The role of MRD in CLL

PAOLO GHIA

Strategic Research Program on CLL - Lab of B Cell Neoplasia
Division of Experimental Oncology

Università Vita-Salute San Raffaele - Milano
IRCCS Istituto Scientifico San Raffaele - Milano
Increasing CRs & MRD Negativity in First-Line CLL

- **MRD-positive CR (or sample not available)**
- **MRD-negative CR**

**B**: bendamustine; **C**: Cyclophosphamide; **F**: Fludarabine; **M**: mitoxantrone; **R**: rituximab

*Clinical responses only.*

**Patients (%)**

<table>
<thead>
<tr>
<th>Year</th>
<th>Technique</th>
<th>LOD/Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>Morphology Qualitative</td>
<td>LOD 1-10%</td>
</tr>
<tr>
<td>1980</td>
<td>2/3-CLR Flow or IgH-PCR Qualitative</td>
<td>LOD 0.1-1%</td>
</tr>
<tr>
<td>1990</td>
<td>ERIC Flow RQ-PCR-CLR Quantitative</td>
<td>LOD $10^{-5} - 10^{-4}$</td>
</tr>
<tr>
<td>2000</td>
<td>RFCM Quantitative</td>
<td>LOD $10^{-6} - 10^{-5}$</td>
</tr>
<tr>
<td>2010</td>
<td>FC</td>
<td>LOD 10 - 6</td>
</tr>
<tr>
<td>2020</td>
<td>FC</td>
<td>LOD 10 - 4</td>
</tr>
</tbody>
</table>

**Alemtuzumab**

**Fludarabine**

**Chlorambucil**

**Bendamustine**

**Mitoxantrone**

**Rituximab**

**Chlorambucil**

**Fludarabine**

**Bendamustine**

**Mitoxantrone**

**Rituximab**
Minimal Residual Disease: practical aspects

According to current international definitions undetectable MRD (also known as MRD negativity) equals a quantitative detection of less than 1 CLL cell in 10,000 leukocytes (MRD level < 10\(^{-4}\)).

Hallek et al, Blood 2018

<table>
<thead>
<tr>
<th>Sensitivity threshold (Lower limit of quantification)</th>
<th>&lt; 1 cell cell/10,000 leucocytes (10(^{-4}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods of detection</td>
<td>Multicolour- Flow Cytometry, ASO-qPCR, High Throughput Sequencing (NGS)</td>
</tr>
<tr>
<td>Target tissue</td>
<td>Screening in Peripheral blood, Confirmation in Bone Marrow</td>
</tr>
</tbody>
</table>
Minimal Residual Disease: practical aspects

According to current international definitions undetectable MRD (also known as MRD negativity) equals a quantitative detection of less than 1 CLL cell in 10,000 leukocytes (MRD level < 10^{-4}).

Hallek et al, Blood 2018

Ghia & Rawstron, Leukemia 2018

<table>
<thead>
<tr>
<th>Sensitivity threshold (Lower limit of quantification)</th>
<th>&lt; 1 cell cell/10,000 leucocytes (10^{-4})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods of detection</td>
<td>Multicolour- Flow Cytometry</td>
</tr>
<tr>
<td></td>
<td>ASO-qPCR</td>
</tr>
<tr>
<td></td>
<td>High Throughput Sequencing (NGS)</td>
</tr>
<tr>
<td>Target tissue</td>
<td>Screening in Peripheral blood</td>
</tr>
<tr>
<td></td>
<td>Confirmation in Bone Marrow</td>
</tr>
</tbody>
</table>
# 4 (or more) -colour flow cytometry

<table>
<thead>
<tr>
<th></th>
<th>4-colour</th>
<th>6-colour</th>
<th>6-colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tubes</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Approximate antibody cost</td>
<td>1</td>
<td>0.75</td>
<td>0.37</td>
</tr>
<tr>
<td>Number of cells for 0.01%</td>
<td>8 million</td>
<td>4 million</td>
<td>2 million</td>
</tr>
<tr>
<td>Number of cells for 0.001%</td>
<td>20 million</td>
<td>10 million</td>
<td>5 million</td>
</tr>
<tr>
<td>Acquisition time for 0.01%</td>
<td>20 minutes</td>
<td>10 minutes</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

*Source: Rawstron et al, Leukemia 2016*
Additional methods...may be used in the future

Next generation sequencing (NGS)

Dilutional analysis (Milan) assessing high-throughput sequencing: analysis of 3 CLL cases diluted into leucocytes from leucodepletion filters in serial 1:10.

