Recovery of uninvolved heavy/light chain pair immunoparesis in newly diagnosed transplant-eligible myeloma patients complements the prognostic value of minimal residual disease detection

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Abstract

Immunoparesis (IP) in multiple myeloma (MM) patients can be measured by classic assessment of immunoglobulin (Ig) levels or by analysis of the uninvolved heavy/light chain pair of the same immunoglobulin (uHLC) by the Hevylite® assay. In this study we evaluate the prognostic value of recovery from IP measured by classic total Ig and uHLC assessment in newly diagnosed MM transplant-eligible (NDMM-TE) patients with intensive treatment and its association with minimal residual disease (MRD). Patients were enrolled and treated in the PETHEMA/GEM2012MENOS65 trial and continued in the PETHEMA /GEM2014MAIN trial. Total Ig (IgG, IgA and IgM) and uHLC were analyzed in a central laboratory at diagnosis, after consolidation treatment and after the first year of maintenance. MRD was analyzed by next-generation flow cytometry after consolidation (sensitivity level $2x10^{-6}$). We found no differences in progression-free survival (PFS) between patients who recovered and patients who didn't recover from IP after consolidation when examining classic total Ig and uHLC. However, after the first year of maintenance, in contrast to patients with classic IP, patients with recovery from uHLC IP had longer PFS than patients without recovery, with hazard ratio of 0.42 (95% confidence interval [CI]: 0.21-0.81; *P*=0.008). Multivariate analysis with Cox proportional-hazards regression models confirmed recovery from uHLC IP after the first year of maintenance as an independent prognostic factor for

PFS, with an increase in C-statistic of 0.05 (95% CI: -0.04 to 0.14; *P*<0.001) when adding uHLC IP recovery. Moreover, we observed that MRD status and uHLC IP recovery affords complementary information for risk stratification. In conclusion, recovery from uHLC IP after 1 year of maintenance is an independent prognostic factor for PFS in NDMM-TE patients who receive intensive treatment. Immune reconstitution, measured as recovery from uHLC IP, provides complementary prognostic information to MRD assessment (*clinicaltrials gov. Identifiers: NCT01916252* and *NCT02406144*).

Introduction

Immunoparesis (IP) is a very common finding in multiple myeloma (MM) patients at diagnosis and it is defined as the suppression of polyclonal uninvolved immunoglobulins (Ig). Currently we can measure it in two ways. On the one hand, we can analyze classic IP by measurement of total IgG, IgA and IgM levels by nephelometry or turbidimetry. On the other hand, Hevylite® assay can detect different heavy/light chain (HLC) pairs, so it can measure immunoparesis of the opposite pair of heavy and light chain to the one involved, which is the pair with the same heavy chain and the opposite light chain. Both types of IP are very common in MM at diagnosis and occur in 80-90% of patients.[1-6]

Several studies have shown that patients with classic IP at diagnosis have worse prognosis than patients without IP, in terms of progression-free survival (PFS) and overall survival (OS).[1,2] Suppression of the uninvolved HLC pair of the same isotype (uHLC) and mostly severe suppression of uHLC (<50% of lower limit of normality) at diagnosis, has also been associated with poor prognosis.[4-7]

Recovery from classical IP has been analyzed in a few studies, mainly, but not only, after autologous stem cell transplantation (ASCT). All of these studies showed that patients who recovered from IP during or after treatment had better prognosis than patients who didn't recover from it. However, these are observational and retrospective studies and included patients over a wide period of time, who therefore underwent non-homogeneous treatment.[8-13]

Although uHLC IP recovery has not been properly studied, some papers have reported poor prognosis for patients with an abnormal ratio of involved/uninvolved HLC at follow-up or persistence of uHLC IP after treatment in heterogeneously treated patients.[14-16]

Minimal residual disease (MRD) in MM patients is actually the most important prognostic factor during evolution of the disease and probably will be the main driver of clinical trials in the near future.[17,18] However not all MRD-negative patients have the same prognosis, and therefore other evolutive prognostic factors are needed.[19]

The goal of this work is to evaluate the prognostic value of IP recovery by measurement of classic total Ig and uHLC and to assess the prognostic benefit of adding IP recovery information to MRD in newly diagnosed MM transplant-eligible (NDMM-TE) patients treated with a fixed-duration approach within the GEM2012 and GEM2014 clinical trials.

Methods

Patients and treatment

We included NDMM-TE patients enrolled and treated in the PETHEMA/GEM2012MENOS65 trial (*clinicaltrials gov. Identifier: NCT01916252*) who continued with the PETHEMA/GEM2014MAIN clinical trial (*clinicaltrials gov. Identifier: NCT02406144*). In the first trial, patients received six cycles of VRD-GEM (bortezomib, lenalidomide, dexamethasone) induction, ASCT conditioned with melphalan or busulfan plus melphalan and consolidation with two cycles of VRD-GEM. Afterwards, patients who achieved at least minimal response were enrolled in the second trial that randomly assigned them to maintenance with lenalidomide and low-dose dexamethasone (Rd) or Rd plus ixazomib for 2 years. Those patients who didn't achieve MRD negativity after 2 years of maintenance received 3 more years of Rd to complete a total of 5 years of maintenance. Response assessment was performed according to International Myeloma Working Group (IMWG) criteria.[20] No differences in efficacy between arms were found in the main analysis of this trial.[21]

Informed consent for both trials was obtained before patient participation. Each study site's independent ethics committee reviewed and approved both protocols. The study was designed and conducted in accordance with the Declaration of Helsinki.

Immunoglobulin analysis

Analysis of classic Ig (IgG, IgA and IgM) by turbidimetry and uHLC analyzed by Hevylite® assay (The Binding Site Group Ltd, Birmingham, UK) was performed in a central laboratory in samples at diagnosis, after consolidation and after the first year of maintenance treatment. We considered IP at diagnosis to be when one or more uninvolved total Ig (classic IP) or uHLC (uHLC IP) were under the lower limit of normal (LLN). We considered recovery from classic IP to be when one or all Ig suppressed at diagnosis reached at least the LLN; recovery from uHLC IP was considered when uHLC reached at least LLN plus 10% in patients with suppressed uHLC at diagnosis.

In the PETHEMA/GEM2012MENOS65 trial, 458 patients were included of which 332 patients entered the PETHEMA/GEM2014MAIN clinical trial. We included a total of 245 patients in this study, those who had samples available at any of the three analyzed time points. Patients with light chain only MM (LCMM) were included in the analysis for classic Ig but were not included in the analysis for uHLC.

For classic total Ig, we analyzed 234 patients at diagnosis, 233 after consolidation and 173 after the first year of maintenance. For uHLC, we analyzed 202 patients at diagnosis, 207 after consolidation and 154 after the first year of maintenance (Figure 1).

Minimal residual disease analysis

MRD was evaluated after consolidation treatment by next-generation flow cytometry (NGF)[17] with an estimated sensitivity level of $2x10^{-6}$.

Outcomes

The main outcome examined in this study was PFS of patients with and without IP recovery after the first year of maintenance when measuring classic total Ig and uHLC. In addition, we explored PFS of patients according to IP recovery and MRD status.

PFS was defined as the time from the time point we are evaluating (when groups are built) until progression, relapse or death occurred.

Statistical analysis

Continuous variables are summarized with mean and range while categorical variables are summarized by number of patients and percentage. Survival analysis for PFS were conducted by Kaplan-Meier methodology and differences between survival curves were tested for statistical significance using the two-sided log-rank test. Univariate and multivariate analysis were conducted using Cox proportional-hazards regression models, restricting the number of variables included according to the number of events. Concordance of each model was evaluated by Harrell's C index and comparison between c-index values was done by *t* test for related samples analysis. A *P* value <0.05 indicated statistical significance. All statistical analyses were performed using IBM SPSS Statistics software (version 25.0).

Results

The main characteristics of the 245 patients included (those who had samples available at any of the 3 points) are summarized in Table 1. Of note, 54.8% of patients evaluated had high-risk cytogenetics because we included those patients who had not only del17p, t(4;14), t(14;16) but also those with +1q21 cytogenetic alterations. Median PFS in these 245 patients was not reached after a median follow-up of 84 months and overall survival at 3 years was 94.6%. Number of PFS events (relapse, progression or death) was 84, two before maintenance treatment, 34 after consolidation and before first year of maintenance and 48 after the first year of maintenance.

Immunoparesis at diagnosis

At diagnosis we found classic IP measured by total Ig in 86.7% of patients (203/234) and uHLC IP in 94.5% of patients (191/202). No significant differences in PFS were found between patients with or without IP at diagnosis for both

