

## Mass spectrometry vs immunofixation for treatment monitoring in multiple myeloma

Noemí Puig,<sup>1,\*</sup> María-Teresa Contreras,<sup>2,\*</sup> Cristina Agulló,<sup>2</sup> Joaquín Martínez-López,<sup>3</sup> Albert Oriol,<sup>4</sup> María-Jesús Blanchard,<sup>5</sup> Rafael Ríos,<sup>6</sup> Jesús Martín,<sup>7</sup> María-Belén Iñigo,<sup>8</sup> Anna Sureda,<sup>9</sup> Miguel-Teodoro Hernández,<sup>10</sup> Javier de la Rubia,<sup>11</sup> Verónica González-Calle,<sup>1</sup> Isabel Krsnik,<sup>12</sup> Valentín Cabañas,<sup>13</sup> Luis Palomera,<sup>14</sup> José-María Moraleda,<sup>13</sup> Joan Bargay,<sup>15</sup> María-Teresa Cedena,<sup>3</sup> Bruno Paiva,<sup>16</sup> Laura Rosiñol,<sup>17</sup> Joan Bladé,<sup>17</sup> Jesús San Miguel,<sup>16</sup> Juan-José Lahuerta,<sup>18</sup> and María-Victoria Mateos<sup>1</sup>

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

### Key Points

- EXENT&FLC-MS is more sensitive than IFE in detecting the M-protein of MM patients in serum, both at baseline and during treatment monitoring.
- EXENT&FLC-MS is more accurate than IFE to predict patients' outcome.

Monitoring of the monoclonal protein (M-protein) by electrophoresis and/or immunofixation (IFE) has long been used to assess treatment response in multiple myeloma (MM). However, with the use of highly effective therapies, the M-protein becomes frequently undetectable, and more sensitive methods had to be explored. We applied IFE and mass spectrometry (EXENT&FLC-MS) in serum samples from newly diagnosed MM patients enrolled in the PETHEMA/GEM2012MENOS65 obtained at baseline (n = 223), and after induction (n = 183), autologous stem cell transplantation (n = 173), and consolidation (n = 173). At baseline, the isotypes identified with both methods fully matched in 82.1% of samples; in the rest but 2 cases, EXENT&FLC-MS provided additional information to IFE with regards to the M-protein(s). Overall, the results of EXENT&FLC-MS and IFE were concordant in >80% of cases, being most discordances due to EXENT&FLC-MS<sup>+</sup> but IFE<sup>-</sup> cases. After consolidation, IFE was not able to discriminate 2 cohorts with different median progression-free survival (PFS), but EXENT&FLC-MS did so; furthermore, among IFE<sup>-</sup> patients, EXENT&FLC-MS identified 2 groups with significantly different median PFS (P = .0008). In conclusion, compared with IFE, EXENT&FLC-MS is more sensitive to detect the M-protein of patients with MM, both at baseline and during treatment, and provides a more accurate prediction of patients' outcome. This trial was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as #NCT01916252.

Submitted 3 December 2021; accepted 7 February 2022; prepublished online on *Blood Advances* First Edition 14 February 2022; final version published online 31 May 2022. DOI 10.1182/bloodadvances.2021006762.

\*N.P. and M.T.C. contributed equally to this study.

Requests for data sharing may be submitted to María-Victoria Mateos (mvmateos@usal.es).

The full-text version of this article contains a data supplement.

© 2022 by The American Society of Hematology. Licensed under Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0), permitting only noncommercial, nonderivative use with attribution. All other rights reserved.

## Introduction

The monoclonal protein (M-protein), secreted by the tumor plasma cells in patients with multiple myeloma (MM), has long been used as a biomarker to evaluate treatment response.<sup>1</sup> In fact, treatment monitoring in MM is mainly based on the identification and quantification of the M-protein by electrophoresis and/or immunofixation (IFE) in serum and urine samples.<sup>2</sup> Although the clinical value of these methods has been broadly demonstrated, the current use of highly active therapies has significantly increased the proportion of patients in whom the M-protein becomes undetectable by IFE during and after treatment.<sup>3-5</sup> In fact, recent data from the PETHEMA/GEM2012MENOS65 trial showed that standard response criteria were no longer prognostically meaningful after consolidation.<sup>6</sup> Thus, there is an urgent need to adjust the sensitivity of the techniques used for response assessment to the current treatment efficacy. In this report, we explore the results of mass spectrometry (MS) as an alternative method to detect the M-protein in serum in patients with MM, by comparing them with those obtained using IFE.

