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ORIGINAL PAPER

Haematological Malignancy – Clinical

Serum mass spectrometry for treatment monitoring in patients with multiple myeloma receiving ARI0002h CAR T-cells

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Summary

Chimeric antigen receptor (CAR) T-cell therapies have increased the patients with relapsed/refractory multiple myeloma (RRMM) in whom standard electrophoretic techniques fail to detect the M-protein. Quantitative immunoprecipitation mass spectrometry (QIP-MS) can accurately measure serum M-protein with high sensitivity, and identify interferences caused by therapeutic monoclonal antibodies. Here, we investigate the outcome of QIP-MS in 33 patients treated with the academic BCMA-directed CAR T-cell ARI0002h (Cesnicabtagene Autoleucel). QIP-MS offered more detailed insights than serum immunofixation (sIFE), identifying glycosylated M-proteins and minor additional peaks. Moreover, the potential interferences owing to daratumumab or tocilizumab treatments were successfully detected. When analysing different assay platforms during patient's monitoring after ARI0002h administration, we observed that QIP-MS showed a high global concordance (78.8%) with sIFE, whereas it was only moderate (55.6%) with bone marrow (BM)-based next-generation flow cytometry (NGF). Furthermore, QIP-MS consistently demonstrated

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the lowest negativity rate across the different timepoints (27.3% vs. 60.0% in months 1 and 12, respectively). Patients with QIP-MS(+)/BM-based NGF(−) showed a non-significant shorter median progression free survival than those with QIP-MS(−)/BM-based NGF(−). In summary, we show the first experience to our knowledge demonstrating that QIP-MS could be particularly useful as a non-invasive technique when evaluating response after CAR T-cell treatment in MM.

KEYWORDS
CAR T-cell therapy, mass spectrometry, multiple myeloma

INTRODUCTION

The presence of a monoclonal protein (M-protein) in serum and/or urine is one of the main biomarkers in patients with multiple myeloma (MM), as a surrogate of tumour burden.[1] M-protein monitoring is mainly performed through protein electrophoresis (SPEP), immunofixation (IFE) and/or free light chains (sFLC).[2] However, the introduction of effective treatments such as chimeric antigen receptor (CAR) T-cell therapy has increased the proportion of patients, in whom the M-protein becomes undetectable using these methods.[3–5] Moreover, treating MM with therapeutic monoclonal antibodies (t-mAbs) can hinder their interpretation.[6] In contrast, mass spectrometry (MS) can specifically detect the M-protein without these interferences, discriminating among immunoglobulins based on their mass-to-charge (*m*/*z*) ratio.[7] MS also allows the identification of M-proteins with post-translational modifications (PTMs). Glycosylation, based on the attachment of an oligosaccharide to M-proteins, is the most prevalent.[8] MS has been already explored for diagnosing monoclonal gammopathies and for evaluating treatment response, often associated with cellular or molecular evaluation of bone marrow (BM).[9–11] The easy availability of serum and urine samples, and the possibility of objectively identify the M-protein, may be differential advantages of MS.[12]

Recent data from the academic B-cell maturation antigen (BCMA)-directed CAR T-cell therapy ARI0002h (Cesnicabtagene Autoleucel) have shown its safety and efficacy in patients with relapsed/refractory MM (RRMM), with an overall response rate of 100%, including 67% of complete response (CR).[13] A high proportion of patients achieved a negative minimal residual disease (MRD) measured by next-generation flow cytometry (NGF) compared to those with CR, highlighting the need for alternatives to monitor treatment response.

We herein investigate the quantitative immunoprecipitation MS (QIP-MS) as a complementary serum-based method for identifying the serum M-protein in patients with RRMM after ARI0002h CAR-T cell therapy.

METHODS

Study design

Thirty-three patients with RRMM treated with ARI0002h between June 2020 and January 2021 were enrolled: 30 patients in the setting of the CARTBCMA-HCB-01 clinical trial (ClinicalTrials.gov, NCT04309981, and EudraCT, 2019-001472-11),[13] and three as compassionate use. CARTBCMA-HCB-01 was a single-arm, open-label study done in five academic centres in Spain. Main inclusion criteria included: aged between 18 and 75 years, had undergone at least two prior lines of treatment (including a proteasome inhibitor, an immunomodulating agent and an anti-CD38 monoclonal antibody), and had measurable disease (defined as serum and/or urine M-protein levels exceeding 10 g/L or 200 mg/24 h or free light chain levels exceeding 100 mg/L) based on the International Myeloma Working Group (IMWG) criteria.[14,15] Patients with a previous BCMA-directed therapy and an estimated glomerular filtration rate below 50 mL/min were excluded. Patient characteristics were obtained from electronic medical records. The study protocol was approved by the Ethics Committee of Hospital Clínic de Barcelona.

Samples and procedures

Blood samples were collected at the following timepoints: baseline (at inclusion; before ARI0002h administration), and in months 1, 3, 6, 9 and 12 after ARI0002h administration. The presence of the M-protein was concurrently evaluated using serum IFE (sIFE) on the Hydrasys 2 platform with the Hydragel 4 IF kit (Sebia Inc., Lisses, France), and QIP-MS using the EXENT^{R} Solution platform (The Binding Site, part of Thermo Fisher Scientific). IFE in urine (uIFE) was also performed with the same method as sIFE, but with a previous concentration using Amicon Ultra-4 centrifugal filters (Merck Millipore, Cork, IRL). For QIP-MS, immunoglobulins were initially purified from serum using paramagnetic beads coated with specific polyclonal sheep antibodies targeting human immunoglobulin G (IgG), IgA, IgM heavy chains, and total κ and ʎ light chains through the EXENT-iP500 liquid handler. Subsequently, QIP-MS was conducted using the matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) EXENT-iX500 device, and mass spectra ranging from 5000 to 32 000 *m*/*z* ratio were recorded. The EXENT-iQ500 was used to analyse QIP-MS results. The +2-charge state was utilized for interpretation. The *m*/*z* ratio of the M-protein was determined in the baseline for each patient as a personalized tumour marker. The follow-up samples with the same baseline protein-M were considered as QIP-MS(+). At the end of the study, the number of samples in which paired QIP-MS and sIFE were performed was 165. Of these, 33 were at baseline

and 132 during the other timepoints. For QIP-MS and uIFE, the total number of paired samples was 146, with 30 at baseline and 116 at follow-up.