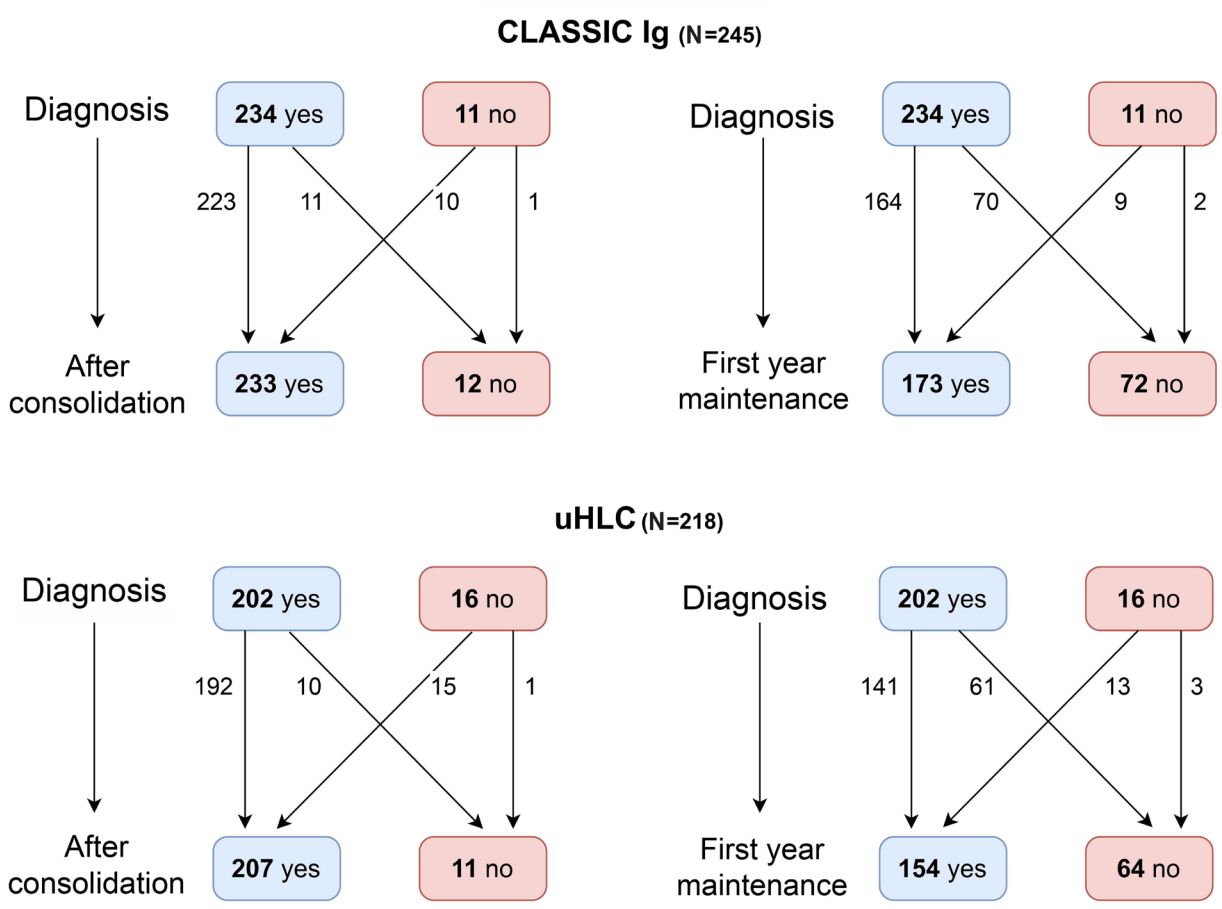


Figure 1. Flowchart showing patients analyzed and not analyzed from diagnosis to post-consolidation and from diagnosis to after the first year of maintenance for classic immunglobulin G and for uninvolved heavy/light chain. IgG: immunoglobulin G; uHCL: uninvolved heavy/light chain.

methods, but it should be pointed out that the number of patients without IP was very small. For this analysis PFS was measured from diagnosis and we found hazard ratio (HR) =0.56 (95% CI: 0.26-1.22; *P*=0.14) for classic Ig and HR=0.49 (95% CI: 0.12-2.01; *P*=0.32) for uHLC.

Immunoparesis recovery

For IP recovery we analyzed only patients with IP at diagnosis, and we investigated IP recovery both after consolidation treatment and after the first year of maintenance. After consolidation we analyzed samples of 233 patients for classic total Ig levels, but we excluded patients without Ig analysis at diagnosis (10 patients) and also those who didn't have IP at diagnosis (28 patients), so IP recovery was evaluated in the remaining 195 patients. For uHLC we analyzed samples from 207 patients, but we excluded those without information at diagnosis (15 patients) and those who didn't have IP at diagnosis (11 patients), so we evaluated uHLC IP recovery in 181 patients. We found recovery of classic IP in 44.6% of patients (87/195) and recovery of uHLC in 49.2% of patients (89/181) who had IP at diagnosis. We found no significant differences in PFS between patients who recovered and patients who didn't recover from IP, for both methods, at this time point (PFS for this analysis was measured from the end of consolidation). For classic Ig we found a HR=0.93 (95% CI: 0.61-1.61; *P*=0.99) and for uHLC we found a HR=0.82 (95% CI: 0.50- 1.36; *P*=0.45).

