Il ruolo della Malattia Minima Residua

Pellegrino Musto

LEUCEMIA LINFATICA CRONICA:
L'INNOVATIVITÀ TERAPEUTICA ED OLTRE...

28-29 MARZO 2023 BOLOGNA ROYAL HOTEL CARLTON

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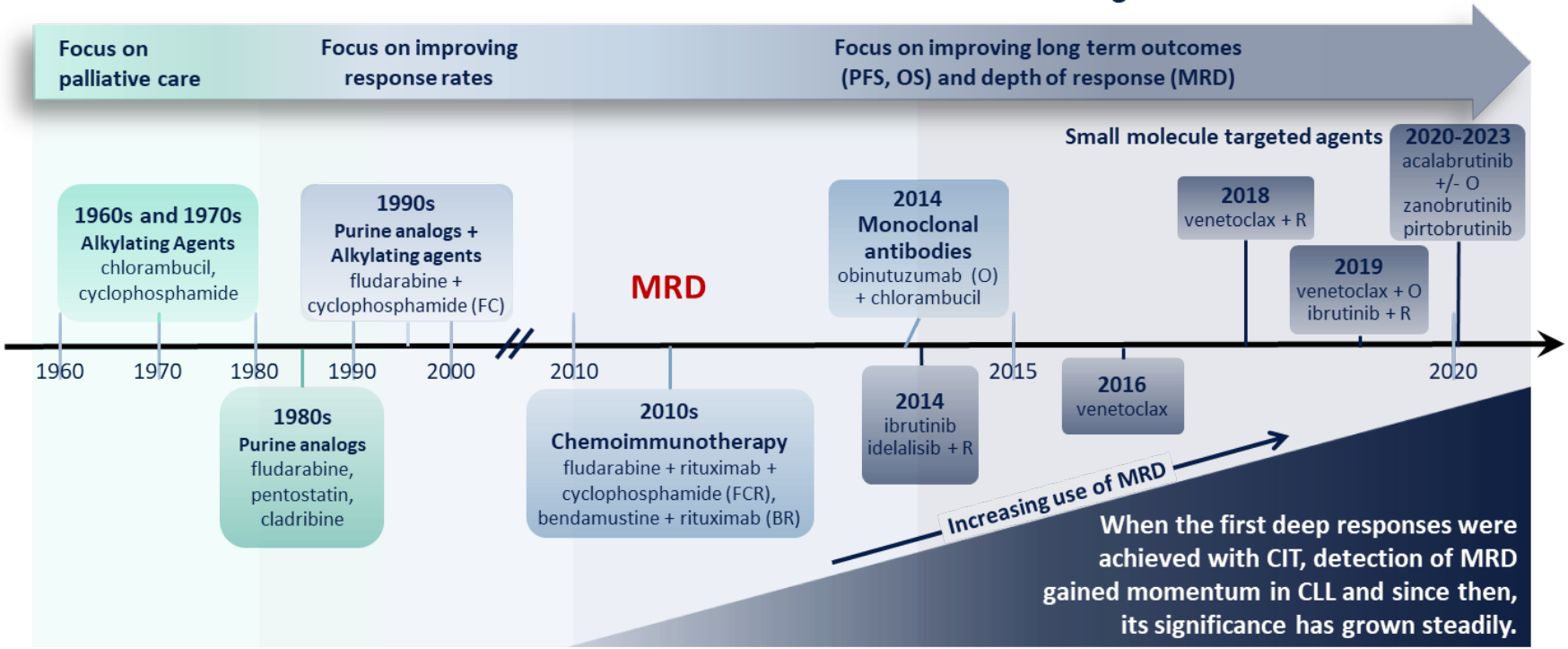
As deeper responses are attained with new agents, measurement of MRD has evolved as an important endpoint in clinical studies as it has been shown to predict long-term outcomes

Evolution of MRD Involvement in CLL Treatment Paradigm



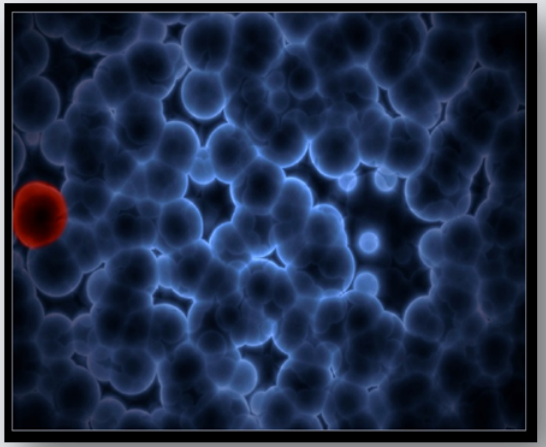
CIT=Chemoimmunotherapy. CLL=Chronic Lymphocytic Leukemia. MRD=Minimal Residual Disease. O=Obinutuzumab. OS=Overall Survival. PFS=Progression Free Survival. R=Rituximab. R/R=Relapsed/Refractory.

