The prognostic value of multiparameter flow cytometry minimal residual disease assessment in relapse multiple myeloma

by Bruno Paiva, Mauricio Chandia, Noemi Puig, Maria-Belen Vidriasles, Jose J. Perez, Lucia Lopez-Corral, Enrique M. Ocio, Ramon Garcia-Sanz, Norma C. Gutierrez, Ana Jimenez-Ubieto, Juan-Jose Lahuerta, Maria Victoria Mateos, and Jesus F. San Miguel

Haematologica 2014 [Epub ahead of print]

doi:10.3324/haematol.2014.115162

Publisher's Disclaimer
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
The prognostic value of multiparameter flow cytometry minimal residual disease assessment in relapse multiple myeloma

Running head: Prognostic value of MRD in relapse myeloma

*Bruno Paiva 1, *Mauricio Chandia 2, Noemi Puig 2, Maria-Belen Vidriales 2, Jose J. Perez 2, Lucia Lopez-Corral 2, Enrique M. Ocio 2, Ramon Garcia-Sanz 2, Norma C. Gutierrez 2, Ana Jimenez-Ubieto 3, Juan-Jose Lahuerta 3, Maria-Victoria Mateos 2, Jesus F. San Miguel 1

* Both authors contributed equally

1Clinica Universidad de Navarra, Centro de Investigacion Medica Aplicada (CIMA), Pamplona, 2Hospital Universitario de Salamanca, Instituto de Investigacion Biomedica de Salamanca, IBMCC (USAL-CSIC), Salamanca, Spain; 3Hospital 12 de Octubre, Madrid. Spain

Correspondence

Jesus F. San Miguel, M.D; Ph.D.,
Clinica Universidad de Navarra; Centro de Investigacion Medica Aplicada (CIMA)
Av. Pio XII 36, 31008 Pamplona, Spain

E-mail: sanmiguel@unav.es
Achieving deep levels of remission is one of the pre-requisites to reach long-term survival in solid tumors and hematological malignancies, and this has also been proved in newly-diagnosed multiple myeloma (MM) patients particularly in the era of novel agents. (1-3) Accordingly, both the Spanish and UK groups have shown the prognostic value and clinical relevance of minimal residual disease (MRD) monitoring by multiparameter flow cytometry (MFC) in both newly-diagnosed transplant candidates and elderly MM patients treated with novel agents.(4-6) However, the value of the depth of response in the relapse setting has been far less investigated as compared to the up-front setting.(7-11) In fact, data exploring different outcomes between patients achieving very good partial response (VGPR) or complete response (CR) with salvage therapy is scanty (7-11), and there is no information regarding the prognostic value of achieving immunophenotypic or molecular responses at relapse. If MRD-negativity would translate into superior outcomes similarly to that observed in newly-diagnosed patients, then it could become a desirable end-point for clinical trials exploring new drugs for relapse/refractory patients.

In the present study we focused on a total of 52 patients that after clinical relapse achieved CR with salvage therapy. Patients were divided into two categories: 21 rescued with novel agents followed by allogeneic stem cell transplantation (alloSCT; n=21), and 31 patients rescued and achieving CR with novel agents (in all except six) followed or not by autologous stem cell transplantation (autoSCT) (11 and 14 cases, respectively).

All samples were collected after informed consent was given by each individual, according to the local ethical committees and the Helsinki Declaration protocol. Median follow-up was of 2.7 years (32 months). Multiparameter flow cytometry (MFC) studies were performed on BM samples using 4-color monoclonal antibody combinations (FITC
Plasma cells (PCs) were initially identified on the basis of strong CD38 expression and intermediate side scatter signals; discrimination between clonal and normal PCs was performed by the recognition of aberrant phenotypic expression profiles such as simultaneous down-regulation of CD19 and CD45, with or without over-expression of CD56. For patients in whom CD45 or CD19 was positively expressed, lack of CD19 or CD45, respectively, dim CD38 intensity and/or bright CD56 staining (equal or higher than that of natural-killer cells) allowed identification of clonal PCs in the vast majority of cases; in selected patients (n=7), CD56 was replaced by CD28, CD81 or CD117 since these markers were known to be more informative according to the baseline phenotypic evaluation. Data acquisition was performed in FACSCalibur and FACSCantoll flow cytometers (Becton Dickinson Biosciences – BDB – San Jose, CA) using the FACSDiva 6.1 software (BDB), and a two-step acquisition procedure allowing for $2 \times 10^5$ leucocytes/tube to be selectively stored. Data analysis was performed using the Paint-a-Gate (BDB) and the Infinicyt software (Cytognos SL, Salamanca, Spain). Patients were defined as being MRD-negative when less than 20 clonal PCs were detectable by MFC, at a sensitivity level of $10^{-4}$. Time-to progression (TTP) was measured from the moment of MRD assessment to the date of progression or last visit; overall survival (OS) was calculated from MRD assessment until the date of death from any cause or until the last date at which the patient was known to be alive. Curves were plotted by the Kaplan-Meier method, and the log-rank test was used to estimate the statistical significance of differences observed between curves. The Cox proportional-hazards model was used to estimate hazard ratios (HR) and 95% confidence intervals (CI), as well as to perform a multivariate analysis including patients’ MRD status (dichotomized into negative/positive), type of treatment (dichotomized into yes/no): autoSCT, novel agents; proteasome inhibition, immune modulators, as well as the event of extramedullary relapses (dichotomized into yes/no). The $X^2$ test was used to estimate
the statistical significance of differences observed between groups. For all statistical analyses, the SPSS software (version 15.0; SPSS Inc., Chicago, IL) was used.

