Minimal Residual Disease assessment in Multiple Myeloma by Next-Generation Sequencing

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MRD Meta-Analysis in Myeloma
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Why to assess MRD in Myeloma?

The main rationale is the correlation response/outcome

<table>
<thead>
<tr>
<th>Response</th>
<th>CR</th>
<th>nCR</th>
<th>PR</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS Medians EFS, months</td>
<td>61</td>
<td>40</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>Medians OS, months</td>
<td>NR</td>
<td>NR</td>
<td>61</td>
<td>15</td>
</tr>
</tbody>
</table>

Lahuerta et al. *JCO* 2008
Why to assess MRD in Myeloma?

Treatment advances have increased the likelihood of achieving CR.

Induction regimen

- ORR
- ≥VGPR
- CR/nCR

Why to assess MRD in Myeloma?

However, a large majority of pts with CR eventually relapse, suggesting that undetectable, but clinically meaningful MRD may be present.

![Graph showing patients responding to different induction regimens](https://via.placeholder.com/577)

**Induction regimen**

- ORR
- ≥VGPR
- CR/nCR

**Patients responding (%)**

Why to assess MRD in Myeloma?

Diagnosis $10^{12}$

CR $10^{10}$

80% HD trial
MRD: What are the techniques?

(Old) Gold Standard: Multiparametric Flow Cytometry

Myeloma cells present a specific phenotype / normal PC

This phenotype is stable during evolution
MRD: What are the techniques?

Next-Generation Sequencing

Myeloma cells present unique Ig gene rearrangements

These clonal rearrangements are stable during evolution
**NGS: Technical principles**

**Functional Allele**

**Locus IGK 2p11**

**Non-Functional allele**

**Locus IGH 14q32**

**Locus IGK 2p11**
NGS: Technical principles

Non B cell Leukocytes
Normal B cells
Myeloma cells

FREQUENCY OF MYELOMA CLONE AMONG B CELLS = $S_L / (S_L + S_R)$

NUMBER OF MYELOMA MOLECULES PER LEUKOCYTE = $S_L \times (N_R / S_R) / N_{TOT}$

QUANTITATE DNA

$D = $ Amount of DNA
$K = $ DNA per Cell

$N_{TOT} = D/K = $ Total Number of Leukocytes

EXTRACT DNA

ADD KNOWN QUANTITY OF REFERENCE IgH

AMPLIFY IgH MOLECULES

SEQUENCE MOLECULES

$N_R = $ Number of Reference Molecules

$S_B = $ Number of B Cell Reads

$S_L = $ Number of Leukemia Reads

$S_R = $ Number of Reference Reads

TCGATAGGATTGAGG
ATGATTAGGATTGAGG
ACGTGATTGATTGAGG
TAGATTGATTGAGG
CGCTGATTGATTGAGG
AGCTGATTGATTGAGG

GCATTGATTGATTGAGG
ACATTGATTGATTGAGG
CCATTGATTGATTGAGG
CGATTGATTGATTGAGG
CGATTGATTGATTGAGG
CGATTGATTGATTGAGG
CGATTGATTGATTGAGG
Is MRD clinically pertinent?
Clinical utility of immunoglobulin heavy chain gene rearrangement identification for tumour cell detection in multiple myeloma

Agneta Swedin,1 Stig Lenhoff,1 Tor Olofsson,2 Brit Theruesson2 and Jan Westin1
1Division of Haematology, Department of Medicine, and 2Blood Centre, University Hospital, Lund, Sweden

High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cyometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma

BLOOD, 19 JANUARY 2012 - VOLUME 119, NUMBER 3

Minimal Residual Disease Assessed by Multiparameter Flow Cytometry in Multiple Myeloma: Impact on Outcome in the Medical Research Council Myeloma IX Study