## Methods

We included the first 223 out of the 458 newly diagnosed transplant-eligible patients with MM enrolled the PETHEMA/GEM2012MENOS65 trial (#NCT01916252), an open-label, phase 3 trial encompassing the administration of 6 induction cycles of bortezomib, lenalidomide, and dexamethasone and autologous stem-cell transplantation (ASCT) followed by 2 consolidation cycles of bortezomib, lenalidomide, and dexamethasone.<sup>7</sup> Each study site's independent ethics committee approved the protocol, and informed consent forms were required prior to patient enrollment. The study was conducted according to the Declaration of Helsinki. Patients' characteristics are detailed in supplemental Table 1. Serum samples were analyzed at baseline ( $n = 223$ ) and after induction ( $n = 183$ ), ASCT ( $n = 173$ ), and consolidation ( $n = 173$ ).

The presence of an M-protein in serum was assessed in parallel by IFE on the Hydrasys 2 instrument using the Hydragel 9 kit (Sebia Inc.) and by MS using the EXENT system (The Binding Site; Birmingham, UK). Pooled normal serum was used as a negative control. First, the EXENT-iP500 liquid handler purified the immunoglobulins through paramagnetic beads coated with polyclonal sheep antibodies specific for human immunoglobulin G (IgG), IgA, or IgM heavy chains, and for total  $\kappa$  and  $\lambda$  light chains (EXENT-MS). Paramagnetic beads specifically recognizing free  $\kappa$  and free  $\lambda$  light chains were also employed (EXENT&FLC-MS). Then, analysis with the EXENT-iX500 matrix-assisted laser desorption/ionization-time of flight device was carried out, and mass spectra from 5000 to 32 000 mass-to-charge ratio were collected. Spectra were reviewed using the in-house software. The +2 charge state was used for interpretation. The mass-to-charge ratio of the M-protein was identified in baseline samples and was used as a patient-specific tumor marker in the subsequent ones.

Statistical analyses were performed using the GraphPad Prism v.9. Progression-free survival (PFS) was defined as time from sample collection until disease progression or death from any cause, and curves were constructed using the Kaplan-Meier method and the (2-sided) log-rank test.

