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Single-point and kinetics of peripheral residual disease by mass spectrometry to predict outcome in patients with high risk smoldering multiple myeloma included in the GEM-CESAR trial

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Single-point and kinetics of peripheral residual disease by mass spectrometry to predict outcome in patients with high risk smoldering multiple myeloma included in the GEM-CESAR trial

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Runnig head: PRD by mass-spec in HR smoldering myeloma

Authors´contributions: NP, CA, TC, MVM and JSM, conceived the analysis and designed the analysis protocol.
JML, PRO, VGC, MSG, AO, NCG, RRT, LR, MAA, JB, APGR, AA, FE, MBI, JdlR, AIT, FdA, LP, MTH, JLJ, MR, AGM, EMO, BP, NP, MTC, JB and JJL provided study samples or patients
NP, CA, TC and SC analyzed the mass-spec data and the M-protein kinetics
NP, CA, TC, MVM, JML, LR, JdlR, bp, NP, MTC, JB, JJL and JSM analyzed and interpreted data
All authors contributed to the writing and final approval of the manuscript.

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Data sharing statement

If required, these data can be obtained via the corresponding author.

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Abstract

The value of quantitative immunoprecipitation mass spectrometry (QIP-MS) to identify the M-protein is being investigated in patients with monoclonal gammopathies but no data are yet available in high-risk smoldering myeloma (HRsMM). We have therefore investigated QIP-MS to monitor peripheral residual disease (PRD) in 62 HRsMM patients enrolled in the GEM-CESAR trial. After 24 cycles of maintenance, detecting the M-protein by MS or clonal plasma cells by NGF identified cases with a significantly shorter median PFS (mPFS; MS: not reached *vs* 1,4 years, p=0.001; NGF: not reached *vs* 2 years, p=0.0002) but reaching CR+sCR did not discriminate patients with different outcome. With NGF as a reference, the combined results of NGF and MS showed a high negative predictive value (NPV) of MS: 81% overall and 73% at treatment completion. When sequential results were considered, sustained negativity by MS or NGF was associated with a very favorable outcome with a mPFS not yet reached *vs* 1.66 years and 2.18 years in cases never attaining PRD or minimal residual disease (MRD) negativity, respectively. We can thus conclude that 1) the standard response categories of the IMWG do not seem to be useful for treatment monitoring in HRsMM patients, 2) MS could be used as a non-invasive, clinical valuable tool with the capacity of guiding timely bone marrow evaluations (based on its high NPV with NGF as a reference) and 3) similarly to NGF, sequential results of MS are able identify a subgroup of HRsMM patients with long-term disease control. *This study was registered at www.clinicaltrials.gov (ClinicalTrials.gov identifier: NCT02415413).*

Introduction

The prognosis of patients with multiple myeloma (MM) achieving optimal responses to therapy is known to be heterogeneous, and different progression-free (PFS) and overall (OS) rates are observed among cases in CR or better (CR+sCR).[1] This has been explained in numerous studies by the persistence of minimal residual disease (MRD) detected in the bone marrow (BM) of some of these patients and being responsible for the observed but unexpected progressions.[1] Acknowledging this situation, the International Myeloma Working Group (IMWG) included guidelines for the definition of MRD negativity as a category of response in 2016, recommending the evaluation of a BM sample either with next generation sequencing (NGS) or with next generation flow cytometry (NGF) with a minimum sensitivity level of 10^{-5} in all patients achieving at least a complete response (CR). [2] A large body of data supports the clinical value of detecting residual disease in the BM using NGS and NGF in patients with MM.[3] In fact, attaining MRD negativity is currently selected as the main endpoint in the majority of clinical trials. However, the use of BM samples to evaluate MRD requires an invasive procedure that cannot be performed very frequently and that could also limit the accuracy of the results. Although a logical alternative to the BM could be the use of peripheral blood (PB) instead, some correlative studies have revealed that 10^{-5} is insufficient to make comparable the results obtained in BM and PB. In fact, Sanoja-Flores *et al.* using NGF in paired BM and PB samples from 137 newly diagnosed MM patients after active treatment, showed that in 55 out of the 91 (60%) patients with detectable MRD in the BM, circulant tumor plasma cells could not be identified. [4] Similarly, among the 28 MRD positive follow-up samples detected by NGS by Mazzoti and colleagues, 18 (64%) did not present detectable circulating tumor DNA.[5] For this reason, highly sensitive approaches are being investigated for the detection of residual disease in PB, including mass spectrometry (MS). MS is a method able to identify the presence of M-protein in serum based on the specific aminoacidic sequence of each patient paraprotein and thus its unique m/z ratio.[6] In fact, we recently reported that, as compared IFE, Immunoglobulin Isotypes (GAM) assay for the mass spectrometry (MS) EXENT® Analyser is more sensitive to detect the M-protein in the serum of MM

patients, both at baseline and during treatment monitoring and that, most importantly, MS is more accurate than IFE to predict patients' outcome. [7] Different MS-based technologies are in development, although due to their high throughput, those based on matrix-assisted laser desorption ionization time of flight MS (MALDI-TOF-MS) are the most suitable for routine clinical practice. [6,8,9]

In the context of the GEM-CESAR trial for HRsMM patients, we have evaluated the presence of residual disease in serum samples by MS to ascertain its value as a complement to MRD that could overcome the limitations of BM based disease evaluation.