Rawstron et al, Leukemia 2016
Minimal Residual Disease: practical aspects

... According to current international definitions undetectable MRD (also known as MRD negativity) equals a quantitative detection of less than 1 CLL cell in 10000 leukocytes (MRD level < 10^{-4}).

Hallek et al, Blood 2008

| Sensitivity threshold (Lower limit of quantification) | < 1 cell cell/10,000 leucocytes (10^{-4}) |
| Methods of detection | Multicolour- Flow Cytometry  
ASO-qPCR  
High Throughput Sequencing (NGS) |
| Target tissue | Screening in Peripheral blood  
Confirmation in Bone Marrow |
Is MRD useful in CLL?

Available data has shown that undetectable MRD at the end of induction treatment is a strong predictor of PFS and OS.....

FCR in first-line CLL (phase 2 MDACC study)

<table>
<thead>
<tr>
<th>Overall survival</th>
<th>N</th>
<th>Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRD negative</td>
<td>106</td>
<td>73</td>
</tr>
<tr>
<td>MRD positive</td>
<td>139</td>
<td>59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PFS</th>
<th>N</th>
<th>Prog-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR MRD negative</td>
<td>95</td>
<td>51</td>
</tr>
<tr>
<td>CR MRD positive</td>
<td>89</td>
<td>25</td>
</tr>
<tr>
<td>PR MRD negative</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>PR MRD positive</td>
<td>41</td>
<td>5</td>
</tr>
</tbody>
</table>

CR, complete remission; FC, fludarabine + cyclophosphamide; FCR, FC + Rituximab; PR, partial remission.

MRD negativity can be achieved in elderly patients

CLL11: Chlorambucil versus R-chlorambucil versus G-chlorambucil

G-Clb MRD negative: 87 87 87 80 68 88 57 45 37 28 19 8 4 1 0
G-Clb MRD positive: 144 134 133 127 89 54 38 26 10 7 3 0 0 0

Novel molecules for the treatment of CLL

Cavallini et al, EHA 2016, poster #1091; Davies et al, poster #421

Adapted from Byrd J, et al. J Clin Oncol 2014
Updated Efficacy and Safety from the Phase 3 Resonate-2 Study: Ibrutinib As First-Line Treatment in Patients ≥65 Years with CLL/SLL

Ibrutinib (n=136) vs Chlorambucil (n=133)

Follow-up 18-4 months ➔ 48 months

CR rates continue to improve:
7% @12 mo ➔ 18% @29 mo ➔ 24% @ 48 mo

- PFS @ 18 mo: 90% ➔ PFS @ 48 mo: 76%
- PFS benefit across all sub-groups
- (Fit patients: median PFS FCR ➔ 55 mo; BR ➔ 42 mo)

Progression-Free Survival

Overall Survival

n=55 crossed over to ibrutinib following PD

OS rates at 48 months were 86% with ibrutinib and 76% with chlorambucil.