Of the 173 patients with available sample after the first year of maintenance, nine were excluded because of lack of sample at diagnosis, 21 had no IP at diagnosis and ten had an event for PFS before the end of first year of maintenance, so a total of 133 patients were investigated for classic IP recovery. A total of 124 of 154 patients were evaluated for uHCL IP recovery (10 patients without analysis of uHCL at diagnosis, 11 patients without uHCL IP at diagnosis and 9 patients who had an event for PFS before the end of the first year of maintenance were excluded). We observed recovery from classic IP in 53.8% (70/133) and recovery from uHLC immunoparesis in 63.2% of patients (77/124) who had IP at diagnosis. We found no differences in PFS between patients who recovered and patients who didn't recover from classic IP with a HR=1.22 (95% CI: 0.65-2.30; *P*=0.538; Figure 2A). However, patients with recovery from uHLC IP after 1 year of maintenance had longer PFS than patients who didn't recover from uHLC IP (*P*=0.008) with a HR=0.42 (95% CI: 0.21-0.81; Figure 2B). For both analyses PFS was measured from the end of the first year of maintenance.

Recovery from IP for both techniques was associated with depth of response. For classic total Ig measurements, after the first year of maintenance, 57.4% of patients in complete response (CR) (58/101) had recovery from IP while only 33.3% of patients in very good partial responsse (VGPR) (9/27) recovered from classic IP (*P*=0.026). For uHLC after the first year of maintenance, IP recovery occurred in 65.9% of patients in CR (60/91) as compared to 53.6% of patients in VGPR (15/28), but this difference was not statistically significant (*P*=0.236).

We analyzed other prognostic factors for PFS measured from the end of the first year of maintenance, but, in univariate analysis, only MRD status after consolidation and high-risk cytogenetics at diagnosis were statistically significant, in addition to recovery from uHLC IP after the first year of maintenance. We created a multivariate analysis model for PFS with uHLC IP recovery at the end of the first year of maintenance, MRD status after consolidation and high-risk cytogenetics found at diagnosis. As seen in Table 2, all three variables had independent prognostic value for PFS. Harrell's C concordance index for this model was 0.62 (95% CI: 0.55-0.71) without uHLC IP recovery and 0.67 (95% CI: 0.58-0.76) after adding uHLC IP recovery to the model. This difference, 0.05 (95% CI: -0.04 to 0.14), was statistically significant (*P*<0.001).

As almost all the patients had uHLC IP at diagnosis, if we analyze uHLC values at the first year of maintenance without

Table 1. Main characteristics of patients at diagnosis.

Characteristics	All, N=245
Sex, N (%) Male Female	 135 (55.1) 110 (44.9)
Age in years, mean (range)	56.1 (31-65)
Type of MM, N (%) IgG IgA LCMM	 160 (65.3) 58 (23.7) 27 (11.0)
LDH, N (%) Normal High	 207 (87.3) 30 (12.7)
ISS stage, N (%) 1 2 3	 104 (43) 80 (33.1) 58 (23.9)
Durie-Salmon stage, N (%) 1 2 3 Substage A Substage B	 31 (12.7) 99 (40.4) 115 (46.9) 242 (98.8) 3 (1.2)
High risk cytogenetics, N (%) Yes No	 115 (54.8) 95 (45.2)
Classic Ig immunoparesis, N (%) Yes No	 203 (86.7) 31 (13.2)
uHLC immunoparesis, N (%) Yes No	 191 (94.5) 11 (5.4)

MM: multiple myeloma; LCMM: light chain MM; LDH: lactate dehydrogenase; ISS: international staging system; Ig: immunoglobulin; uHCL: uninvolved heavy/light chain.

taking into account uHLC levels at diagnosis (N=163), similar results are obtained. Patients with uHLC at the end of the first year of maintenance above the cutoff value (LLN plus 10%) had longer PFS than patients with uHLC below cut-off value with HR=0.45 (95% CI: 0.25-0.84; *P*=0.009). In a multivariate model, together with MRD status and high-risk cytogenetics, all three factors were statistically significant and Harrell's C concordance index for this model was 0.62 (95% CI: 0.55-0.71) before including uHLC levels after the first year of maintenance and 0.67 (95% CI: 0.59-0.75) after including it, this difference was also statistically significant (0.05 [95% CI: -0.03 to 0.13); *P*<0.001).

Association of minimal residual disease status with immunoparesis recovery

We also analyzed the prognostic value of the association of MRD status after consolidation and uHLC IP recovery at the end of the first year of maintenance. We evaluated the association in the 122 patients where both data was available: 45 patients were MRD-negative and recovered from uHLC IP, 24 patients were MRD-negative but didn't recover from uHLC IP, 31 patients were MRD-positive but recovered from uHLC IP and 22 patients were MRD-positive and didn't recover from uHLC IP. PFS for this analysis was measured from the end of the first year of maintenance.