## Results and discussion

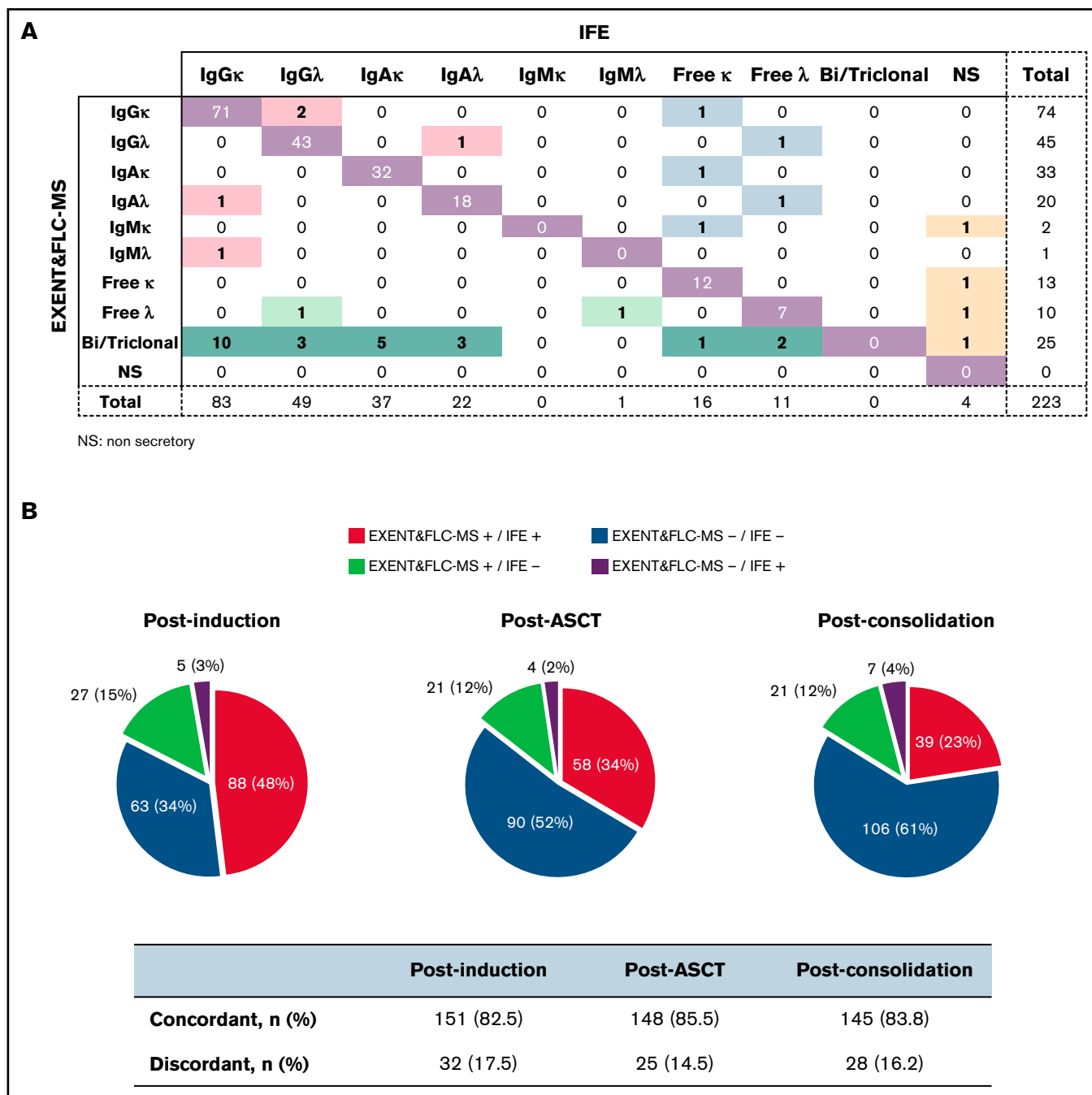
First, we compared the results obtained with EXENT&FLC-MS and IFE at baseline (Figure 1A). Both techniques provided fully matched results in 82.1% (183/223) of cases. Further analysis of the 40 remaining samples showed that: (1) in 24 cases (dark green), both methods concurred with the isotype of the main clone but EXENT&FLC-MS identified minor additional peaks; (2) 4 patients deemed non-secretory by IFE were found to have an M-protein by EXENT&FLC-MS (yellow); (3) in 5 cases (blue), EXENT&FLC-MS detected the heavy and light chain of the M-protein but IFE only the corresponding light chain, and in 2 (light green) IFE identified heavy and light chains but EXENT&FLC-MS only identified the light chain; and (4) the 5 cases highlighted in pink corresponded to complete isotyping discrepancies. These absolute isotyping discrepancies, although difficult to explain, have already been described by Mills et al.<sup>8</sup>

We analyzed the evolution of the results in 12 follow-up samples obtained from the 24 cases in which, as compared with IFE, EXENT&FLC-MS identified minor additional peaks besides the main clone. Whereas in 6 of them both the main and the minor peaks persisted, in 4 cases only the main peak remained (and the minor peaks disappeared); most importantly, in 2 cases, only the minor peaks exclusively identified by EXENT&FLC-MS at diagnosis persisted as a marker of disease. However, further studies are needed to ascertain whether these minor peaks represent additional tumor clones. We identified glycosylated M-proteins in 6.3% of cases ( $n = 14$ ), a feature more commonly described in certain diagnoses (AL amyloidosis<sup>9,10</sup> and cold agglutinin disease<sup>11</sup>) and also associated with a higher risk of progression in monoclonal gammopathy of unknown significance.<sup>12</sup>