Burger et al, EHA 2018
A new Frontier: Searching for MRD with novel inhibitors

HELIOS (BRI versus BR)

ORR (investigator assessment)

OR = 87.2% versus 66.1% (p<0.0001)

CR/CRi

PR

<table>
<thead>
<tr>
<th></th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibrutinib + BR</td>
<td>53.3%</td>
</tr>
<tr>
<td>Placebo + BR</td>
<td>58.9%</td>
</tr>
</tbody>
</table>

OR = 87.2% versus 66.1% (p<0.0001)

PR = 33.9% vs 7.2% (p<0.0001)

CR/CRi

58.9% vs 33.9% (p<0.0001)

3-yr update

BR, bendamustine + rituximab;
CRi, CR with incomplete marrow recovery; OR, overall response.
Improved ORR and CR Rates with Ibrutinib-Obinutuzumab by IRC and Investigator Assessments

- In the high-risk population, IRC-assessed ORR rates were 90% vs. 68% with ibrutinib-obinutuzumab vs. chlorambucil-obinutuzumab; CR/CRi rates were 14% vs. 4%, respectively

---

IRC Assessment

<table>
<thead>
<tr>
<th>Response</th>
<th>CR 19%</th>
<th>CRi 4%</th>
<th>nPR 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>65%</td>
<td>6%</td>
<td></td>
</tr>
</tbody>
</table>

ORR 88%

<table>
<thead>
<tr>
<th>Response</th>
<th>CR 83%</th>
<th>CRi 8%</th>
<th>nPR 21%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>66%</td>
<td>6%</td>
<td></td>
</tr>
</tbody>
</table>

ORR 73%

---

INV Assessment

<table>
<thead>
<tr>
<th>Response</th>
<th>CR 37%</th>
<th>CRi 41%</th>
<th>nPR 3%</th>
<th>PR 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>42%</td>
<td>4%</td>
<td>8%</td>
<td></td>
</tr>
</tbody>
</table>

ORR 91%

<table>
<thead>
<tr>
<th>Response</th>
<th>CR 16%</th>
<th>CRi 16%</th>
<th>nPR 3%</th>
<th>PR 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>61%</td>
<td>4%</td>
<td>8%</td>
<td></td>
</tr>
</tbody>
</table>

ORR 81%

---

P<0.01 for both ORR and CR/CRi rates; P<0.05 for ORR and P<0.001 for CR/CRi rates; One patient in each arm had a best response of progressive disease. CR, complete response; CRi, complete response with incomplete bone marrow recovery; nPR, nodular partial response; ORR, overall response rate; PR, partial response; SD, stable disease.
Higher Rates of Undetectable MRD\textsuperscript{a} with Ibrutinib-Obinutuzumab

<table>
<thead>
<tr>
<th>MRD Analysis, n (%)</th>
<th>Ibrutinib-Obinutuzumab N=113</th>
<th>Chlorambucil-Obinutuzumab N=116</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undetectable MRD in ITT population</td>
<td>MRD test performed\textsuperscript{b}</td>
</tr>
<tr>
<td>Bone marrow and/or peripheral blood</td>
<td>39 (35)</td>
<td>101 (89)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>23 (20)</td>
<td>92 (81)</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>34 (30)</td>
<td>90 (80)</td>
</tr>
</tbody>
</table>

- All cases of undetectable MRD in bone marrow observed in patients with complete (CR/CR\textsubscript{i}) or partial response (nPR/PR) by both IRC and investigator assessment
- In the high-risk population, undetectable MRD in bone marrow and/or peripheral blood was achieved in 27% with ibrutinib-obinutuzumab vs. 15% with chlorambucil-obinutuzumab

\textit{ITT, intent to treat}
\textsuperscript{a}Defined as <1 CLL cell per 10,000 leukocytes as measured by flow cytometry at a central laboratory;
\textsuperscript{b}Bone marrow aspirate and/or peripheral blood samples were collected from patients at cycle 9 and in patients with CR/CR\textsubscript{i} for assessment of MRD; patients with PR/nPR were evaluated for MRD using peripheral blood samples, with bone marrow assessment to confirm MRD status if undetectable MRD in blood.
Phase III ILLUMINATE study: PFS as assessed by IRC
In the intention-to-treat population

Moreno et al., ASH 2018; abstract 691

- Estimated 30-mo PFS: 79% vs. 31%
- In non-del(17p): 74% reduction in risk of PD or death with I+G

Median follow-up, 31.3 months

High Risk Population
(uIGHV, del(11q), del(17p) and/or TP53 mutation)

