


# Characterisation and prognostic impact of immunoparesis in relapsed multiple myeloma

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Received 11 November 2019; accepted for publication 24 December 2019

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This study was presented at the American Society of Hematology 61<sup>st</sup> Annual Meeting at Orlando, Florida on 12/9/2019 [Abstract # 4359].

## Summary

Characterisation and prognostic impact of immunoparesis in relapsed multiple myeloma (MM) is lacking in the current literature. We evaluated 258 patients with relapsed MM, diagnosed from 2008 to 2015, to investigate the prognostic impact of deep immunoparesis on post-relapse survival. On qualitative immunoparesis assessment, no, partial and full immunoparesis was present in 9%, 30% and 61% of patients, respectively. Quantitative immunoparesis was assessed by computing the average relative difference (ARD) between polyclonal immunoglobulin(s) and corresponding lower normal limit(s), with greater negative values indicating deeper immunoparesis. The median ARD was  $-39\%$ , with an optimal cut-off of  $-50\%$  for overall survival (OS) by recursive partitioning analysis. Deep immunoparesis (ARD  $\leq -50\%$ ) was associated with a higher tumour burden at first relapse compared to none/shallow [ARD  $> -50\%$ ] immunoparesis. The OS ( $P = 0.007$ ) and progression-free survival (PFS;  $P < 0.001$ ) differed significantly between the deep and none/shallow immunoparesis groups. Kaplan–Meier estimates for 3-year OS were 36% and 46%, and for 2-year PFS were 17% and 27%, respectively. On multivariable analysis (MVA) for PFS, both qualitative and quantitative immunoparesis retained negative prognostic impact independently. However, only quantitative immunoparesis was independently prognostic for OS on MVA. Depth of immunoparesis in relapsed MM is an important prognostic factor for post-relapse survival in the era of novel agents and continuous therapy.

Multiple myeloma (MM) is a common haematological malignancy that arises from monoclonal plasma cells in the bone marrow. These malignant plasma cells typically secrete monoclonal immunoglobulin, either in the form of an intact immunoglobulin molecule (e.g., IgG- $\kappa$ ) or free light chain [ $\kappa$  or  $\lambda$ ] or both. Immunoparesis, suppression of polyclonal immunoglobulins, is an established hallmark of MM and its precursor states such as monoclonal gammopathy of undetermined significance (MGUS) and smouldering MM (Pruzanski *et al.*, 1980; Wang, 1987; Kyle *et al.*, 2003). Presence and depth of immunoparesis at MM diagnosis has a negative prognostic impact on overall survival (OS) in the era of novel agents (Kastritis *et al.*, 2014; Heaney *et al.*, 2018). Although prior studies have shown that immunoparesis is a risk factor for infections in patients with MM (Hargreaves *et al.*, 1995), recent studies have shown that the negative prognostic impact of immunoparesis on OS is not entirely explained by

infection-related mortality (Kastritis *et al.*, 2014; Heaney *et al.*, 2018).

Despite the availability of several effective drug classes including proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) and monoclonal antibodies (mAbs), patients with MM experience multiple disease relapses requiring sequential lines of treatment. Frontline use of PIs and alkylating agents, like cyclophosphamide, can lead to suppression of uninvolved immunoglobulins in addition to reducing the monoclonal protein burden (Ravi *et al.*, 2017). Hence, immunoparesis in relapsed MM can be due to the underlying disease/microenvironment, prior anti-myeloma therapies, or a combination of both. Little is known regarding the characterisation and prognostic impact of immunoparesis in the setting of relapsed MM. A prior study of 47 patients with relapsed/refractory MM demonstrated a negative prognostic impact of severe ( $>50\%$ ) suppression of heavy-/light-chain matched-

pairs on OS (Ludwig *et al.*, 2016). Suppression of one or two uninvolved immunoglobulins was not associated with a poor prognosis in that study; however, the study was limited due to a small sample size. Furthermore, although we have standardised tools for risk stratification of MM at diagnosis (Palumbo *et al.*, 2015), dynamic prognostic assessment based on updated information at relapse is warranted and important for optimal

c Full immunoparesis (suppression of all polyclonal immunoglobulins).

d Quantitative immunoparesis was assessed in two steps:

Calculating the relative difference (RD) between each polyclonal immunoglobulin and their LLN using the following formula:

$$\frac{\text{Level of polyclonal immunoglobulin at first relapse} - \text{Corresponding LLN}}{\text{Corresponding LLN}}$$

risk-stratification and management. We hypothesised that immunoparesis in relapsed MM is associated with high tumour burden and an inferior post-relapse survival in the era of novel agents. To test our hypothesis, we queried our institutional MM database at the Cleveland Clinic to identify all patients experiencing first relapse requiring an additional line of therapy and with adequate data on pre-treatment immunoglobulin levels.