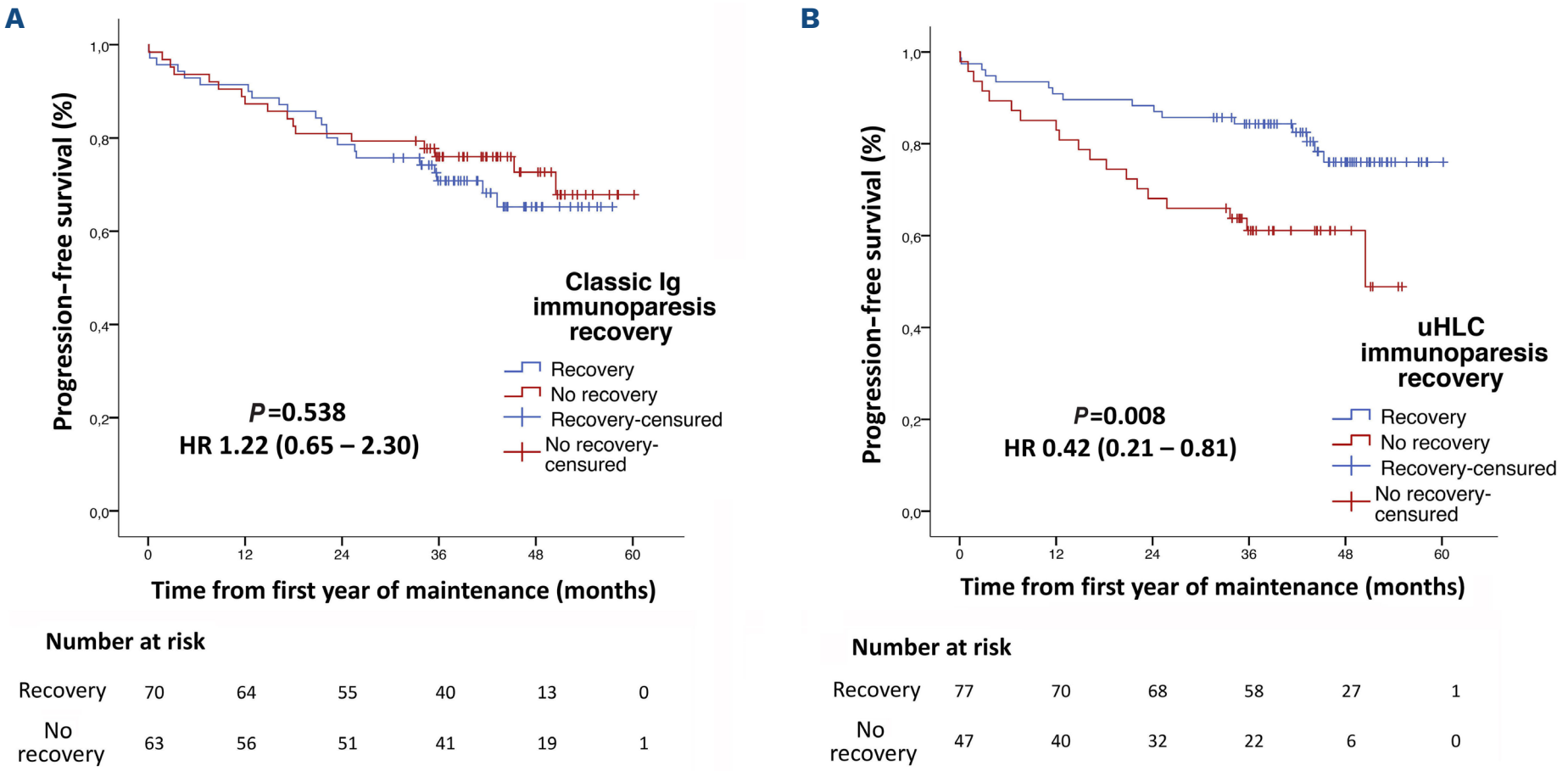


Figure 2. Progression-free survival according to immunoparesis recovery after the first year of maintenance. (A) Progression-free survival for classic immunglobulin G (IgG) immunoparesis recovery and (B) for uninvolved heavy/light chain (uHLC) immunoparesis recovery. HR: hazard ratio (95% confidence interval).

Table 2. Univariate and multivariate analysis for progression-free survival from first year of maintenance (M1).

Variables	Univariate			Multivariate		
	P	HR	CI 95%	*P*	HR	CI 95%
Durie-Salmon stage(I-II *vs.* III), N=185	0.081	0.609	0.35-1.06	-	-	-
High-risk cytogenetics, N=161	0.018	0.471	0.25-0.88	0.034	0.450	0.21- 0.94
LDH (normal *vs.* high), N=178	0.105	0.533	0.25-1.14	-	-	-
ISS stage (I *vs.* II-III), N=182	0.059	0.558	0.30-1.02	-	-	-
Conventional response at M1 (CR *vs.* VGPR), N=179	0.107	0.579	0.30-1.12	-	-	-
MRD status after consolidation, N=182	0.002	0.396	0.22-0.70	0.036	0.466	0.23-0.95
uHLC IP recovery at M1, N=124	0.010	0.416	0.21-0.81	0.026	0.449	0.22-0.91

HR: hazard ratio; CI: confidence interval; LDH: lactate dehydrogenase; ISS: international staging system; CR: complete reposnse; VGPR: very good partial response; MRD: minimal residual disease; uHCL: uninvolved heavy/light chain; IP: immunoparesis.