MRD in the BM was investigated by NGF (median sensitivity of 1×10^{-6}) in months 1, 3, 6, and 12 after ARI0002h administration. It was performed using two-tube eight-colour flow cytometry according to the EuroFlow platform, using a FACSCanto (BD Biosciences, San Jose, CA, USA) flow cytometer and Infinicyt 2.0 software (Cytognos SL, Salamanca, Spain). Any detectable level of MRD greater than 1×10^{-6} was considered positive. The number of samples in which QIP-MS and BM-based NGF were performed simultaneously was 112, all of them at follow-up. Finally, a total of 107 samples were analysed simultaneously by QIP-MS, sIFE and BM-based NGF.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism 8.3 (GraphPad Software, San Diego, CA) or SAS System 9.4 (SAS Institute, Cary, NC) and R 4.3.0 (RStudio, PBC, Boston, MA). The graphics were created with GraphPad Prism 8.3 or Biorender.com. Progression-free survival (PFS) was defined as time between ARI0002h administration and disease progression or death from any cause. Overall survival (OS) was defined as time between ARI0002h administration and death from any cause. A landmark analysis was performed for both PFS and OS according to the sample collection timepoint. The log-rank test (two-sided) and Kaplan–Meier were employed to construct survival curves and analyse the results, as well Cox proportional hazards regression analysis for hazard ratios (HR) calculations. A $p < 0.05$ was considered statistically significant.

RESULTS

Features of enrolled patients

The main characteristics of the 33 patients are in Table 1. The median age at inclusion was 60 years; and nearly the half of the patients presented plasmacytomas, with 21.2% showing an extramedullary location. Prior exposure to lenalidomide, bortezomib and anti-CD38 antibodies was universal with a median of three prior lines of therapy, including 54.8% of triple-class refractory patients. The mean serum M-protein was 18.8 g/L, with 21% secreting only light chain M-protein. The median follow-up of the patients was 27.9 months (95% CI 26.9–28.8).

TABLE 1 Main features of patients with RRMM treated with the academic BCMA-directed CAR T-cell therapy ARI0002h at inclusion in this study.

	Patients enrolled (*n* = 33)
Age, years (median, IQR)	60 (52.5–65.5)
Sex (*n*, %)	
Female	14/33 (42.4)
Male	19/33 (57.6)
ISS stage (*n*, %)	
I	7/29 (24.1)
II	10/29 (34.5)
III	12/29 (41.4)
Plasmacytomas (*n*, %)	15/33 (45.5)
Extramedullary plasmacytomas (*n*, %)	7/33 (21.2)
High risk cytogenetics [*t* (4;14), *t* (14;16), del17p/*TP53* mutations] (*n*, %)	10/33 (30.3)
Number of previous lines of treatment (median, IQR, range)	3 (3–5, 1–10)
Lenalidomide exposed (*n*, %)	33/33 (100)
Bortezomib exposed (*n*, %)	33/33 (100)
Anti-CD38 exposed (*n*, %)	33/33 (100)
Daratumumab exposed (*n*, %)	32/33 (96.7)
Carfilzomib exposed (*n*, %)	17/33 (51.5)
Pomalidomide exposed (*n*, %)	19/33 (57.6)
Triple refractory* (*n*, %)	17/31 (54.8)
Penta refractory** (*n*, %)	3/31 (9.7)
Previous autologous haematopoietic stem-cell transplantation (*n*, %)	30/33 (90.9)
Previous allogeneic haematopoietic stem-cell transplantation (*n*, %)	5/33 (15.2)
Previous autologous and allogeneic haematopoietic stem-cell transplantation (*n*, %)	4/33 (12.1)
Bone marrow malignant plasma cells, % (mean, SD)	26.5 (31.9)
Serum monoclonal protein, g/L (mean, SD)	18.8 (22.5)

Abbreviation: ISS, international staging system.

Note: Triple refractory*: refractory to bortezomib, lenalidomide and an anti-CD38 antibody. Penta refractory**: refractory to bortezomib, lenalidomide, an anti-CD38 antibody, carfilzomib and pomalidomide.

QIP-MS provides information about glycosylation and minor additional peaks during M-protein isotyping

In baseline serum samples, a main peak in the MS spectra corresponding to the M-protein-secreting clone could be identified in all patients (100%; 33/33). These results were compared with sIFE results regarding the M-protein heavy and light chain isotype (Table 2). Both techniques provided fully matched information about the M-protein heavy and light chain isotypes in 97% (32/33) of the cases (green). One patient was known to have an M-protein with a IgDλ isotype. However, upon conducting sIFE and QIP-MS, we only identified the λ light chain of the M-protein by both techniques, as reagents to detect the IgD were not included. On the contrary, there was one discrepancy (3%; 1/33) between sIFE and QIP-MS regarding isotyping. In this patient, sIFE identified both M-protein heavy and light chains, while QIP-MS only detected a peak corresponding to the light chain (grey). Although difficult to explain this mismatch

TABLE 2 Comparison of serum M-protein isotyping at baseline between QIP-MS and IFE. Concordance in M-protein heavy and light chain isotyping between both assays are coloured in green. A discordance caused because sIFE identified the M-protein heavy and light chain isotype, while QIP-MS only detected the light chain isotype is coloured in grey. In this case, the sIFE deemed the M-protein as IgAʎ, while the QIP-MS result was free ʎ. Additional information provided by QIP-MS regarding the presence of glycosylation in the M-protein is in yellow, while the additional information regarding the presence of minor peaks besides the main M-protein is in blue. QIP-MS for M-protein isotyping was performed in a single centre in a centralized manner, while sIFE was performed at each patient's original centre.