Mark Korthals1,2, Nina Sehnke1, Ralf Kronenwett1,2, Thomas Schroeder1, Tobias Strapatsas1, Guido Kobbe1, Rainer Haas1, Roland Fenk1,2
1Department of Haematology, Oncology and Clinical Immunology, Klinikum Rechts der Isar, Technische Universität München, Germany
2Department of Functional Genomics and Medical Imaging, University Magdeburg, Magdeburg, Germany

Molecular Monitoring of Minimal Residual Disease in the Peripheral Blood of Patients with Multiple Myeloma

BLOOD, 15 MAY 2014 - VOLUME 123, NUMBER 20

Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma

Joaquin Martinez-Lopez1, Juan J. Lahuent1, Franca Pizzuto1, Marcos Gonzalez1, Santiago Barro1, Rosa Ayala1, Niemi Puig1, Maria A. Menthban1, Bruno Paiva1, Li Wend1, Cristina Jimenez1, Maria Josefa1, Martin Mooij1, Teresa Caden1, Immaculada Repol1, Maria Victoria Mateos1, Laura Rosil1, Abo Osi1, Maria J. Blanchard1, Pablo Martinez1, Joan Blasco1, Jesus Santamaria1, Mateu Fadurn1, and Ramon Garzia-Senz1
1Hospital Universitario 12 de Octubre, Madrid, Spain; 2Serpenta, Inc., San Francisco, CA; 3Hospital Universitario de Salamanca-BSAL, BIMOC-CIBIC, Salamanca, Spain; 4Cinco de Mayo University of Navarra, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Pamplona, Spain; 5Hospital Clinic i Provincial de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; 6Hospital Germans Trias i Pujol, Barcelona, Spain; 7Hospital Ramón y Cajal, Madrid, Spain; and 8Hospital Clínico de Madrid, Madrid, Spain
Significant Impact of Minimal Residual Disease (MRD) Status On Survival Outcomes In pts (pts) With Multiple Myeloma (MM) Who Achieve Complete Response (CR): A Meta-Analysis

• A total of 405 published articles with MRD
  – 25 articles recently published articles
• Of these, 21 reported overall survival (OS) or progression-free survival (PFS) results, as well as MRD status
• Overall, 2,208 pts were evaluated for MRD
• Nine publications reported conventional CR at the time of MRD measurement. Six represented unique data sets.

Munshi N et al., JAMA Oncol, in press
Refining the literature search

Exclusion criteria

• Publications were excluded if they:
  - Only included patients with relapsed and/or refractory MM
  - Included patients who had undergone allogenic SCT
  - Assessed MRD in apheresis product
  - Reported on the same study population used in an already-included trial

• Analysis was restricted to techniques with a detection limit of \( \leq 0.01\% \)

MM, multiple myeloma; MRD, minimal residual disease; SCT, stem cell transplant.
21 articles retrieved in total

- Any response-achieving patients (n = 13 studies)
- CR-achieving patients (n = 4 studies)

OS, overall survival; PFS, progression-free survival.

Munshi N et al., JAMA Oncol, in press
The effect of MRD status on PFS and OS (All patients)

Munshi N et al., JAMA Oncol, in press
The effect of MRD status on PFS and OS
(All patients)

Munshi N et al., JAMA Oncol, in press
The effect of MRD status on PFS and OS (CR patients)

Munshi N et al., JAMA Oncol, in press
The effect of MRD status on PFS (CR patients)

CR-achieving patients

χ² (adjusted) = 35.85; \( P < 0.0001 \)

Number at risk by year:

<table>
<thead>
<tr>
<th>Year</th>
<th>MRD-negative (n=389)</th>
<th>MRD-positive (n=155)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>389</td>
<td>155</td>
</tr>
<tr>
<td>2</td>
<td>359</td>
<td>129</td>
</tr>
<tr>
<td>4</td>
<td>301</td>
<td>86</td>
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<tr>
<td>6</td>
<td>211</td>
<td>51</td>
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<tr>
<td>8</td>
<td>155</td>
<td>33</td>
</tr>
<tr>
<td>10</td>
<td>96</td>
<td>19</td>
</tr>
<tr>
<td>12</td>
<td>65</td>
<td>12</td>
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<tr>
<td></td>
<td>35</td>
<td>7</td>
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<td>16</td>
<td>7</td>
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<tr>
<td></td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>0</td>
</tr>
</tbody>
</table>