Patients with both favorable factors (MRD-negative and recovery from uHLC IP) had better PFS than patients with both unfavorable factors (MRD-positive without recovery from uHLC IP) with HR=0.23 (95% CI: 0.09-0.61; *P*=0.001; Figure 3A, B). Both groups of patients with just one favorable factor (MRD-negative without recovery from uHLC IP or MRD-positive with recovery from uHLC IP) have a similar prognosis and their prognosis is intermediate between the other two groups (Figure 3A). If these two groups of patients with just one favorable factor are joined into a single group and this is compared to the other groups, it has a HR=0.47 (95% CI: 0.19-1.15; *P*=0.091) compared with the "both favorable factors" group and a HR=0.48 (95% CI: 0.22-1.03; *P*=0.054) compared with the "both unfavorable factors" group (Figure 3B).

Discussion

In this study we confirm the independent prognostic value of recovery from uHLC IP after the first year of maintenance in the setting of NDMM-TE patients treated within a clinical trial with intensive fixed-duration treatment. Furthermore, uHLC IP recovery at the end of the first year of maintenance provides complementary prognostic value to MRD detection. There was no prognostic value for recovery from classic IP after the first year of maintenance in these patients.

Previous results for the prognostic value of uHLC and HLC ratio during follow-up are consistent with our findings and recovery from uHLC IP appears to be a solid prognostic marker in MM.[14-16] This may be due to the important impact of dysregulation of immune system in MM patients at diagnosis that affects mainly T cells and dendritic cells, but also B cells and NK cells.[22] Recovery from uHLC IP is a good marker for B-cell immune reconstitution in our population and confers good prognosis. Other prognostic markers of B-cell immune reconstitution have previously been reported such as the presence of oligoclonal bands, percentage of mature B cells in bone marrow or even recovery from classic IP.[23,24] In previous studies[8-13] recovery from classic IP was found to be an important prognostic factor for PFS and OS in MM. Therefore, the lack of prognostic value for recovery from classic IP in this study is surprising. A possible explanation is that previous studies included patients non-homogeneously treated, during a wide period of time and mostly with less intensive treatment. It is possible that the intensity of the treatment in this trial prevented an increase of classic total Ig levels but did not prevent uHLC recovery after the first year of maintenance. Measurement of uHLC appears to be a more sensitive method to detect immune reconstitution than measurement of classic Ig, at least in our scenario of intensive treatment. As far as we know, this is the first study to compare immunoparesis recovery of uHLC *versus* classic Ig.

After consolidation treatment, neither recovery from classic Ig nor uHLC IP had any prognostic value. This time point may be too early to evaluate recovery from IP. Previous studies, evaluating classic Ig in patients who underwent ASCT with less intensive induction, showed that the best time point to evaluate prognosis of recovery from IP was 1 year after transplant.[9,10,12] Maintenance treatment in this trial, which is less intensive than previous treatment (induction, transplant and consolidation), allowed patients to recover from uHLC IP but not classic Ig IP after the first year. Maybe with a longer duration of maintenance treatment or maybe with a longer interval after stopping maintenance treatment, recovery from classic Ig IP will achieve a good prognostic value. After the first year of maintenance, we saw no difference in the prognostic value of recovery from uHLC or classic Ig IP between treatment arms (Rd *vs*. Rd plus Ixazomib), so time after transplant may be more important than the intensity of maintenance treatment when considering recovery from IP.

The proportion of patients with uHLC IP at diagnosis in our series was very high (94,6%) and we have confirmed that similar results, in terms of prognosis, are observed if we evaluate only the level of uHLC at the end of the first year of maintenance instead of uHLC IP recovery compared to baseline. Therefore, in clinical practice, if information on uHLC value at diagnosis is not available, uHLC levels after the first year of maintenance alone can be used with similar prognostic value.

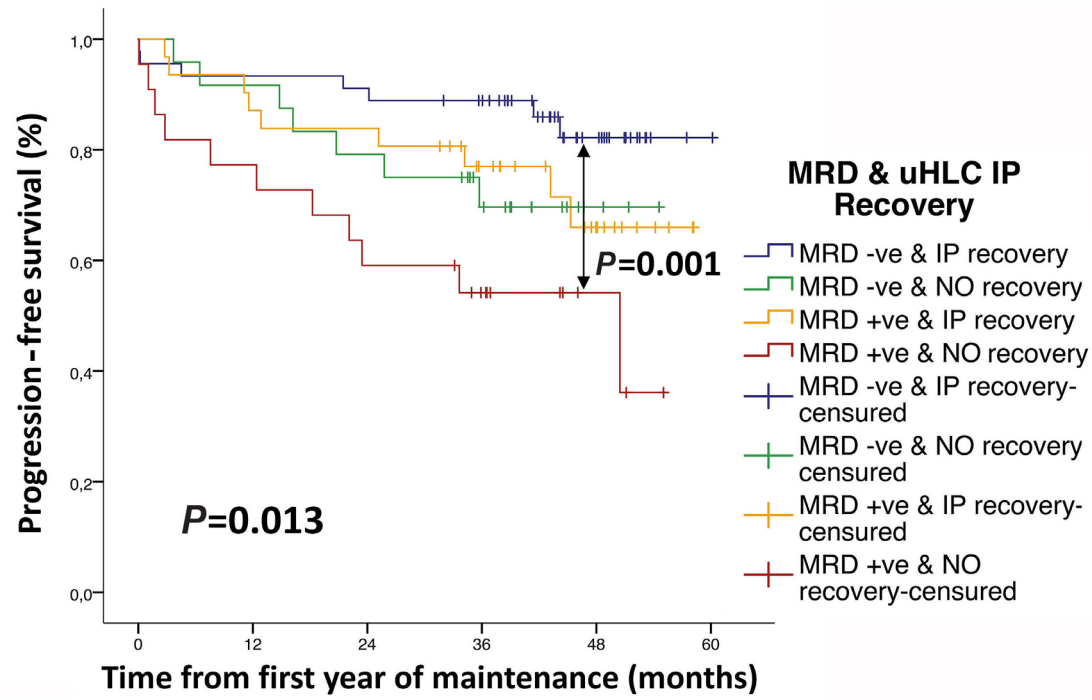
Treatment-induced IP could be an important limitation for using IP recovery (classic or uHLC) as a prognostic factor. Anti-CD38 antibodies, which are now used in the first line treatment of transplant-eligible and transplant-ineligible patients, produce an important decrease of Ig levels. In addition, bispecific antibodies and chimeric antigen receptor (CAR) T cells produce a severe IP that usually needs prophylactic treatment with intravenous Ig.[25,26] Most of the patients undergoing these treatments will probably not achieve recovery of Ig levels, whether measured by classic Ig or uHLC. Maybe we will need to look for small increases in uHLC levels or whether uHLC levels follow a positive trend to know which patients undergo immune reconstitution and have better prognosis. Other immune prognostic markers should also be sought to give further information. Supportive prophylactic treatment with intravenous Ig may further complicate interpretation of changes in uHLC levels, especially in IgG MM patients. Further studies to understand uHLC behavior when we use anti-CD38 antibodies, bispecific antibodies or CAR T-cell therapy are needed.

In this study we can see that the prognostic value of uHLC IP recovery complements the prognostic value of an MRD result at a single time point. It is known that the prognostic value of sustained MRD negativity (for 12 or 24 months) is clearly better than the prognostic value of just one MRD-negative result.[27] Some patients with a single MRD-negative result become MRD-positive after a short period of time and may suffer a relapse over the following months.[19] Immune

reconstitution may play an important role in sustaining MRD negativity over time, but other factors also seem to be implicated.[28] Patients with very aggressive disease, as those with two or more high-risk cytogenetic abnormalities and those with high level of circulating tumor cells at baseline lose MRD negativity more frequently.[29,30] The relationship between immune reconstitution, measured with uHLC IP recovery or other markers, evolution of MRD measured by NGF or next-generation sequencing, and aggressiveness markers of myeloma needs to be explored in future studies.

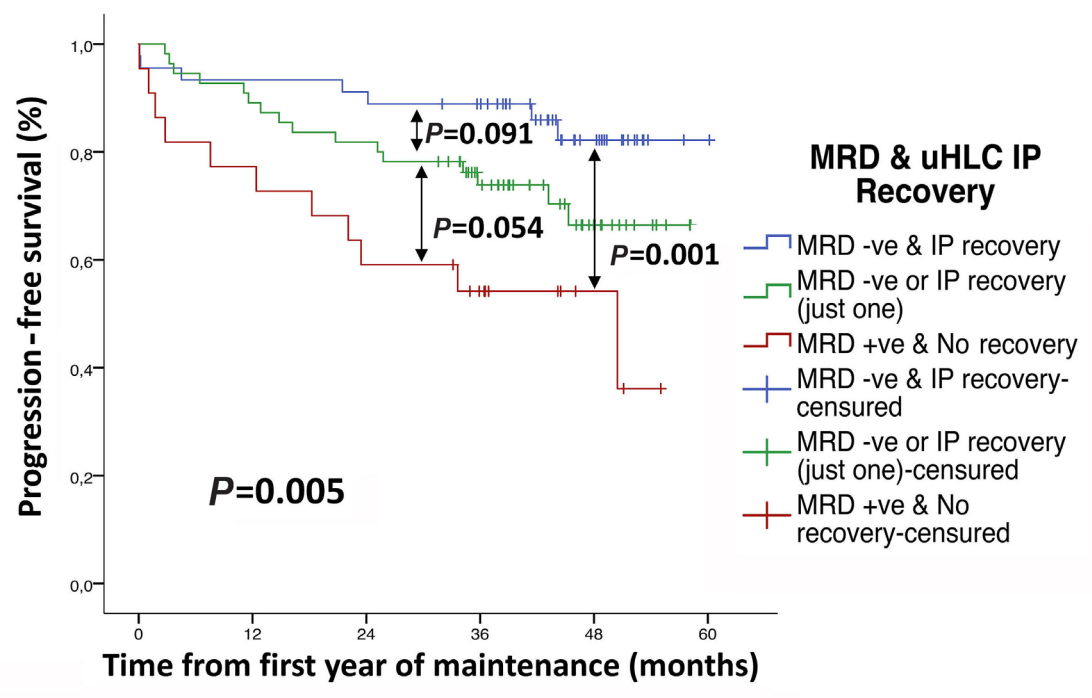
In conclusion, recovery from uHLC IP is an independent prognostic factor for NDMM-TE patients who receive intensive continuous treatment. In addition, uHLC IP recovery

A



Number at risk						
MRD –ve & IP rec	45	42	41	38	17	1
MRD –ve & No IP rec	24	22	19	13	3	0
MRD +ve & IP rec	31	27	26	19	9	0
MRD +ve & No IP rec	22	17	13	9	3	0

B



Number at risk						
MRD –ve & IP rec	45	42	41	38	17	1
MRD –ve & IP rec (just one)	55	49	45	32	12	0
MRD +ve & No IP rec	22	17	13	9	3	0

Figure 3. Progression-free survival according to minimal residual disease status after consolidation and uninvolved heavy/light chain immunoparesis recovery after the first year of maintenance. (A) Progression-free survival (PFS) of the 4 combinations of minimal residual disease MRD (positive or negative) and uninvolved heavy/light chain (uHLC) immunoparesis (IP) (recovery or no recovery). (B) Comparison of PFS of patients with both favorable factors (MRD-negative & uHLC IP recovery), patients with only 1 favorable factor (MRD-negative or uHLC IP recovery) and patients with both unfavorable factors (MRD-positive & absence uHLC IP recovery). -ve: negative; +ve: positive, rec: recovery.

complements the prognostic value of an MRD result at a single time point, but further studies in patients treated with new therapies and longitudinal analysis of uHLC recovery alongside MRD are needed.

Disclosures

LR has received honoraria for lectures from Janssen, BMS-Celgene, Amgen, Takeda, Sanofi and GSK. NP has received honoraria for lectures and consultancy fees from The Binding Site. BP has received honoraria derived from lectures and/or participation on advisory boards from Adaptive, Amgen, Becton Dickinson, BMS-Celgene, GSK, Janssen, Roche, Sanofi and Takeda; has received research funding from BeiGene, BMS-Celgene, GSK, Roche, Sanofi and Takeda; has received consultancy fees from BMS-Celgene, Janssen, Sanofi and Takeda. RR-T has received honoraria for lectures and consultancy fees from Amgen, BMS, GSK, Janssen, Sanofi and The Binding Site; and has participated in advisory board meetings at Beckton-Dickinson, GSK and Janssen. M-VM has received honoraria derived from lectures and/or participation on advisory boards from Janssen, Celgene, Amgen, Takeda, AbbVie, GlaxoSmithKline, Oncopeptides, Sanofi, Pfizer, Roche, and Stemline; and has received consultancy fees from The Binding Site. J-JL has received consultancy fees and/or has participated on advisory board of BMS, Sanofi, Amgen and Janssen; and has received travel expenses from Celgene and Pfizer. JS-M has participated on advisory boards and consulting services for Abbvie, Amgen, BMS-Celgene, GSK, Haemalogix, Janssen, Karyopharm, MSD, Novartis, Pfizer, Takeda, Regeneron, Roche, Sanofi, and SecuraBio. All other authors have no conflicts of interest to disclose.

Contributions

SL, M-TH and JS-M conceived and designed the study. M-APP and LM-G analyzed the samples. SL and M-TH analyzed and interpreted data and performed the statistical analysis. JDC and RAF reviewed statistical analysis. SL, M-TH, JS-M, BP and JM-L reviewed and interpreted the results. SL, M-TH and JS-M wrote the paper. All authors provided patients, and reviewed and approved the manuscript.

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Data-sharing statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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