- Estimated 30-mo PFS: 77% vs. 16%
- In high risk without del(17p): 84% reduction in risk of PD or death
- In unmutated IGHV without del(17p): 85% improvement in PFS vs G-Clb

Moreno et al., ASH 2018; abstract 691
MRD with Venetoclax: Venetoclax in R/R CLL

Venetoclax monotherapy\(^1,^\dagger\)

- ORR: 86%
- N=78

Venetoclax monotherapy in del(17p) CLL\(^2,^\ddagger\)

- ORR: 74%
- Investigator
- ORR: 79%
- IRC

Venetoclax + rituximab\(^3,^\dagger\)

- ORR: 86%
- N=49

**MRD negative (BM):**
- 35% of CR/CRis\(^1\)
- 80% of CR/CRis; 47% of PRs
- 79% within 7 months of therapy\(^3\)

**MRD negative (PB):**
- 18/45 evaluated patients
- (17% of ITT population; median time to PB MRD was 8.8 months)\(^2\)
- 51%

*Figure adapted from Table 2 in the manuscript; \(^\dagger\) Percentages are based on the entire sample size.

Venetoclax in R/R CLL with del(17p)(Ph 2 study M13-982)

PFS according to MRD

- 24-month PFS estimates of MRD in blood by flow cytometry:
  - MRD(neg) CR/CRi: 100% (n=22)
  - MRD(pos) CR/CRi: 86% (n=9)
  - MRD(neg) nPR/PR: 83% (n=26)
  - MRD(pos) nPR/PR: 62% (n=40)

A Phase 3 study (MURANO): VR vs BR in R/R CLL

Superior PFS with VR maintained with 1 more year of follow-up

- Median follow-up of 36.0 months (range 0.0-48.6);
- VR: 36.1 months, BR: 35.9 months
A Phase 3 study (MURANO): VR vs BR in R/R CLL

Improved Response Rates for VR vs BR

Of 42 INV-assessed CRs discrepant in VenR arm, 28 due to residual CT scan nodes 15–30 mm diameter; 88% of these were PB MRD negative

As of 8 May 2017

Seymour et al., ASH 2017 (oral presentation)
A Phase 3 study (MURANO): VR vs BR in R/R CLL

Impact of MRD on long-term clinical outcomes

PB uMRD rates higher with VenR than BR at EOCT

uMRD status at EOCT highly predictive of prolonged PFS

Difference in uMRD rates: **49.0%**

Kater et al., ASH 2018; abstract 695

EOCT, end of combination therapy

VenR (n=194), BR (n=195)

- Kater et al., ASH 2018; abstract 695
- EOCT, end of combination therapy
Venetoclax and rituximab (Phase 1 M13-365)

Disposition of patients who discontinued with deep response

Brander et al., ASH 2018; abstract 183
An old frontier: MRD in clinical trials

Available data has shown that undetectable MRD at the end of induction treatment is a strong predictor of PFS and OS.....

Surrogate endpoint

MRD can replace PFS as a primary endpoint in clinical trials

The vast improvement in RD detection over the last two decades has now led to the concept that low RD levels are a desirable and achievable goal of CLL therapy.

Undetectable minimal residual disease (MRD) in patients with chronic lymphocytic leukaemia (CLL) in clinical complete remission (= MRD response rate) after induction therapy may be used as an intermediate endpoint for licensure in randomised well controlled studies designed to show superiority in terms of PFS.
**IS THIS THE END OF CHEMOTHERAPY?**

**CLL13-TRIAL OF THE GCLLSG in cooperation with HOVON, Nordic CLL Study Group and SAKK (GAIA)**

Previously untreated Fit CLL patients (N=920)  
(CIRS ≤6 and normal creatinine clearance)

- **FCR** or **BR**^
- **ABT-199** + Rituximab
- **ABT-199** + Obinutuzumab
- **ABT-199** Obinutuzumab Ibrutinib

Follow-up for progression and survival

- 2 primary endpoints
  - Rate of MRD negativity
  - PFS

Obinutuzumab: 6 cycles  
Venetoclax: 12 cycles  
Ibrutinib: 36 cycles or MRD\textsuperscript{neg}

