# Prospective evaluation of minimal residual disease in the phase II FORTE trial: a head-to-head comparison between multiparameter flow cytometry and next-generation sequencing

Stefania Oliva,[a] Elisa Genuardi,[b] Laura Paris,[c] Mattia D'Agostino,[a,b] Jennifer Rogers,[d] Delia Rota-Scalabrini,[e] Allison P. Jacob,[f] Francesca Patriarca,[g] Mario Luppi,[h] Paola Bertazzoni,[i] Cristina Velluti,[b] Andrea Capra,[b] Elona Saraci,[b] Marco Rossi,[j] Alessandro Allegra,[k] Roberto Mina,[a,b] Massimo Gentile,[l] Ilan R. Kirsch,[f] Angelo Belotti,[m] Michele Cavo,[n,o] Benedetto Bruno,[a,b] Pellegrino Musto,[p,q] Mario Boccadoro,[b] Elena Zamagni,[n,o] and Francesca Gay[a,b,*]

[a]Division of Hematology, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Torino, Italy
[b]Division of Hematology, Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy
[c]Division of Hematology, ASST Papa Giovanni XXIII, Bergamo, Italy
[d]Multiple Myeloma Research Foundation (MMRF), Norwalk, CT, USA
[e]Multidisciplinary Oncology Outpatient Clinic, Candiolo Cancer Institute, FPO - IRCCS, Torino, Italy
[f]Adaptive Biotechnologies, Seattle, WA, USA
[g]Hematologic Clinic and Transplant Center, University Hospital of Central Friuli, DAME, University of Udine, Udine, Italy
[h]Dipartimento di Scienze Mediche e Chirurgiche Materno Infantili e dell'Adulto, UNIMORE, UOC Ematologia, Azienda Ospedaliero-Universitaria di Modena, Modena, Italy
[i]ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy
[j]SOC Ematologia, Department of Oncology/Hematology, Azienda Ospedaliera Pugliese-Ciaccio and Department of Experimental and Clinical Medicine, Magna Graecia University, Catanzaro, Italy
[k]Division of Hematology, Department of Human Pathology in Adulthood and Childhood "Gaetano Barresi", University of Messina, Messina, Italy
[l]UOC Ematologia, AO Cosenza, Cosenza, Italy
[m]Department of Hematology, ASST Spedali Civili di Brescia, Brescia, Italy
[n]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna, Italy
[o]Dipartimento di Scienze Mediche e Chirurgiche, Università di Bologna, Bologna, Italy
[p]Department of Precision and Regenerative Medicine and Ionian Area, "Aldo Moro" University School of Medicine, Bari, Italy
[q]Hematology and Stem Cell Transplantation Unit, AOU Consorziale Policlinico, Bari, Italy

eClinicalMedicine 2023;60: 102016

Published Online 9 June 2023
https://doi.org/10.1016/j.eclinm.2023.102016

## Summary

**Background** Limited data are available on the concordance between multiparameter flow cytometry (MFC) and next-generation sequencing (NGS) for minimal residual disease (MRD) detection in a large trial for multiple myeloma (MM) patients.

**Methods** MRD was explored in the FORTE trial for transplant-eligible MM patients randomised to three carfilzomib-based induction-intensification-consolidation treatments and carfilzomib-lenalidomide (KR) *vs* R maintenance. MRD was assessed by 8-colour 2nd-generation flow cytometry in patients with ≥very good partial response before maintenance. NGS was performed in case of suspected complete response (CR) in a correlative subanalysis. Biological/prognostic concordance between MFC and NGS, conversion to MRD negativity during maintenance, and 1-year/2-year sustained MRD negativity were explored.

**Findings** Between September 28, 2015 and December 22, 2021, 2020 samples were available for MFC and 728 for the simultaneous MFC/NGS correlation in the "suspected CR population". Median follow-up was 62 months. Biological agreement was 87% at the $10^{-5}$ and 83% at the $10^{-6}$ cut-offs. A remarkable prognostic concordance was observed: hazard ratios in MFC-MRD and NGS-MRD-negative *vs* -positive patients were 0.29 and 0.27 for progression-free survival (PFS) and 0.35 and 0.31 for overall survival, respectively ($p < 0.05$). During maintenance, 4-year PFS was 91% and 97% in 1-year sustained MFC-MRD-negative and NGS-MRD-negative patients ($10^{-5}$), respectively, and 99% and 97% in 2-year sustained MFC-MRD-negative and NGS-MRD-negative patients, regardless of treatment

*Corresponding author. Division of Hematology, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino and Division of Hematology, Department of Molecular Biotechnology and Health Sciences, University of Torino, via Genova 3, 10126, Torino, Italy.
*E-mail address:* francesca.gay@unito.it (F. Gay).

received. The conversion rate from pre-maintenance MRD positivity to negativity during maintenance was significantly higher with KR *vs* R both by MFC (46% *vs* 30%, p = 0.046) and NGS (56% *vs* 30%, p = 0.046).

**Interpretation** The significant biological/clinical concordance between MFC and NGS at the same sensitivity suggests their possible use in the evaluation of one of the currently strongest predictors of outcome.

**Funding** Amgen, Celgene/Bristol Myers Squibb, Multiple Myeloma Research Foundation.



**Keywords:** Newly diagnosed multiple myeloma (NDMM); Minimal residual disease (MRD); Multiparameter flow cytometry (MFC); Next-generation sequencing (NGS); Autologous stem-cell transplantation (ASCT)

### Research in context

#### Evidence before this study

The assessment of minimal residual disease (MRD) has been implemented in all the recent clinical trials for the treatment of multiple myeloma (MM). This approach derives from the results of several meta-analyses and a recently published consensus on the use and reporting of results of different MRD techniques. These works showed that MRD is an excellent clinical endpoint, which can be evaluated at different stages of the disease (at diagnosis and relapse), in young or old patients with standard-risk or high-risk cytogenetics. From a methodological point of view, MRD is now standardly assessed in the bone marrow by using flow cytometry (next-generation flow [NGF]) and molecular (next-generation sequencing [NGS]) techniques. However, it remains to be determined which technique should be preferred in clinical practice. Several studies demonstrated that MRD plays a role in the assessment of patients achieving at least a very good partial response/complete response, but how this parameter can then modify patient treatment is still being tested. In addition, MRD assessment also allows for the reclassification of patients into high-risk and low-risk subgroups.

#### Added value of this study

In a large number of paired samples from the FORTE trial, we showed a good biological correlation between multiparameter flow cytometry (MFC)/NGF and NGS by using the same limit of detection with both techniques. This biological concordance was associated with a good clinical concordance in terms of the impact of MRD negativity *vs* positivity on progression-free survival (PFS) and overall survival, by performing the two techniques after a median follow-up of 62 months. Moreover, MFC and NGS showed a similar impact on PFS of 1-year and 2-year sustained MRD negativity at a sensitivity of $10^{-5}$, with an impressive PFS rate of 2-year sustained MRD negativity. The impact of sustained MRD negativity was independent of previous treatment, showing its possible routine clinical assessment for treatment decision-making. Finally, during the maintenance phase, the use of carfilzomib in combination with lenalidomide *vs* lenalidomide alone was associated with a significantly higher rate of conversion from MRD positivity to negativity assessed by both MFC and NGS.

#### Implications of all the available evidence

Our results confirmed that MRD detections by MFC and NGS are comparable predictors of long-term outcomes in patients with MM and that achieving and sustaining MRD negativity assessed by either technique was correlated with prolonged survival. MRD analysis should now be implemented in all clinical trials, in order to shed light on many unresolved issues in the treatment of multiple myeloma, such as the optimal duration of maintenance treatment, the role of delayed transplantation, the option to intensify treatment to convert patients to a MRD negative status, and, finally, the possibility to use MRD assessment for early approval of new anti-MM therapies.