## Patients and methods

### Patients

This study was approved by the Cleveland Clinic Institutional Review Board and was conducted in accordance with federal regulations and the principles of the Declaration of Helsinki. We reviewed all consecutive patients in our prospectively maintained MM database who were diagnosed and treated at the Taussig Cancer Center at Cleveland Clinic from 1 January 2008 to 31 December 2015 and followed for relapse or death until 31 May 2019. Patients who experienced first relapse requiring an additional line of therapy were included in our analysis. Patients with primary refractory disease, continued first remission at latest follow-up, and insufficient data on immunoglobulin levels at first relapse were excluded.

Immunoparesis was defined as suppression of polyclonal immunoglobulins, i.e., reduction of polyclonal IgG, IgA, and/or IgM below the lower limit of normal (LLN). Immunoglobulins IgG, IgA, and IgM were measured by nephelometry using polyclonal goat anti-IgG, -IgA, and -IgM antisera, respectively, on an IMMAGE 800 Immunochemistry instrument (Beckman Coulter, Brea, CA, USA). Immunoparesis assessment was performed by qualitative and quantitative methods, as described earlier in the setting of amyloid light-chain (AL) amyloidosis and MM (Ludwig *et al.*, 2016; Muchtar *et al.*, 2017). Qualitative immunoparesis was defined as the number of suppressed polyclonal immunoglobulins below the LLN, with three categories:

a No immunoparesis (all polyclonal immunoglobulins above the LLN).

b Partial immunoparesis (suppression of at least one but not all polyclonal immunoglobulins).

Calculating the mean of all RDs to obtain the average RD (ARD) for each patient.

### Statistical analysis

The primary objective of the analysis was to determine if qualitative or quantitative immunoparesis at first relapse were prognostic for progression-free survival (PFS) or OS (primary endpoints), or for best response in second remission (secondary endpoint). Additional objectives were to assess the relationship between immunoparesis at diagnosis and immunoparesis at first relapse, and to determine the individual prognostic impact of IgG, IgA, and IgM immunoparesis at first relapse. Recursive partitioning analysis with a log-rank splitting method was used to identify an optimal cut-off of ARD that was prognostic for OS. Baseline characteristics between the resulting two groups were compared with Wilcoxon rank-sum test, chi-squared test, or Fisher's exact test. Cox proportional hazards analysis was used to identify prognostic factors for PFS and OS. The study variables assessed as prognostic factors were immunoparesis at first relapse (qualitative and quantitative), age at first relapse (per 10-year increase), sex, race (Caucasian vs. Others), International Staging System (ISS) Stage at diagnosis (ISS Stage III vs. ISS Stage I/II), bone marrow plasma cells at diagnosis (%), metaphase cytogenetics at diagnosis (normal vs. abnormal), best response in first remission [less than very good partial response (VGPR) vs. VGPR or better], frontline autologous haematopoietic cell transplantation, time from diagnosis to first relapse ( $\leq 12$  vs.  $> 12$  months), pattern of first relapse (clinical vs. biochemical), and relapse with respect to therapy (on therapy vs. on observation). Biochemical relapse was defined as per the International Myeloma Working Group (IMWG) consensus criteria (Kumar *et al.*, 2016). Patients having new end-organ damage as per IMWG criteria (Kumar *et al.*, 2016) or with new extramedullary disease were categorised as clinical relapse irrespective of serological markers. Stepwise Cox analysis, with a variable entry criterion of  $P < 0.10$  and a variable retention criterion of  $P < 0.05$ , was used to identify multivariable prognostic factors for PFS and OS. Fluorescence *in situ* hybridisation (FISH) cytogenetics was not included due to missing data in 46% of patients. Two variations of the model were assessed, one with quantitative immunoparesis (ARD  $\leq -50\%$  vs. ARD

>−50%) and one with qualitative immunoparesis (no vs. partial vs. full). Cox results were summarised as hazard ratio (HR) and 95% confidence interval (CI). Association between qualitative immunoparesis at first relapse and other variables were assessed with Cochran-Mantel-Haenszel correlation test (qualitative immunoparesis at diagnosis), Jonckheere-Terpstra test (quantitative immunoparesis at diagnosis), and Cochran-Armitage test (ARD >−50% vs. ≤−50%). Analyses were done with Statistical Analysis System (SAS®) software, version 9.4 (SAS Institute, Inc., Cary, NC, USA). All statistical tests were two-sided and  $P < 0.05$  was used to indicate statistical significance.