Methods. Sixty-two eligible patients were included. The diagnosis of SMM was based on the 2010 IMWG criteria and the high-risk of progression was established according to either the Mayo 2008 and/or the PETHEMA models.[10–12] Since the trial was designed in 2013, ultra-high-risk SMM cases were also included. [13] GEM-CESAR is a non-randomized, open-label, multicenter phase II trial in which patients received induction with six 4-week cycles of KRd (carfilzomib, lenalidomide and dexamethasone), high-dose melphalan followed by ASCT, consolidation with two further cycles of KRd and up to 2 years of maintenance with Rd. Patients presenting with biochemical progression were offered to receive rescue therapy with daratumumab, pomalidomide and dexamethasone. Each study site´s independent ethics committee approved the protocol, and informed consent forms were required prior to patient enrollment. The study was conducted according to the Declaration of Helsinki.

Next Generation Flow Cytometry. MRD was assessed using the NGF method developed by EuroFlow for highly sensitive and standardized MRD detection in MM after induction, three months after ASCT and at the end of the treatment phase, after completing maintenance.[14] MRD studies were centralized in the three laboratories of the Spanish Myeloma Group.

Quantitative Immunoprecipitation Mass Spectrometry. Serum samples were analyzed using Immunoglobulin Isotypes (GAM) assay for the MS EXENT® analyzer. First, the EXENT-iP500 liquid handler purified the immunoglobulins

through paramagnetic beads coated with polyclonal sheep antibodies specific for human IgG, IgA or IgM heavy chains, and for total kappa and lambda light chains. Then, samples were washed, eluted (20mM TCEP in 5%(v/v) acetic acid) and spotted onto MALDI plates with HCCA matrix. Subsequently, analysis with the EXENT-iX500 MALDI-TOF device was carried out and mass spectra from 5000 to 32000 mass to charge ratio (m/z) were collected. The +2 charge state was used for the interpretation of spectra by the EXENT-iQ software. The m/z of the M-protein identified in baseline samples was used as a patient-specific tumor marker in the subsequent samples.

End Points and Assessments of the trial. The primary endpoint of the trial was MRD negativity rate 3 months after-ASCT; secondary endpoints included standard response rates and sustained MRD negativity rate at 3, 4 and 5 years after-ASCT. We have assessed and compared the results and clinical value in terms of PFS of SPEP/IFE, NGF and MS to identify residual disease (the M-protein in serum samples by SPEP/IFE and MS and clonal plasma cells in bone samples by NGF) 3 months after-ASCT and after two years of maintenance, at single time points and considering the kinetics of the results.

Statistical analyses. GraphPad Prims v.9 was used for all statistical analyses. Differences in sensitivity were tested by the Fishers' exact test. Confidence intervals were determined using exact binomial distribution. PFS was defined as the time since inclusion in the trial to the development to biochemical progression defined by biochemical relapse/progressive disease according to the IMWG criteria; under this term we also included ultrasensitive MRD relapse defined by reappearance of MRD confirmed at least 2 months apart. Curves were constructed using the Kaplan-Meier method and the (two-sided) log-rank test. Dynamics of residual disease were analyzed from post-induction to treatment completion.

Results

Patients. Baseline demographic and disease characteristics are represented in Table 1. Out of the 90 patients included in the trial we had serum samples

available for further analyses in 62 (68.8%), 61 (67.7%), 61 (67.7%), 51 (57%) and 35 (38.8%) of them, at the time points post-induction, ASCT, consolidation, after one year of maintenance and at treatment completion, respectively. We focused our analysis in these group of samples (and patients). Besides progression, some samples were missed due to the Covid-19 pandemic difficulties.

Performance of SPEP/IFE, mass-spec and NGF to detect residual disease. As a first step, we compared the ability of the three methods to detect the presence of residual disease at the different stages of the treatment schema (ie, the M-protein in serum using MS or SPEP/IFE and clonal plasma cells in BM by NGF; figure 1). During intensive treatment (post-induction, after ASCT and post-consolidation), MS was the technique detecting residual disease in the highest proportion of patients; after the first year of maintenance both NGF and MS equaled and NGF showed the highest percentage of positive cases at treatment completion, after 24 cycles of maintenance. Notably, as stated above, the number of samples available for analysis was lower during maintenance.

As detailed in figure 1, MS identified the M-protein in 46/62 (74%) post-induction, 33/61 (54%) post-ASCT and 29/61 (48%%) post-consolidation, 11/51 (22%) 1st year after maintenance and 7/35 (20%) after the 2nd year of maintenance.

Analysis of the combined results of mass-spec with SPEP/IFE or NGF post-ASCT and after 24 cycles of maintenance. We then paired the results obtained with MS with those from one the alternative techniques (SPEP/IFE or NGF) and analyzed the combined results two by two, post-ASCT and after two years of maintenance.