\*<65 years of age  
^>65 years of age
CAPTIVATE phase 2 study: Ibrutinib + Venetoclax
3 Cycles of Ibrutinib Reduces TLS Risk and Bulky disease

After 3 cycles of ibrutinib lead-in:
- 36 of 40 patients (90%) with high baseline TLS risk shifted to medium or low risk
- 7 of 37 patients (19%) with medium baseline TLS risk plus CrCl <80 mL/min shifted to low risk
- Hospitalization was avoided in 59 patients following shift from baseline TLS risk

No patients developed clinical TLS

Laboratory TLS reported as AEs in 2 patients (neither met Howard criteria)
- 1 additional lab TLS not reported as AE but met Howard criteria

*Data after ibrutinib lead-in not available for 5 patients who discontinued prior to cycle 4.
High rates of undetectable MRD (77%) in PB after 6 cycles of I+V
- Confirmed undetectable MRD* in 11 of 14 patients (79%) after 12 cycles of I+V

*Confirmed undetectable MRD defined as undetectable MRD serially over at least 3 cycles in PB, and undetectable MRD in both PB and BM. BM MRD was assessed per protocol after C15 for all patients who reached this time point as of the data extract.
MRD in CLL: irrespective of known poor risk factors?

CLL8

<table>
<thead>
<tr>
<th></th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR IGHV M patients</td>
<td>113</td>
</tr>
<tr>
<td>FC IGHV M patients</td>
<td>117</td>
</tr>
<tr>
<td>FCR IGHV UM patients</td>
<td>197</td>
</tr>
<tr>
<td>FC IGHV UM patients</td>
<td>195</td>
</tr>
</tbody>
</table>

MDACC

<table>
<thead>
<tr>
<th></th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGHV-M, MRD neg</td>
<td>35</td>
</tr>
<tr>
<td>IGHV-M, MRD pos</td>
<td>34</td>
</tr>
<tr>
<td>IGHV-UM, MRD neg</td>
<td>35</td>
</tr>
<tr>
<td>IGHV-UM, MRD pos</td>
<td>66</td>
</tr>
</tbody>
</table>

IGHV, immunoglobulin heavy chain; M, mutated; MDACC, MD Anderson Cancer; UM, unmutated.

MRD negativity = undetectable MRD

- ASO-PCR, allele-specific oligonucleotide polymerase chain reaction;
- MRD negativity defined as <1 CLL cell/10,000 leukocytes ($10^{-4}$); NGS, next-generation sequencing.

Lower level of residual disease, generally associated with longer time to progression (i.e. less likely to relapse)

MRD Negativity: Not “One Size Fits All”

iwCLL, International Workshop on CLL; NCI, National Cancer Institute.

Adapted from Peter Hillmen.
A new frontier: MRD in general practice?

MRD-guided treatment?

Can MRD be used to guide treatment decisions?

Patients could stop treatment when achieving MRD negativity

Patients who achieve a CR but are still MRD positive may benefit from additional treatment? Maybe treated to MRD negativity?

If a patient reverts to MRD positive without evidence of disease progression, do they benefit from re-treatment?

Ghia P. Hematology 2012
The present and future of MRD in CLL
Laboratory of B Cell Neoplasia
Lydia Scarfò, Athanasios Pseftokgas, Alessandra Rovida, Chartomatsidou Elisavet, Pamela Ranghetti, Silvia Bonfiglio

Strategic Research Program on CLL
Lydia Scarfò, Maria Colia, Silvia Heltai, Virginia Sgarlato, Eloise Scarano
ERIC is proud to announce that it currently has over 850 Members representing more than 64 countries and these numbers are growing as we speak!

ADVANTAGES OF JOINING:

- Membership is free of charge
- Everyone is welcome and eligible for membership
- It is quick and easy to become a member - takes just two minutes of your time!
- Joining ERIC will allow you to be informed (via our newsletters and webpage)

Societatea Romana de Hematologie - 2018