## Results

### *Baseline characteristics*

A total of 527 newly diagnosed patients were identified in our MM database between January 2008 and December 2015. Among them, 258 patients experiencing first relapse and with adequate data on immunoparesis formed the study cohort. The Consolidated Standards of Reporting Trials (CONSORT) diagram for patient selection is shown in Supplementary Appendix S1. The baseline characteristics of the study cohort at diagnosis and at first relapse are shown in Table I. The median (range) age at diagnosis and at first relapse were 62 (22–86) and 64 (26–87) years, respectively. The monoclonal protein was an intact immunoglobulin in 76% and light-chain-only in 24% of patients. Among 192 patients with intact immunoglobulin MM, 120 had IgG paraprotein, 68 had IgA paraprotein, and two each had IgM and IgD paraprotein. Among 228 patients with available data on ISS Stage at diagnosis, 33% were Stage III and 34% were Stage II. A total of 140 patients had available FISH data at diagnosis, with 31% having a high-risk signature [defined as the presence of t(4;14), t(14;16), t(14;20), or deletion(17p)]. Data on immunoglobulin measurements at diagnosis were available for 214 patients. On qualitative analysis, 11 patients (5%) had no immunoparesis at diagnosis, 35 (16%) had partial immunoparesis, and 168 (79%) had full immunoparesis. On quantitative analysis, the median (range) ARD at diagnosis was −58 (−92 to + 805)%, with negative values indicating greater immunoparesis. A total of 77% were exposed to bortezomib and 76% to lenalidomide prior to first relapse, with 85% of patients relapsing on therapy [IMiD or PI maintenance ( $\pm$  steroid) in 64%]. Clinical relapse with end-organ damage or extramedullary disease was seen in 44% of patients. The treatment regimens initiated at first relapse by quantitative immunoparesis subgroups (deep vs. none/shallow) is shown in Supplementary Appendix S2.

### *Immunoparesis at first relapse: characterisation and correlation with other parameters*

On qualitative immunoparesis assessment, 24 patients (9%) had no immunoparesis at first relapse, 76 (29%) had partial immunoparesis, and 158 (61%) had full immunoparesis. On

quantitative immunoparesis assessment, the median (range) ARD at first relapse was −39 (−92 to + 241)% for the entire cohort. The proportion of patients with full immunoparesis at first relapse by qualitative method was 76%, 60%, and 46% in IgA, IgG and light-chain MM, respectively ( $P = 0.002$ ). The median ARD (quantitative method) at first relapse was −42%, −37%, and −27% in patients with IgA, IgG and light-chain MM, respectively ( $P = 0.13$ ). The relationship between qualitative and quantitative immunoparesis at first relapse is shown in Fig 1. The median (range) ARD at first relapse for patients with no, partial and full immunoparesis was + 67 (+15% to + 241)%, +15 (−44% to + 208)% and −58 (−92% to −14)%, respectively ( $P < 0.001$ ).

Based on recursive partitioning analysis for ARD at relapse, an optimal cut-point of −47% was prognostic for OS, which was rounded to −50% for ease of interpretation. This rounding only impacted the categorisation of two patients. Hence, patients were analysed in two groups reflecting the depth of quantitative immunoparesis: ≤−50% (deep immunoparesis;  $n = 103$ , 40%) and >−50% (none/shallow immunoparesis;  $n = 155$ , 60%). The baseline characteristics of the two groups are shown in Table I. Patients with ARD ≤−50% (compared to ARD >−50%) had a significantly higher incidence of abnormal karyotype at diagnosis, <VGPR in first remission, relapse within a year of diagnosis, end-organ damage or extramedullary disease (clinical progression) at first relapse, elevated lactate dehydrogenase (LDH) at first relapse, elevated involved/uninvolved serum free light-chain ratio at first relapse, and ISS Stage II/III at first relapse. There was a trend towards higher bone marrow plasma cells at diagnosis in the deep immunoparesis (ARD <−50%) group. Notably, there was no significant difference in the incidence of high-risk FISH cytogenetics at diagnosis, ISS Stage at diagnosis, and relapse on therapy *versus* observation between the two groups.

### *Immunoparesis at first relapse: impact on survival and response*

The median (range) follow-up of surviving patients from first relapse was 34 (3–124) months. In patients with no, partial and full immunoparesis on qualitative assessment OS was not different among groups ( $P = 0.09$ ), whereas PFS was ( $P = 0.008$ ). The estimated 3-year OS in patients with no, partial and full immunoparesis was 60%, 42% and 40%, respectively; with respective 2-year PFS being 36%, 25% and 20%. Both OS ( $P = 0.007$ ) and PFS ( $P < 0.001$ ) differed significantly between patients with none/shallow (ARD >−50%) *versus* deep (ARD ≤−50%) immunoparesis on quantitative assessment. In the ARD >−50% and ARD ≤−50% groups, the 3-year OS estimate was 47% and 36%, respectively. The respective 2-year PFS estimate was 27% and 17%. The Kaplan–Meier curves for PFS and OS with qualitative and quantitative immunoparesis groups are shown in Fig 2 and

Table I. Clinical and demographic characteristics of the study cohort.