		QIP-MS											
		IgGκ	IgGʎ	IgAκ	IgAʎ	IgMκ	IgMʎ	IgDκ	IgDʎ	Free κ	Free ʎ	Minor peaks	Glycosylation
sIFE	IgGκ	**8**	0	0	0	0	0	0	0	0	0	0	**2**
	IgGʎ	0	**6**	0	0	0	0	0	0	0	0	**2**	0
	IgAκ	0	0	**4**	0	0	0	0	0	0	0	0	0
	IgAʎ	0	0	0	**5**	0	0	0	0	0	**1**	**1**	0
	IgMκ	0	0	0	0	**0**	0	0	0	0	0	0	0
	IgMʎ	0	0	0	0	0	**0**	0	0	0	0	0	0
	IgDκ	0	0	0	0	0	0	**0**	0	0	0	0	0
	IgDʎ	0	0	0	0	0	0	0	**0**	0	0	0	0
	Free κ	0	0	0	0	0	0	0	0	**4**	0	**2**	0
	Free ʎ	0	0	0	0	0	0	0	0	0	**5**	**1**	0

Abbreviations: QIP-MS, quantitative immunoprecipitation mass spectrometry; sIFE, serum immunofixation.

Note: Bold values is used to highlight those values that are different from 0, making it easier to identify the conditions where there are actual cases.

could be caused by a sIFE reader-dependent mistake, as previously described.[3,16]

A glycosylated M-protein was detected in 6.3% (2/32) by QIP-MS (yellow) as additional information in patients with concordant results, although the isotype was concordantly deemed as IgGκ by sIFE and QIP-MS. Despite the substantial concordance in the isotyping, QIP-MS can also provide more information than sIFE identifying minor additional peaks. QIP-MS identified minor additional peaks in 18.2% (6/33) of the patients (blue), though the main M-protein isotype was concordant with sIFE.

With the aim of investigating the evolution of those cases where QIP-MS provided additional information, samples over time were analysed. The glycosylated M-protein was eliminated in one patient after ARI0002h treatment, while remained in the other patient until the last collected sample (month 6). Regarding the six patients with minor additional peaks besides the main M-protein-secreting clone, three different situations were identified: (i) In three patients, the main clone disappeared in month 1, while the minor peaks persisted. Over time, these minor peaks also became undetectable within the first 3 months. (ii) In two patients, the minor peaks were eliminated, but the M-protein-secreting clone persisted throughout all timepoints. (iii) One patient successfully eliminated main clone and minor peaks in month 1, without reappearance.

Monitoring of t-mAb interferences by QIP-MS

Daratumumab is an anti-CD38 t-mAb of IgGκ isotype that can cause false positive interferences on sIFE in patients with IgGκ M-protein. The +2-charge (*z*) state of daratumumab's κ light chain shows an *m/z* ratio of 11690.0 by MS and this can be used to identify interferences.[17,18] Therefore, the presence of an IgGκ peak with the *m/z* ratio of 11690.0 was evaluated. Although 97% (32/33) of patients were exposed to daratumumab, the time elapsed since the last administration varied widely among them. Before ARI0002h administration, the peak corresponding to daratumumab was identified in 31.3% (10/32) of the patients by QIP-MS. In those patients, the median time elapsed from the last administration was 1.73 months. This peak persisted in 80% (8/10) and in 40% (4/10) of patients in months 1 and 3, respectively. The median time from the last daratumumab administration and the last QIP-MS identification in these patients was 3.4 months.

Tocilizumab is a humanized t-mAb of IgGκ isotype directed against interleukin-6 receptor, used for treating the cytokine release syndrome (CRS).[19] In our cohort, 87.9% (29/33) of patients developed CRS, with no grade 3 or higher events. The median onset of CRS was 7 days after ARI0002h administration, and 72.4% (21/29) of patients received tocilizumab. A method for quantifying of tocilizumab by liquid chromatography tandem-MS using a tryptic digestion without IgG purification has been described.[20] However, to our knowledge, the tocilizumab *m/z* ratio at the +2-charge state by QIP-MS has not been yet reported. In 66.7% (14/21) of patients treated with tocilizumab, apart from detecting the M-protein secreted by the main malignant clone, an IgGκ monoclonal peak with *m/z* ratio of 11750.0 was observed in month 1 after ARI0002h administration. This IgGκ peak was different from the M-protein-secreting clone in all patients, and did not appear at baseline in any case. Importantly, the presence of this peak was exclusive to those patients treated with tocilizumab and it only persisted in

one patient in month 3. In fact, the half-life of tocilizumab has been reported to be approximately 6 days.[21] Therefore, this IgGκ peak with *m/z* ratio of 11750.0 could correspond to tocilizumab.

Overall, the ability of QIP-MS to track these t-mAbs was crucial for clarifying potential false positive results using sIFE. A case of simultaneous presence of daratumumab and tocilizumab interferences is exemplified in Figure 1.

QIP-MS, IFE and BM-based NGF as assay platforms

We then compared QIP-MS and sIFE during the follow-up of patients treated with ARI0002h (Figure 2A). A total of 132 serum samples were concurrently analysed by QIP-MS and sIFE. Both were concordant in 78.8% of samples (104/132). Discrepancies between QIP-MS and sIFE were observed in a total of 17 patients. In 11 of them, the discrepancies were transient, while in six were persistent. On the basis of the higher sensitivity of QIP-MS,[3,22,23] most discordances (20/28) were due to QIP-MS(+)/sIFE(–) results, except for eight cases (28.6%). In three of them, samples were mistakenly deemed as sIFE(+) owing to the interference of daratumumab, tocilizumab or both. Of note, in other three cases, the QIP-MS(–)/sIFE(+) discrepancy was due to the presence of bands in sIFE corresponding to clones different from the original M-protein, but with the same M-protein heavy and light chain isotype. This could correspond to the already described oligoclonal bands appearing after immune reconstitution.[3,24] The last two discrepancies, although difficult to explain since the QIP-MS result was clearly negative, could be due to sIFE reader-dependent errors. Interestingly, six patients with QIP-MS(–) and transient mistakenly deemed sIFE(+) showed positive outcomes, with a median PFS of 21.7 months (CI 95% 12.2–31.3).