When we analyzed the results of SPEP/IFE and MS (figure 2a), we found out that at the two time points analyzed both methods were highly in agreement and that, among discordances, all were due to IFE-/MS+ samples except for 4 cases IFE+/MS-. In detail, 72% of the results were concordant (29% $IFE^{+}MS^{+}$ and 43% $IFE^{-}MS^{-}$) and 28% discordant (25% $IFE^{-}MS^{+}$ and 3% $IFE^{+}MS^{-}$) post-ASCT, and 82% were concordant (9% $IFE^{+}MS^{+}$ and 73% $IFE^{-}MS^{-}$) and 18% discordant (12% $IFE^{-}MS^{+}$ and 6% $IFE^{+}MS^{-}$ at the end of the treatment. Overall,

out of the 274 samples analyzed, 74.8% of the results were concordant (26.3% $IFE^{+}MS^{+}$ and 48.5% $IFE^{-}MS^{-}$) and 25.2% discordant (4.4% $IFE^{+}MS^{-}$ and 20.8% $IFE^{-}MS^{+}$).

Similarly, we analyzed the results of NGF and MS (figure 2b), finding now that approximately two thirds of the results were concordant; among discordances, the majority of them post-ASCT were MS+/NGF- while MS-/NGF+ at treatment completion. These results reflect the respective sensitivities of NGF and MS at the two time points investigated, already specified above. In detail, 71% of the results were concordant (34% $NGF^{+}MS^{+}$ and 37% $NGF^{-}MS^{-}$) and 29% discordant (8% $NGF^{+}MS^{-}$ and 21% $NGF^{-}MS^{+}$) post-ASCT and 74% were concordant (14% $NGF^{+}MS^{+}$ and 60% $NGF^{-}MS^{-}$) and 26% discordant (20% $NGF^{+}MS^{-}$ and 6% $NGF^{-}MS^{+}$) at the end of the treatment. Overall, out of the 274 samples analyzed, 75% of the results were concordant (33% $NGF^{+}MS^{+}$ and 42% $NGF^{-}MS^{-}$) and 23% discordant (9.5% $NGF^{+}MS^{-}$ and 14% $NGF^{-}MS^{+}$).
Taking the results of NGF as a reference, the negative predictive value (NPV) of MS was 81% overall. Furthermore, the NPV of MS *vs* NGF was 83% and 73% post-ASCT and after 2 years of maintenance, respectively (figure 2b).

Response rates and clinical value of SPEP/IFE, mass-spec and NGF at the main end-points of the trial. The primary endpoint of the trial was the rate of undetectable residual disease three months after ASCT assessed by NGF with a sensitivity level of 10^{-5}, which in the ITT analysis was reached in 62% of the patients (56/90) as recently published by Mateos *et al*. (accepted in JCO, in press). Number of patients analyzed and rates of undetectable residual disease using SPEP/IFE, MS and NGF at the main time points evaluated are detailed in Table 2.
Please note that, in spite of not being the same cohort due to the lack of serum samples available for MS analysis in all cases, the rates of CR+sCR and MRD negativity are quite comparable to those reported by Mateos *et al*. at the same time points (accepted in JCO, in press).
Based on the previous results, we found out that reaching CR+sCR according to the IMWG categories of standard response did not discriminate two cohorts of patients with significantly different outcome in terms of PFS at any of the

main end-points analyzed (post-ASCT and after 2 years of maintenance; figure 3a).

According to the recommendations of the IMWG, we then analyzed the clinical value of MS and NGF in paired samples obtained from the group of patients reaching CR+sCR.[2] When focusing in these patients (42/61 [69%] post-ASCT and 28/35 [80%] at treatment completion), both PRD and MRD statuses were able to discriminate two cohorts with significantly different PFS, after ASCT and at treatment completion after two years of maintenance. As shown in figure 3b, three months post-ASCT, the median PFS (mPFS) was not reached in PRD or MRD negative patients *vs* 4.1 years in PRD positive cases (p=0.0021) and 4.6 years in MRD positive cases (p=0.04); at treatment completion, the mPFS in PRD or MRD negative patients was not reached *vs* 1.1 years in PRD positive cases (p<0.0001) and 2.05 in MRD positive cases, (p=0.0005).

Impact of peripheral residual disease and minimal residual disease dynamics in patient's outcome. Finally, we analyzed the dynamics of PRD by MS during the period of treatment and evaluated their impact on patient's outcome. Thirty-five patients with paired BM and PB samples available at least at two of the 5 time points analyzed were included in this landmark analysis performed from the end of the treatment after completing the 24 cycles of maintenance. Sustained PRD negativity was observed in 12 pts (34%), and sustained positivity in 6 (17%). In 16 pts (46%) PRD converted from positive to negative, and in 1 (3%) from negative to positive. Whereas sustained PRD+ was associated with a very short mPFS of 1.66 years, patients with sustained PRD- or converting from PRD+ to PRD- displayed a very favorable outcome with mPFS not reached and significantly different compared to the sustained PRD+ group (p<0,0001; figure 4). Interestingly, the only patient that converted from PRD- to PRD+ had a very poor outcome with a mPFS of 3.8 months.