## Introduction

The rates of progression-free survival (PFS) and overall survival (OS) in patients with multiple myeloma (MM) have significantly improved due to deeper and more sustained responses associated with the use of novel triplets or quadruplets, as compared with standard approaches.[1–5]

The second-generation proteasome inhibitor carfilzomib showed a good efficacy and safety profile in relapsed/refractory MM patients,[6] thus providing the rationale to investigate its incorporation in upfront induction and consolidation therapies for newly diagnosed (ND), transplant-eligible (TE) patients. In the phase II randomised FORTE clinical trial, we reported the superiority of carfilzomib-lenalidomide-dexamethasone plus autologous stem-cell transplantation (KRd plus ASCT) over both carfilzomib-cyclophosphamide-dexamethasone plus ASCT (KCd plus ASCT) and 12 cycles of KRd without ASCT (KRd12) in terms of PFS, OS, and minimal residual disease (MRD) negativity

rates. Moreover, maintenance treatment with carfilzomib-lenalidomide (KR) showed a higher efficacy than lenalidomide alone (R).[7]

Evidence from metanalyses and international consensus papers confirmed the association between MRD status and survival outcomes.[8–11] Both next-generation sequencing (NGS) and multiparameter flow cytometry (MFC) proved to be valid and standardised methods to detect MM residual cells in the bone marrow (BM) and showed that the higher was the sensitivity achieved, the better was the outcome.[12,13] Besides, the standardisation of imaging-MRD by positron emission tomography/computed tomography (PET/CT) is also important, given the spatial heterogeneity of MM. Nevertheless, the MM community is wondering how and when MRD evaluation is going to enter clinical practice, what the optimal technique is, and whether MRD by MFC and NGS yield comparable prognostic information.

Here we report the MRD analysis of the FORTE trial, to correlate biological and prognostic results by MFC and NGS in NDTEMM patients.

## Methods

### Study design and participants

NDTEMM patients were enrolled in the phase II multicentre FORTE trial, with a first randomisation (R1) to receive KRd plus ASCT, KRd12, or KCd plus ASCT as induction-intensification-consolidation treatment. Patients received either 4 induction cycles of KRd followed by ASCT and 4 consolidation cycles of KRd; 12 cycles of continuous KRd without ASCT; or 4 induction cycles of KCd followed by ASCT and 4 consolidation cycles of KCd. At the end of consolidation, patients were randomised (R2) to receive KR *vs* R maintenance treatment until disease progression or intolerance (Supplementary methods and Fig. S1). Clinical results have been recently published.[7]

This analysis was conducted in accordance with the protocol of the UNITO-MM-01/FORTE trial (see the Redacted trial protocol in the Supplementary Appendix), which was approved by the ethics or institutional review boards at each of the participating centres and registered at ClinicalTrials.gov (NCT02203643). All patients gave written informed consent before entering the study, which was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines.

### Randomisation and masking

At enrolment, patients were randomly assigned (1:1:1) to one of the three induction-intensification-consolidation groups. A block randomisation (block size 12), stratified according to International Staging System (ISS) stage (I *vs* II or III) and age (<60 years *vs* 60–65 years), was generated at enrolment by a computer programme and implemented into a web-based procedure by the investigator or designated research staff. Patients who did not experience unacceptable toxicity or progression during the induction, intensification, and consolidation phases were eligible for maintenance treatment. Maintenance randomisation was balanced with a permuted block (block size 8) and was stratified according to induction-intensification-consolidation treatment in a 1:1 ratio.

### Procedures and outcomes

Specifications of procedures and outcomes have been reported previously[7] and are detailed in the Supplementary methods. MRD analyses were centralised in the laboratory of the University of Torino, Division of Hematology (Italy). If more than one tube was collected for the BM MRD evaluation, MFC or NGS material was randomly selected from the different tubes.

*MFC*. MFC status was assessed in patients with at least a very good partial response (VGPR) first at pre-maintenance and then every 6 months during maintenance treatment until progressive disease (PD). An optional time point was planned after induction treatment. MFC was performed on BM aspirates according to consensus guidelines on MRD sample staining in MM, data acquisition, and analysis (8 colours, 2 tubes).[14,15] Samples were processed after the red blood cell ("bulk") lysis with ammonium chloride and stained with specific antibodies (Supplementary Table S1). Data were collected using a Navios flow cytometer and analysed with Kaluza software (Beckman Coulter, Brea, US-CA). We aimed to acquire ≥3.5 million cells.

A subset of patients (n = 73) was included in a subanalysis at pre-maintenance. We applied the next-generation flow (NGF) protocol with specific antibodies (Supplementary Table S1b), following the standard guidelines suggested by the Black Swan project.[16] We aimed to acquire ≥5 million cells per tube. We evaluated the limit of quantitation (LOQ) and the limit of detection (LOD) of both MFC and NGF MRD methods, which were calculated as ≥50 and 20 out of the total acquired nucleated cells, respectively. This allowed the discrimination between positive and negative samples. The cut-off for MFC-MRD positivity was set at ≥20 clonal plasma cells out of the total of nucleated cells, with a sensitivity of $\geq 10^{-5}$.

*NGS*. The NGS research in the FORTE trial was supported by a grant from the Multiple Myeloma Research Foundation (MMRF; Norwalk, US-CT) in a pre-planned (n = 246) subset of patients with at least a suspected complete response (CR) at pre-maintenance. NGS was subsequently monitored every 6 months during maintenance treatment until PD (as performed by MFC). We referred to the clonoSEQ® assay (v2.0, Adaptive Biotechnologies, Seattle, US-WA) by sending stored samples to the Seattle laboratory. A final report with qualitative and quantitative MRD results was provided by Adaptive Biotechnologies (Supplementary methods).

### Statistical analysis

The concordance of MRD results obtained by MFC and NGS was evaluated comparing the MRD count (log10) based on Spearman's rank tests ($r_s$) in paired samples with the same LOD. This concordance was also evaluated in terms of binary assessment (positivity *vs* negativity), by calculating the proportion of samples with concordant results (MRD negativity by MFC and NGS or MRD positivity by MFC and NGS) over all samples that were simultaneously assessed.

The conversion rate was determined to be the proportion of patients who were MRD positive after consolidation and turned to MRD negativity during maintenance treatment. 1-year and 2-year sustained MRD negativities were defined as 2 consecutive MRD-negative test results, the first at the pre-maintenance time point (after induction or after consolidation) and the second respectively at least 12 and 24 months apart.

Analyses of PFS and OS were performed by MRD status at the $10^{-5}$ cut-off as per intention to treat (ITT) analysis, namely including all patients eligible for the main analysis of the trial. Patients who did not achieve a VGPR were considered positive for MRD by both MFC and NGS. Patients who missed MRD testing by MFC were also considered positive for MRD. Patients who achieved a CR or stringent CR (sCR) were excluded from the NGS analysis if their samples were not available for MRD testing. ITT populations are further detailed in the Supplementary methods. Landmark analyses were performed to ensure that the MRD status was considered well defined and fixed at time 0 of the follow-up period, in order to reduce the immortal time bias (Supplementary methods).

Time-to-event data were analysed using the Kaplan–Meier method. The Cox proportional hazards model was used to estimate the hazard ratios (HRs). Rates were compared by using the Fisher's Exact Test. The logistic model was used to compute odds ratios (ORs) with confidence intervals (CIs) and p-values.

To account for potential confounders, the Cox and logistic models were adjusted for relevant baseline prognostic factors.

The statistical analysis was performed using R (v.4.1.0). The data cut-off was 17/2/2022.

### Role of the funding source

The funders of the UNITO-MM-01/FORTE had no role in study design, data collection, data formal analysis, data interpretation, or writing of this report.

## Results

### Patient characteristics and correlation analysis of MFC and NGS samples

*MFC*

MFC-MRD was evaluated in 371/397 (93%) ≥VGPR patients, 73 of whom were analysed using the NGF protocol and 3 (2%) were not evaluable because of inadequate specimen processing and/or instrument setup; 26 samples of patients who achieved a VGPR were missing and were well balanced in the three arms. Patient characteristics in the MFC population according to ITT are detailed in Table 1.

Between September 28, 2015 and December 22, 2021, 2020 samples were analysed by MFC during the entire course of treatment (333 by NGF), out of which 654 at pre-maintenance (127 by NGF; 202 after induction-mobilisation, 139 after ASCT, and 313 after consolidation) and 1366 during maintenance (206 by NGF). A sensitivity of $10^{-5}$ was reached in 1685/1687 (99.8%) samples analysed by MFC, and a sensitivity of at least $2.5 \times 10^{-6}$ in 295/333 (88.5%) samples analysed by NGF. The median LOD of MFC at $10^{-5}$ was 0.00065% (interquartile range [IQR] 0.00061%–0.00071%) *vs* 0.00021% (IQR 0.000187%–0.00022%) of NGF ($p < 0.001$).