Then, we analyzed the results of both methods during treatment monitoring (Figure 1B). As in previous publications,<sup>8,13,14</sup> there was substantial concordance between EXENT&FLC-MS and IFE at the 3 time points analyzed: 82% post-induction, 86% post-ASCT, and 84% post-consolidation. Most discordances were due to cases EXENT&FLC-MS<sup>+</sup> but IFE<sup>-</sup> (15% post-induction, 12% post-ASCT, and 12% post-consolidation), although there were 16 samples from 14 patients found to be EXENT&FLC-MS<sup>-</sup> but IFE<sup>+</sup> (5, 4, and 7 post-induction, ASCT, and consolidation, respectively). After a median follow-up of 5 years, only 2 out of these 14 patients had progressed.

Overall, 14 samples (from 11 patients) were deemed positive based exclusively on the analysis of free light chains by MS (6 post-induction, 6 post-ASCT, and 2 post-consolidation). Thus, the overall results of EXENT&FLC-MS (which includes the analysis of free light chains) were almost identical to those of EXENT-MS (that excludes them) (supplemental Figure 1A). Of note, 8 of these 14 samples were obtained from 5 patients with free light chain MM according to IFE.

When we investigated the clinical correlation of the results of both techniques separately, we observed that IFE was able to discriminate 2 subgroups of patients with significantly different median PFS after induction (IFE<sup>+</sup> = 5.78 years vs IFE<sup>-</sup> not reached) and post-ASCT (IFE<sup>+</sup> = 4.98 years vs IFE<sup>-</sup> not reached) but not post-consolidation (Figure 2A), thus confirming recent data published by Jiménez-Ubieto et al.<sup>6</sup> In contrast, according to previous reports,<sup>15,16</sup> the results of both EXENT-MS (supplemental Figure



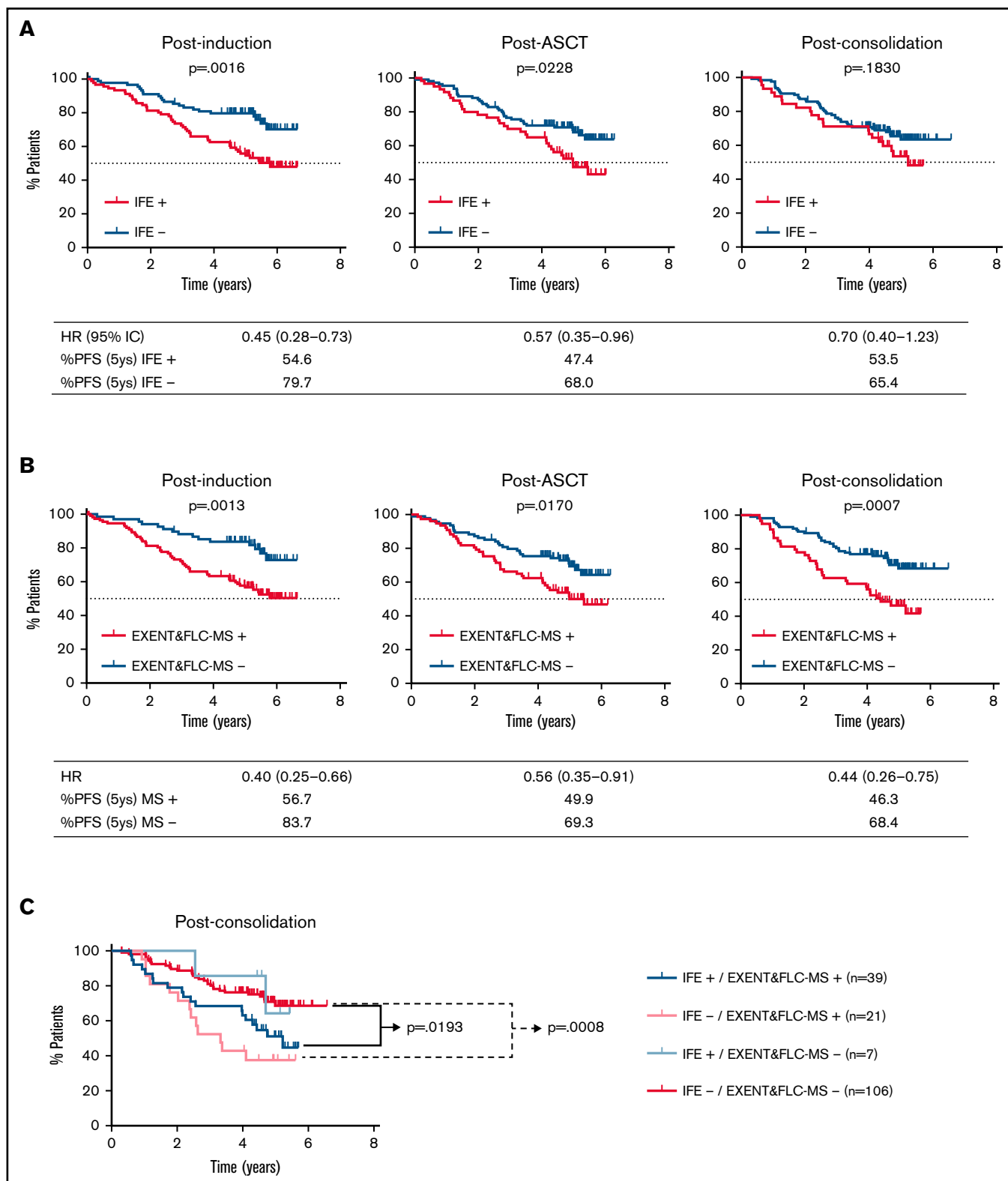
**Figure 1. Comparison between EXENT&FLC-MS and IFE results.** (A) M-protein(s) isotyping at baseline. (B) Detection of the M-protein post-induction, post-ASCT, and at the end of consolidation.