Sustained MRD negativity during the same period was observed in 8 patients (23%), and sustained positivity in 11 (31%). In 15 patients (43%) MRD converted from positive to negative, and in 1 (3%) from negative to positive. Whereas sustained MRD positivity was associated with a very short mPFS of 2.1 years, patients with sustained MRD- or who converted from MRD+ to MRD-

displayed a very favorable outcome with mPFS not reached and significantly different compared to those with sustained or converting to MRD+.
Please note that the figures above cannot be directly compared with those from the whole cohort reported by Mateos et al (accepted in JCO, in press), where the analysis of the rate of sustained MRD negativity was calculated in patients MRD negative after ASCT that maintained the negative status 4 and 5 years afterwards. Unfortunately, we do not have serum samples from the patients at those time points to carry out the appropriate comparison.

Discussion

In this study, patients with HRsMM either by the Mayo or the Pethema models were treated with an intensive schema based on KRd induction and consolidation, ASCT and maintenance with Rd limited to two years.[11,12] Response assessment was based on MRD analysis in BM samples by NGF and the primary endpoint of the trial was the rate of MRD negativity three months post-ASCT in the ITT population. In 61 out of the total 90 patients included in the trial, we have analyzed and compared treatment response post-ASCT and at treatment completion using standard and MRD methods (SPEP/IFE in serum and NGF in BM, respectively) and MS, as a highly sensitive method to detect the presence of the patient's MP in serum samples.

From the results of our analysis, we have learnt that in this cohort of patients, 1) out of the three methods applied to detect residual disease, SPEP/IFE showed the lowest rates of positive results, MS the highest during intensive treatment and both NGF and MS performed similarly during maintenance, 2) the results of SPEP/IFE do not associate with the clinical behavior of the disease in terms of PFS whereas MS and NGF both predict patient's outcome with similar accuracy and, 3) the clinical value of MRD and PRD analysis is improved when not only a single time-point is considered but the dynamics of the results are taken into account.

In terms of the ability of the three methods to detect residual disease, SPEP/IFE showed the lowest rates of positive results, MS the highest during intensive treatment and both NGF and MS behave similarly during maintenance. This

pattern (MS > NGF > SPEP/IFE) was maintained throughout, except at the end of the treatment, where the lower number of samples analyzed (n=35) could also be influencing our findings. We and others have shown the higher sensitivity of MS as compared to SPEP/IFE, further confirmed in this cohort or patients[7,15–18]. MS identified residual disease in a higher number of cases as compared to NGF at most of the time points analyzed, an unexpected finding considering our previous results in patients included in the GEM2012-2014 clinical trials (submitted). Besides the potential limitations associated with MRD assessment in BM samples (hemodilution and patchy infiltration), more than 60% of the patients analyzed had an IgG MM and the longer half-life of IgG could also be having a role by increasing the detection rate of not yet cleared M-protein at the initial stages of the treatment.[18] In this regard, analysis of MS+/NGF- cases (n=13) showed that 8 of them were obtained from patients with an IgG MM. Besides this, it is important to note that the higher detection rate of MS was not clinically misleading in this case, but also translated into a clinical value, similar to that observed with NGF. Finally, we acknowledge the fact that, the target of serum-based techniques (the M-protein) is different from that of bone-marrow based techniques (clonal/tumor plasma cells) and therefore, discrepant results are expected and their meaning should be always cautiously interpreted in the context of their clinical translation.

Despite the significant increase in treatment efficacy fortunately observed in the last years in patients with MM, treatment response continues to be assessed following guidelines largely unchanged.[2] Only the introduction of the stringent complete response (CR) category not long ago aimed to be an advancement in that regard, although its clinical value has not been fully consolidated.[19,20] Currently, a very high proportion of patients reach the best category of standard response (CR or stringent CR) during or after treatments and the GEM/PETHEMA group has been able to show that among them, only patients found to be MRD negative in the BM benefit from a more favorable outcome.[1] The lack of discriminative capacity (all patients reach CR) together with its limited sensitivity (highlighted by the fact that only the MRD results are clinically determinant), are both translating into that classical response categories do not longer associate with a different clinical value. This was also recently shown in

the context of the GEM2012MAIN trial where depth of standard response criteria after treatment intensification had limited prognostic value in transplant eligible MM patients.[21] In this study, likely due to the same reasons stated above, standard response criteria did not associate with a significant prognostic value in terms of PFS at any of the time-points analyzed.

Analysis of the results obtained post-ASCT and after 2 years of maintenance with MS and SPEP/IFE show that concordances (MS^+/IFE^+ and MS^-/IFE^-) increase with the progress of the therapy due to an increasingly higher number of double negative cases induced by the treatment. Accordingly, a lower number of discordances is identified, mostly cases MS^+ but $SPEP/IFE^-$ due to the higher sensitivity of the former. The higher sensitivity of MS as compared to SPEP/IFE is the reason explaining the clinical value associated with the results of mass-spec but not with SPEP/IFE. When we analyzed the combined results of MS and NGF, the figures remain quite similar after ASCT and 2 years of maintenance (2/3 of the cases concordant and 1/3 discordant) but whereas the majority of discordances post-ASCT were due to cases MS^+/NGF^- in contrast, at the end of the treatment they were mostly due to MS^-/NGF^+ cases. Importantly, both methods were in agreement in the majority of cases and discordances are explained by capacity of both techniques to identify residual disease in this specific cohort of patients. It is also worth to stress here that despite the expected discordances between two methods with different targets, the results of both of them were associated with patients' outcome. Also, taking NGF as the gold standard method for MRD detection, the NPV of MS at the two time points analyzed was high enough (82% post-ASCT and 75% at treatment completion) to consider the results of MS as a factor to determine the most appropriate moment to carry out a BM aspiration with the aim of confirming MRD negativity in patients with MM.