- 3-year PFS: 70% (MRD\(^{-}\)) vs. 46% (MRD\(^{+}\))
- 5-year PFS: 48% (MRD\(^{-}\)) vs. 27% (MRD\(^{+}\))
- Majority of MRD-positive patients progressed by 6 years; nearly 50% of MRD-negative patients progression free

Munshi N et al., JAMA Oncol, in press
The effect of MRD status on OS (CR patients)

CR-achieving patients

\[ \chi^2 \text{ (adjusted)} = 15.06; \quad P < 0.0001 \]

Number at risk by year:

<table>
<thead>
<tr>
<th>Year</th>
<th>MRD-negative (n=362)</th>
<th>MRD-positive (n=134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>362</td>
<td>134</td>
</tr>
<tr>
<td>1</td>
<td>359</td>
<td>131</td>
</tr>
<tr>
<td>2</td>
<td>331</td>
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<td>3</td>
<td>274</td>
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<td>4</td>
<td>218</td>
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<td>5</td>
<td>138</td>
<td>35</td>
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<tr>
<td>6</td>
<td>76</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

- Median OS was not reached for MRD-negative pts versus 82 months for MRD-positive pts)
- OS @ 3-years, 94% versus 80%
- OS @ 7-years, 67% versus 47%
- OS @ 5-years, 80% versus 61%

CR, complete response; MRD, minimal residual disease; NR, not reached; OS, overall survival.

Conclusions of the meta-analysis

MRD is definitely predictive of both longer PFS and OS

Most of the available results are from MFC

MFC has a quite low sensitivity \((10^{-4})\)

Following question:

→ Would a higher sensitivity have a better predictivity?
Higher sensitivity: NGS

Martinez-Lopez J et al., Blood 2014;123:3073
IFM DFCI 2009 Trial
700 patients < 66y,
Newly diagnosed symptomatic MM

3 RVD

5 RVD

MEL200 + ASCT

2 RVD

12 months Lenalidomide maintenance

* Primary objective = 7-color Flow, Secondary objective = Molecular
IFM 2009 trial

54% Conventional CR

289 patients analyzed by NGS

Applicability of NGS: 92% (8% clone ID failure)

Median follow-up: 44 months
P-value (trend): p<0.0001

Patients without progression (%)

N at risk (events)

<10^{-6} 87 (0) 87 (0) 87 (2) 85 (2) 83 (6) 74 (4) 54 (3) 31 (0) 8
[10^{-6};10^{-5}] 31 (0) 31 (1) 30 (2) 28 (0) 27 (4) 22 (1) 17 (2) 8 (1) 4
[10^{-5};10^{-4}] 49 (0) 49 (2) 47 (2) 45 (2) 43 (7) 34 (4) 22 (6) 8 (0) 2
[10^{-4};10^{-3}] 79 (0) 79 (9) 70 (11) 59 (9) 50 (11) 38 (6) 28 (9) 6 (3) 0
P-value (trend) : p<0.0001

Patients without progression (%)

N at risk (events)