*NGS*

NGS was performed in 246 patients with suspected CR. Patient characteristics according to ITT NGS results are shown in Supplementary Table S2. All baseline samples passed the quality control (QC) analysis, and the molecular marker was identified in 228/246 (92%) patients. A total of 833 samples were sequenced for NGS MRD analysis during the entire course of treatment, out of which 17 (2%) failed the QC analysis. A sensitivity of $10^{-5}$ was reached in 787/833 (94%) samples, and a sensitivity of $10^{-6}$ in 625/833 (75%) samples (Supplementary Fig. S2), with the sample amount input being significantly associated with the probability to achieve a sensitivity of $10^{-6}$ *vs* not to achieve it [median number of B-cells 3.354.251 (IQR 2.905.833–3.568.719) *vs* 919.097 (422.595–1.894.532), respectively; $p < 0.001$]. The median LOD was $0.610 \times 10^{-6}$ ($0.546–2.519 \times 10^{-6}$) for NGS samples, $2.793 \times 10^{-6}$ (1.252–4.852) for samples at the $10^{-5}$ sensitivity cut-off, and indeterminate for samples at the $10^{-6}$ cut-off.

*Correlation between MFC and NGS*

A total of 728 samples were available for the correlation between MFC and NGS in the "suspected CR population": 589 at $10^{-5}$ and 139 at $10^{-6}$, based on the sensitivity achieved by the two techniques (MFC at $10^{-5}$ *vs* NGF at $10^{-6}$). Findings regarding NGS were detailed in a final report provided by Adaptive Biotechnologies. The two methods were concordant in 513 (87%) analyses at the $10^{-5}$ cut-off and in 115 (83%) at the $10^{-6}$ cut-off. Discordances between the two methods were found in 76 (13%) paired samples at $10^{-5}$ and in 24 (17%) at $10^{-6}$. In particular, 59/487 (12%) MFC-negative samples were NGS positive at $10^{-5}$ and 22/105 (21%) NGF-negative samples were NGS positive at $10^{-6}$, while only 17/445 (4%) NGS-negative samples were MFC positive at $10^{-5}$ (only 2 at $10^{-6}$), but all of them were NGS positive at the

| Characteristic | FORTE: all (n = 474) | MFC Neg (n = 310) | MFC Pos (n = 164) | p-value (MFC Neg *vs* Pos) |
|---|---|---|---|---|
| **Demographics** | | | | |
| Median age (IQR) | 57 (51–62) | 56.5 (51–62) | 58 (52–62) | 0.27 |
| Sex, female, No. (%) | 212 (45) | 148 (48) | 64 (39) | 0.08 |
| **Disease characteristics, No. (%)** | | | | |
| Isotype, BJ | 71 (15) | 49 (16) | 22 (13) | 0.31 |
| Isotype, IgA | 81 (17) | 54 (17) | 27 (16) | – |
| Isotype, IgD | 3 (1) | 3 (1) | – | – |
| Isotype, IgG | 298 (63) | 187 (60) | 111 (68) | – |
| Ns | 21 (4) | 17 (5) | 4 (2) | – |
| Light chain, kappa | 295 (64) | 193 (64) | 102 (65) | 0.83 |
| Light chain, lambda | 165 (36) | 110 (36) | 55 (35) | – |
| **ISS, No. (%)** | | | | |
| Stage I | 240 (51) | 162 (52) | 78 (48) | 0.006 |
| Stage II | 152 (32) | 107 (35) | 45 (27) | – |
| Stage III | 82 (17) | 41 (13) | 41 (25) | – |
| **R-ISS, No. (%)** | | | | |
| Stage I | 127 (31) | 90 (33) | 37 (25) | 0.05 |
| Stage II | 247 (59) | 159 (59) | 88 (60) | – |
| Stage III | 42 (10) | 21 (8) | 21 (14) | – |
| *Missing* | 58 | 40 | 18 | – |
| **LDH > ULN, No. (%)** | 61 (13) | 33 (11) | 28 (17) | 0.06 |
| **CTCs > 0.07%, No. (%)** | 130 (32) | 65 (25) | 65 (47) | <0.001 |
| **CA by FISH, No. (%)** | | | | |
| del(17p13.1) | 61 (15) | 28 (10) | 33 (23) | <0.001 |
| del(1p32.3) | 44 (11) | 33 (13) | 11 (8) | 0.18 |
| t(4;14) | 65 (16) | 35 (13) | 30 (21) | 0.03 |
| t(14;16) | 21 (5) | 14 (5) | 7 (5) | 1 |
| gain(1q21) (3 copies) | 129 (32) | 86 (33) | 43 (31) | 0.02 |
| amp(1q21) (≥4 copies) | 52 (13) | 25 (10) | 27 (19) | – |
| High-risk CA per IMWG[a] | 133 (33) | 73 (27) | 60 (43) | 0.002 |
| 1+ HRCA[b] | 243 (61) | 154 (59) | 89 (65) | 0.329 |
| 2+ HRCA[b] | 105 (27) | 56 (22) | 49 (36) | 0.01 |

Abbreviations: MFC, multiparameter flow cytometry; Neg, negative; Pos, positive; IQR, interquartile range; No., number; BJ, Bence-Jones; Ns, not specified; ISS, International Staging System; R-ISS, Revised ISS; LDH, lactate dehydrogenase; ULN, upper limit of normal; CTCs, circulating tumour plasma cells; CA, cytogenetic abnormalities; FISH, fluorescence *in situ* hybridisation; del, deletion; t, translocation; amp, amplification; IMWG, International Myeloma Working Group; HRCA, high-risk cytogenetic abnormalities. [a]High-risk cytogenetics were defined in accordance with the International Myeloma Working Group (IMWG) criteria: presence of t(4;14) and/or t(14;16) and/or del(17p). [b]1+ HRCA was defined as the presence of at least 1 of the following high-risk cytogenetic abnormalities: del(17p), t(4;14), t(14;16), del(1p), gain(1q), or amp(1q). 2+ HRCA was defined as the presence of at least 2 high-risk cytogenetic abnormalities.

***Table 1:*** **Patient characteristics at baseline in the MFC population and comparison with the general population.**

higher sensitivity of $10^{-6}$ (Fig. 1). The concordance between the two techniques was confirmed at different time points (Supplementary Fig. S3).

## Clinical correlation between MFC and NGS: MRD impact on survival

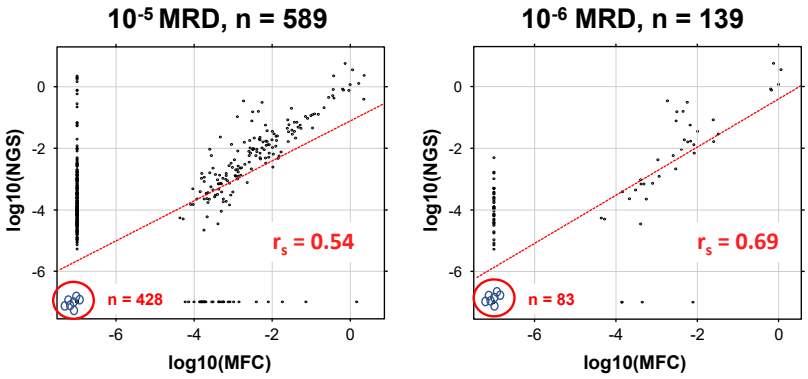
The biological concordance between the two MRD methods translated into a clinical and prognostic concordance: after a median follow-up of 62 months (IQR 55–68) from R1, patients who were MRD negative showed prolonged PFS and OS *vs* patients who were MRD positive at the $10^{-5}$ cut-off.

### *MFC*

The 4-year PFS was 78% *vs* 40% in MFC-negative *vs* MFC-positive patients, respectively (HR 0.29, 95% CI 0.2–0.4, p < 0.0001; Fig. 2a); the 4-year OS was 93% *vs* 75% in MFC-negative *vs* MFC-positive patients (HR 0.35, 95% CI 0.22–0.57, p < 0.0001, Fig. 2c).

### *NGS*

The 4-year PFS was 83% *vs* 46% in NGS-negative *vs* NGS-positive patients, respectively (HR 0.27, 95% CI 0.18–0.39, p < 0.0001; Fig. 2b); the 4-year OS was 94% *vs*




| | | | | NGS | | Observed agreement |
|---|---|---|---|---|---|---|
| | | | | MRD Pos, n (%) | MRD Neg, n (%) | |
| MRD Neg $10^{-5}$, n (%) | Flow cytometry | MRD Pos | 102 | 85 (83) | 17 (17) | 87% |
| | | MRD Neg | 487 | 59 (12) | 428 (88) | |
| MRD Neg $10^{-6}$, n (%) | NGF | MRD Pos | 34 | 32 (94) | 2 (6) | 83% |
| | | MRD Neg | 105 | 22 (19) | 83 (81) | |

***Fig. 1:*** **MFC-NGS correlation in patients with at least a suspected CR. Abbreviations**: MFC, multiparameter flow cytometry; NGS, next-generation sequencing; CR, complete response; MRD, minimal residual disease; $r_s$, Spearman's rank coefficient; Pos, positivity; Neg, negativity; NGF, next-generation flow.