Although the clinical value of reaching MRD negativity is broadly proven [3], various papers have been recently published regarding the value of the dynamics of MRD. [22–24] Whereas achieving sustained MRD negativity seems to be the most accurate predictor for long-term disease control, conversions from positive to negative o vice versa have also shown to have an added clinical

value. [23]We have also confirmed this in our paper. In fact, maintaining or converting to MRD or PRD negativity identified a subgroup of cases with a very favorable outcome and with a mPFS not yet reached after a median follow-up of 65 months; in contrast, sustained positivity or conversions from negative to positive, especially by MS, identify patients with an imminent risk of progressive disease.

Due to the timing of the trial, there were 18 out of the 62 (29%) patients included in our study that presented with one of the SLiM-CRAB criteria: 5 (27.8%) had ≥60% clonal plasma cells in the bone marrow, 10 (55.5%) a ratio of involved/uninvolved serum FLC >100 and 3 (16.7%) had more than one focal lesion on MRI. This represents almost a third of the analyzed cohort that, together with the limited number of samples/cases analyzed, could be explaining the short PFS found in the whole series and specially in those MRD or PRD positive.

In the present work we show for the first time that, as opposed to SPEP/IFE, MS is a clinical valuable monitoring tool in PB at all stages of the treatment in patients with HRsMM. These findings mainly relate to the higher sensitivity of MS as compared to SPEP/IFE and they could justify the introduction of a new serological MS-based response category. Furthermore, due to the comparable clinical value of MS and NGF and the high NPV of MS taking NGF as a reference method (specially at later stages of the treatment), MS could also be used as a gateway to perform a BM aspiration/biopsy for MRD assessment. Finally, MS and NGF dynamics during treatment are both able to identify a subgroup of HRsMM that with imminent risk of progressive disease and with a long-term disease control.

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Tables

Table 1: Baseline demographic and disease characteristics of all the patients included in the study.

Age in years, median (range)	59 (40-70)
Male, n (%)	34 (54.8%)
Isotype, n (%)	
IgG	42 (68%)
IgA	20 (32%)
Light-chain	6 (7%)
Amount of M-protein (mean, range)	2.9 g/dL (0.3-8.6)
Bone marrow infiltration (mean, range)	27.4% (5.5-78)
Cytogenetics, n (%)	
High-risk	18 (29%)
Standard risk	40 (65%)
Unknown	4 (6%)
High-risk, n (%)	
Mayo	6 (10%)
Pethema	21 (34%)
Both	17 (27%)
Ultra-high-risk (SLiM-CRAB criteria)	
BMPC ≥60%	5 (27.8%)
I/U FLC >100	10 (55.5%)
>1 focal lesion by MRI	3 (16.7%)
M-spike by mass-spectrometry	**100%**

Table 2: Number of patients analyzed and rates of undetectable residual disease using SPEP/IFE, mass-spec and NGF. The results corresponding to the main end point of the trial are in highlighted in bold. MRD^{neg} (minimal residual disease negativity rate) refers to the percentage of cases with absence of phenotypically abnormal plasma cells in the bone marrow using EuroFlow recommendations (with a minimum sensitivity of 1 cell in 10^5 nucleated cells). PRD^{neg} (peripheral residual disease negativity rate) refers to the percentage of patients with undetectable M-protein in serum samples using the Immunoglobulin Isotypes (GAM) assay for the MS EXENT analyzer, with a limit of detection of 0.015g/L.

	Post-induction	**Post-ASCT**	**Post-Consolidation**	**After 1 year maintenance**	**After 2 years maintenance**
	n=62	n=61	n=61	n=51	n=35
≥CR	28 (45%)	**41 (67%)**	45 (74%)	42 (82%)	30 (86%)
PRD^{neg}	16 (26%)	**28 (46%)**	32 (52%)	40 (78%)	28 (80%)
MRD^{neg}	17 (27%)	**36 (58%)**	37 (61%)	40 (78%)	23 (66%)