1B) and EXENT&FLC-MS (Figure 2B) retained clinical value at the 3 time points, so that patients with undetectable disease by either of them displayed a statistically significantly longer PFS as compared with positive cases.

Finally, we analyzed the clinical value of the combined results of IFE and EXENT&FLC-MS at the end of consolidation (Figure 2C). Importantly, among IFE<sup>-</sup> patients (ie, in complete response or better; n = 127), EXENT&FLC-MS was able to segregate 2 groups with significantly different PFS from consolidation (median PFS

of 3.32 years in the 21 cases EXENT&FLC-MS<sup>+</sup> vs not reached; *P* = .0008). This result, in accordance with Nandakumar et al,<sup>17</sup> confirms that the higher sensitivity of EXENT&FLC-MS is also associated with a meaningful clinical value.

Overall, these data show that, as compared with IFE, EXENT&FLC-MS is able to better identify and characterize the M-protein of patients with MM in baseline samples and detects residual disease in a higher proportion of cases during treatment monitoring. This translates to a meaningful clinical value in terms of PFS throughout



**Figure 2. PFS after induction, post-ASCT, and at the end of consolidation.** (A) IFE status. (B) EXENT&FLC-MS status. (C) combined IFE and EXENT&FLC-MS status. HR, hazard ratio.

the treatment that IFE fails to show post-consolidation, and furthermore, the results of EXENT&FLC-MS identify 2 groups of patients among those IFE<sup>-</sup> with significantly different PFS. Further studies comparing the results of MS with bone marrow-based MRD methods, such as next-generation flow and next-generation sequencing, are warranted.

## Acknowledgments

This study was supported by grants from the Centro de Investigación Biomédica en Red—Área de Oncología—del Instituto de Salud Carlos III CIBERONC, CB16/12/00369, CB16/12/00400, CB16/12/00233, and CB16/12/00284, Instituto de Salud Carlos III/Subdirección General de Investigación Sanitaria FIS no. PI15/01956, PI15/02049, PI15/02062, PI18/01709, PI18/01673, and PI19/01451, the Cancer Research UK, FCAECC, and AIRC under the Accelerator Award Program (EDITOR).

## Authorship

Contribution: N.P., M.T.C., and M.-V.M. conceived the analysis; N.P., M.T.C., M.-V.M., L.R., J.B., J.S.M., and J.-J.L. designed the analysis protocol; N.P. and M.T.C. analyzed the MS data and the M-protein kinetics; N.P., M.T.C., and M.-V.M. analyzed and interpreted data; N.P. and M.T.C. performed statistical analysis; N.P., M.T.C., and M.-V.M. wrote the manuscript; and all authors provided study material or patients and reviewed and approved the manuscript.