Despite the distinct origin, we also compared uIFE and serum QIP-MS results. As in previous reports,[24,25] a substantial concordance rate (93.3%; 28/30) was observed at baseline. However, this concordance rate was lower in the different monitoring timepoints (Figure S1). Most discordances (88.4%; 38/43) after ARI0002h administration were due to uIFE(–)/QIP-MS(+). Notably, among the five patients showing uIFE(+)/QIP-MS(–) results, three were indeed false positive results, caused by the presence of bands in uIFE corresponding to minor clones different to the original M-protein-secreting one. The other two uIFE(+)/QIP-MS(–) discrepancies might be attributable to uIFE reader-dependent errors.

A total of 112 BM samples were analysed using NGF, and 88.4% (99/112) were considered evaluable. Most not evaluable BM samples were due to haemodilution, which mainly happened in the early monitoring timepoints (months 1 and 3). In months 1 and 3, 96.0% (24/25) and 93.1% (27/29) of evaluable samples were negative by BM-based NGF, respectively. In months 6 and 12, BM-based NGF negativity rates were 87.0% (20/23), and 81.8% (18/22). We subsequently compared the results of BM-based NGF with those obtained using serum QIP-MS (Figure 2B). Concordance between QIP-MS and BM-based NGF was only observed in 55.6% (55/99) of the samples. However, the concordance rate progressively increased over time. The discordances were due to QIP-MS(+)/BM-based NGF(–) results in all cases, except one.

A comparison during treatment monitoring between by sIFE, QIP-MS and BM-based NGF was performed (Figure 3). Methods based on detecting M-proteins in peripheral blood showed a lower negativity rate than BM-based NGF. This difference was higher during the first months after ARI0002h administration. Of note, QIP-MS revealed the lowest negativity rate through the timepoints. However, the ongoing increase in the QIP-MS negativity rate (27.3% vs. 60.0% in months 1 and 12, respectively) and the consistent pattern of BM-based NGF were outlined, in accordance with the deepening of responses reported in patients treated with other BCMA-directed CAR T-cell treatments.[26,27] A simplified comparison of these three methods is shown in Figure S2.

Outcomes and clinical value of QIP-MS

Only a non-significant shorter median PFS was observed in patients with a detectable M-protein by QIP-MS in months 1 (12.91 vs. 19.15 months; HR 1.31 [95% CI 0.55–3.14] and $p = 0.56$) and 3 (10.55 vs. 16.59 months, HR 1.48 [95% CI 0.64–3.21] and $p = 0.377$) (Figure 4).

Regarding the clinical value of BM-based NGF in our cohort, only 1 and 2 patients presented a BM-based NGF(+) in month 1 and month 3, respectively, which complicates this analysis in relation to the patients' outcome (Figure S3). This fact also affects the interpretation of combining BM-based NGF(+) and QIP-MS results. However, patients with QIP-MS(+)/BM-based NGF(–) showed a non-significant shorter median PFS than those with QIP-MS(–)/BM-based NGF(–) in month 1 (15.18 vs. 22.7 months, $p = 0.575$) and month 3 (11.24 vs. 16.76 months, $p = 0.363$).

DISCUSSION

Proteomics assessment using MS-based techniques to monitor MM treatment response, disease progression, and MRD is under evaluation, being a subject of high priority in the IMWG.[22] Our main objective was to investigate QIP-MS as a highly sensitive serum-based technique for identifying M-protein in patients with RRMM after treatment with ARI0002h CAR T-cell therapy.

QIP-MS could accurately define the *m/z* ratio of the peak corresponding to the M-protein-secreting clone and other minor additional peaks, which were undetectable by electrophoretic methods. This detection was more precisely than sIFE, ensuring more sensitive and specific results as previously reported.[26] Regarding isotyping, the M-protein could be characterized in 100% of enrolled patients. Furthermore,