<table>
<thead>
<tr>
<th>N x 10^2</th>
<th>N x 10^1</th>
<th>N x 10^0</th>
<th>N x 10^1</th>
<th>N x 10^2</th>
<th>N x 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10^-6</td>
<td>86 (0)</td>
<td>86 (0)</td>
<td>86 (0)</td>
<td>86 (0)</td>
<td>86 (5)</td>
</tr>
<tr>
<td>10^-6;10^-5</td>
<td>29 (0)</td>
<td>29 (0)</td>
<td>29 (0)</td>
<td>29 (0)</td>
<td>28 (5)</td>
</tr>
<tr>
<td>10^-5;10^-4</td>
<td>23 (0)</td>
<td>23 (0)</td>
<td>23 (0)</td>
<td>23 (1)</td>
<td>22 (3)</td>
</tr>
<tr>
<td>10^-4;10^-3</td>
<td>40 (0)</td>
<td>40 (0)</td>
<td>40 (0)</td>
<td>40 (6)</td>
<td>33 (9)</td>
</tr>
</tbody>
</table>

MRD at post-maintenance

Months since randomization

P-value (trend) : p<0.0001

IFM 2009 trial
IFM 2009 trial
FCM Negative Patients

P-value: $p<0.0001$

P-value: $p<0.0001$

N at risk (events)

<table>
<thead>
<tr>
<th></th>
<th>MRD neg ($&lt;10^{-6}$)</th>
<th>MRD positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>87 (0)</td>
<td>159 (0)</td>
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<tr>
<td></td>
<td>87 (0)</td>
<td>159 (12)</td>
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<tr>
<td></td>
<td>87 (2)</td>
<td>147 (15)</td>
</tr>
<tr>
<td></td>
<td>85 (2)</td>
<td>132 (11)</td>
</tr>
<tr>
<td></td>
<td>83 (6)</td>
<td>120 (22)</td>
</tr>
<tr>
<td></td>
<td>74 (4)</td>
<td>94 (11)</td>
</tr>
<tr>
<td></td>
<td>54 (3)</td>
<td>67 (17)</td>
</tr>
<tr>
<td></td>
<td>31 (0)</td>
<td>22 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 (6)</td>
</tr>
</tbody>
</table>
IFM 2009 trial
FCM Negative Patients

P-value : p<0.0001

Patients without progression (%)

Negative (<10^-6)

Positive

Months since randomization

N at risk (events)

<table>
<thead>
<tr>
<th>MRD neg (&lt;10^-6)</th>
<th>86</th>
<th>86</th>
<th>86</th>
<th>86</th>
<th>86</th>
<th>86</th>
<th>77</th>
<th>61</th>
<th>36</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRD positive</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>83</td>
<td>64</td>
<td>45</td>
<td>11</td>
<td>5</td>
</tr>
</tbody>
</table>
### MRD by NGS vs MFC

<table>
<thead>
<tr>
<th>NGS</th>
<th>FCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Highly sensitive ($&gt; 10^{-6}$)</td>
<td>- Less sensitive ($10^{-5}$)</td>
</tr>
<tr>
<td>- Standardized</td>
<td>- Many different panels</td>
</tr>
<tr>
<td>- Informative in 92% of pts</td>
<td>- Informative in 100% of pts</td>
</tr>
<tr>
<td>- Frozen samples</td>
<td>- Fresh samples</td>
</tr>
</tbody>
</table>
Conclusions

The highest sensitivity is the most discriminant
\[ \rightarrow 10^{-6} \] is required

NGS is probably the technique of choice

MFC should be used in case of clone ID failure

MRD could (should?) be the main objective of future trials

MRD could identify cured patients
Conclusions

Limitations: MRD evaluates MM in one BM region

→ What about other regions?

Solutions:

• cfDNA sequencing analyses? → ongoing
• Imaging techniques: PET-TDM+++
PFS according to PET-SUV post ASCT PET/CT
in patients achieving CR

PET-SUV
100% reduction
61%

PET-SUV
< 100% reduction
30%

Logrank P-value = .0195

Months
0 12 24 36 48 60
IFM 2009 trial

PET-CT normalisation before maintenance
Impact on PFS (62% normalised)

p < 0.001
Conclusions

New concepts in assessment of response in MM

- IMWG consensus (Kumar et al., Lancet Oncol, in press)
  - MRD in BM with the most sensitive technique
  - PET-TDM