Towards a comprehensive multimodal minimal residual disease assessment in multiple myeloma: the role of circulating cell-free DNA to define the extent of disease spreading

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I have nothing to disclose
Despite the clear clinical impact of achieving MRD negativity at the level of $10^{-6}$, biological relapses may still occur at a significant rate.

1) QUALITY OF BM SAMPLE
The marrow aspiration can lead to significant blood "contamination" and underestimation of PCs burden.

2) QUALITY OF RESIDUAL CELLS
A certain amount of residual and undetectable cells still remains and influence prognosis.

3) SPATIAL HETEROGENEITY OF MM
A cardinal feature of MM disease that can lead to misleading MRD results derived from a single BM biopsy.

Bal S. et al., BJH 2019
Rasche L et al., Nat Comm 2019
Kumar et al., Lancet Oncol 2016
Ledergor G et al., Nat Med 2020
Goicoechea I et al., Blood 2020
Perrot et al., Blood 2018
Paiva et al., JCO 2019
Moreau et al., Blood 2019
AIM of the study

LIQUID BIOPSY: a valuable opportunity to both profile MM disease and to possibly implement minimal residual disease assessment through a less invasive patients’ monitoring

1) Identify the level of concordance between cfDNA-BM-PET at diagnosis
2) Monitoring cfDNA-BM-PET during follow-up
EXPERIMENTAL PLAN
StreaMMing project

AT BASELINE on 139 patients

- Genomic quantitative and qualitative profiling by Ultra Low Pass WGS both on gDNA and cfDNA

FOLLOW-UP MONITORING of 22 patients

- MRD by NGS on BM and Whole body FDG-PET/CT every 6 months
- ULPWGS on cfDNA every months
Patients’ cohort description

<table>
<thead>
<tr>
<th>N. Patients</th>
<th>78</th>
<th>%</th>
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<tbody>
<tr>
<td>AGE (M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65yr</td>
<td>26</td>
<td>31,3</td>
</tr>
<tr>
<td>&lt;65yr</td>
<td>57</td>
<td>68,7</td>
</tr>
<tr>
<td>SEX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>43,5</td>
</tr>
<tr>
<td>F</td>
<td>31</td>
<td>33,5</td>
</tr>
<tr>
<td>B2M (M)</td>
<td>3,1 (1,2-13,7)</td>
<td></td>
</tr>
<tr>
<td>Alb (M)</td>
<td>3,4 (2,4-5,35)</td>
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<tr>
<td>Creatinine (M)</td>
<td>0,88 (0,48-8)</td>
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<tr>
<td>Clearance &gt;50</td>
<td>3</td>
<td>7,5</td>
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<tr>
<td>HB &gt;105</td>
<td>36</td>
<td>43,4</td>
</tr>
<tr>
<td>PLT &gt;150</td>
<td>12</td>
<td>14,4</td>
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<tr>
<td>PC &gt;60%</td>
<td>31</td>
<td>41,3</td>
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<tr>
<td>LDH</td>
<td>8</td>
<td>11,4</td>
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<tr>
<td>IgG</td>
<td>55</td>
<td>79,7</td>
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<tr>
<td>IgA</td>
<td>11</td>
<td>14,6</td>
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<tr>
<td>BJ</td>
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<tr>
<td>IgD</td>
<td>1</td>
<td>1,3</td>
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<tr>
<td>FLC k</td>
<td>52</td>
<td>69,3</td>
</tr>
<tr>
<td>FLC l</td>
<td>22</td>
<td>29,7</td>
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<tr>
<td>Calcio &gt;105</td>
<td>11</td>
<td>14,8</td>
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<tr>
<td>PCR &gt;.05</td>
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<tr>
<td>t(4;14)</td>
<td>11</td>
<td>14,3</td>
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<tr>
<td>t(14;16)</td>
<td>5</td>
<td>6,4</td>
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<tr>
<td>t(14;20)</td>
<td>2</td>
<td>3,3</td>
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<tr>
<td>t(6;14)</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>10</td>
<td>22,7</td>
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<th>Induction therapy</th>
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<tr>
<td>PI triplets (VTD, VRD, VCD, VMP)</td>
<td>55</td>
<td>70,5</td>
</tr>
<tr>
<td>CD38mAb-VCD/VRD</td>
<td>14</td>
<td>17,9</td>
</tr>
<tr>
<td>RD</td>
<td>9</td>
<td>11,5</td>
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<th>RESPONSE</th>
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<tr>
<td>≥VGPR</td>
<td>41</td>
<td>58,6</td>
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<tr>
<td>&lt;VGPR</td>
<td>29</td>
<td>41,4</td>
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<td>single</td>
<td>16</td>
<td>25,4</td>
</tr>
<tr>
<td>double</td>
<td>42</td>
<td>66,7</td>
</tr>
<tr>
<td>no TX</td>
<td>16</td>
<td>25,4</td>
</tr>
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| MEDIAN FOLLOW-UP: 19 m (3-37 months) |
Does cfDNA mirror the BM clone @ diagnosis?