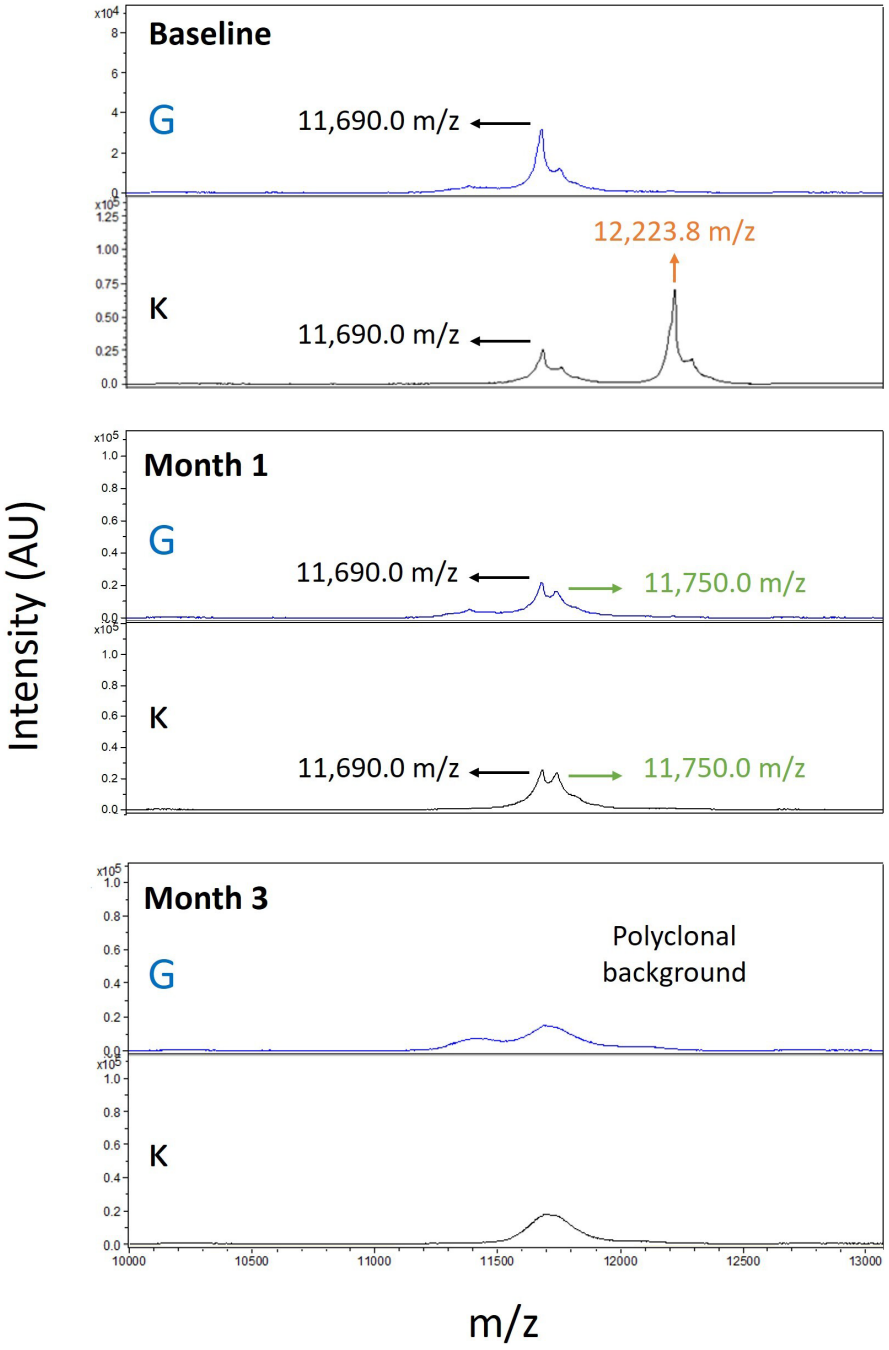
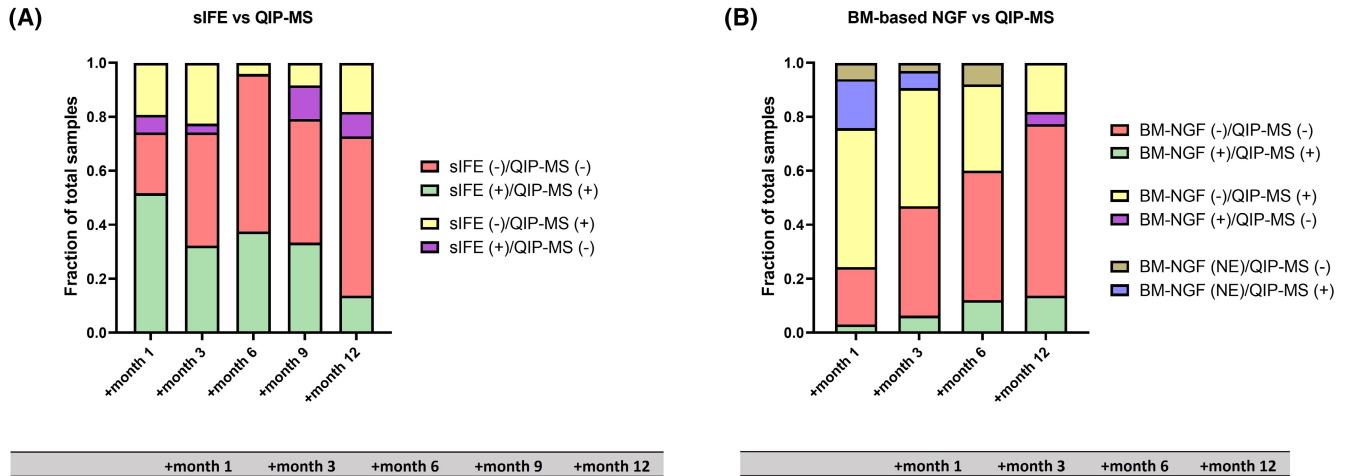


FIGURE 1 Serum mass spectrum of a patient with a free κ light chain isotype multiple myeloma at three different timepoints of the ARI0002h treatment monitoring. Representative example of the quantitative immunoprecipitation mass spectrometry (QIP-MS) ability to identify the M-protein and therapeutic monoclonal antibodies (t-mAb) interferences in a patient with a free κ light chain isotype multiple myeloma. The M-protein (orange arrow) and daratumumab (black arrow) were initially detected, just before ARI0002h administration. The small peak near daratumumab is attributed to a matrix adduct. The +2-charge state of daratumumab's chains have a mass-to-charge (*m/z*) ratio of 11690.0. The M-protein disappeared after the treatment and was undetectable in months 1 and 3. Daratumumab and tocilizumab (green arrow) were detected in month 1. The +2-charge state of tocilizumab's chains have a *m/z* ratio of 11750.0. This serum was mistakenly deemed as positive for serum immunofixation (sIFE) owing to the interferences caused by t-mAbs. In month 3, the QIP-MS was deemed as negative because only polyclonal background was detected. AU, arbitrary units.

additional information was obtained in eight patients, including two patients with glycosylated M-proteins and six with minor additional peaks. M-protein glycosylation may influence the development of amyloid deposits in patients with light chain amyloidosis. In fact, monoclonal light chain glycosylation has been reported as a risk factor of progression to MM and amyloidosis.[28,29] However, the detection of minor additional peaks besides the main M-protein-secreting clone has been already described in MM, but their clinical relevance remains unclear.[3] In some instances, these peaks may represent oligoclonal bands after immune reconstitution.[30,31]



	+month 1	+month 3	+month 6	+month 9	+month 12
Concordant, n (%)	23 (74.2)	23 (74.2)	23 (95.8)	19 (79.2)	16 (72.7)
Discordant, n (%)	8 (25.8)	8 (25.8)	1 (4.2)	5 (20.8)	6 (27.3)

	+month 1	+month 3	+month 6	+month 12
Concordant, n (%)	8 (32.0)	15 (51.7)	15 (65.2)	17 (77.3)
Discordant, n (%)	17 (68.0)	14 (48.3)	8 (34.8)	5 (22.7)

FIGURE 2 Comparison between sIFE, QIP-MS and BM-based NGF to evaluate the response to ARI0002h treatment. Detection of M-protein by serum immunofixation (sIFE) and quantitative immunoprecipitation mass spectrometry (QIP-MS) (A), or bone marrow (BM)-based next-generation flow cytometry (NGF) and QIP-MS (B). The concordance and discordance rates between sIFE and BM-based NGF were calculated over evaluable BM samples. NE, not evaluable.