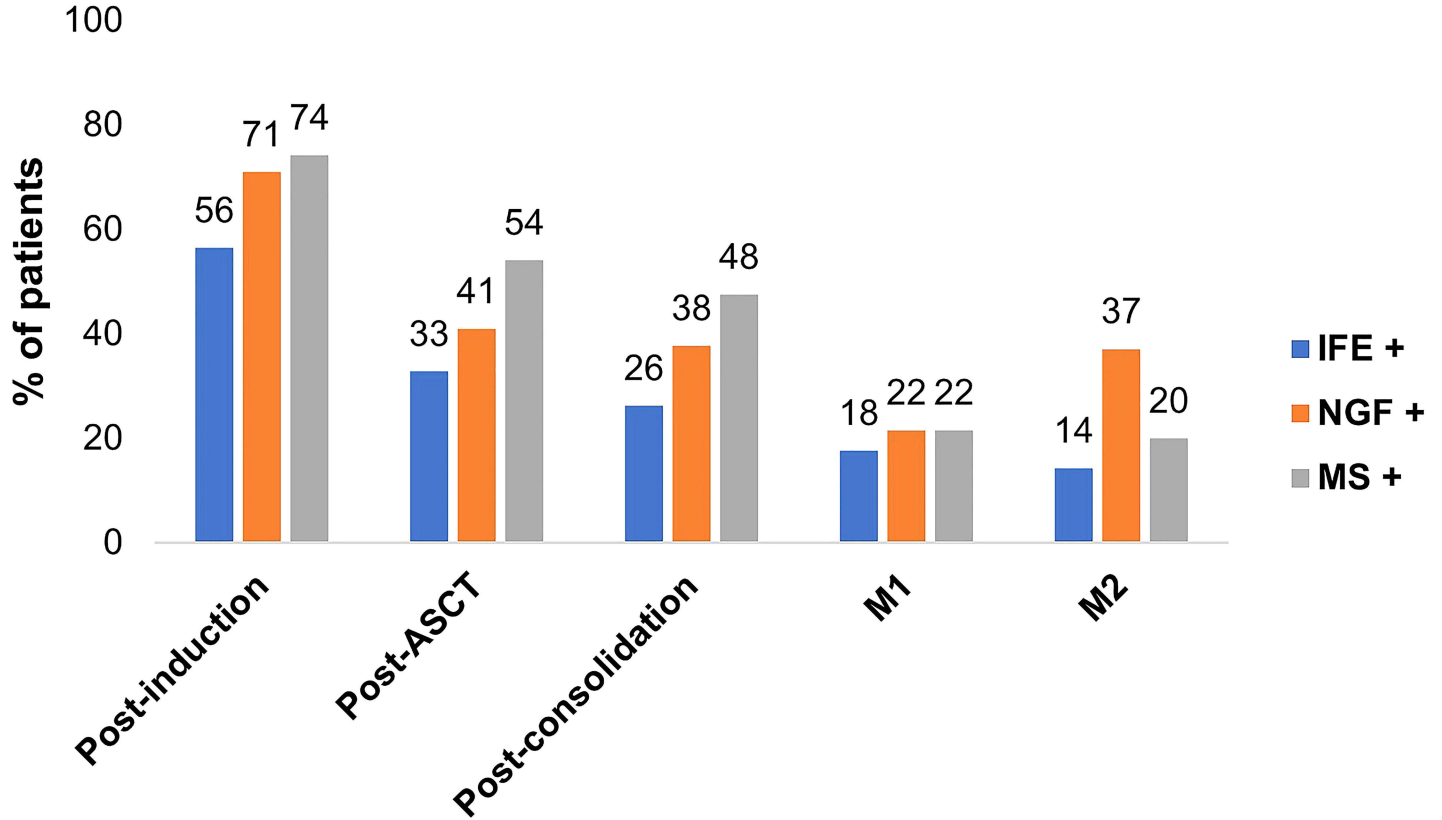
Figure legends

Figure 1: Percentages of patients with detectable residual disease by SPEP/IFE (blue bars), masss-spectrometry (MS; grey bars) and Next Generation Flow (NGF; orange bars) at the 5 time points analyzed in the trial (post-induction, after high-dose chemotherapy and ASCT, post-consolidation, after 1 year of maintenance and at treatment completion after 2 years of maintenance)

Figure 2: Analysis of the combined results of mass-spectrometry (MS) with SPEP/IFE or Next Generation Flow (NGF) post-ASCT and at after 24 cycles of maintenance. Percentages of concordant and discordant results, sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of MS considering SPEP/IFE **(A)** and NGF **(B)** as a reference.

Figure 3. Progression-free survival after ASCT (left column) and at treatment completion after two years of maintenance (right column) according to the results of a) SPEP/IFE in all patients, b) Next Generation Flow (NGF; minimal residual disease [MRD]) and mass-spectrometry (MS; peripheral residual disease [PRD]) in patients in complete response (CR) or stringent CR (sCR)

Figure 4: Landmark analysis of progression-free survival (PFS) based on a) minimal residual disease (MRD) or b) peripheral residual disease (PRD) kinetics from randomization to treatment completion after 24 months of maintenance


100
80
60
40
20
0
% of patients
56
71
74
33
41
54
26
38
48
18
22
22
14
37
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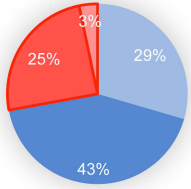
Post-induction
Post-ASCT
Post-consolidation
M1
M2
IFE +
NGF +
MS +

a)

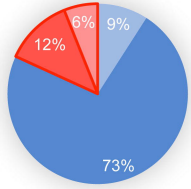
Post-ASCT



IFE/MS AGREEMENT **72% (44/61)**
IFE/MS DISAGREEMENT **28% (17/61)**

p value	<0.0001
Sensitivity	0.9000
Specificity	0.6341
PPV	0.5455
NPV	0.9286

End of the treatment



IFE/MS AGREEMENT **82% (27/33)**
IFE/MS DISAGREEMENT **18% (6/33)**

p value	0.0519
Sensitivity	0.6000
Specificity	0.8571
PPV	0.4286
NPV	0.9231

Post-ASCT

		IFE	
		Negative	Positive
MS	Negative	26 (43%)	2 (3%)
	Positive	15 (25%)	18 (29%)