First, we focused on the more standard treatment population of the 31 patients attaining CR after salvage therapy in the non-alloSCT setting (Table 1). Among them, 13 achieved MRD-negative (42%) whereas in the remaining 18 (58%) cases persistent MRD was detected by MFC (median of 0.2% clonal PC among total BM leukocytes; range: 0.01% - 1.67%). MRD-negative cases showed a median TTP of 75 months, whereas 17 of the 18 MRD-positive CR patients progressed with a median TTP of only 14 months (HR: 2.8, 95% CI: 1.0 – 7.6; P = .039) (Figure 1A). Similar results were observed while specifically analyzing patients’ TTP during the first 3-years after MRD assessment (Figure 3B), with median TTP for MRD-negative cases not yet reached vs. 14 months among MRD-positive patients (P = .037). On the multivariate analysis for TTP that included in addition to patients’ MRD status the type of treatment being used (i.e.: autoSCT, novel agents; proteasome inhibition, immune modulators) and the event of extramedullary relapses, MRD status showed a HR of 2.96 (95% CI: 0.8 – 10.7) and a P-value = 0.098; all other variables were non-significant and showed inferior P-values (data not shown). Additional studies in larger series of patients are therefore warranted to assess the independent prognostic value of MRD monitoring over the type of salvage therapy and type of relapse in MM (i.e.: medullary vs. extramedullary). Afterwards, we focused on the 21 patients in CR after alloSCT, and observed that 10 (48%) failed to eradicate MRD (median of 0.12% clonal PC among total BM leukocytes; range: 0.01% - 0.7%). Surprisingly, there were no significant differences according to the presence vs. absence of MRD in TTP (P = .77) among patients in CR after alloSCT. These observations led us to investigate whether this phenomenon could be associated with a higher incidence of extramedullary relapses (and therefore potentially missed on BM monitoring) after alloSCT. Accordingly, a total of 11 extramedullary relapses were observed among patients in CR after alloSCT, 7 of which among MRD-
negative patients. By contrast, only 2 out of 31 patients in the non-alloSCT setting had extramedullary relapses (both MRD-positive).

In summary, we show that CR patients after salvage therapy constitute a heterogeneous subgroup with approximately half of the cases showing persistent MRD and early relapse (approximately 1-year). Patients with MRD-negativity experience significantly prolonged TTP outside of the alloSCT setting, and further studies with larger series of patients are warranted to confirm if MRD-negativity could become an end-point for novel drugs being tested in relapsed/refractory patients. The likelihood of extramedullary relapses even among MRD-negative patients after alloSCT suggests that at least for this particular therapeutic strategy, response assessment should include combined medullar and extramedullary (PET/CT) measure of MRD.
Acknowledgments: This study was supported by the Cooperative Research Thematic Network grants RD12/0036/0058 of the Red de Cancer (Cancer Network of Excellence); Instituto de Salud Carlos III, Spain, Instituto de Salud Carlos III/Subdirección General de Investigación Sanitaria (FIS: PI060339; 06/1354; 02/0905; 01/0089/01-02; PS09/01897/01370; G03/136; Sara Borrell: CD13/00340); and Asociación Española Contra el Cáncer (GCB120981SAN), Spain. The study was also supported internationally by the International Myeloma Foundation Junior Grant Proposal and the Multiple Myeloma Research Foundation research fellow award.


Conflict of interest: There are no conflicts of interest to disclose
References


Table 1. Minimal residual disease (MRD) rates and prognostic value according to the different treatment schemas used on relapsing multiple myeloma patients.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MRD positive n=28; (54%)</th>
<th>MRD negative n=24; (46%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy without SCT, n=20</td>
<td>14 (70%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Conventional, n=6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Novel agents, n=14</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Chemotherapy with autoSCT, n=11</td>
<td>4 (47%)</td>
<td>7 (63%)</td>
</tr>
<tr>
<td>Chemotherapy with alloSCT, n=21</td>
<td>10 (48%)</td>
<td>11 (52%)</td>
</tr>
</tbody>
</table>

SCT: stem cell transplantation
Figure legends.

Figure 1. (A) Time-to progression (TTP) of multiple myeloma (MM) patients in complete response (CR) after salvage chemotherapy with or without autoSCT (n=31), according to the absence (MRD-negative) vs. presence of phenotypically aberrant clonal plasma cells (MRD-positive). (B) Specific analysis of TTP during the first 3-years after MRD assessment among MM patients in CR after salvage chemotherapy with or without autoSCT. (C) TTP of MM patients in CR after salvage chemotherapy with alloSCT (n=21), according to patients’ MRD status.
A

HR: 2.8, 95% CI: 1.0 – 7.6; P = .03

Time to progression (%)

Time from MRD assessment (months)

MRD-positive: 18 7 2 1 1 1 1 1 1
MRD-negative: 13 5 2 2 1 1 1 1 1 0

B

HR: 3.0, 95% CI: 1.0 – 9.2; P = .03

Time to progression (%)

Time from MRD assessment (months)

MRD-positive: 18 12 7 5
MRD-negative: 13 13 5 3
c

\[ P = 7.1 \]

\begin{tabular}{l|cccccc}
  & Time from MRD assessment (months) \\
  & 0 & 20 & 40 & 60 & 80 & 100 & 120 \\
MRD-positive: & 10 & 1 & 0 & 0 & 0 & 0 & 0 \\
MRD-negative: & 11 & 4 & 1 & 1 & 1 & 1 & 1 \\
\end{tabular}