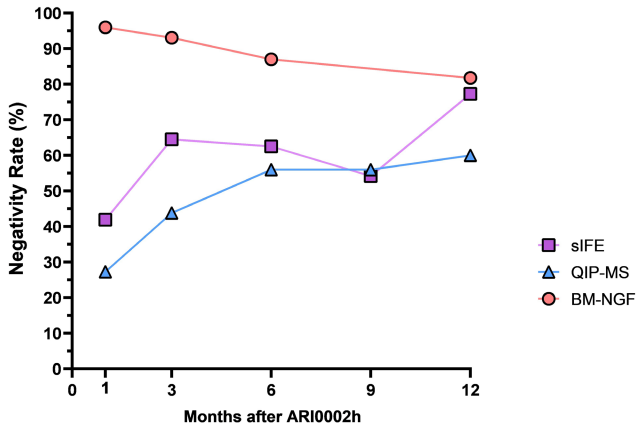


FIGURE 3 Evaluation of the negativity rate showed by sIFE, QIP-MS and BM-based NGF along ARI0002h treatment monitoring. BM-NGF, bone marrow-based next-generation flow cytometry; QIP-MS, serum quantitative immunoprecipitation mass spectrometry; sIFE, serum immunofixation.

The utilization of t-mAbs in the treatment of MM leads to a growing need to discriminate between these drugs and the M-proteins. QIP-MS offers greater specificity compared to sIFE for identifying interferences caused by t-mAbs. Each M-protein showed a specific amino acid sequence determined during somatic recombination, allowing single-clone tracking overtime. We successfully tracked the peaks corresponding to daratumumab and tocilizumab by QIP-MS, identified with an *m/z* ratio of 11690.0 and 11750.0, respectively. The *m/z* ratio of daratumumab has been extensively described,[17,18,32] due to its common use in the treatment of MM patients. By contrast, tocilizumab has been unfrequently used in MM, but it is very effective in managing CRS associated with CAR-T therapy or COVID-19.[19] Here we have identified the *m/z* ratio of tocilizumab, which has not been reported yet to our knowledge. Regarding t-mAbs clearance, it was reported that the median time of daratumumab disappearance analysed by QIP-MS was 6.1 months,[32] while in our hands it was 3.4 months. Our results about tocilizumab clearance were concordant to those previously reported.[21]

An important concordance between sIFE and serum QIP-MS results was here described, in agreement with previous reports.[3] Despite this important concordance, the utility of QIP-MS in the characterization of IFE false positive results and other interferences was highlighted. In fact, oligoclonal peaks, probably related to immune reconstitution, can be deemed as false positive by sIFE. In this context, QIP-MS is unequivocal in distinguishing this kind of peaks.[30,31]

Regarding QIP-MS performed in urine samples, a concordance of 91% with uIFE has been reported determining the presence of monoclonal peaks.[24] Although, we only performed QIP-MS in serum samples, we compared these results with uIFE and we observed a 62.6% of global concordance between them. The distinct origin of the samples could explain the differences between the concordance rates.

Besides electrophoretic assays, high-sensitivity techniques to evaluate MRD such as BM-based NGF are considered crucial to assess response after CAR T-cell therapies.[33] However, our data showed a high discordance between QIP-MS and BM-based NGF, especially in the initial timepoints. Recently, Paiva et al. studied the dynamics of MRD and clinical responses during BCMA-directed CAR-T cell therapy in RRMM and concluded that MRD is reached faster in the BM than the CR in peripheral blood.[34] In our case, we observed that the negativity rate of BM-based NGF was much higher compared to serum QIP-MS. In fact, the half-life of the M-proteins enables them to be detected in the serum even after M-protein-secreting malignant clones have been eliminated from the BM.[34,35] Also, the potential

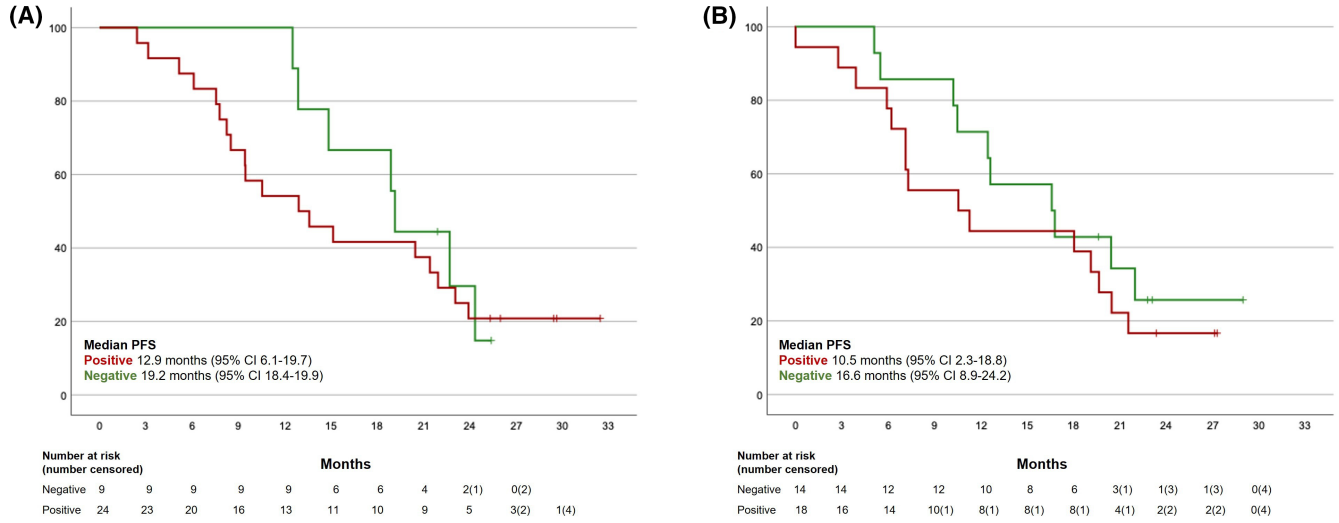


FIGURE 4 PFS after ARI0002h treatment in terms of QIP-MS status. PFS in terms of QIP-MS status in month 1 (A) or 3 (B) after ARI0002h CAR T-cell therapy.

production of the M-protein in extramedullary lesions could also explain this fact.