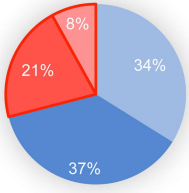
End of the treatment

		IFE	
		Negative	Positive
MS	Negative	24 (73%)	2 (6%)
	Positive	4 (12%)	3 (9%)

Overall concordance: 74.8% (205/274); 72 (26.3%) $IFE^{+}MS^{+}$, 133 (48.5%) $IFE^{-}MS^{-}$
Overall discordance: 25.2% (69/274); 12 (4.4%) $IFE^{+}MS^{-}$, 57 (20.8%) $IFE^{-}MS^{+}$

b)

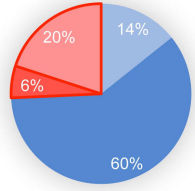
Post-ASCT



NGF/MS AGREEMENT **71% (44/61)**
NGF/MS DISAGREEMENT **29% (18/61)**

p value	0.0007
Sensitivity	0.8077
Specificity	0.6389
PPV	0.6176
NPV	0.8214

End of the treatment



NGF/MS AGREEMENT **74% (26/35)**
NGF/MS DISAGREEMENT **26%(9/33)**

p value	0.0331
Sensitivity	0.4167
Specificity	0.9130
PPV	0.7143
NPV	0.7500

Post-ASCT

		NGF	
		Negative	Positive
MS	Negative	23 (37%)	5(8%)
	Positive	13 (21%)	21 (34%)

End of the treatment

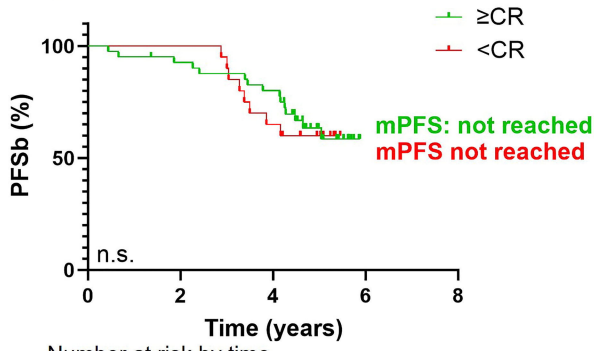
		NGF	
		Negative	Positive
MS	Negative	21 (60%)	7 (20%)
	Positive	2 (6%)	5 (14%)

Overall concordance: 75% (207/274); 90 (33%) $NGF^{+}MS^{+}$, 117 (42%) $NGF^{-}MS^{-}$
Overall discordance: 23.5% (67/274); 28 (9.5%) $NGF^{+}MS^{-}$, 39 (14%) $NGF^{-}MS^{+}$