1. Kay NE. Blood. 2006;107:848. 2. Montserrat E. Blood. 2005;105:2-3. 3. Burger JA, O'Brien S. Nat Rev Clin Oncol. 2018;15(8):510-527.

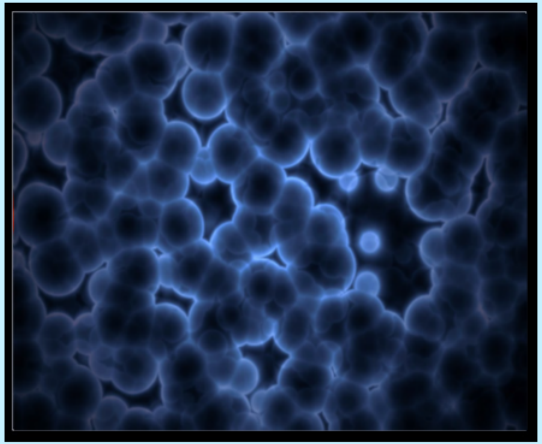
Minimal Residual Disease (MRD) Definition

Minimal Residual Disease

MRD is the presence of persistent, low level cancer cells after treatment, even in patients with no clinically measurable disease by routine imaging and laboratory screening (CR)



Undetectable MRD (uMRD)



Defined by iwCLL as <1 CLL cell per 10,000 leukocytes ($<10^{-4}$) and is routinely measured in the blood or bone marrow[1]

CLL=Chronic Lymphocytic Leukemia. CR=Complete Response. MRD=Minimal Residual Disease.

1. Hallek M, et al. Blood 2018; 131:2745–2760.
2. 2. Thompson PA & Wierda WG. Blood 2016; 127: 279-286.
3. 3. Wierda WG et al. Leukemia 2021; 35.3059-3072.
4. 4. Al Sawaf et al. Clin Adv Hematol Oncol 2022; 20:97-103.

Leukemia (2021) 35:3059–3072
https://doi.org/10.1038/s41375-021-01241-1

REVIEW ARTICLE

Chronic lymphocytic leukemia

Measurable residual disease in chronic lymphocytic leukemia: expert review and consensus recommendations

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Received: 17 April 2020 / Revised: 17 March 2021 / Accepted: 23 March 2021 / Published online: 24 June 2021
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Abstract

Assessment of measurable residual disease (often referred to as "minimal residual disease") has emerged as a highly sensitive indicator of disease burden during and at the end of treatment and has been correlated with time-to-event outcomes in chronic lymphocytic leukemia. Undetectable-measurable residual disease status at the end of treatment demonstrated independent prognostic significance in chronic lymphocytic leukemia, correlating with favorable progression-free and overall survival with chemoimmunotherapy. Given its utility in evaluating depth of response, determining measurable residual disease status is now a focus of outcomes in chronic lymphocytic leukemia clinical trials. Increased adoption of measurable residual disease assessment calls for standards for nomenclature and outcomes data reporting. In addition, many basic questions have not been systematically addressed. Here, we present the work of an international, multidisciplinary, 174-member panel convened to identify critical questions on key issues pertaining to measurable residual disease in chronic lymphocytic leukemia, review evaluable data, develop unified answers in conjunction with local expert input, and provide recommendations for future studies. Recommendations are presented regarding methodology for measurable residual disease determination, assay requirements and in which tissue to assess measurable residual disease, timing and frequency of assessment, use of measurable residual disease in clinical practice versus clinical trials, and the future usefulness of measurable residual disease assessment. Nomenclature is also proposed. Adoption of these recommendations will work toward standardizing data acquisition and interpretation in future studies with new treatments with the ultimate objective of improving outcomes and curing chronic lymphocytic leukemia.

Table 2 Recommended nomenclature for reporting measurable residual disease in CLL.

Recommended	Rationale
Measurable residual disease (MRD)	Replaces "minimal" residual disease as a more objective term
Undetectable-MRD (U-MRD)	As a general term, replaces MRD negative or MRD- as a more accurate term in cases where MRD threshold is not specified
MRD4, MRD5, etc.	Specifies upper limit of disease (e.g., MRD4 denotes <0.01%/<10^{-4} disease, MRD5 < 0.001%/<10^{-5} disease, etc) for an individual sample or for a group of patients in clinical trial reporting
Detectable (d) or undetectable (u) within an MRD category	Detectable = residual disease is below the stated threshold but measurable above the next MRD threshold. Undetectable = residual disease is not detectable, but the assay/sample is not suitable for detection of disease at the next threshold MRD4d: < 0.01%/10^{-4} but ≥0.001%/10^{-5} MRD4u: < 0.01%, assay limit of detection does not reach 0.001%/10^{-5}
Always report assay method (e.g., Flow) and analysis technique (e.g., ERIC-FC)	Results may differ by assay method even for assays with identical sensitivity
Always report tissue assayed (e.g., PB, BM)	MRD may differ in different tissues from the same patient/timepoint
In clinical trials, always report MRD rate as percentage U-MRD in ITT population	Avoids confusion with the rate in the MRD-tested population, e.g., MRD4 rate = number of patients with <0.01% MRD as a percentage of the ITT population

BM bone marrow, *CLL* chronic lymphocytic leukemia, *Flow* flow cytometry, *ITT* intention-to-treat, *PB* peripheral blood.