Variable	Entire cohort (N = 258)	ARD $\leq$ -50% (n = 103)	ARD $>$ -50% (n = 155)	P
<b>At diagnosis</b>				
Age, years, median (range)	62 (22–86)	62 (22–86)	62 (30–86)	0.66
Male sex, n (%)	131 (51)	47 (46)	84 (54)	0.18
Caucasian race, n (% of 256, 101 and 155, respectively)	205 (80)	85 (84)	120 (77)	0.19
MM subtype, n (%)				
IgG	120 (47)	47 (46)	73 (47)	0.56
IgA	68 (26)	30 (29)	38 (25)	
LCO	63 (24)	22 (21)	41 (27)	
Others	7 (3)	4 (4)	3 (2)	
Bone marrow plasma cells, %, median (range) (n = 237, 98 and 139, respectively)	56 (2–100)	60 (2–100)	50 (2–100)	0.07
ISS Stage, n (% of 228, 93 and 135, respectively)				
I	75 (33)	27 (29)	48 (36)	0.22
II	77 (34)	29 (31)	48 (36)	
III	76 (33)	37 (40)	39 (29)	
High-risk cytogenetics by FISH*, n (% of 140, 60 and 80, respectively)	43 (31)	19 (32)	24 (30)	0.83
Abnormal metaphase cytogenetics, n (% of 212, 82 and 130, respectively)	38 (18)	21 (26)	17 (13)	0.020
<VGPR (PR/MR/SD) in first remission, n (% of 251, 101 and 150, respectively)	83 (33)	48 (48)	35 (23)	<0.001
>12 months from diagnosis to first relapse, n (%)	204 (79)	68 (66)	136 (88)	<0.001
Relapse on therapy, n (% of 255, 101 and 154, respectively)	217 (85)	86 (85)	131 (85)	0.99
<b>At first relapse</b>				
Age, years, median (range)	64 (26–87)	63 (26–86)	65 (37–87)	0.67
Serum M-protein, g/dl, median (range) (n = 254, 100 and 154, respectively)	0.51 (0–6.40)	0.55 (0–5.63)	0.49 (0–6.40)	0.06
Involved/uninvolved sFLC ratio (range) (n = 253, 100 and 153, respectively)	17.3 (0.4–6105.6)	59.2 (0.4–6105.6)	10.9 (0.6–636.1)	<0.001
Serum creatinine, mg/dl, median (range) (n = 257, 103 and 154, respectively)	0.95 (0.47–15.28)	0.95 (0.50–15.28)	0.98 (0.47–6.85)	0.65
Haemoglobin, g/l, median (range)	118 (52–164)	109 (52–159)	121 (67–164)	<0.001
ISS Stage, n (% of 169, 72 and 97, respectively)				
I	109 (64)	37 (51)	72 (74)	0.001
II	34 (20)	16 (22)	18 (19)	
III	26 (15)	19 (26)	7 (7)	
LDH > UNL (% of 187, 79 and 108, respectively)	51 (27)	28 (35)	23 (21)	0.032
Clinical relapse** (% of 252, 100 and 152, respectively)	112 (44)	52 (52)	60 (39)	0.05

ARD, average relative difference; FISH, fluorescence *in situ* hybridisation; ISS, International Staging System; LCO, light-chain only; LDH, lactate dehydrogenase; MR, minimal response; PR, partial response; SD, stable disease; sFLC, serum free light-chain; UNL, upper limit of normal; VGPR, very good partial response.

\*High-risk FISH abnormality was defined by the presence of deletion(17p), t(4;14), t(14;16), and/or t(14;20).

\*\*Clinical relapse was defined as relapse with CRAB (hypercalcaemia, renal insufficiency, anaemia or bone disease) features or extramedullary disease or both.

Fig 3, respectively. Among patients with clinical relapse those with deep immunoparesis on quantitative assessment (ARD  $<$ -50%) had a significantly worse PFS and OS compared to the none/shallow immunoparesis group (ARD  $\geq$ -50%) [Supplementary Appendix S3].

Factors prognostic for OS on multivariable analysis (MVA) using the quantitative model were depth of quantitative immunoparesis (HR for ARD  $\leq$ -50% vs. ARD  $>$ -50%: 1.72, 95% CI 1.24–2.40;  $P = 0.001$ ), <VGPR in first remission (HR for <VGPR vs.  $\geq$ VGPR: 0.60, 95% CI 0.42–0.87;  $P = 0.007$ ), and clinical relapse (HR for clinical vs. biochemical relapse: 3.16, 95% CI 2.27–4.40;  $P < 0.001$ ). Qualitative immunoparesis was not prognostic for OS on MVA. Factors

prognostic for PFS on MVA in the quantitative model were depth of quantitative immunoparesis (HR for ARD  $\leq$ -50% vs. ARD  $>$ -50%: 1.82, 95% CI 1.35–2.44;  $P < 0.001$ ), age at initial relapse (HR per 10-year increase: 1.17, 95% CI 1.02–1.34;  $P = 0.020$ ), ISS Stage III at diagnosis (HR for ISS Stage III vs. ISS Stage I/II: 1.52, 95% CI 1.12–2.07;  $P = 0.007$ ), and clinical relapse (HR for clinical vs. biochemical relapse: 2.39, 95% CI 1.77–3.22;  $P < 0.001$ ). Factors prognostic for PFS on MVA in the qualitative model were full immunoparesis (HR for full vs. no immunoparesis: 2.03, 95% CI 1.13–3.64;  $P = 0.018$ ), along with the other variables mentioned above in the quantitative model (age at first relapse, ISS Stage III at diagnosis and clinical relapse). Results of