130/139 (93.5%)

cfDNA genomic profiles are identical to BM clone in most of the patients

cfDNA originates from the same BM clone
cfDNA genomic profiles can be different from BM clone

PET: no EMD cases, but several focal lesions with osteolysis (M= 4; range: 1-15 vs. M=2; range: 0-2)
Higher SUV max (M= 5,5 vs. 3,6)19

9/139 (6,5%)
gDNA and cfDNA profiles are different

They originates from different clones!

→Possible SPATIAL HETEROGENEITY?
cfDNA tumor fraction reflects BM tumor burden

cfDNA tumour fraction (TF) at diagnosis was significantly lower as compared to gDNA TF

cfDNA TF
M = 3,2% (range: 0,4 – 40,6%)
gDNA TF
M = 74,4% (range: 5,9 – 97,1%)

cfDNA tumor fraction correlates with the percentage of CD138/CD38 positive cells in the bone marrow

Pearson correlation: 0.2948796
p-value = 0.03042
A higher rate of tumoral cfDNA spreaded into blood stream correlates with a poor prognosis

According to the cfDNA TF median (M) value, patients can be stratified in high cfDNA TF (M = 10.65%; range: 3.2-40.6) vs. patients with low cfDNA TF (M = 1.2%; range: 0.4-3.2)
Tumor cfDNA decreases after induction therapy, but may re-emerge during disease course.

During follow-up, the cfDNA tumor fraction fluctuations monitored monthly in 22 patients.
Sex: M  BM PCs: 15%
Age 69 yrs  Genomic CNAs: Gain 1q, Gain chr17
ISS stage II  Induction: 4 cyc Dara-VRD (VGPR) – ASCT (VGPR) – CONS (VGPR) – Dara-Len Mant (CR 06/05/21)

**PET lesions: 3**  SUV max: 4.4

**ID CLONOTYPE**  IGK V1D-J4 30%

**cfDNA TF**  22.9%

**PET lesions: 0**  SUV max: 0

**NGS POSITIVE**  1,3x10^-5

**NGS NEGATIVE**  LOD 10^-5

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D  
12/09/19

VGPR  
17/12/19  +3m

PRE-ASCT  
24/02/20  +6m

PRE-MANT  
22/09/20  +12m
Conclusions

- cfDNA reflects the tumor burden in most of the patients and might resume spatial heterogeneity in a small subgroup
- High amount of tumor cfDNA released into peripheral blood correlates with poor prognosis
- Although BM biopsy still remains the gold standard, cfDNA might be considered a suitable and less invasive marker: however, more comparative studies needed, to define sensitivity and test threshold in order to avoid false negative results
- Ongoing studies are trying a) to improve sensitivity of cfDNA by increase the number of markers to be tested along the genome (e.g. mutations) and b) since the release of cfDNA might be influenced by BM microenvironment, we are exploring features and mechanisms that could make a microenvironment more permissive to cfDNA release
Acknowledgements

Multiple Myeloma Research Unit
Prof. Michele Cavo

MOLECULAR BIOLOGY LAB
Carolina Terragna
Marina Martello
Enrica Borsi
Silvia Armuzzi
Ilaria Vigliotta
Barbara Taurisano
Ignazia Pistis

BIOINFO NERDs
Vincenza Solli
Andrea Poletti
Gaia Mazzocchetti

CYTOGENETIC LAB
Nicoletta Testoni
Giulia Marzocchi

DATA ANALYSIS and MANAGEMENT
Giada Giulia Riso
Simona Barbato
Federica Pedali

IMMUNOLOGY LAB
Mario Arpinati
Gabriella Chirumbolo

CLINICAL RESEARCH UNIT
Elena Zamagni
Paola Tacchetti
Lucia Pantani
Katia Mancuso
Serena Rocchi
Ilaria Rizzello
Gabriella De Cicco
Alessio Fusco
Margherita Ursi

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA