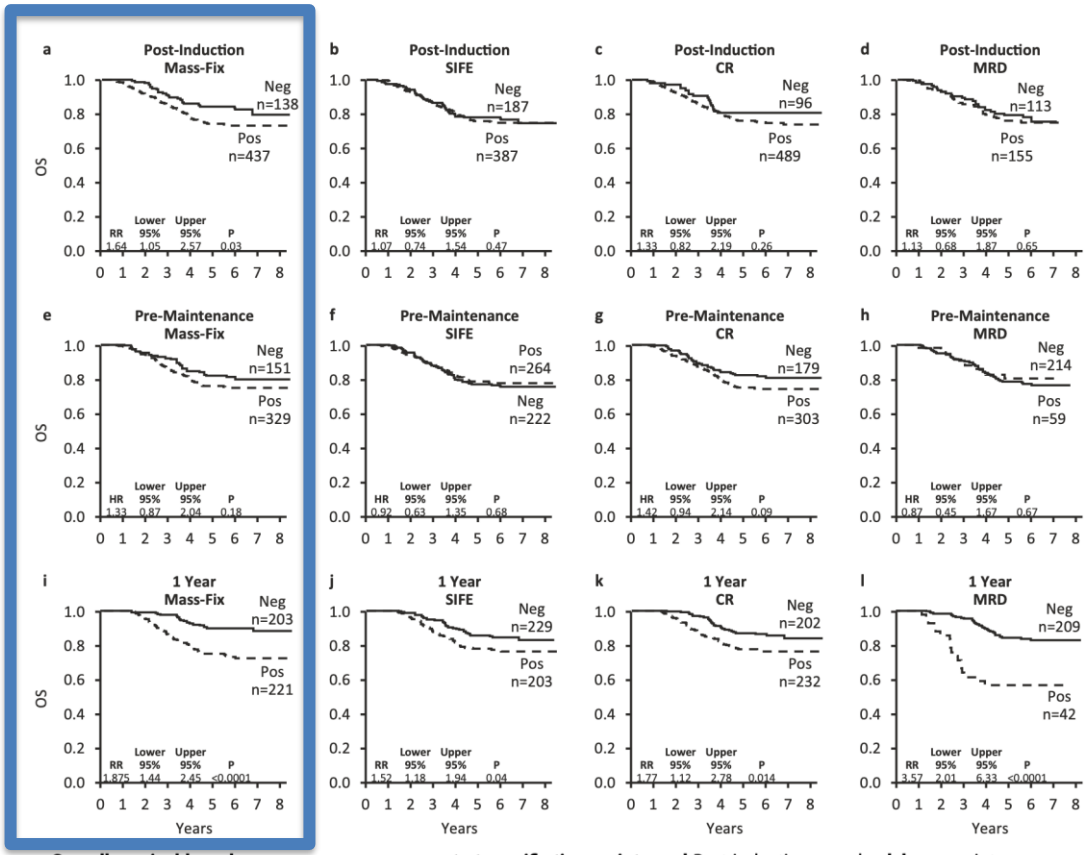


Overall survival based on response measurement at specific time points. a–d Post-induction sample; **d–h** pre-maintenance sample; and **i–l** 1 year post enrollment sample.

Value of tests at post-induction & at 1-year
Multivariate

	PFS RR (95%CI)	P	OS RR (95%CI)	P
Post-induction samples				
MRD positive	1.50 (1.1, 2.1)	0.017	-	-
Mass-Fix positive	-	NS	1.64 (1.05, 2.57)	0.03
<CR *	-	NS	-	-
SIFE positive	-	NS	-	-
1-year sample s				
MRD positive	3.0 (1.9, 4.7)	<0.001	2.8 (1.5, 5.1)	0.001
Mass-Fix positive	1.6 (1.1, 2.3)	0.012	1.9 (1.0, 3.6)	0.036
<CR *	-	NS	NS	NS
SIFE positive	-	NS	NS	NS

Post-induction:

- **MRD was only response measure predictive for PFS**
- **Mass-Fix was only response measure predictive for OS**

At 1YR measure,

- **Mass-Fix and MRD predicted for PFS and OS**

* CR was assessed by individual investigators using IMWG criteria including serum, urine, and bone marrow.

Conclusions

1. Post-induction: Mass-Fix was the only response measure to be prognostic for OS
2. At 1YR measure, Mass-Fix predicted for PFS and OS independent of MRD status by high-resolution flow
3. Mass-Fix provides a convenient and non-invasive means of predicting for myeloma outcomes
4. Future directions will include comparison of Mass-Fix by next generation sequencing and to determine whether Mass-Fix can detect early relapse

Mass spectrometry vs immunofixation for treatment monitoring in multiple myeloma

Noemí Puig,[1,*] María-Teresa Contreras,[2,*] Cristina Agulló,[2] Joaquín Martínez-López,[3] Albert Oriol,[4] María-Jesús Blanchard,[5] Rafael Ríos,[6] Jesús Martín,[7] María-Belén Iñigo,[8] Anna Sureda,[9] Miguel-Teodoro Hernández,[10] Javier de la Rubia,[11] Verónica González-Calle,[1] Isabel Krsnik,[12] Valentín Cabañas,[13] Luis Palomera,[14] José-María Moraleda,[15] Joan Bargay,[15] María-Teresa Cedena,[3] Bruno Paiva,[16] Laura Rosiñol,[17] Joan Bladé,[17] Jesús San Miguel,[16] Juan-José Lahuerta,[16] and María-Victoria Mateos[1]

[1]Hematology Department, IBSAL, Instituto de Biología Molecular y Celular del Cáncer–Consejo Superior de Investigaciones Científicas (IBMCC-CSIC), Centro de Investigación Biomédica en Red de Cáncer (CIBERONC); [2]Clinical Biochemistry Department, Hospital Universitario de Salamanca, Salamanca, Spain; [3]Hospital Universitario 12 de Octubre, Complutense University, i+12, CNIO, CIBERONC CB16/12/00369, Madrid, Spain; [4]Institut Català d'Oncologia, Institut Josep Carreras Hospital Germans i Trials, Barcelona, Spain; [5]Hematology Department, Hospital Ramón y Cajal, Madrid, Spain; [6]Hematology Department, Hospital Universitario Virgen de las Nieves, Ibs. GRANADA, CIBERESP, Granada, Spain; [7]Hospital Universitario Virgen del Rocío, Sevilla, Spain; [8]Hospital Clínico San Carlos, Madrid, Spain; [9]Institut Català d'Oncologia-I'Hospitalet, IDIBELL, Universitat de Barcelona, Barcelona, Spain; [10]Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain; [11]Department of Hematology, University Hospital La Fe and School of Medicine and Dentistry, Catholic University of Valencia, CIBERONC CB16/12/00284, Valencia, Spain; [12]Hospital Puerta de Hierro, Madrid, Spain; [13]Hospital Universitario Virgen de la Arrixaca, IMIB, Murcia, Spain; [14]Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain; [15]Hospital Son Llatzer, Palma de Mallorca, Spain; [16]Clínica Universidad de Navarra, CIMA, IDISNA, CIBERONC CB16/12/00369, Pamplona, Spain; [17]Hospital Clínic, Institut d'Investigacions Biomédiques August Pi I Sunyer (IDIBAPS), Barcelona, Spain; and [18]Instituto de Investigación del Hospital Universitario 12 de Octubre, CIBERONC, Madrid, Spain

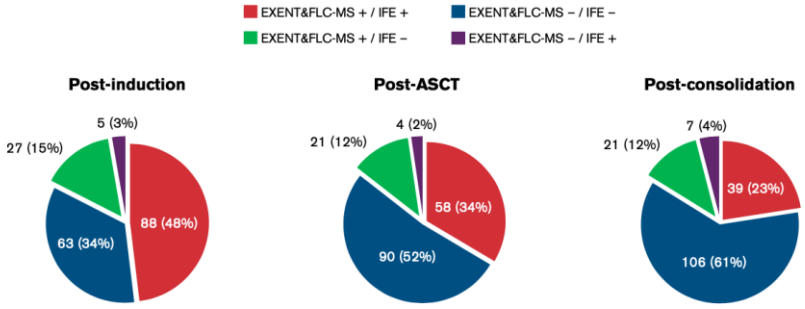
A

IFE

EXENT&FLC-MS	IgGκ	IgGλ	IgAκ	IgAλ	IgMκ	IgMλ	Free κ	Free λ	Bi/Triclonal	NS	Total
IgGκ	71	2	0	0	0	0	1	0	0	0	74
IgGλ	0	43	0	1	0	0	0	1	0	0	45
IgAκ	0	0	32	0	0	0	1	0	0	0	33
IgAλ	1	0	0	18	0	0	0	1	0	0	20
IgMκ	0	0	0	0	0	0	1	0	0	1	2
IgMλ	1	0	0	0	0	0	0	0	0	0	1
Free κ	0	0	0	0	0	0	12	0	0	1	13
Free λ	0	1	0	0	0	1	0	7	0	1	10
Bi/Triclonal	10	3	5	3	0	0	1	2	0	1	25
NS	0	0	0	0	0	0	0	0	0	0	0
Total	83	49	37	22	0	1	16	11	0	4	223

NS: non secretory

B



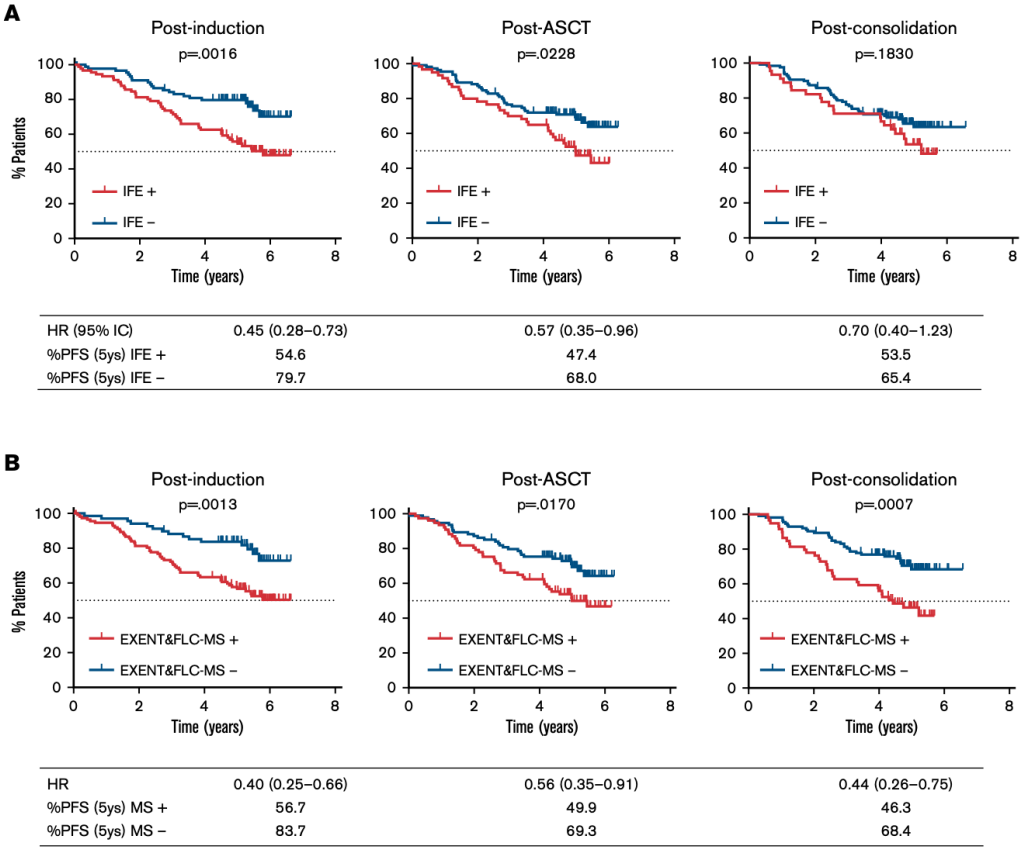
	Post-induction	Post-ASCT	Post-consolidation
Concordant, n (%)	151 (82.5)	148 (85.5)	145 (83.8)
Discordant, n (%)	32 (17.5)	25 (14.5)	28 (16.2)

Comparison between EXENT&FLC-MS and IFE results. **(A)** M-protein(s) isotyping at baseline. **(B)** Detection of the M-protein post-induction, post-ASCT, and at the end of consolidation.

Mass spectrometry vs immunofixation for treatment monitoring in multiple myeloma

Noemí Puig,[1,*] María-Teresa Contreras,[2,*] Cristina Agulló,[2] Joaquín Martínez-López,[3] Albert Oriol,[4] María-Jesús Blanchard,[5] Rafael Ríos,[6] Jesús Martín,[7] María-Belén Iñigo,[8] Anna Sureda,[9] Miguel-Teodoro Hernández,[10] Javier de la Rubia,[11] Verónica González-Calle,[1] Isabel Krsnik,[12] Valentín Cabañas,[13] Luis Palomera,[14] José-María Moraleda,[15] Joan Bargay,[15] María-Teresa Cedena,[3] Bruno Paiva,[16] Laura Rosiñol,[17] Joan Bladé,[17] Jesús San Miguel,[16] Juan-José Lahuerta,[16] and María-Victoria Mateos[1]

[1]Hematology Department, IBSAL, Instituto de Biología Molecular y Celular del Cáncer–Consejo Superior de Investigaciones Científicas (IBMCC-CSIC), Centro de Investigación Biomédica en Red de Cáncer (CIBERONC); [2]Clinical Biochemistry Department, Hospital Universitario de Salamanca, Salamanca, Spain; [3]Hospital Universitario 12 de Octubre, Complutense University, i+12, CNIO, CIBERONC CB16/12/00369, Madrid, Spain; [4]Institut Català d'Oncologia, Institut Josep Carreras Hospital Germans i Trials, Barcelona, Spain; [5]Hematology Department, Hospital Ramón y Cajal, Madrid, Spain; [6]Hematology Department, Hospital Universitario Virgen de las Nieves, Ibs. GRANADA, CIBERESP, Granada, Spain; [7]Hospital Universitario Virgen del Rocío, Sevilla, Spain; [8]Hospital Clínico San Carlos, Madrid, Spain; [9]Institut Català d'Oncologia-I'Hospitalet, IDIBELL, Universitat de Barcelona, Barcelona, Spain; [10]Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain; [11]Department of Hematology, University Hospital La Fe and School of Medicine and Dentistry, Catholic University of Valencia, CIBERONC CB16/12/00284, Valencia, Spain; [12]Hospital Puerta de Hierro, Madrid, Spain; [13]Hospital Universitario Virgen de la Arrixaca, IMIB, Murcia, Spain; [14]Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain; [15]Hospital Son Llatzer, Palma de Mallorca, Spain; [16]Clínica Universidad de Navarra, CIMA, IDISNA, CIBERONC CB16/12/00369, Pamplona, Spain; [17]Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona, Spain; and [18]Instituto de Investigación del Hospital Universitario 12 de Octubre, CIBERONC, Madrid, Spain



PFS after induction, post-ASCT, and at the end of consolidation.
(A) IFE status. **(B)** EXENT&FLC-MS status.

Mass spectrometry vs immunofixation for treatment monitoring in multiple myeloma

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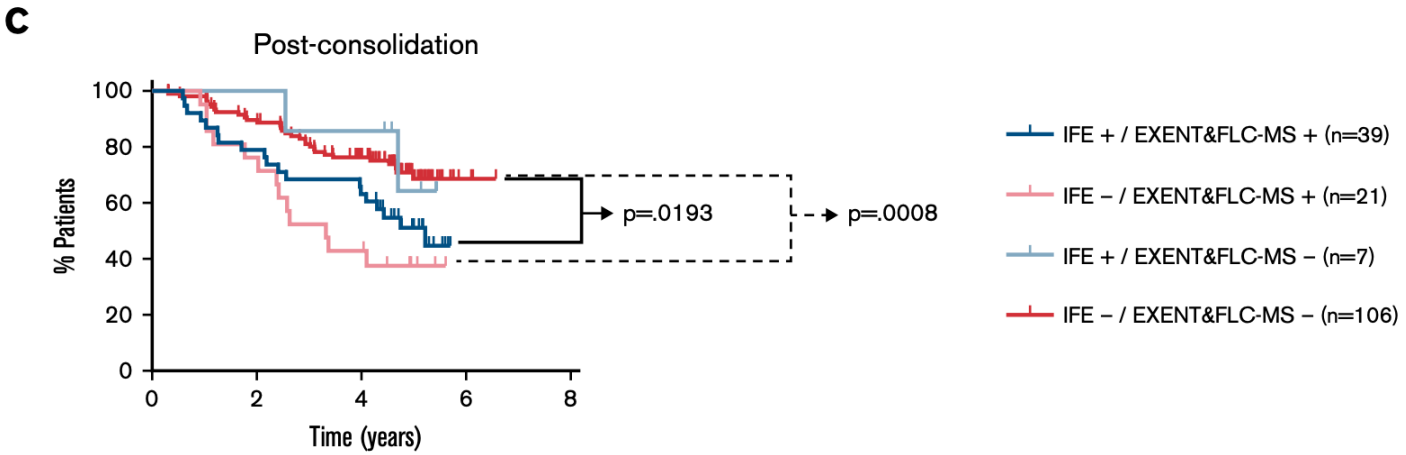

PFS after induction, post-ASCT, and at the end of consolidation. (C) combined IFE and EXENT&FLC-MS status. HR, hazard ratio.

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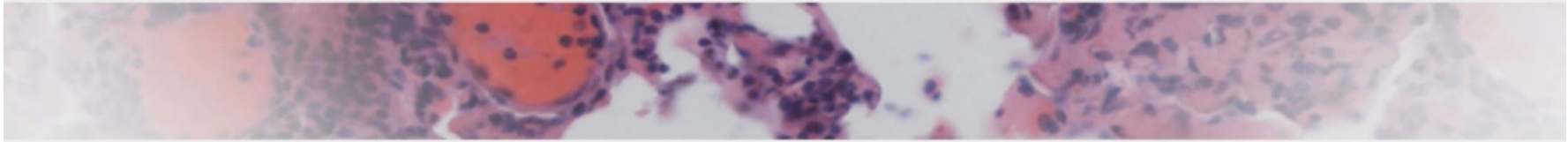
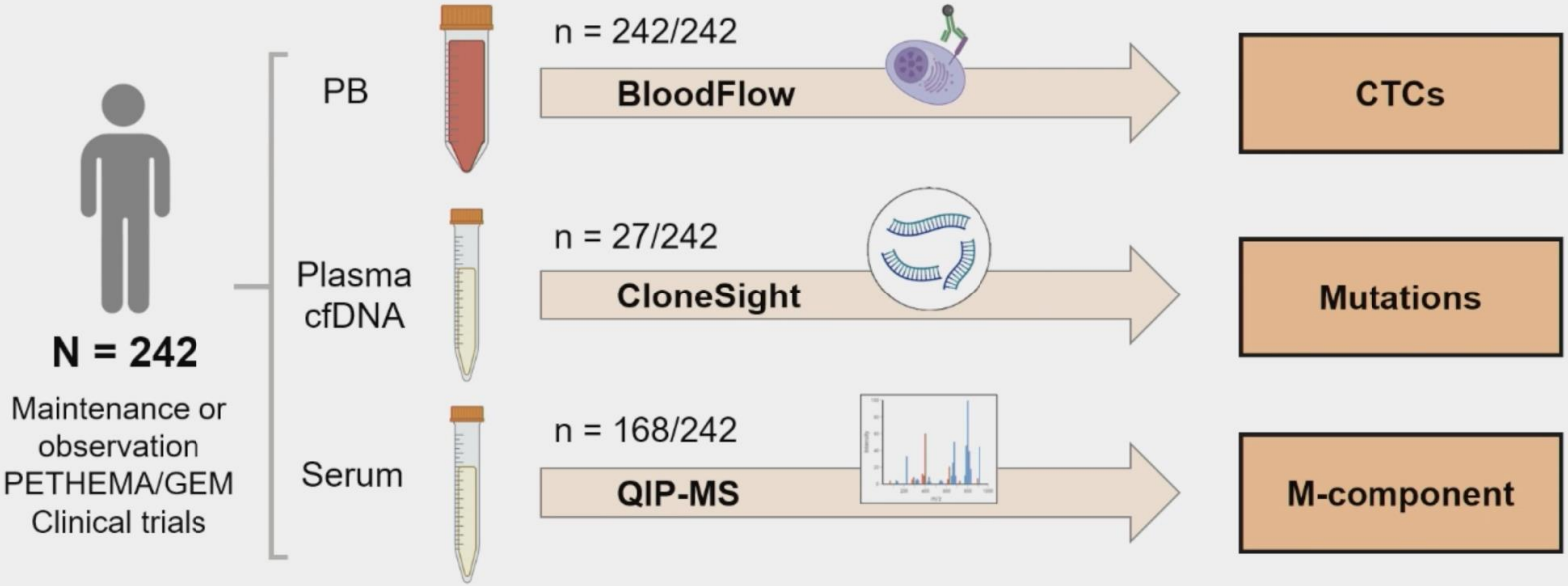


Minimally Invasive Assessment of Measurable Residual Disease (MRD) in Multiple Myeloma (MM)

M Lasa, L Notarfranchi, C Agullo, N Buenache, **C Gonzalez**, A Zherniakova, S Castro, J J Perez, V González de la Calle, M T Cedena, S Barrio, A Martin-Muñoz, L Burgos, D Alignani, M J Calasanz, P Rodriguez-Otero, L Rosiñol, F De Arriba, E M. Ocio, A Oriol, L Palomera, Y Gonzalez, A Sureda, M T Hernandez, M E Clavero Sanchez, A Ibañez, C Gomez, A Orfao, M V Mateos, J J Lahuerta, J Blade, J San Miguel, J Martinez-Lopez, N Puig and B Paiva,

on behalf of the PETHEMA/GEM cooperative group

Investigate the complementarity and prognostic value of new multimodal minimally invasive MRD assessment in MM



Minimally invasive response assessment in PB using BloodFlow

Median CTCs/µL, 0.016 (range 0.0003 - 0.29)

506 paired samples		Bone Marrow	
		NGF-	NGF+
Periph Blood	BloodFlow-	358 (70.8%)	103 (20.3%)
	BloodFlow+	2 (0.4%)	43 (8.5%)

22% of patients relapsed thus far

79% concordance BM (NGF) & PB (BloodFlow)

Minimally invasive response assessment in PB using BloodFlow

Median CTCs/µL, 0.016 (range 0.0003 - 0.29)

506 paired samples		Bone Marrow	
		NGF-	NGF+
Periph Blood	BloodFlow-	358 (70.8%)	103 (20.3%)
	BloodFlow+	2 (0.4%)	43 (8.5%)

1 of the 2 patients relapsed thus far

79% concordance BM (NGF) & PB (BloodFlow)

Minimally invasive MRD assessment in cfDNA and serum

CloneSight and QIP-MS respectively yielded high PPV and NPV

CloneSight

48 paired samples		Bone Marrow	
		NGF-	NGF+
cfDNA	CloneSight -	14 (29.2%)	29 (60.4%)
	CloneSight +	0 (0%)	5 (10.4%)

40% concordance BM & PB

QIP-MS

249 paired samples		Bone Marrow	
		NGF-	NGF+
Serum	QIP-MS-	154 (61.8%)	34 (13.7%)
	QIP-MS+	18 (7.2%)	43 (17.3%)

79% concordance BM & PB

BloodFlow and QIP-MS showed more balanced NPV and PPV

CloneSight showed the highest PPV but low NPV

BloodFlow	CloneSight	QIP-MS
78% NPV 96% PPV	33% NPV 100% PPV	82% NPV 70.5% PPV

BloodFlow and QIP-MS showed more balanced NPV and PPV

CloneSight showed the highest PPV but low NPV

BloodFlow

78% NPV
96% PPV

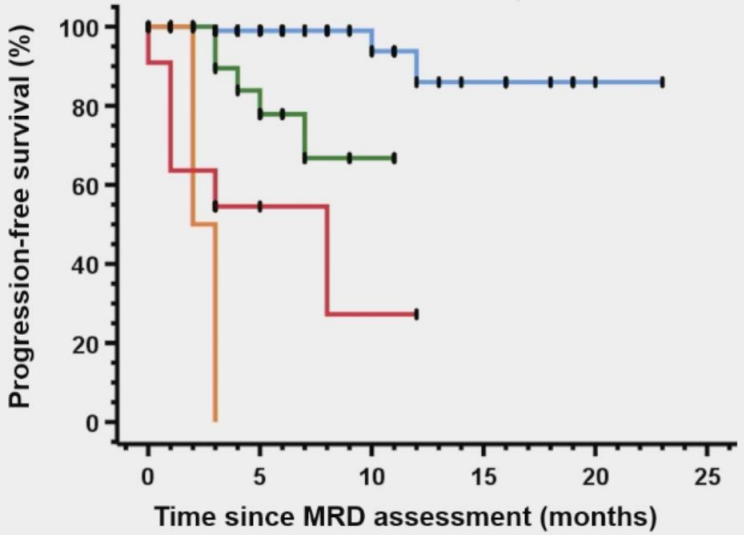
QIP-MS

82% NPV
70.5% PPV

Are these methods complementary for improved prediction of PFS?

Complementarity between BloodFlow and QIP-MS

3/129 (2%) double negative MRD patients progressed thus far



MRD BF & QIP-MS	No.	Median PFS	PFS @1y	Hazard ratio
-/-	129	NR	98%	-
-/+	26	NR	80%	9.8 (P = .002)
+/-	2	2 mo	0%	97.7 (P < .001)
+/+	11	8 mo	46%	29.5 (P < .001)

Double-negative MRD detection in PB and serum using BloodFlow and QIP-MS achieved a **NPV of 84%** (ie, MRD negativity in BM using NGF)

Conclusions

- BloodFlow, CloneSight and QIP-MS are empowered to detect MRD with high sensitivity in PB, cfDNA and serum
- The presence of CTCs was systematically associated with dismal PFS
- BloodFlow and Clonesight showed very high PPV and QIP-MS achieved the highest NPV
- The complementarity between these methods enabled the identification of multimodal MRD negative patients with very low risk of relapse
- This study paves the way towards minimally invasive MRD assessment in MM patients on maintenance or observation

P-074

Personalized mass spectrometry as a tool for minimal residual disease detection in the blood of myeloma patients

Somayya Noori[1], Charissa Wijnands[2], Vincent Bonifay[3], Theo Luider[1], Pierre Sonigo[3], Thomas Dejoie[4], Jolein Gloerich[2], Alain van Gool[2], Martijn van Duijn[1], Joannes Jacobs[2]

[1]Erasmus Medical Center; [2]Radboudumc; [3]Sebia; [4]Centre Hospitalier Universitaire

Introduction: Multiple myeloma (MM) is a clonal plasma cell disorder found in the bone marrow that produces a monoclonal immunoglobulin (M-protein). Blood based M-protein diagnostics allows monitoring of disease activity, but with limited sensitivity. Minimal residual disease (MRD) status is a powerful prognostic biomarker. Lack of sensitivity prevents MRD detection by conventional blood based assays. Bone marrow based assays such as next-generation sequencing (NGS) are highly sensitive in measuring MRD. However, bone marrow biopsies introduce a risk of non-representative sampling and are invasive, which limits repeated testing. Frequent MM monitoring during remission could provide actionable information on disease activity and treatment response. Earlier detection of disease progression could lead to early intervention and, potentially, patient survival benefits. The aim of this study was to perform M-protein monitoring on blood samples of MM patients with sensitive targeted mass spectrometry. **Methods:** We have developed a targeted mass spectrometry-based MRD blood-test (MS-MRD) that detects clonotypic peptides originating from the variable region of the M-protein. Absolute M-protein quantification (g/L) was performed based on the M-protein peptide and an internal standard, and the data were evaluated for early increases in disease activity. MS-MRD was performed on 926 longitudinally collected sera of 41 MM patients from the IFM 2009 trial (ClinicalTrials.gov number: NCT01191060). **Results:** Based on unique patient-specific M-protein peptides, absolute M-protein quantification was feasible in all 41 patients with 1000 times higher sensitivity compared to electrophoretic M-protein quantification. For patients with confirmed progression within the serum sample collection period, MS-MRD revealed the increase of MM disease activity on average 455 days earlier than the progression detected with currently used routine diagnostics (p≤0.0001). **Conclusions:** MS-MRD blood-testing is feasible in all patients with multiple myeloma and it has similar sensitivity and prognostic value compared to NGS-MRD evaluation performed on bone marrow. The MS-MRD blood-test paves the way for dynamic MRD monitoring to allow detection of early disease relapse. This minimally invasive MRD test may proof to be well suited to facilitate future clinical implementation of MRD-guided therapy.

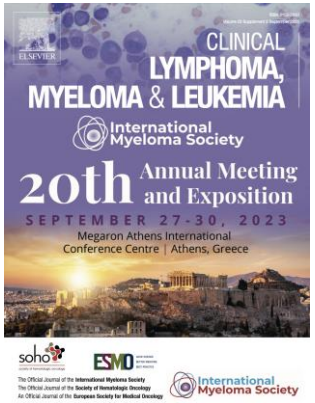
P-085

The use of clonotypic mass spectrometry for post-AHCT blood-based measurable residual disease monitoring in patients with light chain multiple myeloma

Michael Slade[1], Abir Khaled[2], Julie Fortier[1], Mariya Liyasova[2], Mark Fiala[1], Zac McDonald[2], Sarah Kelley[1], Kate Breberina[2], Nisha Owens[2], Zachary Crees[1], Mark Zaydman[1], Mark Schroeder[1], Keith Stockerl-Goldstein[1], Ravi Vij[1]

[1]Washington University School of Medicine in St. Louis; [2]Rapid Novor

Introduction: Given the invasive nature of bone marrow sampling, there is growing interest in blood-based methods of measurable residual disease (MRD) testing for patients with multiple myeloma (MM). We previously reported data utilizing clonotypic mass spectrometry (MS) in patients with intact immunoglobulin M-protein (Slade, ASH 2022). However, 10-20% of patients present with light chain (LC) M-protein and data on the feasibility of clonotypic MS in these patients is limited. **Methods:** Given the invasive nature of bone marrow sampling, there is growing interest in blood-based methods of measurable residual disease (MRD) testing for patients with multiple myeloma (MM). We previously reported data utilizing clonotypic mass spectrometry (MS) in patients with intact immunoglobulin M-protein (Slade, ASH 2022). However, 10-20% of patients present with light chain (LC) M-protein and data on the feasibility of clonotypic MS in these patients is limited. **Results:** Of 17 patients analyzed, 15 had a measurable clonotypic signature at diagnosis while 2 did not and were excluded from further analysis. Fourteen patients were White and 8 were male. Three, 8 and 4 patients were R-ISS stage I, II and III. The median age at AHCT was 59 (range: 45 – 76). The median difference in FLC (dFLC) at diagnosis was 265 mg/dL (range: 43 – 1449) and median EasyM was 5.62 arbitrary units (AU) (range 0.16 – 207.86). The lower limit of quantification varied given the unique clonotypic signature of each M-protein, with a median of 0.0088 AU (range: 0.00094 – 0.033) and median % quantifiable reduction in EasyM of 99.87% (range: 95.86 – 99.99%). At day +100, 14 patients (93%) were in CR. One was in VGPR at day +100. The median dFLC was 0.10 mg/dL (range 0.00 – 0.99). 8 (53%) had no detectable disease by EasyM (MRD-) at day +100; 7 (47%) had residual disease (MRD+). The median % residual EasyM in MRD+ patients was 0.20% of baseline (range 0.02 – 1.12%). Median baseline dFLC (249 vs. 270 mg/dL) and EasyM (0.78 vs. 10.32 AU) were numerically lower in the MRD- group, though these differences did not reach statistical significance. Median follow up was 4.4 years. At last follow up, 4 of 7 patients in the MRD+ group had relapsed and 3 had died. One MRD- patient died in remission from infection on day +619. The remainder of the MRD- patients remain alive and disease-free at a median of 3.9 years (range: 1.0 – 8.3) post-AHCT. **Conclusions:** In this study, we showed that EasyM generates a trackable clonotype in the vast majority (88%) of patients with LC MM and that relapse was numerically higher in patients with normal dFLC and MRD+ by EasyM at day +100 after AHCT. Defining the clinical application of EasyM requires larger studies with additional time-points, but this series demonstrated that it may be a useful tool for disease monitoring in LC MM.



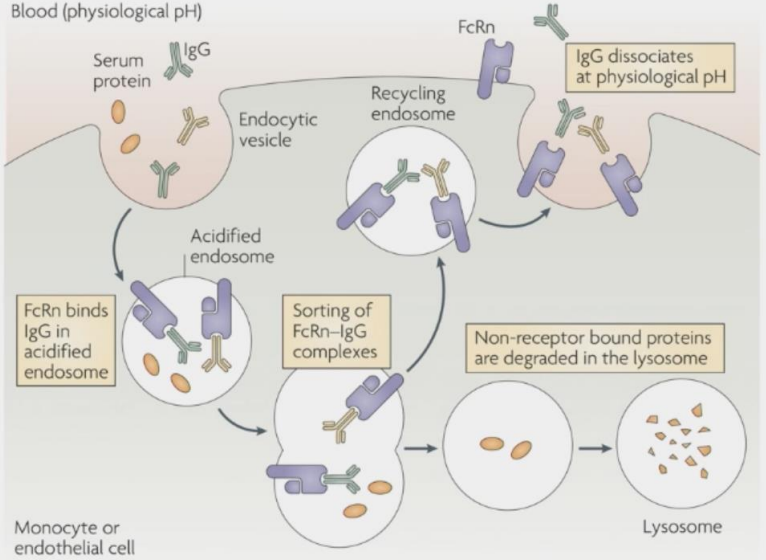
Mass Spectrometry-Based Assessment of M-Protein in Peripheral Blood during Maintenance Therapy in Multiple Myeloma (MM) in the Phase III ATLAS Trial

Tadeusz Kubicki, Dominik Dytfeld, David Barnidge, DJ Sakrikar, Gabriella Lakos, Anna Przybyłowicz-Chalecka, Krzysztof Jamroziak, Paweł Robak, Jarosław Czyż, Agata Tyczyńska, Agnieszka Druzd-Sitek, Krzysztof Giannopoulos, Tomasz Wróbel, Adam Nowicki, Tomasz Szczepaniak, Anna Łojko-Dankowska, Magdalena Matuszak, Lidia Gil, Bartosz Puła, Łukasz Szukalski, Agnieszka Końska, Jan M Zaucha, Jan Walewski, Damian Mikulski, Olga Czabak, Tadeusz Robak, Ken Jiang, Jennifer H Cooperrider, Andrzej J Jakubowiak, Benjamin A Derman

Study Objectives

1. To determine the feasibility of using MS as blood-based MRD evaluation method in the post-transplant setting.

2. To determine the optimal timing of post-ASCT MS assessment for prognostication.



Roopenian, Nature Reviews Immunology 200

Methods

ATLAS trial

Key eligibility criteria:

≤100 days after HSCT
≤ 12 months after diagnosis
≤ 2 induction regimens
≥SD after HSCT

RANDOMIZE

1:1

KRd

Carfilzomib (36 mg/m²)
D1,2,8,9,15,16 – cycles 1-4
D1,2,15,16 – cycles 5-8
Lenalidomide (25 mg)
D1-21 – cycles 1-8
Dexamethasone (20 mg)
D1,8,15,22 – cycles 1-8

MRD (-), SR

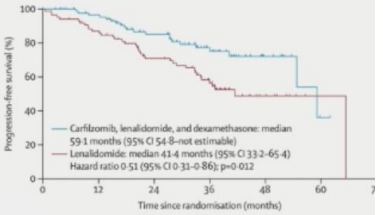
Carfilzomib (36 mg/m²)
D1,2,15,16 – cycles 9-36
Lenalidomide (25 mg)
D1-21 – cycles 9-36
Dexamethasone (20 mg)
D1,8,15,22 – cycles 9-36

Lenalidomide (15 mg)
D1-28 – cycles 9-36

R
Lenalidomide (10 mg/15 mg*)
D1-28 – cycles 1-36
** 10 mg for C1-C3, 15 mg from C4, if tolerated*

LENALIDOMIDE MAINTENANCE

Interim results (PFS)



KRd pts with standard risk (SR) cytogenetics having reached IMWG MRD negativity after C6 converted to R alone after C8

MALDI-TOF MS

Screening | Cycle 6 | Cycle 12 | Cycle 18 | Cycle 24 | Cycle 36 → EXENT® Solution

Dytfeld, Lancet Oncology 2023

Samples evaluation

(n=138 patients from n=180 treated in the ATLAS trial)

	MS	Paired MS/NGS	Paired MS/MFC
Screening	121	70	105
Cycle 6	118	73	106
Cycle 12	114	64	98
Cycle 18	96	57	80
Cycle 24	81	51	71
Cycle 36	55	34	39
Overall	**585**	**349**	**499**

- No baseline diagnostic samples were available.
- Samples were not available for every patient at every timepoint.
- A sample was called positive by MS when the assay confirmed the presence of an M protein, matching the known (diagnostic) isotype.
- Threshold for bone marrow MRD positivity – 10^{-5}.

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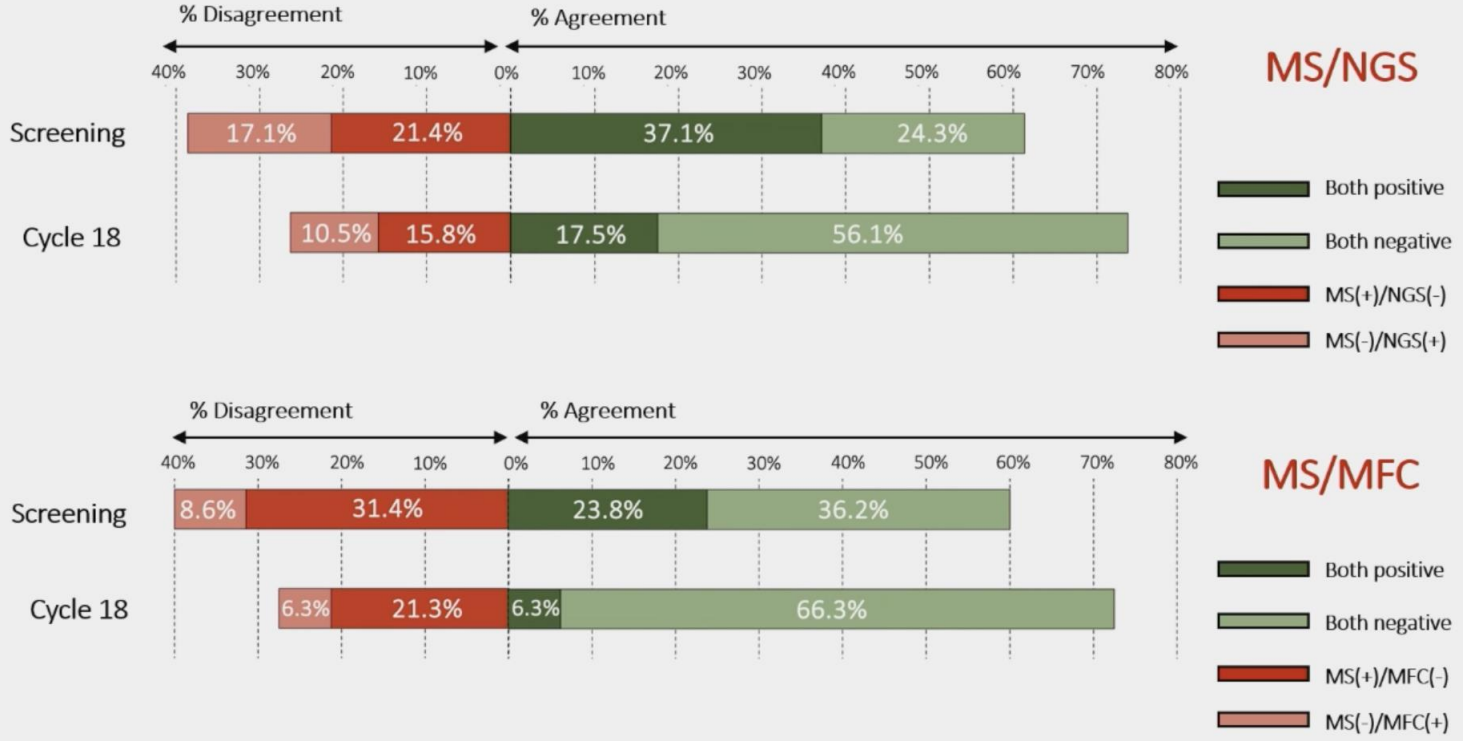
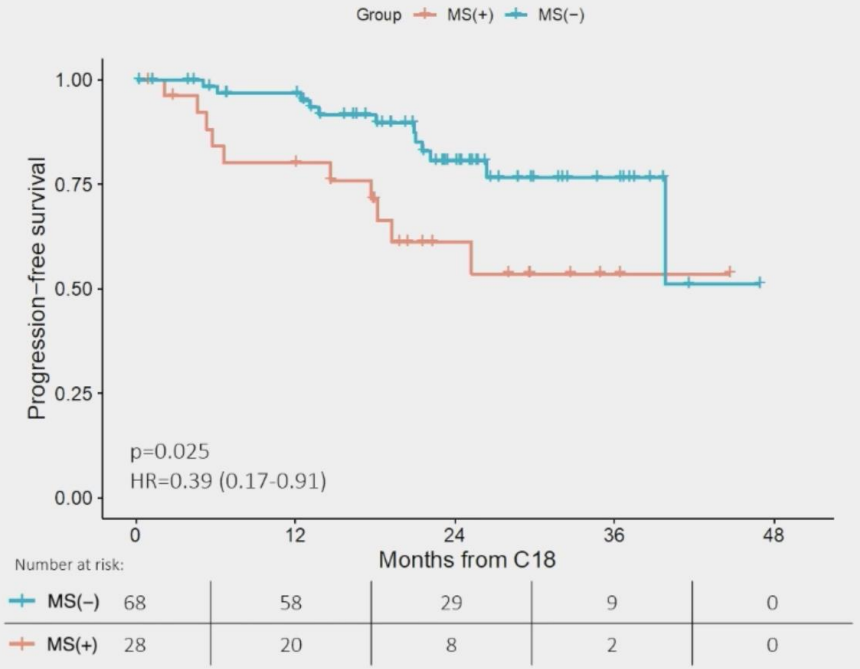
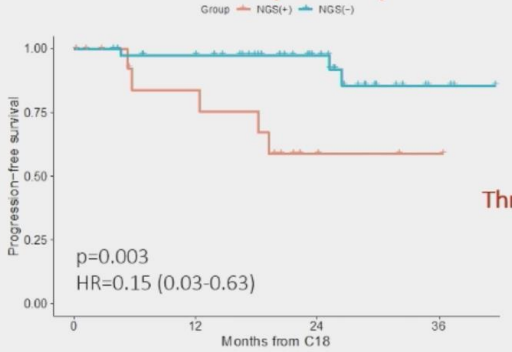
Agreement between the methods increased with time

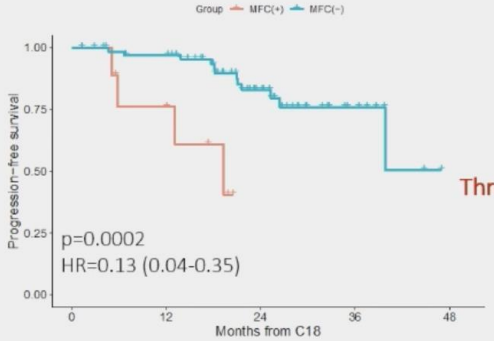


MS (-) status post cycle 18 was associated with superior progression-free survival (PFS)



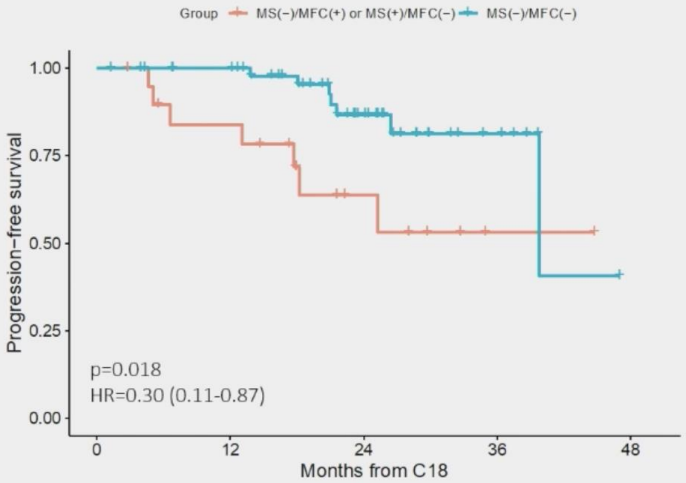


NGS
Threshold: 10^{-5}

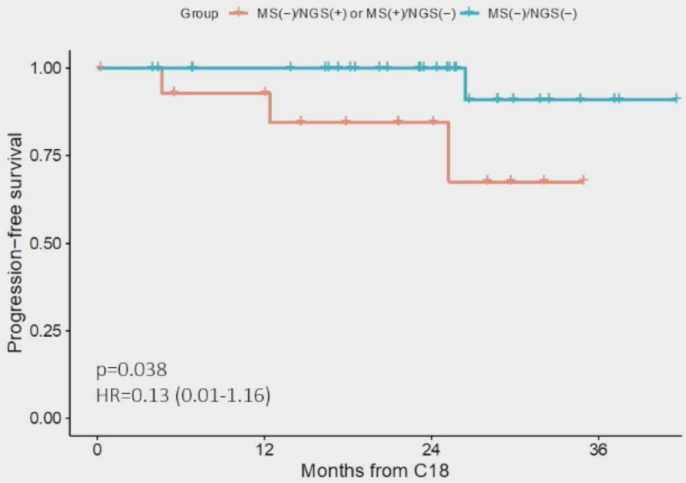


MFC
Threshold: 10^{-5}

Double (MS and MRD) negativity is associated with favorable outcomes



MFC and MS



NGS and MS

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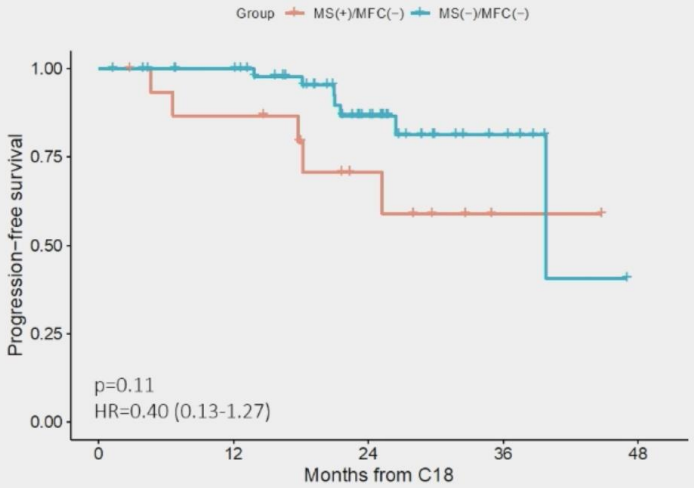
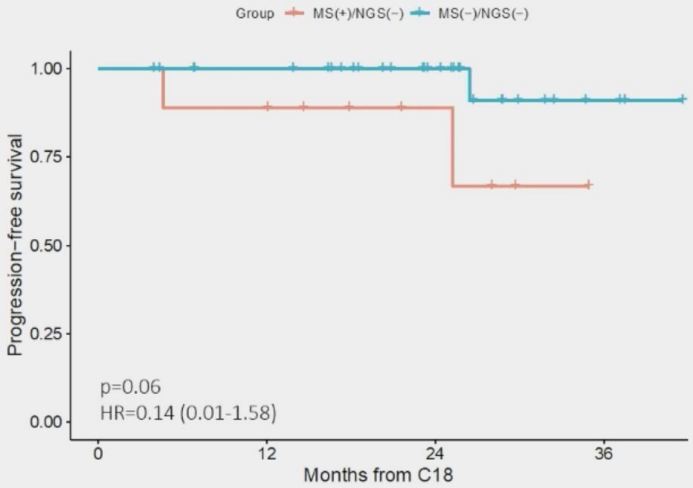
MS results may add prognostic value to MRD negative status



MFC and MS



NGS and MS

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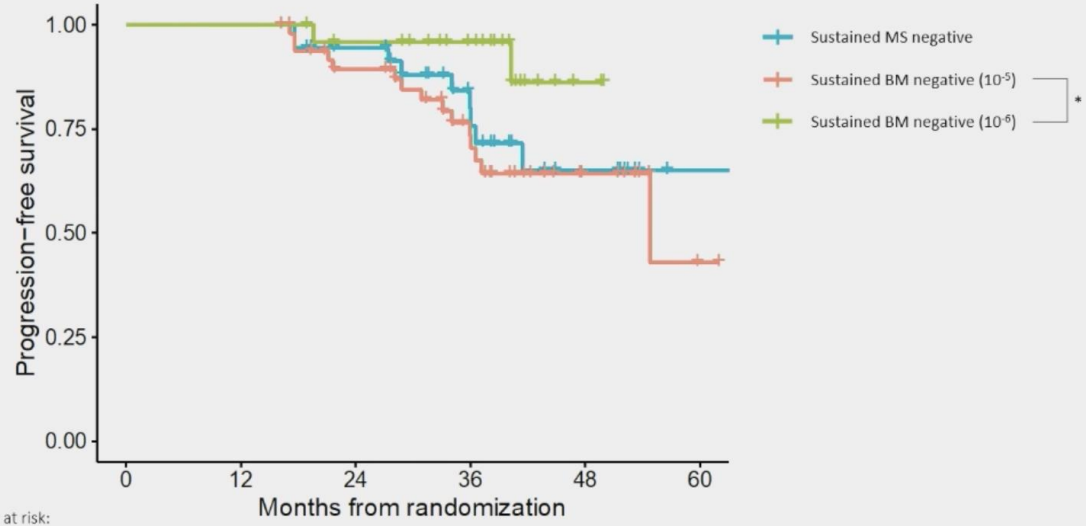
Prognostic implications of sustained (≥12 months) MS negativity



Number at risk:

Sustained MS negative	38	38	31	19	8	1
Sustained BM negative (10^{-5})	50	50	40	24	8	1
Sustained BM negative (10^{-6})	25	25	22	16	2	0

Conclusions

- MS-based disease assessment in the post-ASCT setting may be feasible.
- Prognostic significance of MS negativity increases with time.
- MS is complementing BM-based MRD assessments.
- Further prospective studies are needed to confirm these conclusions.

Polish Myeloma Consortium

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Isolated and Dynamic Peripheral Blood Residual Disease Status Characterized by Mass Spectrometry Predicts Outcome in Patients with High Risk Smoldering Multiple Myeloma Treated in the GEM-CESAR Trial

Noemí Puig, Cristina Agulló, Teresa Contreras, Bruno Paiva, María-Teresa Cedena, José-Juan Pérez, Irene Aires, Joaquín Martínez-López, Paula Rodríguez, Verónica González-Calle, Marta-Sonia González, Norma Gutiérrez, Albert Oriol, Rafael Ríos, Laura Rosiñol, Miguel-Ángel Álvarez, María-José Calasanz, Joan Bargay, Ramón García-Sanz, Ana-Pilar González, Adrián Alegre, Fernando Escalante, Rafael Martínez, Javier de la Rubia, Ana Isabel Teruel, Felipe de Arriba, Luis Palomera, Miguel-Teodoro Hernández, Javier López, Arancha García-Mateo, Enrique M Ocio, Joan Bladé, Jesús F San Miguel, Juan-José Lahuerta and María-Victoria Mateos

On behalf of the Myeloma Spanish Group (GEM/PETHEMA)

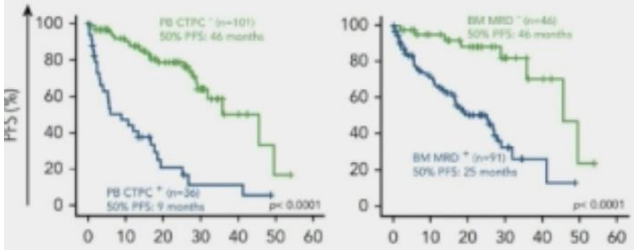
PETHEMA GEM GRUPO ESPAÑOL DE MIELOMA

VNiVERSiDAD D SALAMANCA

EuroFlow a division of ESLHO

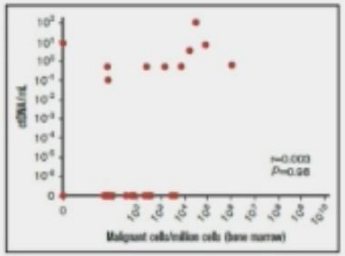
Peripheral blood as alternative sample for MRD analysis in patients with MM

Flow cytometry



Sanoja-Flores L, et al. Blood. 2019 Dec 12;134(24):2218-2222

NGS



Mazzotti C, et al. Blood Adv. 2018 13;2(21):2811-2813

Mass spectrometry

QIP-Mass Spectrometry in High Risk Smoldering Multiple Myeloma Patients Included in the GEM-CESAR Trial: Comparison with Conventional and Minimal Residual Disease IMWG Response Assessment

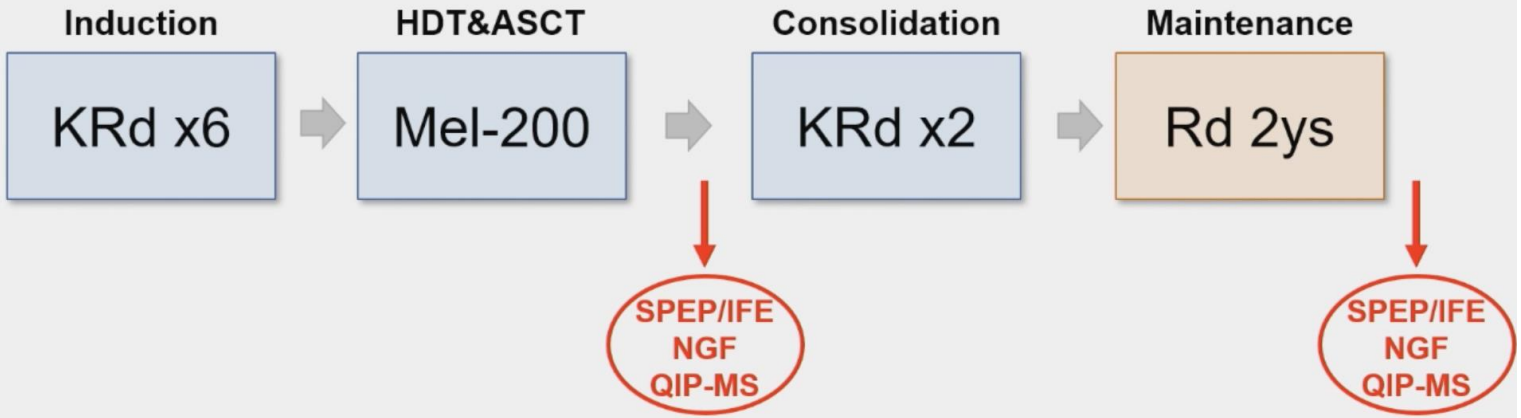
Noemí Puig, Teresa Contreras, Bruno Paiva, María-Teresa Cedena, José-Juan Pérez, Irene Aires, Cristina Agulló, Joaquín Martínez-López, Paula Rodríguez-Otero, Verónica González de la Calle, Marta-Sonia González, Albert Oriol, Norma-Carmen Gutiérrez, Rafael Ríos, Laura Rosiñol, Miguel-Ángel Álvarez, María-José Calasanz, Joan Bargay, Ana Pilar González, Adrián Alegre, Fernando Escalante, Rafael Martínez, Javier de la Rubia, Ana-Isabel Teruel, Felipe de Arriba, Luis Palomera, Miguel-Teodoro Hernández, Javier López, Jesús Martín, Aránzazu García Mateo, Ramón García-Sanz, Enrique M. Ocio, Joan Bladé, Juan-José Lahuerta, Jesús F. San Miguel, María-Victoria Mateos

on behalf of the Spanish Myeloma Group (PETHEMA/GEM)

Puig et al, ASH 2019

GEM CESAR: study design

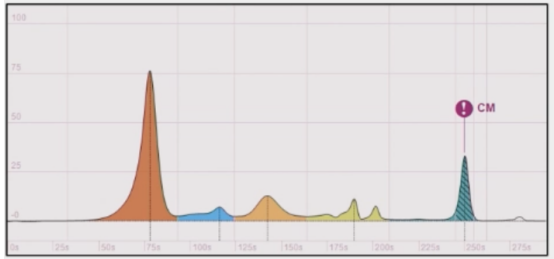
Multicenter, open-label, phase II trial
High-risk smoldering MM patients
n=90



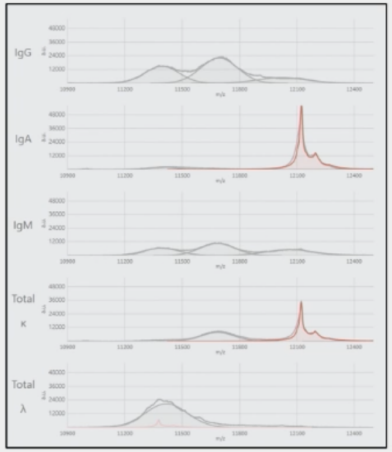
KRD: 4-week cycles of iv carfilzomib (K) at dose 20/36 mg/m^2 days 1-2, 8-9,15-16, oral lenalidomide (R) 25 mg days 1-21 and dexamethasone (D) 40 mg once weekly.
Rd: R at dose of 10 mg on days 1-21 plus dexamethasone at dose of 20 mg once per week for two years

Methods

SPEP/IFE

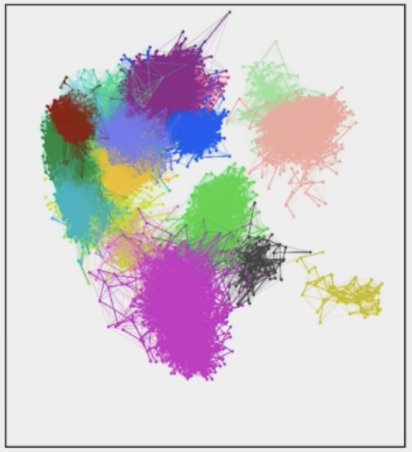


QIP-MS



Carried out with anti IgG/A/M, total κ and total λ beads using the EXENT®Solution (The Binding Site, part of Thermo Fisher Scientific).

NGF



According to the recommendations of the IMWG and the Euroflow guidelines

Patients characteristics

	N=90 (%)
Age in years, median (range)	59 (33-70)
Male	47 (52%)
MM subtype	
IgG	57 (63%)
IgA	27 (30%)
Light-chain	6 (7%)
Amount of MP (mean, g/dL)	2.8
% bone marrow infiltration (mean)	26.9%
Cytogenetics, n (%)	
High-risk	31 (35%)
Standard risk	55 (61%)
Unknown	4 (4%)
High risk	
Mayo/Pethema/Both	7 (8%) 30 (33%) 22 (24%)
Ultra high-risk	31 (34%)
M-spike by QIP-MS	**100%**

Ability to identify persistent disease

	Induction	HDT&ASCT	Consolidation	1 year Maintenance	2 years Maintenance
	n=62	n=61	n=61	n=51	n=35
SPEP/IFE	34 (55%)	**20 (33%)**	16 (26%)	9 (18%)	5 (14%)
QIP-MS	46 (74%)	**33 (54%)**	29 (47.5%)	11 (21.5%)	7 (20%)
NGF	45 (72.5%)	**26 (43%)**	24 (39%)	11 (21.5%)	12 (34%)

Comparative results IFE *vs* QIP-MS

After HDT&ASCT

n = 67

		IFE	
		+	-
QIP-MS	+	19 (28%)	16 (24%)
	-	3 (4%)	29 (43%)

p-value	<0.0001
Positive Predictive Value	0.5429
Negative Predictive Value	0.9063
Sensitivity	0.8636
Specificity	0.6444

After 2 years of maintenance

n = 37

		IFE	
		+	-
QIP-MS	+	5 (13%)	4 (11%)
	-	2 (5%)	26 (70%)

p-value	0.0049
Positive Predictive Value	0.5556
Negative Predictive Value	0.9286
Sensitivity	0.7143
Specificity	0.8667

* Fisher's exact test

Comparative results IFE *vs* QIP-MS

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* Fisher's exact test

Comparative results NGF *vs* QIP-MS

After HDT&ASCT

n = 67

		NGF	
		+	-
QIP-MS	+	24 (36%)	**14 (21%)**
	-	**5 (7%)**	24 (36%)

p-value	**0.0002**
Positive Predictive Value	0.6316
Negative Predictive Value	0.8276
Sensitivity	0.8276
Specificity	0.6316

After 2 years of maintenance

n = 39

		NGF	
		+	-
QIP-MS	+	6 (15%)	**3 (8%)**
	-	**8 (20%)**	22 (56%)

p-value	**0.0475**
Positive Predictive Value	0.6667
Negative Predictive Value	0.7333
Sensitivity	0.4286
Specificity	0.8800

* Fisher's exact test

Comparative results NGF *vs* QIP-MS

After HDT&ASCT

n = 67

		NGF	
		+	-
QIP-MS	+	24 (36%)	14 (21%)
	-	5 (7%)	24 (36%)

p-value	**0.0002**
Positive Predictive Value	0.6316
Negative Predictive Value	**0.8276**
Sensitivity	0.8276
Specificity	0.6316

After 2 years of maintenance

n = 39

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		+	-
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	-	8 (20%)	22 (56%)

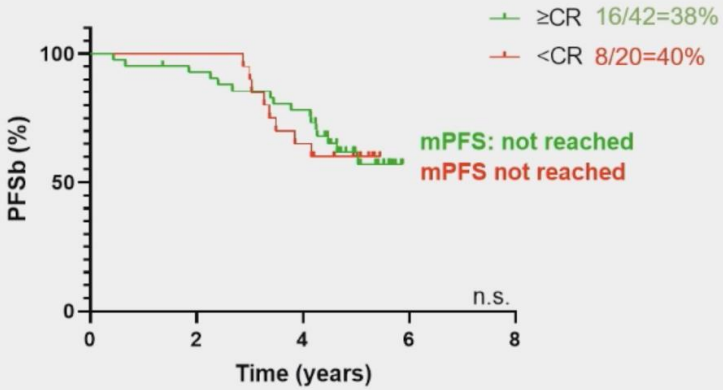
p-value	**0.0475**
Positive Predictive Value	0.6667
Negative Predictive Value	**0.7333**
Sensitivity	0.4286
Specificity	0.8800

* Fisher's exact test

PFS according to IMWG standard response criteria

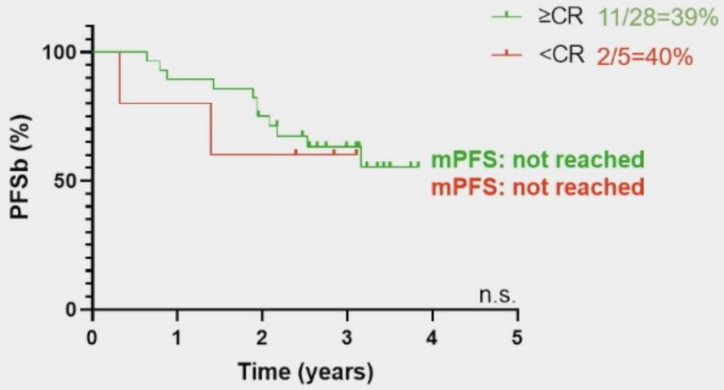
After HDT&ASCT

n = 62



After 2 years of maintenance

n = 33

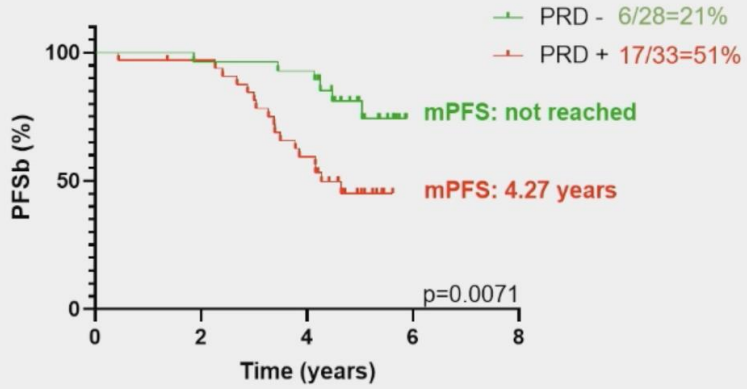


Standard response criteria (≥CR vs <CR) did not separate groups with different PFS

PFS according to QIP-MS

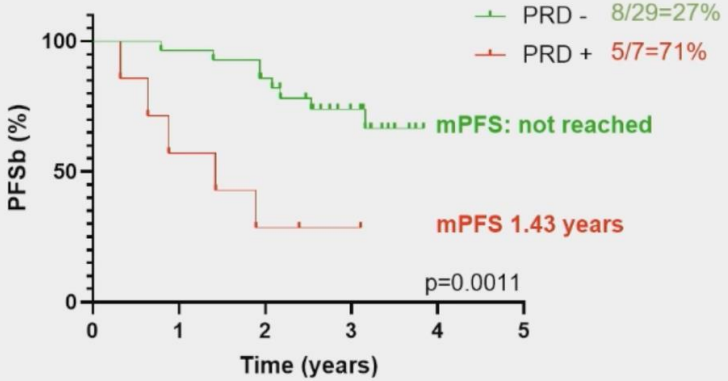
After HDT&ASCT

n = 61



After 2 years of maintenance

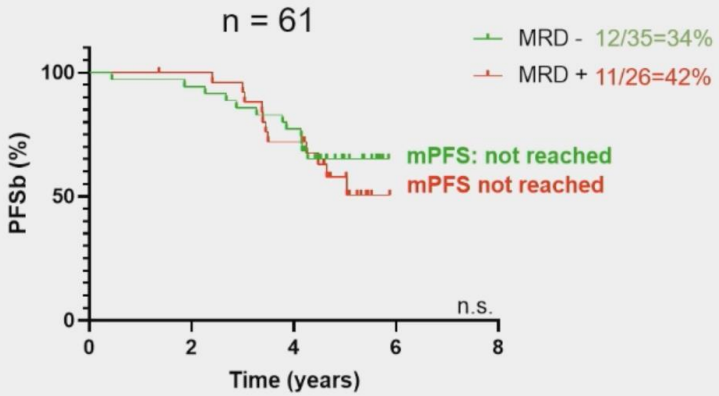
n = 36



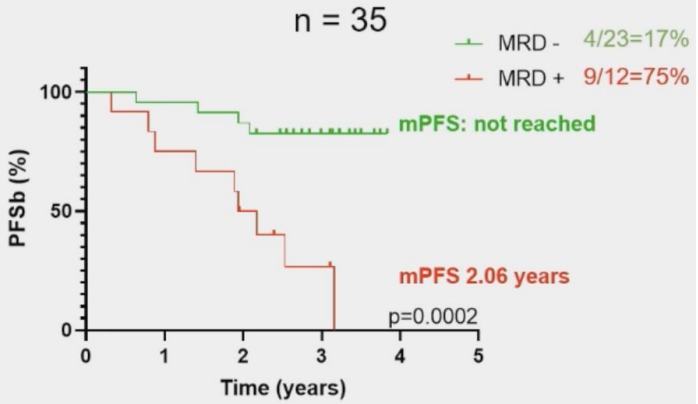
Results obtained with QIP-MS separated two groups with different PFS

PFS according to NGF

After HDT&ASCT



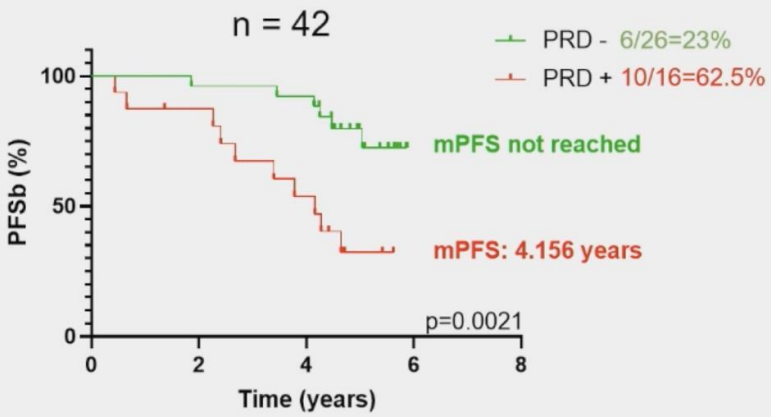
After 2 years of maintenance



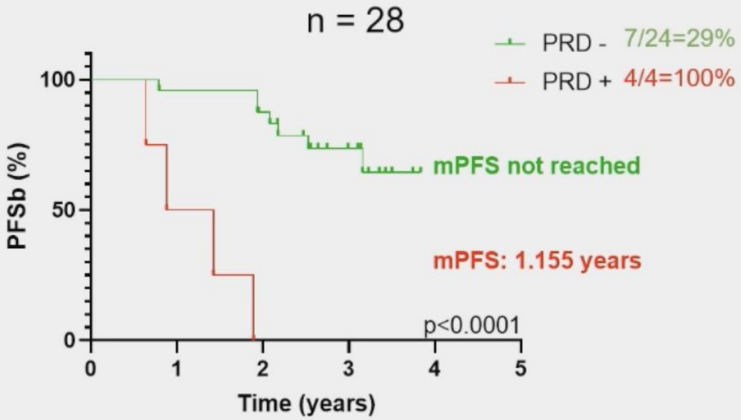
Results obtained with NGF separated two groups with different PFS at the end of treatment

PFS according to QIP-MS in cases ≥CR

After HDT&ASCT



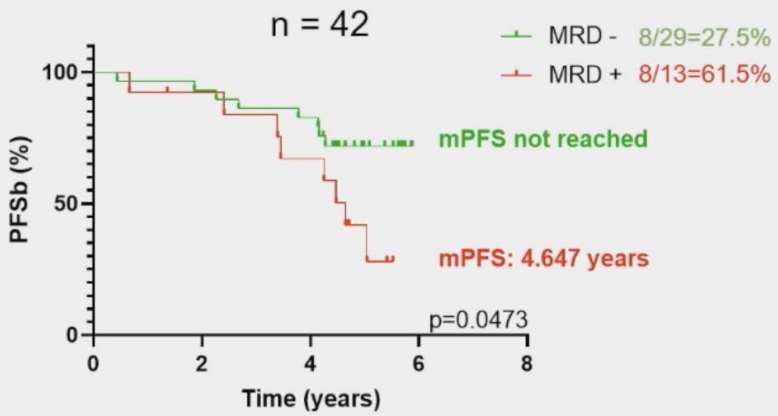
After 2 years of maintenance



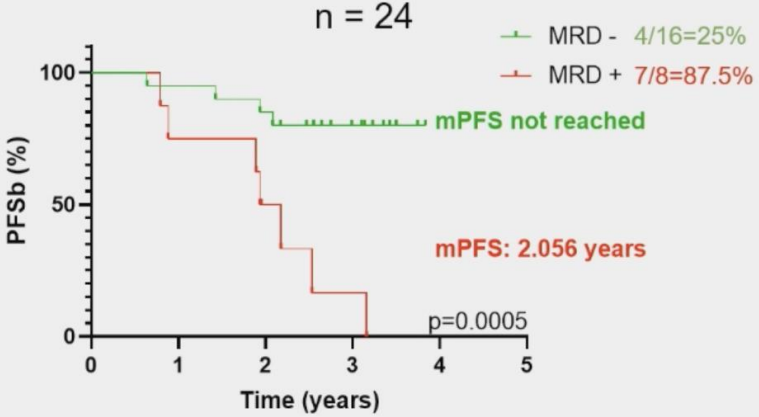
Among patients in ≥CR, results obtained with QIP-MS separated two groups with different PFS

PFS according to NGF in cases ≥CR

After HDT&ASCT



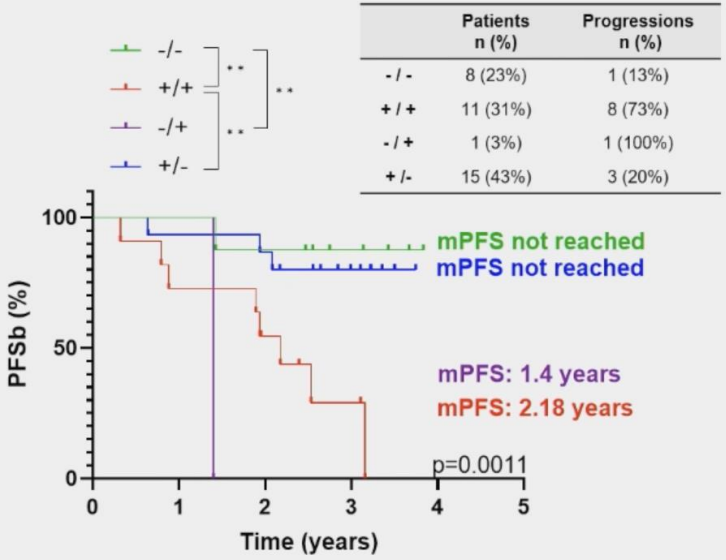
After 2 years of maintenance



Among patients in ≥CR, results obtained with NGF separated two groups with different PFS

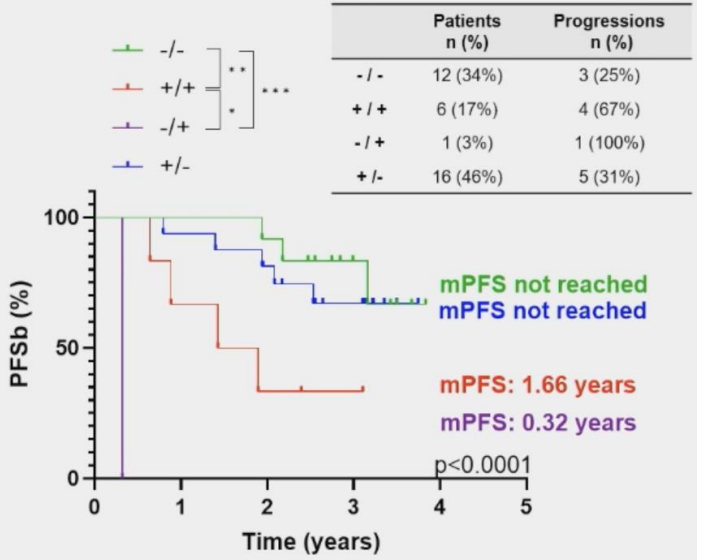
PFS according to QIP-MS and NGF dynamics

MRD dynamics



	Patients n (%)	Progressions n (%)
- / -	8 (23%)	1 (13%)
+ / +	11 (31%)	8 (73%)
- / +	1 (3%)	1 (100%)
+ /-	15 (43%)	3 (20%)

PRD dynamics



	Patients n (%)	Progressions n (%)
- / -	12 (34%)	3 (25%)
+ / +	6 (17%)	4 (67%)
- / +	1 (3%)	1 (100%)
+ /-	16 (46%)	5 (31%)

Analysis of the dynamics of the results obtained by QIP-MS and NGF improved the prediction of patients´outcome

Conclusions

In this cohort of high-risk smoldering MM patients treated intensively with a curative intention,

1. standard CR or better was not associated with clinical outcome
2. mass-spec segregates two groups of patients with different PFS at the 2 time points analyzed (post-ASCT and after 2 years of maintenance) and both in the global cohort as well as in cases in CR or better
3. our data suggest that the dynamics of mass-spec results improve the prediction of patients outcome
4. the results of mass-spec identify a cohort of patients in CR with detectable disease and worse outcome that, if our results are further confirmed, could justify the introduction a new serological response category

CONNECTING LIFE AND SCIENCE

Measurable Residual Disease Status by Clonotypic Mass Spectrometry with EasyM Assay Predicts Outcomes Following AHCT in Multiple Myeloma

Michael J Slade, MD, MS[1], Abir Khaled, PhD[2], Zac McDonald, PhD[2], Mark A Fiala, PhD[1], Mariya Liyasova, PhD[2], Julie M Fortier, PhD[1], Mark A Zaydman, MD, PhD[3], Sarah Kelley, BS[1], Zachary D. Crees, MD[1], Mark A. Schroeder, MD[1], Keith E Stockerl-Goldstein, MD[1], Liqiang Yang, PhD[2] and Ravi Vij, MD, MBBS[1]

[1]Division of Oncology, Washington University School of Medicine, Saint Louis, MO, [2]Rapid Novor, Kitchener, ON [3]Division of Laboratory & Genomic Medicine, Washington University School of Medicine, Saint Louis, MO.

Background and Significance

- MRD tests in clinical use for multiple myeloma require bone marrow (BM) biopsies that are uncomfortable and time-consuming for patients.
- Consequently, there is an urgent unmet need for peripheral blood-based MRD testing that would allow for convenient, repeated assessment of MRD.
- The use of clonotypic mass spectrometry (i.e. the EasyM assay) to track the M-protein "peptide fingerprint" allows for sensitive and specific monitoring of MRD in the peripheral blood.
- The association with EasyM peripheral blood MRD with outcomes is unknown.

Objective

We aim to evaluate the association between reduction in M-protein via the EasyM clonotypic mass spectrometry assay (i.e. EasyM MRD) and progression-free survival (PFS) after autologous hematopoietic cell transplantation.

Patients and Methods

Study Cohort:

- Retrospective cohort of patients who met the following criteria: 1) diagnosis of MM, 2) underwent autologous hematopoietic cell transplant (AHCT), 3) M-protein ≥0.2 g/dL by serum protein electrophoresis (SPEP) or >20 mg/dL (light chain) at diagnosis, and 4) had adequate samples for analysis collected at day +100 after AHCT. For analysis of association between MRD and PFS, only patients with had no detectable M-protein by SPEP at day +100 were included.

Sample Processing

- M-proteins in serum were de-novo sequenced using a combination of multiple protease sample digestion and mass spectrometry (MS) based analysis to identify a unique clonotype for each patients. Each peptide was targeted using a parallel reaction monitoring method applied on an Exploris 120 Orbitrap instrument and spiked protein standard was used for normalization.

Definitions

- The primary endpoint was progression-free survival (PFS), defined as time from AHCT to death or disease recurrence by IMWG criteria
- Percent reduction in EasyM was defined as decrease from baseline EasyM value.

Statistical Analysis

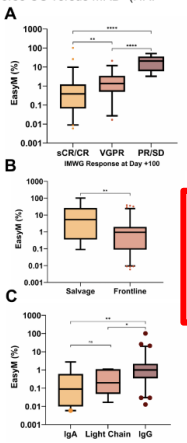
- Demographics and treatment characteristics were reported using standard descriptive statistics.
- EasyM values between groups were comparing using the Mann-Whitney U test
- The optimal cutoff to predict PFS (i.e. EasyM MRD definition) was derived using the conditional inference tree (C-tree) method.
- PFS and overall survival (OS) were evaluated using the EasyM MRD definition via the Kaplan-Meier method and log-rank test.

Results

- 114 patients were included in the analysis (Table 1).
- 86% of patients received IMID/PI-based induction, 10% received quadruplet induction and 90% received frontline AHCT.
- Post-AHCT percent residual EasyM was significantly associated with IMWG response (Fig 1A) and salvage vs. frontline AHCT (Fig 1B).
- 31 patients were removed from the cohort for MRD analysis due to detectable disease (19), poor limit-of-detect (9) or failed clonotype identification (3).
- In the MRD cohort, the post-AHCT percent residual EasyM was significantly higher in patients with IgG versus IgA/LC myeloma (Fig 3C)
- In IgG patients, the optimal MRD cutoff was 1.034% residual EasyM versus 0.048% residual EasyM in IgA/LC patients.
- By these definitions, 46% (40/86) patients were MRD-positive post-AHCT.
- At day +100, MRD+ was associated with significantly shorter PFS versus MRD- (HR: 2.38, 95% C.I. 1.21 – 4.68) versus MRD
- MRD+ was also associated with significantly worse OS versus MRD- (HR: 5.83, 95% C.I. 1.71 – 19.9).

	Full Cohort	MRD Cohort
Patients - (n)	114	86
Age - median (range)	63 (35 - 78)	64 (35 - 78)
Male - n (%)	72 (63)	52 (61)
White - n (%)	100 (88)	76 (88)
M-Protein Isotype - n (%)		
IgG	80 (70)	61 (71)
IgA	19 (17)	19 (22)
Light Chain	15 (13)	6 (7)
R-ISS Stage - n (%)		
Stage I	28 (25)	23 (27)
Stage II	72 (63)	54 (63)
Stage II	14 (12)	9 (10)
High Risk Cyto - n (%)	33 (29)	26 (30)
Upfront AHCT - n (%)	102 (90)	76 (88)
Response at day +100 - n (%)		
sCR/CR	65 (57)	54 (63)
VGPR	37 (32)	32 (37)
PR	10 (9)	0 (0)
SD	2 (2)	0 (0)
Pre-AHCT Induction - n (%)		
Immunomodulatory Drug	101 (89)	77 (90)
Proteasome Inhibitor	111 (97)	83 (97)
Anti-CD38 Antibody	11 (10)	10 (12)

Table 1: Demographics and clinical characteristics for study cohort.



Results

Figure 2: Progression-free survival (Fig 1A) and overall survival (Fig 1B) by EasyM MRD status. MRD status by EasyM was strongly associated with both PFS (HR: 2.38) and OS (HR: 5.83), suggesting this assay may provide useful prognostic data for patients undergoing AHCT for multiple myeloma.

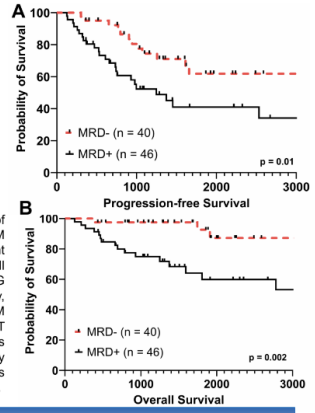


Figure 1: Association of percent residual EasyM post-AHCT with key patient factors. EasyM was well correlated with IMWG response (Fig 1A). Notably, post-AHCT residual EasyM was higher in salvage AHCT (Fig 1B) and in IgG versus IgA/LC myeloma, likely related to the long half-lives of these molecules (Fig 1C).

Conclusions

- Identification of an EasyM clonotype was successfully performed in 97% of patients submitted for analysis.
- Post-AHCT percent residual EasyM was well-correlated with IMWG response criteria and was higher in patients with IgG myeloma.
- MRD status by EasyM post-AHCT was significantly associated with improvements in PFS and OS.
- Validation of proposed MRD cutoffs in otherl cohorts and analysis of EasyM in other treatment settings is required to further understand clinical utility.

Disclosures

EasyM assay was performed by Rapid Novor at no cost and some co-authors on this project are Rapid Novor employees as noted above. Rapid Novor was blinded to clinical data during analysis. None of the other author offers receive financial support or compensation from Rapid Novor or have any ownership stake in the company. Full author disclosures available with abstract submission.

EVALUATION OF EASYM, A CLONOTYPIC MASS SPECTROMETRY ASSAY, AND EUROFLOW MINIMAL RESIDUAL DISEASE ASSESSMENT IN MULTIPLE MYELOMA

JESSIE ZHAO[1,2], TIFFANY T. KHONG[1,2], MALGORZATA GORNIAK[3], ABIR KHALED[4], ZAC MCDONALD[4], JOHN REYNOLDS[1,2], DANIEL WONG[1,2], NICHOLAS BINGHAM[1,2], ANNA M. JOHNSTON[5], OLGA MOTORNA[6], NICHOLAS E. MURPHY[5], HANG QUACH[7], LIQIANG YANG[4], AND ANDREW SPENCER[1,2].

1. Department of Malignant Haematology and Stem Cell Transplantation, The Alfred Hospital, Melbourne, Australia; 2. Australian Centre for Blood Diseases, Monash University, Melbourne, Australia; 3. Alfred Pathology, The Alfred Hospital, Melbourne, Australia; 4. Rapid Novor, Kitchener, ON, Canada; 5. Department of Haematology, Royal Hobart Hospital, Hobart, Australia; 6. Department of Haematology, Eastern Health, Melbourne, Australia; 7. Department of Haematology, St. Vincent's Hospital Melbourne, Melbourne, Australia.

INTRODUCTION AND AIM

- Minimal residual disease (MRD) negativity is the optimal measure of therapeutic response for multiple myeloma (MM) patients and is associated with improved survival
- Bone marrow (BM) MRD assessment with next-generation sequencing (NGS) or next-generation flow cytometry (NGF) can achieve a minimum sensitivity of 10^{-5} however an invasive, single-site BM biopsy is required and may be limited by specimen quality and failure to capture MM's spatial heterogeneity
- EasyM (Rapid Novor, Kitchener, ON) is a peripheral blood-based (PB-based) clonotypic mass spectrometry (MS) assay involving *de novo* amino acid sequencing of the full-length M-protein and quantification of unique peptides with parallel reaction monitoring. EasyM has been compared to 8-colour multiparameter flow cytometry with a sensitivity of 10^{-4} and clonoSEQ,[1,2] but not to NGF to our knowledge
- We aimed to evaluate EasyM in MM patients undergoing sequential NGF (EuroFlow platform) MRD assessment

METHODS

- We retrospectively identified MM patients enrolled in the Australasian Leukaemia and Lymphoma Group MM19 (ACTRN12616000772448) and MM21 (ACTRN12618001490268) trials with measurable M-protein ≥2g/L by serum protein electrophoresis and/or free light chains ≥100mg/L at baseline
  - Briefly, MM19 evaluated the addition of ixazomib to thalidomide and dexamethasone consolidation therapy for 12 months in transplant eligible newly diagnosed MM (TE NDMM) patients undergoing front-line autologous stem cell transplantation (ASCT)
  - MM21 evaluated an intensive salvage approach using daratumumab-lenalidomide-dexamethasone (DRd) as re-induction (DRd x 4 cycles) and post-ASCT consolidation (DRd x 12 cycles followed by R maintenance until disease progression) in TE NDMM patients failing (<partial response as best response) front-line bortezomib-based induction therapy
- NGF MRD status was determined using the standardised 8-colour EuroFlow platform at pre-ASCT, post-ASCT, and end of consolidation timepoints in MM19 and MM21 patients and additionally post-cycle 2 of consolidation in MM21 patients
- Matched serum samples were evaluated with the EasyM assay and additionally post-cycle 7 of consolidation in MM19 patients
- Concordance between NGF and MS was assessed by the Chi-squared and McNemar's tests

RESULTS

Patients

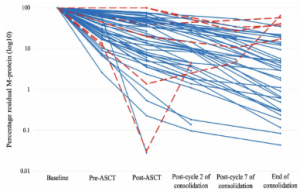
- 62 patients (25 MM19 and 37 MM21) were identified
- 57 (92%) patients underwent MS analysis
- 4 patients had no clonotypic target tryptic peptides to monitor MRD and 1 patient was not sequenced due to a low baseline value

M-Protein Negativity by EasyM

- EasyM was undetectable (MS-) in 3 (5%) patients
- All 3 patients were enrolled on the MM21 trial and the first MS- timepoint was pre-ASCT, post-ASCT, and post-cycle 2 of consolidation
- All 3 patients remain in complete response (CR) at 46-50 months post-ASCT

Sequential MS MRD Monitoring

Figure 1. EasyM MRD kinetics in individual patients



- Rising EasyM levels seen in 6 patients and coincided with relapse in 2 patients and preceded relapse in 4 patients by 3, 15, 25, and 38 months
- 5 patients had matched BM NGF results at time of rising EasyM levels, and of note, 1 patient was NGF MRD negative whilst the other 4 patients were NGF MRD positive

Matched MS and NGF Samples

Figure 2. MM MRD assessment by MS (EasyM) and NGF (Euroflow platform).

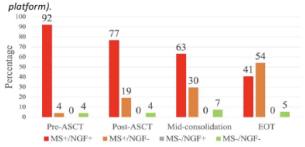


Table 1. McNemar's Test of matched MS (EasyM) and NGF (Euroflow platform) samples.

	NGF+	NGF-	McNemar's Test H0: equal discordancies (+/- = -/+)
All samples (n = 136)			
MS+	91	38	Statistic: 36.026
MS-	0	7	Exact probability: <0.001
Pre-ASCT (n = 25)			
MS+	23	1	Statistic: 0
MS-	0	1	Exact probability: >0.999
Post-ASCT (n = 47)			
MS+	36	9	Statistic: 7.111
MS-	0	2	Exact probability: 0.004
Post-cycle 2 of consolidation (n = 27)			
MS+	17	8	Statistic: 6.125
MS-	0	2	Exact probability: 0.008
End of consolidation (n = 37)			
MS+	15	20	Statistic: 18.050
MS-	0	2	Exact probability: <0.001
CR (n = 32)			
MS+	9	17	Statistic: 15.059
MS-	0	6	Exact probability: <0.001

- 136 serum samples for MS with matched BM for NGF were available, with 25 samples pre-ASCT, 47 post-ASCT, 27 post-cycle 2 of consolidation, and 37 end of consolidation
- 45 samples (33%) were NGF- but only 7 (5%) were MS-
- NGF MRD negativity (NGF-) was 8%, 23%, 37%, and 59% for the 4 timepoints
- 98 samples (72%) were concordant
- McNemar's test p-values were >0.999, 0.004, 0.008, and <0.001 for the 4 timepoints, indicating that MS+/NGF- were more likely than MS-/NGF+ discordances at all timepoints except for pre-ASCT

RESULTS CONT.

- The Chi-squared test for association between MS and NGF was not significant at all 4 timepoints
- Of the 32 matched samples with confirmed CR status, 9 (28%) were MS+/NGF+, 17 (53%) were MS+/NGF-, and 6 (19%) were MS-/NGF- (Chi-squared and McNemar's p-values of 0.149 and <0.001)

CONCLUSIONS

- EasyM appears to be more sensitive than BM NGF; there was poor concordance in samples including in those with confirmed CR, with 53% of samples showing detectable M-protein by EasyM but NGF MRD negativity
- This preliminary data highlights the potential of EasyM for highly sensitive, sequential PB-based clonotypic MS MRD monitoring in MM
- Comparison of larger sample sets and validation through prospective clinical trials is warranted to better assess the clinical utility of EasyM and rationalise BM-based assessment for MM patients

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652. Multiple Myeloma: Clinical and Epidemiological: Poster II
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Conclusions (part 1)

1. Intact protein MS methods and clonotypic peptide MS methods have been developed that show promise for high-throughput M-protein detection and MRD measurements.

2. These MS-based methods to measure M-proteins are applied on peripheral blood, which makes serial sampling possible to guide optimal personalized treatment.

3. They form an attractive alternative to the bone marrow–based methods currently applied for MRD detection

BUT…..

Questions

1. Does the M-protein add value to MRD assessment especially compared to the recommended next-generation bone marrow assays or to new serum-based markers like cell-free DNA and circulating tumor cells?

2. Is one mass spectrometry assay better than the other, or does each offer value in a specific setting?

3. Can these research methods be adapted for more routine use in the clinical lab?

4. What about plasmocytoma?

Conclusions (part 2)

1. In the future, MS will not replace existing MRD tests in bone marrow but will have clinical value as a companion method, especially for monitoring of MRD in blood.

2. This approach is in line with the recent IMWG recommendation that the development of blood-based MRD monitoring should be the ultimate goal, as it would allow for serial sampling without the trauma of repeated bone marrow aspirations and ensures assessment of extramedullary disease, which is not evaluated by bone marrow biopsy.


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