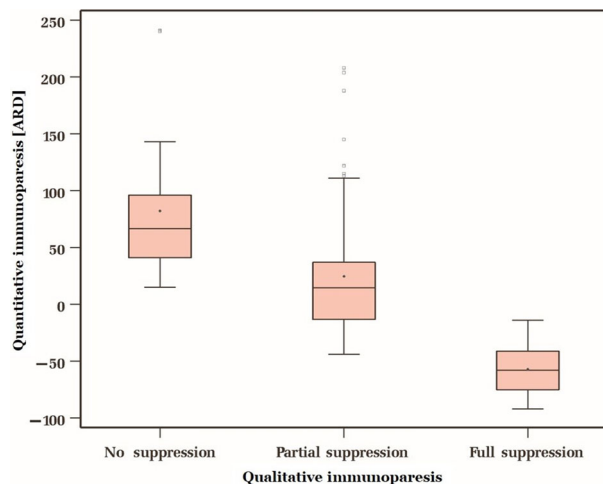


Fig 1. Boxplot showing quantitative immunoparesis at first relapse (y-axis) stratified by qualitative immunoparesis groups (no, partial and full; x-axis). The median (range) average relative difference (ARD) in the no, partial, and full immunoparesis groups were +67 (+15 to +241), +15 (-44 to +208) and -58 (-92 to -14)%, respectively, with higher negative ARD values indicating deeper immunoparesis ( $P < 0.001$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

stepwise Cox multivariable analysis for PFS and OS using both models (qualitative and quantitative) are shown in Table II.

There was no relationship between the depth of immunoparesis (qualitative or quantitative) and best response in second remission [Table III].

#### *Correlation between immunoparesis at diagnosis and at first relapse*

A total of 216 patients had available data at both time-points for quantitative immunoparesis and 214 patients had available data at both time-points for qualitative immunoparesis. On qualitative immunoparesis assessment, full immunoparesis at diagnosis was seen in 45%, 63% and 92% of patients with no, partial and full immunoparesis at first relapse, respectively. On quantitative immunoparesis assessment at diagnosis, deep immunoparesis (ARD  $\leq -50\%$ ) was seen in 25%, 38% and 74% of patients with no, partial and full immunoparesis at relapse, respectively. Spearman correlation between quantitative immunoparesis at diagnosis and at first relapse was 0.49 ( $P < 0.001$ ).

#### *Prognostic impact of IgG, IgA, and IgM immunoparesis at first relapse*

IgG immunoparesis was present in 74% of 136 patients with non-IgG MM. IgA immunoparesis was present in 61% of 191 patients with non-IgA MM. IgM immunoparesis was present in 88% of 256 patients with non-IgM MM. In general, patients with immunoparesis had numerically lower

median PFS and OS compared to those without immunoparesis; however, was statistically significant only for IgM immunoparesis ( $P = 0.004$  for PFS and  $P = 0.048$  for OS). Kaplan–Meier curves for PFS and OS with IgG, IgA and IgM immunoparesis are shown in Supplementary Appendix S4.

## Discussion

Our present study shows that the depth of immunoparesis at first relapse is associated with a higher tumour burden at relapse, as demonstrated by a higher incidence of ISS Stage II/III disease, clinical relapse, elevated LDH, and higher serum free light-chain ratio in patients with deep immunoparesis compared to those with none/shallow immunoparesis. However, there was no relationship between the depth of immunoparesis and high-risk FISH cytogenetics at diagnosis, as well as whether patients relapsed on therapy *versus* observation. Depth of quantitative immunoparesis at a cut-point of  $-50\%$  was prognostic for both PFS and OS from first relapse on MVA.

How does immunoparesis mediate negative outcomes like death or progression in MM? One of the largest studies on prognostic impact of immunoparesis in newly diagnosed MM was published by the UK Medical Research Council (MRC) group from recent (Myeloma IX and XI) and old (Myeloma IV, V, VI, and VIII) MM clinical trials (Heaney *et al.*, 2018). The prognostic impact of immunoparesis at diagnosis was stronger in recent MM trials incorporating PIs and IMiDs compared to older trials in the era of alkylating agents. Furthermore, IgM but not IgG or IgA immunoparesis was prognostic for survival, with a progressive decrease in PFS and OS with decreasing levels of polyclonal IgM, which highlights the importance of measuring immunoparesis depth. In our present study, IgM immunoparesis at first relapse was associated with a significantly lower PFS and OS. However, no significant prognostic impact of IgG or IgA immunoparesis was observed. Of note, this should be interpreted cautiously, as polyclonal IgG and IgA levels in our present study were only available in 53% and 74% of the entire cohort who had non-IgG or non-IgA MM, respectively. Potential mechanisms for adverse prognostic impact of IgM immunoparesis includes reduced immune surveillance, surrogate for extramedullary disease (as polyclonal IgM-secreting plasma cells are located in spleen and lymph nodes compared to polyclonal IgA/IgG-secreting plasma cells that are present in bone marrow), and increased infection risk (Boes, 2000; Heaney *et al.*, 2018). A recent epidemiological study in patients with MGUS has shown that suppression of two or more uninvolved immunoglobulins is associated with a significantly higher rate of progression to symptomatic MM (Landgren *et al.*, 2019). Hence, immunoparesis could potentially reflect altered tumour microenvironment or adverse plasma cell biology. Additionally, whether recovery of polyclonal immunoglobulins after treatment of relapsed MM is a marker for improved outcomes remains an unanswered question.