Conflict-of-interest disclosure: N.P. has received honoraria from Amgen, Celgene, Janssen, Takeda, and The Binding Site; served in a consulting or advisory role for Amgen, Celgene, Janssen, and Takeda; served on a speakers bureau for Celgene; received research funding from Celgene, Janssen, Amgen, and Takeda; received travel, accommodations, and expense fees from Amgen, Celgene, Janssen, and Takeda. B.P. has served as consultancy and received honoraria and research funding and served on a speakers bureau for Amgen, Bristol Myers Squibb, Celgene, Janssen, Novartis, Roche, and Sanofi; received unrestricted grants from Celgene, EngMab, and Takeda; and served as consultancy for Celgene, Janssen, and Sanofi. M.T.C. has received honoraria from Janssen, Celgene, and Abbvie. J.M.-L. served as consultancy, has received honoraria and research funding, and served on a speakers bureau for Amgen, Astellas, Bristol Myers Squibb, Janssen, Novartis, Roche, and Sanofi, and received unrestricted grants from BMS. A.O. served

as consultancy and served on a speakers bureau for Celgene and Amgen and served as consultancy for Janssen, Sanofi, and GSK. R.R. has received honoraria from or or served in an advisory role for Becton-Dickinson, Sanofi, and The Binding Site. J.M. has received registration fees and travel and accommodation fees from Janssen-Cilag, Amgen, Sandoz, and Bio-Rad Laboratories. M.-T.H. served in a consulting or advisory role for Celgene, Janssen, Amgen, Takeda, and GSK and served on a speakers bureau for Janssen, Celgene, and Amgen. J.d.I.R. has served as a consultant and provided expert testimony within the past 2 years for Amgen, Celgene, GSK, Takeda, Janssen, and Sanofi. V.G.C. has received honoraria from Janssen and Celgene and research funding from Janssen (BECA SEHH-JANSSEN ESTANCIAS DE FORMACIÓN EN EL EXTRA-NJERO 2016-2017) and served in a consulting or advisory role for Prothena and Janssen. J.M.M. served in a consultancy or advisory role for Novartis, Gilead, Roche, Sanofi, Jazz, and Takeda. L.R. has received honoraria from Janssen, Celgene, Amgen, and Takeda; J.B. has received honoraria from Janssen, Celgene, Takeda, Amgen, and Oncopeptides. J.S.M. has received consultancy or served in an advisory role for Abbvie, Amgen, Bristol Myers Squibb, Celgene, GlaxoSmithKline, Janssen, Karyopharm, MSD, Novartis, Roche, Sanofi, SecuraBio, and Takeda. J.-J.L. served in a consulting or advisory role for Celgene, Takeda, Amgen, Janssen, and Sanofi and has received travel accommodations and expenses for Celgene. M.-V.M. has received honoraria from and served as membership on an entity's Board of Directors or advisory committees for Janssen, Celgene, Takeda, Amgen, Adaptive, GSK, Sanofi, and Oncopeptides and has received honoraria from membership in Board of Directors or advisory committees for Abbvie, Roche, Pfizer, Regeneron, and Seattle Genetics. The remaining authors declare no competing financial interests.

ORCID profiles: J.M.-L., 0000-0001-7908-0063; A.O., 0000-0001-6804-2221; R.R., 0000-0001-8193-1402; M.-T.H., 0000-0002-6576-7881; J.d.I.R., 0000-0002-8354-768X; V.G.-C., 0000-0002-5493-6707; I.K., 0000-0002-2912-5072; V.C., 0000-0002-6248-2549; J.-M.M., 0000-0001-9080-1466; M.-T.C., 0000-0001-5851-3720; B.P., 0000-0003-1977-3815; J.S.M., 0000-0002-9183-4857; J.-J.L., 0000-0002-3393-9570; M.-V.M., 0000-0003-2390-1218.

Correspondence: María-Victoria Mateos, Hospital Universitario de Salamanca, Paseo de San Vicente 58-182, CP 37007, Salamanca, Spain; e-mail: mvmateos@usal.es.