78% in NGS-negative *vs* NGS-positive patients (HR 0.31, 95% CI 0.17–0.54, p < 0.0001; Fig. 2d).

The favourable impact of MRD negativity on PFS and OS was confirmed in all subgroups, including the high-risk patients, with similar HR results by MFC and NGS in terms of both PFS and OS (Fig. 2e–h). To further confirm the clinical concordance of these two techniques, we performed a subanalysis in 205 patients simultaneously assessed for MRD negativity by MFC and NGS ("suspected CR population"), who showed similar results. Moreover, patients who did not achieve a CR were stratified by the other response categories (=VGPR, and ≤PR; Supplementary Fig. S4). We also performed a PFS analysis comparing patients who were MRD negative by MFC and NGS *vs* MRD positive by MFC and NGS *vs* MRD negative by MFC and positive by NGS *vs* MRD positive by MFC and negative by NGS (Supplementary Fig. S5).

We already reported the rates of MRD negativity by MFC and NGS at pre-maintenance.[7] Here we focused on the impact of MRD negativity by MFC and NGS on PFS in different treatment arms in the ITT analysis. In MRD-negative patients, the 4-year PFS was slightly longer, although not statistically significant, with NGS in the KRd plus ASCT *vs* the KRd12 and KCd plus ASCT arms (Supplementary Table S3a and b).

We explored the impact on PFS of 1-year and 2-year sustained MRD negativity at $10^{-5}$.

*MFC*

We observed a significant impact on 4-year PFS of sustained MRD negativity by MFC at 1 year (HR 0.21, 95% CI 0.12–0.38, p < 0.001) and at 2 years (HR 0.11, 95% CI 0.04–0.29, p < 0.0001).

*NGS*

We also observed an impact on 4-year PFS of sustained MRD negativity by NGS at 1 year (HR 0.002, 95% CI 0.0002–0.04, p < 0.001) and at 2 years (HR 0.05, 95% CI 0.01–0.31, p = 0.0013; Fig. 3).

As previously reported,[7] the outcome of patients who achieved 1-year sustained MRD negativity was superimposable in the three treatment arms and in the KR *vs* R maintenance arms (Supplementary Fig. S6).

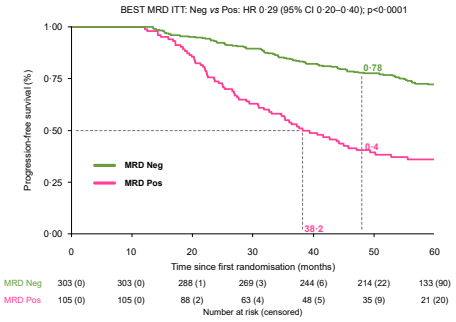
The univariate and multivariate analyses including prognostic factors and their association with the achievement of 1-year and 2-year sustained MRD negativity are reported in Table 2 and Supplementary Table S4. Again, to further confirm the clinical concordance of the two techniques, we performed a subanalysis in patients simultaneously assessed by MFC and NGS ("suspected CR population") for the evaluation of 1-year and 2-year sustained MRD negativity, with almost comparable results (1-year sustained MRD negativity by MFC: HR 0.07, p = 0.0040; by NGS: HR 0.001, p < 0.0001; 2-year sustained MRD negativity by MFC: no events were observed in the sustained MRD negativity group; by NGS: HR 0.04, p = 0.0050).

### Kinetics of conversion to MRD negativity during maintenance treatment and impact on outcome

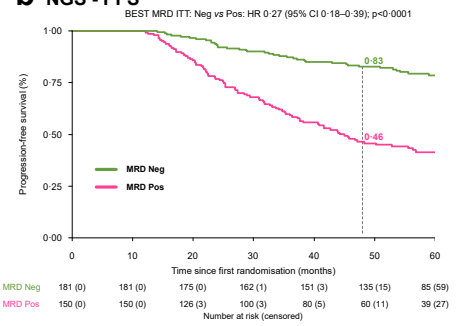
*MFC*

At the time of R2, 65% of randomised patients were MRD negative by MFC at $10^{-5}$ (equally distributed in the

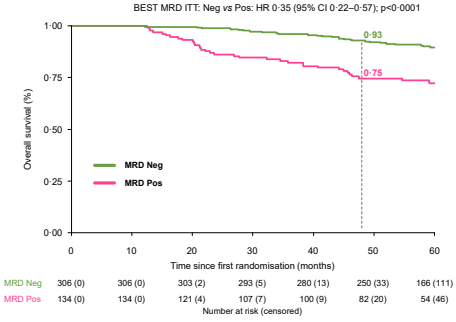
**a** MFC - PFS



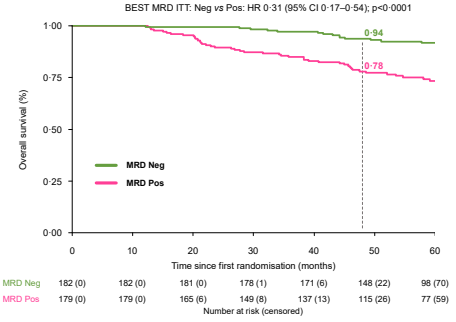

**b** NGS - PFS



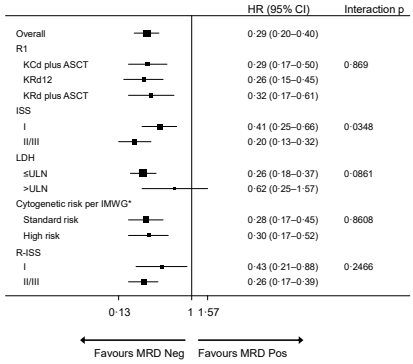

**c** MFC - OS



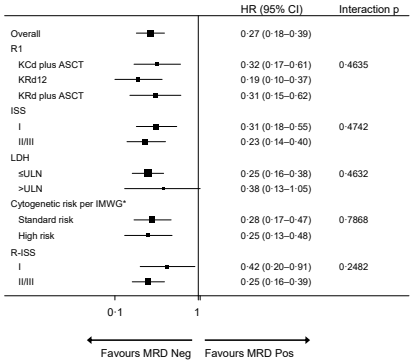

**d** NGS - OS



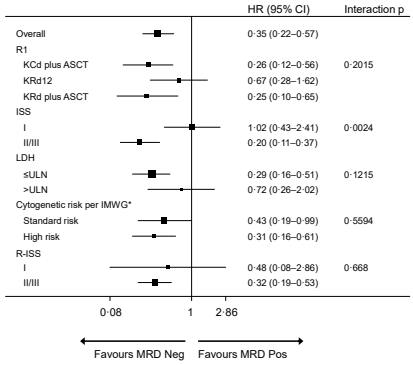

**e** MFC - PFS



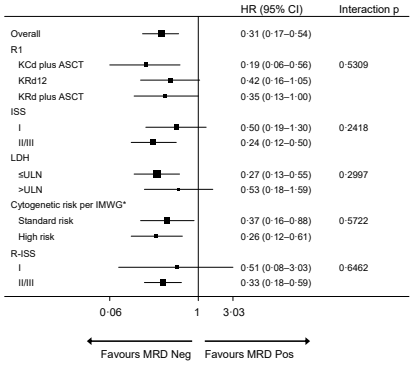

**f** NGS - PFS




**g** MFC - OS




**h** NGS - OS



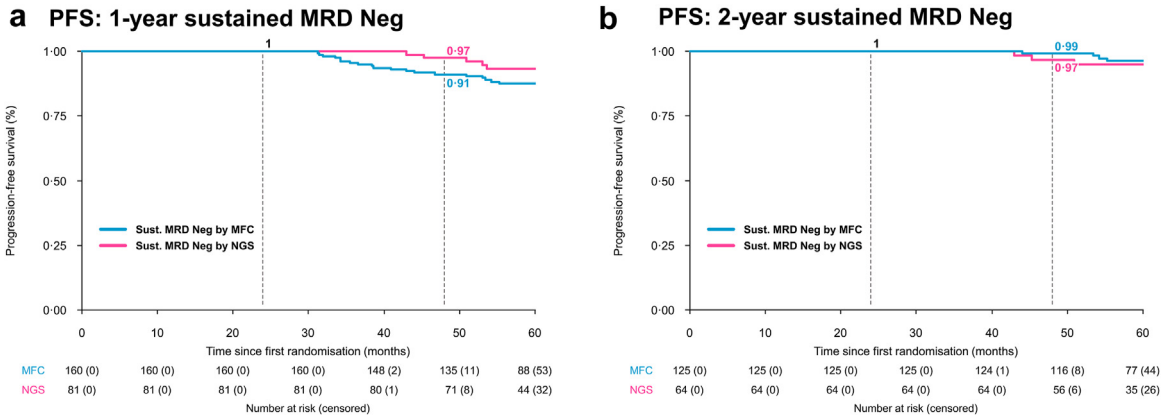



***Fig. 3:* Progression-free survival in patients with sustained MRD negativity by MFC and NGS**. 3a) PFS: 1-year sustained MRD negativity by MFC and NGS. 3b) PFS: 2-year sustained MRD negativity by MFC and NGS. **Abbreviations**: MRD, minimal residual disease; MFC, multiparameter flow cytometry; NGS, next-generation sequencing; PFS, progression-free survival; Neg, negativity; Sust., sustained.