Although other studies have shown the clinical impact of QIP-MS results in patients with monoclonal gammopathies,[3,36] the information provided here by QIP-MS in terms of survival remains unclear in the setting of CAR T-cell treatments. This could be attributed to the sample size or to other experimental reasons. For example, other studies have employed paramagnetic beads specifically designed to recognize free κ and ʎ light chains.[3,36]

In conclusion, here we show that QIP-MS is a feasible non-invasive serum-based technique, useful in the monitoring of MM patients after BCMA-directed CAR T-cell therapy, especially when considering the potential t-mAb interferences or the identification of glycosylated or minor additional peaks.

AUTHOR CONTRIBUTIONS

IOdL, AOC, MER, MJ and CFdL participated in conception and design of the work and participated in the writing of the manuscript draft. IOdL, AOC, KT, GM, EAG, MP, DBR, SN, MUH and MER performed the serum sample collection. IOdL and CA performed the QIP-MS experiments. IOdL, CA and MTC analysed and interpreted the QIP-MS data and the M-protein kinetics. JIA and JY contributed to the analysis of the IFE results. AOC, IZ, VGC, BMA, VC, PRO, NP, JLR, AZ, BP, SI, ALDdC, PCH, MLP, LR, JMM, BP and CFdL contributed to data collection. IOdL, AOC, MER, AZ, BP, SI, ALDdC, LGRL, SV, LGRL, NT, AUI, MM and JSP performed data analysis and interpretation. IOdL, AOC and CFdL have verified the data. All authors revised the article and gave approval of the final version to be published.

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CONFLICT OF INTEREST STATEMENT

AOC declares receiving travel grants for congress attendance from Janssen. MV has received honoraria derived from lectures and participation in boards from: Janssen, BMS, Amgen, GSK, Sanofi, Pfizer, Regeneron, Novartis, Stemline and Oncopeptides. LGRL has received honoraria and travel grants from Janssen, Amgen, BMS, GSK and Sanofi. JMM declares receiving grant research supports from Pfizer, Jazz Pharma; speaker's bureau from Gilead-Kite, Novartis, Bristol-Myers Squibb/Celgene, Roche; travel grants, accommodations and expenses from Jazz Pharma, Gilead-Kite, Janssen, Sandoz; and consulting or advisory Role from Jazz Pharma, Novartis, Sandoz, Rocket Pharmaceuticals. LR has received honoraria from Janssen, BMS, Amgen, Takeda, Sanofi, GSK. VC declares receiving honoraria and advisory boards from Janssen, BMS, Sanofi, Amgen, Glaxo, Pfizer; speakers' bureau from BMS; and financing of scientific research from Janssen. CFL declares receiving grants through his institution from BMS, Janssen, and Amgen; honoraria from Amgen, Janssen, BMS, GSK and Sanofi; support for attending meetings or travel from Janssen, BMS, GSK and Amgen; and participation in data safety monitoring or advisory boards with Janssen, BMS, Amgen, Pfizer and Sanofi.

DATA AVAILABILITY STATEMENT

Individual, de-identified participant data and data dictionary can be made available, with publication, at the request of qualified investigators whose proposed use of the data has been approved by IDIBAPS, after a data access agreement is signed. Requests can be made to the corresponding author.

ETHICS APPROVAL STATEMENT

The study protocol was approved by the Ethics Committee of Hospital Clínic de Barcelona and was done in accordance with the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects.

PATIENT CONSENT STATEMENT

Patients provided written informed consent.

CLINICAL TRIAL REGISTRATION

ClinicalTrials.gov, NCT04309981, and EudraCT, 2019-001472-11.

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REFERENCES

1. Kumar SK, Rajkumar V, Kyle RA, van Duin M, Sonneveld P, Mateos MV, et al. Multiple myeloma. Nat Rev Dis Primers. 2017;3:17046.
2. Tate JR, Graziani MS, Mollee P, Merlini G. Protein electrophoresis and serum free light chains in the diagnosis and monitoring of plasma cell disorders: laboratory testing and current controversies. Clin Chem Lab Med. 2016;54:899–905.
3. Puig N, Contreras MT, Agulló C, Martínez-López J, Oriol A, Blanchard MJ, et al. Mass spectrometry vs immunofixation for treatment monitoring in multiple myeloma. Blood Adv. 2022;6:3234–9.
4. van de Donk NWCJ, Usmani SZ, Yong K. CAR T-cell therapy for multiple myeloma: state of the art and prospects. Lancet Haematol. 2021;8:e446–e461.
5. Perrot A, Lauwers-Cances V, Corre J, Robillard N, Hulin C, Chretien ML, et al. Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. Blood. 2018;132:2456–64.
6. Noori S, Verkleij CPM, Zajec M, Langerhorst P, Bosman PWC, de Rijke YB, et al. Monitoring the M-protein of multiple myeloma patients treated with a combination of monoclonal antibodies: the laboratory solution to eliminate interference. Clin Chem Lab Med. 2021;59:1963–71.
7. Ladwig PM, Barnidge DR, Willrich MAV. Mass Spectrometry Approaches for Identification and Quantitation of Therapeutic Monoclonal Antibodies in the Clinical Laboratory. Clin Vaccine Immunol. 2017;24(5):e00545-16.
8. Hmiel LK, Brorson KA, Boyne MT. Post-translational structural modifications of immunoglobulin G and their effect on biological activity. Anal Bioanal Chem. 2015;407:79–94.
9. Mai EK, Huhn S, Miah K, Poos AM, Scheid C, Weisel KC, et al. Implications and prognostic impact of mass spectrometry in patients with newly-diagnosed multiple myeloma. Blood Cancer J. 2023;13:1.
10. Langerhorst P, Noori S, Zajec M, de Rijke YB, Gloerich J, van Gool AJ, et al. Multiple myeloma minimal residual disease detection: targeted mass spectrometry in blood vs next-generation sequencing in bone marrow. Clin Chem. 2021;67:1689–98.
11. Puig N, Mateos MV, Contreras T, Paiva B, Cedena MT, Pérez JJ, et al. 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. Blood. 2019;134:581.
12. Barnidge DR, Krick TP, Griffin TJ, Murray DL. Using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to detect monoclonal immunoglobulin light chains in serum and urine. Rapid Commun Mass Spectrom. 2015;29:2057–60.
13. Oliver-Caldés A, González-Calle V, Cabañas V, Español-Rego M, Rodríguez-Otero P, Reguera JL, et al. Fractionated initial infusion and booster dose of ARI0002h, a humanised, BCMA-directed CAR T-cell therapy, for patients with relapsed or refractory multiple myeloma (CARTBCMA-HCB-01): a single-arm, multicentre, academic pilot study. Lancet Oncol. 2023;24:913–24.
14. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International myeloma working group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538–e548.
15. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International myeloma working group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17:e328–e346.
16. Mills JR, Kohlhagen MC, Dasari S, Vanderboom PM, Kyle RA, Katzmann JA, et al. Comprehensive assessment of M-proteins using