## References

1. Tate JR. The paraprotein—an enduring biomarker. *Clin Biochem Rev.* 2019;40(1):5-22.
2. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol.* 2016;17(8):e328-e346.
3. Lahuerta J-J, Paiva B, Viduales M-B, et al; GEM (Grupo Español de Mieloma)/PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) Cooperative Study Group. Depth of response in multiple myeloma: a pooled analysis of three PETHEMA/GEM clinical trials. *J Clin Oncol.* 2017;35(25):2900-2910.
4. Martínez-López J, Blade J, Mateos M-V, et al; Programa para el Estudio de la Terapéutica en Hemopatía Maligna. Long-term prognostic significance of response in multiple myeloma after stem cell transplantation. *Blood.* 2011;118(3):529-534.
5. Bertamini L, D'Agostino M, Gay F. MRD assessment in multiple myeloma: progress and challenges. *Curr Hematol Malign Rep.* 2021;16(2):162-171.

6. Jiménez Ubieta A, Paiva B, Puig N, et al. Validation of the International Myeloma Working Group standard response criteria in the PETHEMA/GEM2012MENOS65 study: are these times of change? *Blood*. 2021;138(19):1901-1905.
7. Rosiñol L, Oriol A, Rios R, et al. Bortezomib, lenalidomide, and dexamethasone as induction therapy prior to autologous transplant in multiple myeloma. *Blood*. 2019;134(16):1337-1345.
8. Mills JR, Kohlhagen MC, Dasari S, et al. Comprehensive assessment of M-proteins using nanobody enrichment coupled to MALDI-TOF mass spectrometry. *Clin Chem*. 2016;62(10):1334-1344.
9. Kumar S, Murray D, Dasari S, et al. Assay to rapidly screen for immunoglobulin light chain glycosylation: a potential path to earlier AL diagnosis for a subset of patients [published correction appears in *Leukemia*. 2019;33(4):1060]. *Leukemia*. 2019;33(1):254-257.
10. Mellors PW, Dasari S, Kohlhagen MC, et al. MASS-FIX for the detection of monoclonal proteins and light chain N-glycosylation in routine clinical practice: a cross-sectional study of 6315 patients [published correction appears in *Blood Cancer J*. 2021;11(6):110]. *Blood Cancer J*. 2021;11(3):50-57.
11. Sidana S, Murray DL, Dasari S, et al. Glycosylation of immunoglobulin light chains is highly prevalent in cold agglutinin disease. *Am J Hematol*. 2020;95(9):E222-E225.
12. Dispenzieri A, Larson DR, Rajkumar SV, et al. N-glycosylation of monoclonal light chains on routine MASS-FIX testing is a risk factor for MGUS progression. *Leukemia*. 2020;34(10):2749-2753.
13. Eveillard M, Korde N, Ciardiello A, et al. Using MALDI-TOF mass spectrometry in peripheral blood for the follow up of newly diagnosed multiple myeloma patients treated with daratumumab-based combination therapy. *Clin Chim Acta*. 2021;516:136-141.
14. Kohlhagen MC, Barnidge DR, Mills JR, et al. Screening method for M-proteins in serum using nanobody enrichment coupled to MALDI-TOF mass spectrometry. *Clin Chem*. 2016;62(10):1345-1352.
15. Dispenzieri A, Krishnan AY, Arendt B, et al. MASS-FIX versus standard methods to predict for PFS and OS among multiple myeloma patients participating on the STAMINA trial [abstract]. *JCO*. 2021;39(15 suppl). Abstract 8009.
16. Derman BA, Stefka AT, Jiang K, et al. Measurable residual disease assessed by mass spectrometry in peripheral blood in multiple myeloma in a phase II trial of carfilzomib, lenalidomide, dexamethasone and autologous stem cell transplantation. *Blood Cancer J*. 2021;11(2):19-22.
17. Nandakumar B, Murray DL, Dispenzieri A, et al. Sequential comparison of conventional serum immunofixation (IFE) to mass spectrometry-based assessment (MASS FIX) in patients with multiple myeloma (MM). *Blood*. 2020;136(suppl 1):12-13.