two arms: KR *vs* R). Of 123 MRD-positive patients, 47 (38%) converted to MRD-negative status during maintenance. The conversion rate from MRD positivity to negativity was significantly higher in patients treated with KR *vs* R maintenance: 29/63 (46%) *vs* 18/60 (30%), respectively (OR 2.18, 95% CI 1.01–4.69, p = 0.046), with a constantly higher cumulative incidence of conversion in favour of KR *vs* R over time (Fig. 4). In the MFC subanalysis, patients who converted to MRD-negative status had a 4-year PFS rate of 73% *vs* 44% in those with persistent MRD positivity (HR 0.23, 95% CI 0.11–0.47, p < 0.0001).

*NGS*

In the NGS subanalysis, 48 patients were MRD positive and 21 (44%) converted to MRD-negative status during maintenance: 14/25 (56%) in the KR *vs* 7/23 (30%) in the R arms (OR 3.72, 95% CI 1.02–13.54, p = 0.046), again with a constantly higher cumulative incidence of conversion in favour of KR *vs* R over time (Fig. 4). In the NGS subanalysis, patients who converted to MRD-negative status had a 4-year PFS rate of 84% *vs* 55% in those with persistent MRD positivity (HR 0.19, 95% CI 0.04–0.88, p = 0.034; Supplementary Fig S7).

## Discussion

In this prospective MRD analysis of NDTEMM patients enrolled in the FORTE trial, we demonstrated the applicability of both MFC and NGS techniques in more than 700 paired samples, with 98% of reliable MFC results and 92% of clonoSEQ® marker identification rate, thus confirming the applicability of both techniques for MRD detection. Our NGS results are in line with previous reports, with baseline clonality detection rates ≥90% achieved by either LymphoTrack® or clonoSEQ®.[17–22] The large number of samples analysed in our cohort further confirmed these data.[17,23]

A general good agreement (≥83%) of MRD results was observed in our large sample size, showing that both BM techniques are quite comparable in terms of qualitative results (positivity *vs* negativity). Other head-to-head comparison studies were designed, and their conclusions were similar to those in our analysis, with an agreement >80% between NGS and MFC at the sensitivities of $10^{-5}$ and $10^{-6}$. In the phase III CASSIOPEIA trial, Avet-Loiseau et al. reported an agreement of 83.5% between MFC and NGS, with a sensitivity of $10^{-5}$.[24] Only in a study by Kriegsmann et al., a lower degree of concordance (68% in 125 MRD samples) was

***Fig. 2:* Survival outcomes in the ITT population stratified by MRD status (MFC and NGS; $10^{-5}$)**. 2a) MFC - PFS; 2b) NGS - PFS; 2c) MFC - OS; 2d) NGS - OS; 2e) Subgroup analysis. MFC - PFS; 2f) Subgroup analysis. NGS - PFS; 2g) Subgroup analysis. MFC - OS; 2h) Subgroup analysis. NGS - OS. *High-risk cytogenetics were defined in accordance with the International Myeloma Working Group (IMWG) criteria: presence of t(4;14) and/or t(14;16) and/or del(17p). **Abbreviations**: ITT, intention to treat; MRD, minimal residual disease; MFC, multiparameter flow cytometry; NGS, next-generation sequencing; PFS, progression-free survival; OS, overall survival; Neg, negativity; Pos, positivity; HR, hazard ratio; CI, confidence interval; p, p-value; R1, first randomisation (induction-intensification-consolidation treatment); KCd, carfilzomib-cyclophosphamide-dexamethasone; ASCT, autologous stem-cell transplantation; MEL200, melphalan at 200 mg/m$^2$; KCd plus ASCT, 4 KCd induction cycles, MEL200-ASCT, 4 KCd consolidation cycles; KRd, carfilzomib-lenalidomide-dexamethasone; KRd12, 12 KRd cycles without ASCT; KRd plus ASCT, 4 KRd induction cycles, MEL200-ASCT, 4 KRd consolidation cycles; ISS, International Staging System stage; LDH, lactate dehydrogenase; ULN, upper limit of normal; IMWG, International Myeloma Working Group; FISH, fluorescence *in situ* hybridisation; R-ISS, Revised ISS stage; t, translocation; del, deletion.

reported, due to an undercut LOD (non-assessable MRD) of 9.6% by either one of the two methods that affected the proportion of correlation.[25] Sample quality and haemodilution are important factors that explain discordances and false-negative results. An important difference between the two techniques is the stability of the analyte (gDNA for NGS *vs* cell surface proteins for MFC/NGF). Additionally, since whole blood and BM samples are extracted, the cells do not necessarily need to be intact for NGS analysis as long as the gDNA is still present. Indeed, in our study, 59 MFC-negative samples were NGS positive at a sensitivity of $10^{-5}$ and 22 NGF-negative samples were NGS positive at $10^{-6}$, while only 17 NGS-negative samples were MFC-positive at $10^{-5}$. This discrepancy in favour of NGS can be explained by the presence of haemodiluted samples and other potential effects of pre-analytical sample handling, such as BM collection and processing, time from sampling to analysis, and shipping conditions.[11,14,26,27] A limitation of our study was the lack of information regarding statistically significant patterns that could possibly explain discordant cases of patients who were MRD negative by MFC and MRD positive by NGS (e.g., haemodilution patterns for MFC such as cellularity, mast cells, erythroid precursors, or B-cell precursors). At the same time, the low number of cases did not allow us to evaluate whether the inputs of cells or DNA could shed light on discordant cases of patients who were MRD positive by MFC and MRD negative by NGS. Another issue was the processing of samples from different tubes, with a possible difference in terms of quality, whereas we currently mix all tubes before the storage procedure to homogenise specific samples. Importantly, as recently reported by Costa et al., an early-pull BM aspirate should be used for MRD to avoid haemodilution, ideally followed by enrichment of CD138+ cells; this even in NGS analysis in order to reach a higher input of DNA.[11] Importantly, our study confirmed that, at present, the NGS analysis can only be performed by sending stored internal samples to the Adaptive Biotechnologies laboratory in Seattle (US-CA) and that sequencing and bioinformatic analyses are other crucial procedures that are currently performed by this facility.

Following the biological concordance of MRD samples, an intriguing question concerns the prognostic impact of the achievement of MFC-MRD negativity *vs* NGS-MRD negativity. In our analysis, we observed a clinical prognostic correlation between MFC and NGS in terms of PFS (HR 0.29 and 0.27, respectively) and OS (HR 0.35 and 0.31), thus confirming that both techniques are potent surrogates for survival and can be used in clinical practice to guide treatment decision-making.