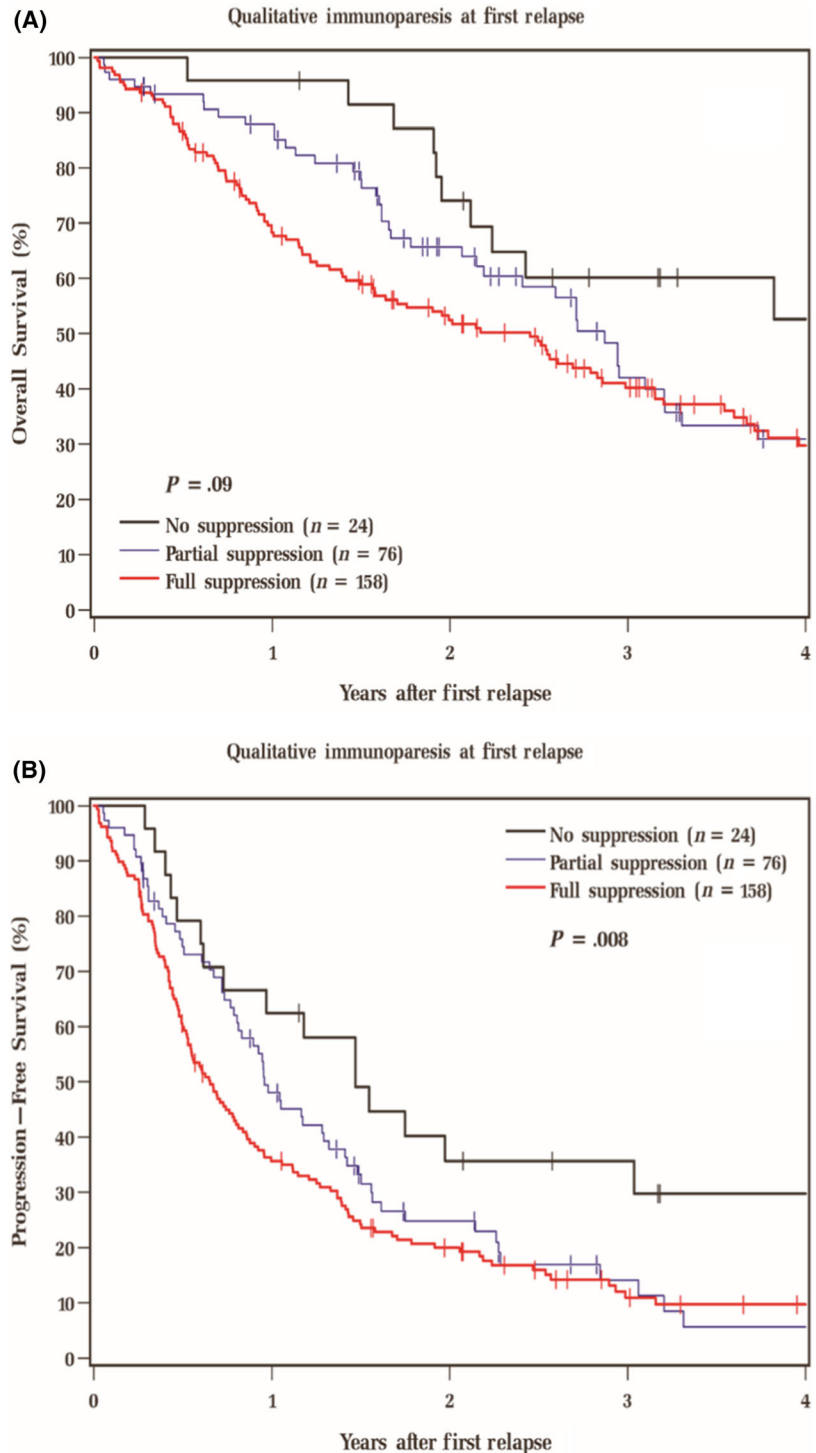


Fig 2. Kaplan–Meier curves showing overall survival (OS; IIA) and progression-free survival (PFS; IIB) calculated from the date of first relapse and compared between different qualitative (no vs. partial vs. full) immunoparesis groups. The 3-year OS estimate in the no, partial, and full immunoparesis groups was 60%, 42% and 40%, respectively, and the 2-year PFS in respective subgroups was 36%, 25%, and 20%. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Another report from the Greek Myeloma Study Group investigated the impact of qualitative immunoparesis on survival in newly diagnosed MM, approximately 40% of whom had received novel agents (Kastritis *et al.*, 2014). Suppression of at least one polyclonal immunoglobulin was associated with a higher disease burden, indicated by higher ISS Stage and extensive bone marrow infiltration. In our present study,

higher depth of immunoparesis at first relapse was associated with a high tumour burden at relapse and short duration of first remission, suggesting that tumour burden and disease biology rather than treatment-specific factors are the primary drivers of immunoparesis in relapsed MM. Furthermore, depth of response at second remission was not associated with pre-treatment immunoparesis at first relapse, which is

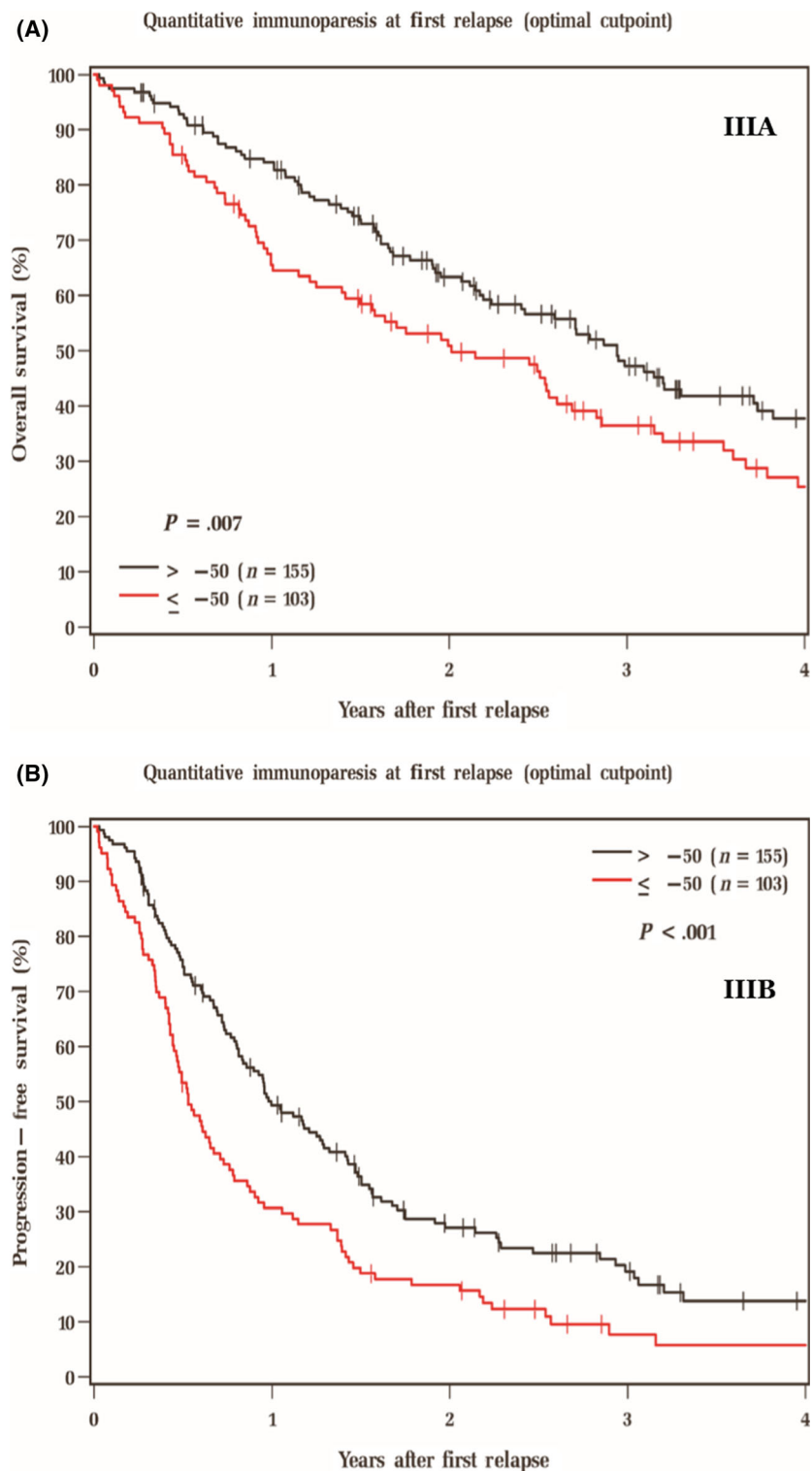


Fig 3. Kaplan–Meier curves showing overall survival (OS; IIIA) and progression-free survival (PFS; IIIB) calculated from the date of first relapse and compared between different quantitative (ARD  $\leq -50\%$  vs.  $> -50\%$ ) immunoparesis groups. The 3-year OS estimate in ARD  $> -50\%$  and ARD  $\leq -50\%$  immunoparesis groups were 47% and 36%, respectively, and the 2-year PFS in respective subgroups were 27% and 17%. ARD represents average relative difference, with higher negative ARD values indicating deeper immunoparesis. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

in line with the Greek study that reported a lack of association between the number of uninvolved immunoglobulins at diagnosis and depth of response at first remission (Kastritis *et al.*, 2014). There was no significant correlation between immunoparesis and presence of high-risk FISH cytogenetics, which is also consistent with prior studies in newly

diagnosed and relapsed/refractory MM (Ludwig *et al.*, 2016; Gao *et al.*, 2019).

Our present study has limitations. First, we did not have data on the frequency and severity of infections, which could have accounted for the difference in survival due to infection-related mortality. However, the UK MRC study has

**Table II.** Multivariable prognostic factors for overall survival (OS) and progression-free survival (PFS) from first relapse.