Nanobody enrichment coupled to MALDI-TOF mass spectrometry. Clin Chem. 2016;62:1334–44.
17. Moore LM, Cho S, Thoren KL. MALDI-TOF mass spectrometry distinguishes daratumumab from M-proteins. Clin Chim Acta. 2019;492:91–4.
18. Eveillard M, Korde N, Ciardiello A, Diamond B, Lesokhin A, Mailankody S, 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;516:136–41.
19. Si S, Teachey DT. Spotlight on Tocilizumab in the treatment of CAR-T-cell-induced cytokine release syndrome: clinical evidence to date. Ther Clin Risk Manag. 2020;16:705–14.
20. Mochizuki T, Shibata K, Naito T, Shimoyama K, Ogawa N, Maekawa M, et al. LC-MS/MS method for the quantitation of serum tocilizumab in rheumatoid arthritis patients using rapid tryptic digestion without IgG purification. J Pharm Anal. 2022;12:852–9.
21. Bondt A, Hoek M, Dingess K, Tamara S, de Graaf B, Peng W, et al. No patient is the same; lessons learned from antibody repertoire profiling in hospitalized severe COVID-19 patients. https://doi.org/10.1101/2022.12.23.22283896
22. Murray DL, Puig N, Kristinsson S, Usmani SZ, Dispenzieri A, Bianchi G, et al. Mass spectrometry for the evaluation of monoclonal proteins in multiple myeloma and related disorders: an international myeloma working group mass spectrometry committee report. Blood Cancer J. 2021;11:24.
23. McDonald Z, Taylor P, Liyasova M, Liu Q, Ma B. Mass spectrometry provides a highly sensitive noninvasive means of sequencing and tracking M-protein in the blood of multiple myeloma patients. J Proteome Res. 2021;20:4176–85.
24. Moonen DH, Kohlhagen M, Dasari S, Willrich MA, Kourelis T, Dispenzieri A, et al. Utilizing mass spectrometry to detect and isotype monoclonal proteins in urine: comparison to electrophoretic methods. Clin Chem. 2023;69:746–53.
25. Fatica EM, Martinez M, Ladwig PM, Murray JD, Kohlhagen MC, Kyle RA, et al. MALDI-TOF mass spectrometry can distinguish immunofixation bands of the same isotype as monoclonal or biclonal proteins. Clin Biochem. 2021;97:67–73.
26. Martin T, Usmani SZ, Berdeja JG, Agha M, Cohen AD, Hari P, et al. Ciltacabtagene Autoleucel, an anti–B-cell maturation antigen chimeric antigen receptor T-cell therapy, for relapsed/refractory multiple myeloma: CARTITUDE-1 2-year follow-up. J Clin Oncol. 2023;41:1265–74.
27. Munshi NC, Anderson LD Jr, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene Vicleucel in relapsed and refractory multiple myeloma. N Engl J Med. 2021;384:705–16.
28. Dispenzieri A, Larson DR, Rajkumar SV, Kyle RA, Kumar SK, Kourelis T, et al. N-glycosylation of monoclonal light chains on routine MASS-FIX testing is a risk factor for MGUS progression. Leukemia. 2020;34:2749–53.
29. Omtvedt LA, Bailey D, Renouf DV, Davies MJ, Paramonov NA, Haavik S, et al. Glycosylation of immunoglobulin light chains associated with amyloidosis. Amyloid. 2000;7:227–44.
30. de Larrea CF, Tovar N, Cibeira MT, Aróstegui JI, Rosiñol L, Elena M, et al. Emergence of oligoclonal bands in patients with multiple myeloma in complete remission after induction chemotherapy: association with the use of novel agents. Haematologica. 2011;96:171–3.
31. de Larrea CF, Tovar N, Teresa Cibeira M, Arostegui JI, Rosinol L, Elena M, et al. Oligoclonal bands in patients with multiple myeloma in complete remission after induction chemotherapy: strong association with the use of novel agents. Blood. 2010;116:2945.
32. Abdallah N, Murray D, Dispenzieri A, Kapoor P, Gertz MA, Lacy MQ, et al. Tracking daratumumab clearance using mass spectrometry: implications on M protein monitoring and reusing daratumumab. Leukemia. 2022;36:1426–8.
33. van de Donk NWCJ, Themeli M, Usmani SZ. Determinants of response and mechanisms of resistance of CAR T-cell therapy in multiple myeloma. Blood Cancer Discov. 2021;2:302–18.
34. Paiva B, Manrique I, Rytlewski J, Campbell T, Kazanecki CC, Martin N, et al. Time-dependent prognostic value of serological and measurable residual disease assessments after Idecabtagene Vicleucel. Blood Cancer Discov. 2023;4:365–73.
35. Landgren O, Hultcrantz M, Diamond B, Lesokhin AM, Mailankody S, Hassoun H, et al. Safety and effectiveness of weekly Carfilzomib, Lenalidomide, dexamethasone, and Daratumumab combination therapy for patients with newly diagnosed multiple myeloma. JAMA Oncol. 2021;7:862–8.
36. Bomsztyk J, Ravichandran S, Giles HV, Wright N, Berlanga O, Khwaja J, et al. Complete responses in AL amyloidosis are unequal – the impact of free light chain mass spectrometry in AL amyloidosis. Blood J. 2024;143:1259–68. https://doi.org/10.1182/blood.2023022399

SUPPORTING INFORMATION

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