Although a MRD threshold of $10^{-6}$ seems to be optimal,[12,13] its applicability is limited by the volume and quality of the BM sample and by the limited access to assays with LOD <$10^{-6}$. Even for the best-validated MRD assays, the threshold of $10^{-6}$ is near the edge of the assay performance capability, thus raising concerns about the use of such a threshold for decision making. In the NGS

| | OR | 95% CI | p-value |
|---|---|---|---|
| **1-year sustained MRD MFC** | | | |
| ISS: II/III *vs* I | 0.87 | (0.57–1.31) | 0.49 |
| LDH: >ULN *vs* ≤ULN | 0.86 | (0.44–1.67) | 0.64 |
| Cytogenetic risk per IMWG[a]: high *vs* standard | 0.79 | (0.48–1.3) | 0.35 |
| gain(1q21) (3 copies) *vs* normal 1q | 0.89 | (0.55–1.46) | 0.65 |
| amp(1q21) (≥4 copies) *vs* normal 1q | 0.63 | (0.29–1.37) | 0.24 |
| R1: KRd12 *vs* KRd plus ASCT | 0.57 | (0.35–0.91) | 0.02 |
| R1: KCd plus ASCT *vs* KRd plus ASCT | 0.38 | (0.23–0.63) | <0.001 |
| CTCs > 0.07% *vs* ≤ 0.07% | 0.36 | (0.21–0.61) | <0.001 |
| *C-index: 0.69* | | | |
| **2-year sustained MRD MFC** | | | |
| ISS: II/III *vs* I | 0.94 | (0.6–1.46) | 0.76 |
| LDH: >ULN *vs* ≤ULN | 0.80 | (0.38–1.67) | 0.54 |
| Cytogenetic risk per IMWG[a]: high *vs* standard | 0.64 | (0.37–1.11) | 0.11 |
| gain(1q21) (3 copies) *vs* normal 1q | 0.86 | (0.51–1.46) | 0.58 |
| amp(1q21) (≥4 copies) *vs* normal 1q | 0.53 | (0.22–1.3) | 0.16 |
| R1: KRd12 *vs* KRd plus ASCT | 0.56 | (0.34–0.91) | 0.02 |
| R1: KCd plus ASCT *vs* KRd plus ASCT | 0.37 | (0.21–0.62) | <0.001 |
| CTCs > 0.07% *vs* ≤ 0.07% | 0.40 | (0.22–0.72) | 0.002 |
| *C-index: 0.69* | | | |
| **1-year sustained MRD NGS** | | | |
| ISS: II/III *vs* I | 1.28 | (0.76–2.16) | 0.35 |
| LDH: >ULN *vs* ≤ULN | 0.54 | (0.23–1.3) | 0.17 |
| Cytogenetic risk per IMWG[a]: high *vs* standard | 0.75 | (0.41–1.38) | 0.35 |
| gain(1q21) (3 copies) *vs* normal 1q | 1.39 | (0.77–2.51) | 0.28 |
| amp(1q21) (≥4 copies) *vs* normal 1q | 1.24 | (0.49–3.12) | 0.65 |
| R1: KRd12 *vs* KRd plus ASCT | 0.76 | (0.42–1.38) | 0.36 |
| R1: KCd plus ASCT *vs* KRd plus ASCT | 0.58 | (0.31–1.07) | 0.08 |
| CTCs > 0.07% *vs* ≤ 0.07% | 0.39 | (0.2–0.76) | 0.005 |
| *C-index: 0.65* | | | |
| **2-year sustained MRD NGS** | | | |
| ISS: II/III *vs* I | 1.15 | (0.65–2.02) | 0.63 |
| LDH: >ULN *vs* ≤ULN | 0.67 | (0.27–1.63) | 0.37 |
| Cytogenetic risk per IMWG[a]: high *vs* standard | 0.58 | (0.29–1.14) | 0.11 |
| gain(1q21) (3 copies) *vs* normal 1q | 1.49 | (0.79–2.84) | 0.22 |
| amp(1q21) (≥4 copies) *vs* normal 1q | 1.63 | (0.63–4.2) | 0.31 |
| R1: KRd12 *vs* KRd plus ASCT | 0.74 | (0.38–1.42) | 0.36 |
| R1: KCd plus ASCT *vs* KRd plus ASCT | 0.59 | (0.30–1.14) | 0.12 |
| CTCs > 0.07% *vs* ≤ 0.07% | 0.44 | (0.22–0.90) | 0.02 |
| *C-index: 0.66* | | | |

Abbreviations: MRD, minimal residual disease; OR, odds ratio; CI, confidence interval; MFC, multiparameter flow cytometry; ISS, International Staging System stage; LDH, lactate dehydrogenase; ULN, upper limit of normal; IMWG, International Myeloma Working Group; amp, amplification; R1, first randomisation (induction-intensification-consolidation treatment); KRd, carfilzomib-lenalidomide-dexamethasone; ASCT, autologous stem-cell transplantation; MEL200, melphalan at 200 mg/m$^2$; KRd12, 12 KRd cycles without ASCT; KRd plus ASCT, 4 KRd induction cycles, MEL200-ASCT, 4 KRd consolidation cycles; KCd, carfilzomib-cyclophosphamide-dexamethasone; KCd plus ASCT, 4 KCd induction cycles, MEL200-ASCT, 4 KCd consolidation cycles; CTCs; circulating tumour plasma cells; NGS, next-generation sequencing; t, translocation; del, deletion. [a]High-risk cytogenetics were defined in accordance with the International Myeloma Working Group (IMWG) criteria: presence of t(4;14) and/or t(14;16) and/or del(17p).

***Table 2:*** **Multivariable logistic regression analysis of factors predicting prolonged 1-year and 2 year-sustained MRD negativity.**

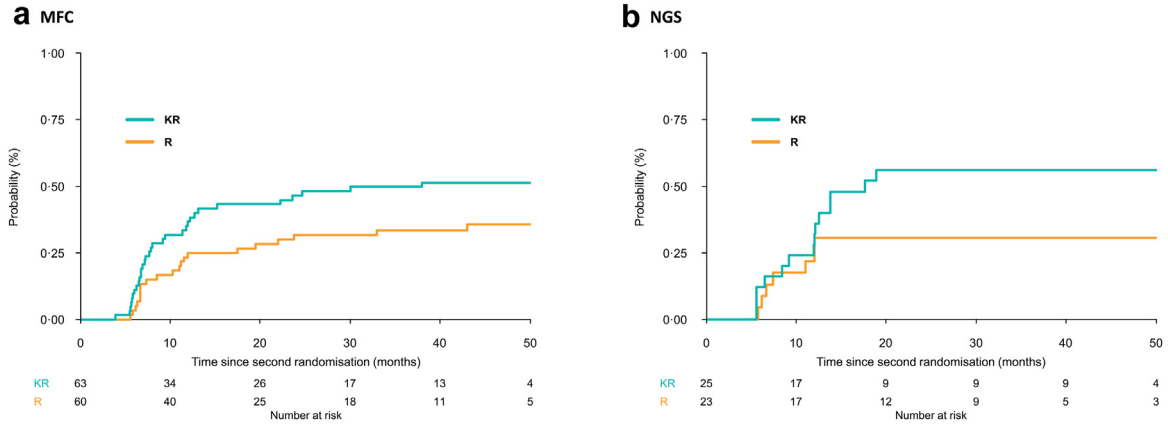



***Fig. 4:*** **Cumulative incidence of conversion from MRD positivity to negativity by MFC (4a) and NGS (4b) during maintenance treatment with KR *vs* R. Abbreviations**: MRD, minimal residual disease; MFC, multiparameter flow cytometry; NGS, next-generation sequencing; KR, maintenance treatment with carfilzomib-lenalidomide; R, maintenance treatment with lenalidomide alone.

analysis, we demonstrated that the $10^{-6}$ cut-off was feasible in 75% of patients *vs* 94% with the $10^{-5}$ cut-off, due to the lower number of B cells (and consequently DNA). This is the reason why we performed a survival analysis using a $10^{-5}$ cut-off, although it was tested down to 6.7 × 1e7 in a work supporting the filing acceptance by the U.S. Food and Drug Administration and completed down to 3 × 1e7 in an additional work.[28] Our choice was also supported by a minimum threshold of $10^{-5}$ recognised in the consensus criteria by the International Myeloma Working Group and the European Medicines Agency.[29,30]

Another important issue for MRD identification is a bias due to patchy BM sampling, which may result in failure to identify the tumour clone and false-negative MRD. PET/CT provides additional prognostic information to BM MRD assessment, with an observed low concordance between MRD in the BM and the resolution of metabolically active disease in PET/CT scans[31–33]: 11%–25% of patients had a residual PET/CT positivity despite the achievement of undetectable BM MRD.[31,32,34] In the companion study by Zamagni et al.,[35] the complementarity between MFC in the BM and imaging MRD by PET/CT was confirmed at pre-maintenance with a 64% of agreement between MFC and PET/CT focal lesions. The best outcomes were observed in patients who achieved MRD negativity by both techniques (survival probability at 24 and 48 months: 95% and 81%, respectively).

In this regard, the evaluation of circulating tumour plasma cells (CTCs) in MM patients can be a potential surrogate marker for the rate of dissemination and overall tumour burden in the BM, possibly overcoming the patchy and heterogenous distributions of bone lesions.[36,37] In a subanalysis of the FORTE trial, CTCs were recently confirmed to be baseline prognosticators and a tool to better stratify high-risk patients,[38,39] particularly those who were more ready to achieve MRD negativity and sustain it at 1 and 2 years. Unfortunately, we did not perform a specific MRD evaluation of patients with high *vs* low levels of CTCs to evaluate a possible correlation between peripheral blood and BM.