Variable	Model 1 Quantitative immunoparesis		Model 2 Qualitative immunoparesis	
	HR (95% CI)	P	HR (95% CI)	P
<b>Models for OS (n = 246; 152 events)</b>				
Immunoparesis:				
Partial/no suppression	NA	NA	1.05 (0.53–2.06)	0.90
Full/no suppression	NA	NA	1.77 (0.94–3.33)	0.08
≤-50/>-50%	1.72 (1.24–2.40)	0.001	NA	NA
Pattern of relapse Clinical*/biochemical	3.16 (2.27–4.40)	<0.001	3.41 (2.44–4.78)	<0.001
Best response in first remission				
<VGPR/≥VGPR	0.60 (0.42–0.87)	0.007	0.64 (0.45–0.92)	0.017
<b>Models for PFS (n = 225; 189 events)</b>				
Immunoparesis:				
Partial/no suppression	NA	NA	1.31 (0.70–2.45)	0.40
Full/no suppression	NA	NA	2.03 (1.13–3.64)	0.018
≤-50/>-50%	1.82 (1.35–2.44)	<0.001	NA	NA
Age at first relapse per 10-year increase	1.17 (1.02–1.34)	0.020	1.16 (1.01–1.32)	0.031
ISS Stage (at diagnosis) III vs. I/II	1.52 (1.12–2.07)	0.007	1.54 (1.13–2.10)	0.006
Type of relapse Clinical*/biochemical	2.39 (1.77–3.22)	<0.001	2.34 (1.72–3.18)	<0.001

CI, confidence interval; HR, hazard ratio; ISS, International Staging System; VGPR, very good partial response.

\*Clinical relapse was defined as relapse with CRAB (hypercalcaemia, renal insufficiency, anaemia or bone disease) features or extramedullary disease or both.

**Table III.** Immunoparesis at first relapse and best response in second remission.

Variable	VGPR or better, n (%)	P
Qualitative immunoparesis		
No suppression (n = 23)	6 (26.1)	0.93
Partial suppression (n = 72)	19 (26.4)	
Full suppression (n = 138)	37 (26.8)	
Quantitative immunoparesis		
>-50% (n = 145)	41 (28.3)	0.46
≤-50% (n = 88)	21 (23.9)	

VGPR: very good partial response.

shown that the negative prognostic impact of IgM immunoparesis in newly diagnosed MM could not be explained by infections. Second, most patients in our present study were not exposed to mAbs, like daratumumab, prior to first relapse. As mAbs are currently being incorporated in the front-line treatment of MM (Mateos *et al.*, 2018; Facon *et al.*, 2019) and can lead to prolonged suppression of polyclonal immunoglobulins, the prognostic impact of immunoparesis at relapse will need to be revisited in future as the treatment landscape evolves. Third, data on FISH cytogenetics and degree of bone marrow infiltration at relapse were lacking, as bone marrow biopsy was not routinely performed in relapsed MM during the time period of our present study. Data from prospective trials in relapsed MM with available information on immunoparesis and other disease-specific variables (e.g., FISH cytogenetics at relapse) should be used to validate our present findings. Finally, as with any

observational study, reverse causation and unmeasured confounding always remain a concern when interpreting prognostic biomarkers.

In conclusion, our present study highlights the negative prognostic impact of immunoparesis depth at first relapse on subsequent relapse or survival in MM in the era of novel agents and continuous therapy. The 3-year OS estimate in patients with deep (ARD ≤-50%) and none/shallow (ARD >-50%) immunoparesis was 47% and 36%, respectively, with deep immunoparesis having an independently negative prognostic impact on MVA. As immunoglobulin levels are routinely monitored in clinical practice, assessment of quantitative immunoparesis can be widely used as a prognostic factor in relapsed MM. Future studies should focus on identifying the predictive role of immunoparesis for subsequent infections in relapsed MM and the prognostic impact of immunoparesis kinetics in the era of anti-myeloma drugs, which ablate malignant as well as normal plasma cells, like CD38- or B cell maturation antigen (BCMA)-targeted therapies.

### Authors' contributions

Rajshekhhar Chakraborty and Faiz Anwer designed the study. Rajshekhhar Chakraborty extracted clinical data and drafted the manuscript. Lisa Rybicki performed statistical analysis. Hayley Dysert maintained the myeloma registry at Cleveland Clinic. Megan O. Nakashima, Robert M. Dean, Beth M. Faiman, Christy J. Samaras, Nathaniel Rosko, and Jason



Valent provided critical input in study design, analysis and manuscript writing and approved the final draft.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix 1.** CONSORT flow diagram demonstrating patient inclusion.

**Appendix 2.** Treatment Regimens at 1<sup>st</sup> Relapse Stratified by Depth of Immunoparesis.

**Appendix 3.** Kaplan-Meier curves for PFS and OS in the subgroup of patients with clinical relapse stratified by depth of immunoparesis.

**Appendix 4.** Kaplan-Meier curves for PFS and OS in patients with non-IgG [A and B], non-IgA [C and D], and non-IgM MM [E and F]. Statistically significant decrease in PFS and OS was noted only with IgM immunoparesis.

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