Post-ASCT

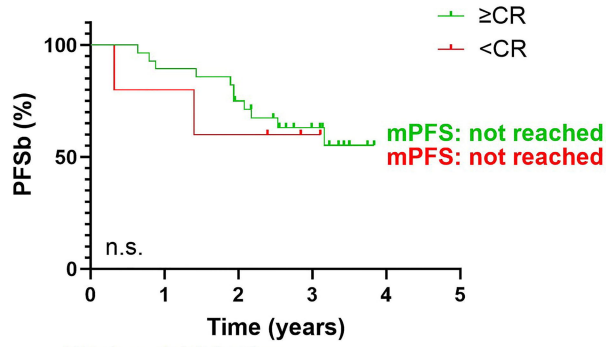
End of the treatment

a) All patients



Number at risk by time

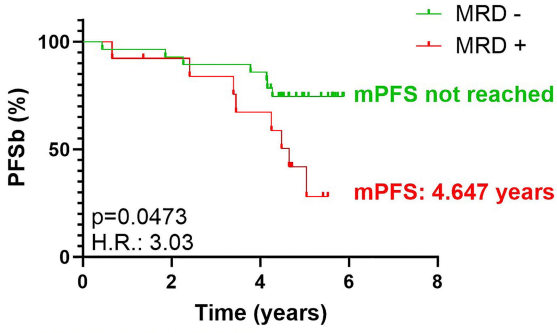
≥ CR	41	38	33	0
< CR	20	20	14	0



Number at risk by time

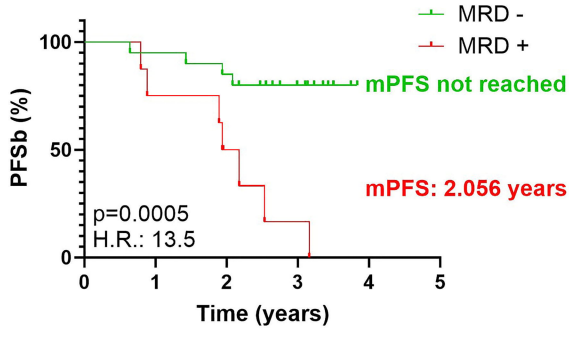
≥ CR	30	27	21	11	0
< CR	5	4	3	1	0

b) Patients in CR or better



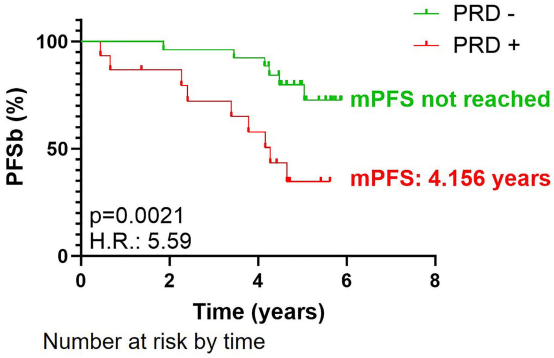
Number at risk by time

MRD -	29	28	25	0
MRD +	13	12	9	0



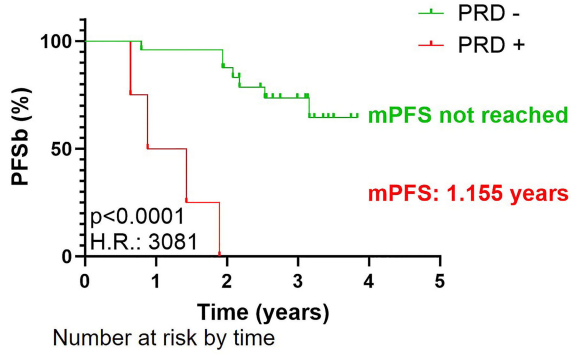
Number at risk by time

MRD -	20	19	18	10	0
MRD +	8	7	4	2	0



Number at risk by time

PRD -	26	25	24	0
PRD +	16	14	9	0

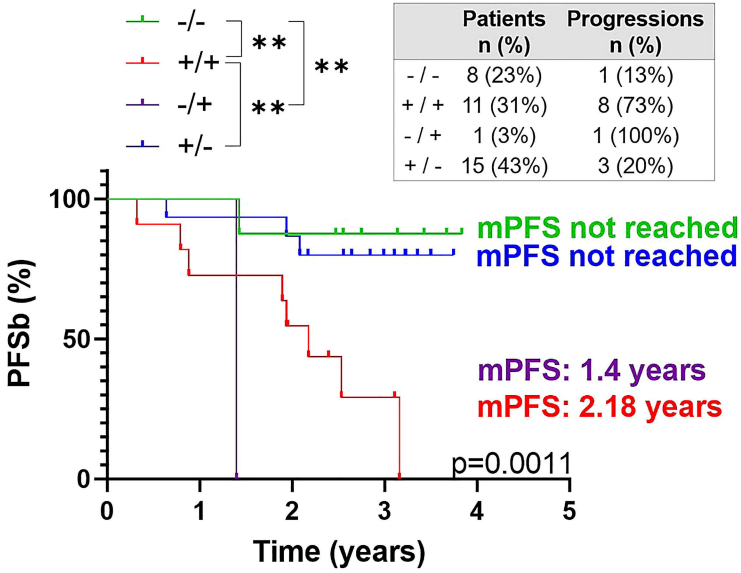


Number at risk by time

MRD -	24	23	21	11	0
MRD +	4	2	0	0	0

a)

MRD kinetics



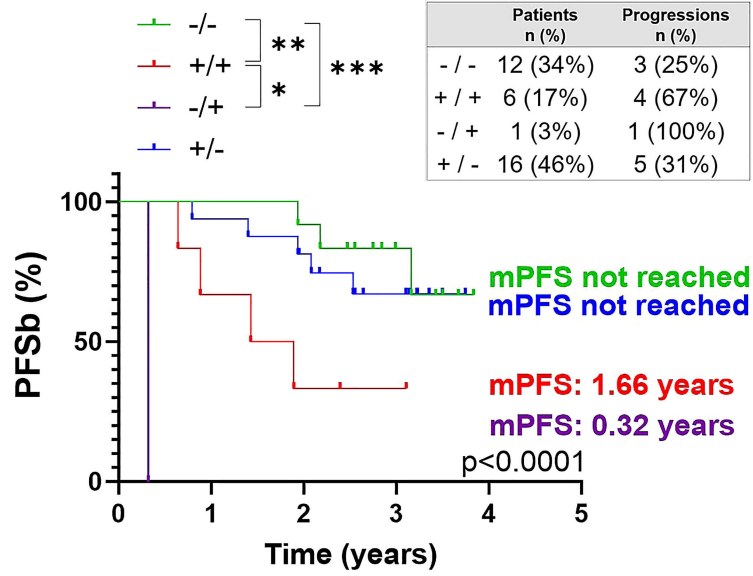
	Patients n (%)	Progressions n (%)
- / -	8 (23%)	1 (13%)
+ / +	11 (31%)	8 (73%)
- / +	1 (3%)	1 (100%)
+ / -	15 (43%)	3 (20%)

Number at risk by time

- / -	8	7	6	3	0
+ / +	11	9	6	5	0
- / +	1	1	0	0	0
+ / -	15	14	14	8	0

b)

PRD kinetics



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

Number at risk by time

- / -	12	12	11	6	0
+ / +	6	5	3	2	0
- / +	1	0	0	0	0
+ / -	16	15	13	8	0