In our analysis, we also demonstrated that a 2-year sustained MRD negativity in the context of maintenance treatment after ASCT-consolidation was associated with an impressive 4-year PFS (99% for patients who achieved sustained MRD negativity by MFC and 97% by NGS). Recently, this was also demonstrated by Diamond et al.: patients who received lenalidomide maintenance and achieved a 2-year sustained MRD negativity had no recorded PD.[40] Moreover, our data are further corroborated by the CASSIOPEIA trial, in which patients who achieved a 1-year or 2-year sustained MRD negativity after induction showed an improved PFS, as compared with patients who did not, regardless of treatment received (1-year sustained MRD negativity: HR 0.20, $p < 0.0001$; 2-year sustained MRD negativity: HR 0.08; $p < 0.0001$).[41] In this regard, a crucial question concerns the optimal duration of maintenance therapy and whether MRD results can be used to determine treatment discontinuation or deintensification in patients with 1-year or 2-year sustained MRD negativity. The ongoing PERSEUS trial (NCT03710603) is investigating daratumumab-lenalidomide maintenance, with daratumumab that was discontinued after 2 years of sustained MRD negativity. The DRAMMATIC trial (NCT04071457) randomised patients with 2-year sustained MRD negativity to receive continuous maintenance with lenalidomide (±daratumumab) *vs* discontinuation.[42]

To conclude, our results confirmed that MRD detections by MFC and NGS are comparable predictors of long-term outcomes in NDTEMM patients and that

achieving and sustaining MRD negativity by either method correlated with prolonged survival. This remarkably suggests the possibility to use both techniques in the evaluation of one of the currently strongest predictors of outcome.

#### Contributors

All authors contributed to the acquisition, analysis, or interpretation of data for this work. All authors critically reviewed the work for important intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors had access to and verified the underlying data. SO, EG, MD, ES, BB, EZ, and FG contributed to conceptualisation, formal analysis, methodology, and writing of the original draft. SO and AC contributed to visualisation. PM, BB, MB, EZ, and FG contributed to supervision. All authors contributed to data curation, investigation, resources, validation, and writing of the manuscript in terms of review and editing. All authors had access to all the data reported in the study and had final responsibility for the decision to submit this manuscript for publication.

#### Data sharing statement

After the publication of this article, data collected for this analysis and related documents will be made available to others upon reasonably justified request, which needs to be written and addressed to the attention of the corresponding author Dr. Francesca Gay at the following e-mail address: francesca.gay@unito.it. The sponsor of the UNITO-MM-01/FORTE trial, the University of Torino (Italy), via the corresponding author Dr. Francesca Gay, is responsible to evaluate and eventually accept or refuse every request to disclose data and their related documents, in compliance with the ethical approval conditions, in compliance with applicable laws and regulations, and in conformance with the agreements in place with the involved subjects, the participating institutions, and all the other parties directly or indirectly involved in the participation, conduct, development, management and evaluation of this analysis.

#### Declaration of interests

SO has received honoraria from Amgen, Celgene/Bristol Myers Squibb, and Janssen; has served on the advisory boards for Adaptive Biotechnologies, Janssen, Amgen, and Takeda.

EG has received speaker honoraria from Werfen.

LP has received honoraria from Celgene, Takeda, Amgen, Bristol Myers Squibb, and Janssen; has served on the advisory boards for Celgene, Bristol Myers Squibb, Amgen, and Janssen; has received consultancy fees from Janssen.

MD has received honoraria for lectures from GlaxoSmithKline, Sanofi, and Janssen; has served on the advisory boards for GlaxoSmithKline, Sanofi, and Bristol Myers Squibb.

APJ is a full-time employee of and has received stock or stock options from Adaptive Biotechnologies.

FP has served on the advisory boards for Celgene, Bristol Myers Squibb, Janssen, Amgen, and GlaxoSmithKline.

ML has received honoraria from Gilead Sciences, Daiichi Sankyo, AbbVie, MSD, Novartis, Jazz Pharmaceuticals, Sanofi, and Grifols; has received support for travel, accommodations, and expenses from Gilead Sciences.

RM has received honoraria from Janssen, Celgene, Takeda, and Amgen; has served on the advisory boards for Janssen, Celgene, Takeda, Bristol Myers Squibb, and Amgen; has received consultancy fees from Janssen, Takeda, and Sanofi.

IRK is a full-time employee of and has received stock or stock options from Adaptive Biotechnologies.

AB has served on the advisory boards for Amgen, Janssen, Takeda, Celgene, and GlaxoSmithKline.

MC has received honoraria from Janssen, Celgene, Amgen, Bristol Myers Squibb, GlaxoSmithKline, Takeda, AbbVie, Sanofi, Pfizer, and Adaptive Biotechnologies; has served on the advisory boards for Janssen, Bristol Myers Squibb, Sanofi, Amgen, GlaxoSmithKline, and Pfizer; has served on the speakers' bureaus for Janssen, Celgene, and Sanofi.

PM has received honoraria from and/or served on scientific boards for AbbVie, Alexion, Amgen, AstraZeneca, Astellas, BeiGene, Bristol Myers Squibb/Celgene, Gilead, GlaxoSmithKline, Incyte, Janssen, Jazz, Novartis, Pfizer, Roche, Sanofi, and Takeda.

MB has received honoraria from Sanofi, Celgene, Amgen, Janssen, Novartis, Bristol Myers Squibb, and AbbVie; has served on the advisory boards for Janssen and GlaxoSmithKline; has received research funding from Sanofi, Celgene, Amgen, Janssen, Novartis, Bristol Myers Squibb, and Mundipharma.

EZ has received honoraria from Janssen, Bristol Myers Squibb, Amgen, and Takeda.

FG has received honoraria from Amgen, Celgene, Janssen, Takeda, Bristol Myers Squibb, AbbVie, and GlaxoSmithKline; has served on the advisory boards for Amgen, Celgene, Janssen, Takeda, Bristol Myers Squibb, AbbVie, GlaxoSmithKline, Roche, Adaptive Biotechnologies, Oncopeptides, bluebird bio, and Pfizer.

The other authors declare no competing financial interests.

#### Acknowledgments

The authors wish to thank all the study participants, referring clinicians, nurses, and data managers for their valuable contributions.

The UNITO-MM-01/FORTE trial was sponsored by the Università degli Studi di Torino (Italy), Department of Molecular Biotechnology and Health Sciences. Amgen and Celgene/Bristol Myers Squibb provided an unrestricted grant to conduct the trial, but had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or publication of this contribution. The research regarding next-generation sequencing in the UNITO-MM-01/FORTE trial was supported by a grant from the Multiple Myeloma Research Foundation (MMRF; Norwalk, US-CT).

The authors were not precluded from accessing data in the study, and they accept responsibility to submit this manuscript for publication.

#### Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102016.

#### References

1 Facon T, Kumar S, Plesner T, et al. Daratumumab plus lenalidomide and dexamethasone for untreated myeloma. *N Engl J Med*. 2019;380:2104–2115.
2 Mateos M-V, Dimopoulos MA, Cavo M, et al. Daratumumab plus bortezomib, melphalan, and prednisone for untreated myeloma. *N Engl J Med*. 2018;378:518–528.
3 Voorhees PM, Kaufman JL, Laubach JP, et al. Depth of response to daratumumab (DARA), lenalidomide, bortezomib, and dexamethasone (RVd) improves over time in patients (pts) with transplant-eligible newly diagnosed multiple myeloma (NDMM): griffin study update. *Blood*. 2019;134:691. Abstract #691 [ASH 2019 61st Meeting].
4 Moreau P, Attal M, Hulin C, et al. Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): a randomised, open-label, phase 3 study. *Lancet*. 2019;394:29–38.
5 San-Miguel J, Avet-Loiseau H, Paiva B, et al. Sustained minimal residual disease negativity in newly diagnosed multiple myeloma and the impact of daratumumab in MAIA and ALCYONE. *Blood*. 2022;139:492–501.
6 Stewart AK, Rajkumar SV, Dimopoulos MA, et al. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2015;372:142–152.
7 Gay F, Musto P, Rota-Scalabrini D, et al. Carfilzomib with cyclophosphamide and dexamethasone or lenalidomide and dexamethasone plus autologous transplantation or carfilzomib plus lenalidomide and dexamethasone, followed by maintenance with carfilzomib plus lenalidomide or lenalidomide alone for patients with newly diagnosed multiple myeloma (FORTE): a randomised, open-label, phase 2 trial. *Lancet Oncol*. 2021;22:1705–1720.

8 Landgren O, Devlin S, Boulad M, Mailankody S. Role of MRD status in relation to clinical outcomes in newly diagnosed multiple myeloma patients: a meta-analysis. *Bone Marrow Transplant*. 2016;51:1565–1568.
9 Munshi NC, Avet-Loiseau H, Rawstron AC, et al. Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma: a meta-analysis. *JAMA Oncol*. 2017;3:28–35.
10 Munshi NC, Avet-Loiseau H, Anderson KC, et al. A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma. *Blood Adv*. 2020;4:5988–5999.
11 Costa LJ, Derman BA, Bal S, et al. International harmonization in performing and reporting minimal residual disease assessment in multiple myeloma trials. *Leukemia*. 2021;35:18–30.
12 Perrot A, Lauwers-Cances V, Corre J, et al. Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. *Blood*. 2018;132:2456–2464.
13 Paiva B, Puig N, Cedena MT, et al. Measurable residual disease by next-generation flow cytometry in multiple myeloma. *J Clin Oncol*. 2020;38:784–792.
14 Stetler-Stevenson M, Paiva B, Stoolman L, et al. Consensus guidelines for myeloma minimal residual disease sample staining and data acquisition. *Cytometry B Clin Cytom*. 2016;90:26–30.
15 Arroz M, Came N, Lin P, et al. Consensus guidelines on plasma cell myeloma minimal residual disease analysis and reporting. *Cytometry B Clin Cytom*. 2016;90:31–39.
16 Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia*. 2017;31:2094–2103.
17 Rustad EH, Hultcrantz M, Yellapantula VD, et al. Baseline identification of clonal V(D)J sequences for DNA-based minimal residual disease detection in multiple myeloma. *PLoS One*. 2019;14:e0211600.
18 Hultcrantz M, Rustad EH, Yellapantula V, et al. Capture rate of V(D)J sequencing for minimal residual disease detection in multiple myeloma. *Clin Cancer Res*. 2022;28:2160–2166.
19 Arcila ME, Yu W, Syed M, et al. Establishment of immunoglobulin heavy (IGH) chain clonality testing by next-generation sequencing for routine characterization of B-cell and plasma cell neoplasms. *J Mol Diagnostics*. 2019;21:330–342.
20 Martinez-Lopez J, Lahuerta JJ, Pepin F, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. *Blood*. 2014;123:3073–3079.
21 Costa LJ, Chhabra S, Godby KN, et al. Daratumumab, carfilzomib, lenalidomide and dexamethasone (Dara-KRd) induction, autologous transplantation and post-transplant, response-adapted, measurable residual disease (MRD)-Based Dara-KRd consolidation in patients with newly diagnosed multiple myeloma (NDMM). *Blood*. 2019;134:860. Abstract #860 [ASH 2019 61st Meeting].
22 Adaptive Biotechnologies Corporation. clonoSEQ® assay technical information. https://www.clonoseq.com/wp-content/uploads/2021/08/PNL-10027-03_clonoSEQ-Technical-Information.pdf.
23 Vij R, Mazumder A, Klinger M, et al. Deep sequencing reveals myeloma cells in peripheral blood in majority of multiple myeloma patients. *Clin Lymphoma Myeloma Leuk*. 2014;14:131–139.e1.
24 Avet-Loiseau H, Bene MC, Wuilleme S, et al. Concordance of post-consolidation minimal residual disease rates by multiparametric flow cytometry and next-generation sequencing in CASSIOPEIA. *Clin Lymphoma Myeloma Leuk*. 2019;19:e3–e4 [Abstract #OAB–004, 17th IMW 2019].
25 Kriegsmann K, Hundemer M, Hofmeister-Mielke N, et al. Comparison of NGS and MFC methods: key metrics in multiple myeloma MRD assessment. *Cancers (Basel)*. 2020;12:2322.
26 Mina R, Oliva S, Boccadoro M. Minimal residual disease in multiple myeloma: state of the art and future perspectives. *J Clin Med*. 2020;9:2142.
27 Cloos J, Harris JR, Janssen JJWM, et al. Comprehensive protocol to sample and process bone marrow for measuring measurable residual disease and leukemic stem cells in acute myeloid leukemia. *J Vis Exp*. 2018;5:56386.
28 Ching T, Duncan ME, Newman-Eerkes T, et al. Analytical evaluation of the clonoSEQ Assay for establishing measurable (minimal) residual disease in acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma. *BMC Cancer*. 2020;20:612.
29 Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*. 2016;17:e328–e346.
30 European Medicines Agency. Guideline on the use of minimal residual disease as a clinical endpoint in multiple myeloma studies. https://www.ema.europa.eu/en/guideline-use-minimal-residual-disease-clinical-endpoint-multiple-myeloma-studies#current-version-section; 2018.
31 Moreau P, Attal M, Caillot D, et al. Prospective evaluation of magnetic resonance imaging and [18F]Fluorodeoxyglucose positron emission tomography-computed tomography at diagnosis and before maintenance therapy in symptomatic patients with multiple myeloma included in the IFM/DFCI 2009 trial. *J Clin Oncol*. 2017;35:2911–2918.
32 Moreau P, Zweegman S, Perrot A, et al. Evaluation of the prognostic value of positron emission tomography-computed tomography (PET-CT) at diagnosis and follow-up in transplant-eligible newly diagnosed multiple myeloma (TE NDMM) patients treated in the phase 3 Cassiopeia study: results of the Cassiopet companion study. *Blood*. 2019;134:692. Abstract #692 [ASH 2019 61st Meeting].
33 Alonso R, Cedena MT, Gómez-Grande A, et al. Imaging and bone marrow assessments improve minimal residual disease prediction in multiple myeloma. *Am J Hematol*. 2019;94:853–861.
34 Rasche L, Alapat D, Kumar M, et al. Combination of flow cytometry and functional imaging for monitoring of residual disease in myeloma. *Leukemia*. 2019;33:1713–1722.
35 Zamagni E, Oliva S, Gay F, et al. Impact of minimal residual disease standardised assessment by FDG-PET/CT in transplant-eligible patients with newly diagnosed multiple myeloma enrolled in the imaging sub-study of the FORTE trial. *eClinicalMedicine*. 2023;60:102017.
36 Sanoja-Flores L, Flores-Montero J, Puig N, et al. Blood monitoring of circulating tumor plasma cells by next generation flow in multiple myeloma after therapy. *Blood*. 2019;134:2218–2222.
37 Chakraborty R, Muchtar E, Kumar SK, et al. Serial measurements of circulating plasma cells before and after induction therapy have an independent prognostic impact in patients with multiple myeloma undergoing upfront autologous transplantation. *Haematologica*. 2017;102:1439–1445.
38 Bertamini L, Oliva S, Rota-Scalabrini D, et al. High levels of circulating tumor plasma cells as a key hallmark of aggressive disease in transplant-eligible patients with newly diagnosed multiple myeloma. *J Clin Oncol*. 2022;40:3120–3131.
39 Garcés J-J, Cedena M-T, Puig N, et al. Circulating tumor cells for the staging of patients with newly diagnosed transplant-eligible multiple myeloma. *J Clin Oncol*. 2022;40(27):3151–3161.
40 Diamond B, Korde N, Lesokhin AM, et al. Dynamics of minimal residual disease in patients with multiple myeloma on continuous lenalidomide maintenance: a single-arm, single-centre, phase 2 trial. *Lancet Haematol*. 2021;8:e422–e432.
41 Avet Loiseau H, Sonneveld P, Moreau P, et al. Daratumumab (DARA) with bortezomib, thalidomide, and dexamethasone (VTd) in transplant-eligible patients (pts) with newly diagnosed multiple myeloma (NDMM): analysis of minimal residual disease (MRD) negativity in cassiopeia Part 1 and Part 2. *Blood*. 2021;138:82–85 [Abstract, ASH 2021 63rd Meeting].
42 Krishnan A, Hoering A, Hari P, Sexton R, Orlowski RZ. Phase III study of daratumumab/rhuph20 (nsc- 810307) + lenalidomide or lenalidomide as post-autologous stem cell transplant maintenance therapyin patients with multiple myeloma (mm) using minimal residual disease todirect therapy duration (DRAMMATIC study): SWOG s1803. *Blood*. 2020;136:21–22 [Abstract #1515, ASH 2020 62nd Meeting].