MRD is Measured via Flow Cytometry, PCR-based Methods, and NGS

FLOW CYTOMETRY

PCR-BASED METHODS

NEXT GENERATION SEQUENCING

TECHNOLOGY	Antibodies identify CLL **cells** similar to a diagnostic flow, with a greater sensitivity (4-6 or more colors)	ASO-PCR identifies **DNA** sequences that are **unique to patient** CLL cells	**Consensus primers and high-throughput sequencing** (HTS) amplify and sequence all clonal **gene mutations & rearrangements** (**DNA**) present in diagnostic samples and track their evolution

Technology Category	Flow Cytometry	ASO-PCR	NGS: ClonoSEQ	NGS: CAPP-seq
Assay Status	iwCLL accepted	iwCLL accepted	iwCLL accepted	Validation underway
Sample Processing	<48 hours	Batched	Batched	Batched
Assay Platform	Universal	Patient-specific	Universal	Universal
Sensitivity	$10^{-4} – 10^{-5}$	$10^{-4} – 10^{-5}$	$10^{-4} – 10^{-6}$	$10^{-3} – 10^{-5}$
Assay Targets	Tumor cell surface markers (i.e. CD19, CD5, CD20, CD38, CD22, CD43, CD79b, CD81)	Ig heavy chain genes (CDR3)	Ig heavy chain genes	Dozens of somatic mutations + Ig heavy/light chain genes
Assay Controls	T-cells	Not required	Not required	Normal specimen
Advantages	Low cost Technical requirements minimal	Robust accuracy and reproducibility Stable samples (frozen)	Robust accuracy and reproducibility High sensitivity	Simultaneous MRD monitoring and clonal evolution
Disadvantages	Live samples required Less robust	Complex set-up Limited to Ig genes	Limited to Ig genes High level of expertise	Normal controls preferred High level of expertise

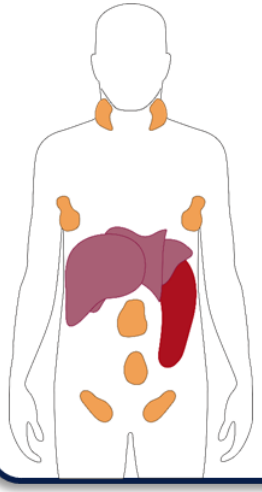
According to iwCLL guidelines, as these techniques have undergone critical evaluation and become well standardized, sensitive multicolor flow cytometry, PCR, or next-generation sequencing may all be used to detect MRD.

ASO=Allele-Specific Oligonucleotide. MRD=Minimal Residual Disease. NGS=Next Generation Sequencing. PCR=Polymerase Chain Reaction.

1. Ghia P. Hematology Am Soc Hematol Educ Program 2012; 2012:97–104. 2. Ritgen M, et al. Haematologica Reports 2005; 1:5–8. 3. Uhrmacher S, et al. Adv Hematol 2010; 2010:272517. 4. Rawstron AC, et al. Leukemia 2007; 21: 956 964. 5. Fürstenau M, et al. HemaSphere 2019; 3: e287. 6. Rawstron AC, et al. Leukemia 2016; 30: 929 936. 7. Wierda WG et al. Leukemia 2021; 35.3059-3072. 8. Al Sawaf et al. Clin Adv Hematol Oncol 2022; 20:97-103.

Measurement of MRD in Bone Marrow vs Peripheral Blood

A challenge in MRD testing is that CLL is a multicompartmental disease involving the BM, PB, LN, liver, and spleen[4]



- After treatment, one or more of these sites may serve as a reservoir for residual disease
- However, current MRD assessment focuses on sampling low level disease from BM and PB.[1,2,3]

	Bone Marrow (BM)	Peripheral Blood (PB)
PROS	✓ Not impacted by different therapy types[1] ✓ More accurate/conclusive results – highest sensitivity source to detect MRD[1,2]	✓ Easy to obtain regular sample of sufficient quantity[3] ✓ Additional samples can be obtained if the original is insufficient quality[3] ✓ Less invasive/painful[3]
CONS	✓ Invasive and painful procedure[3] ✓ Must be tested from first draw – subsequent draws are likely to be contaminated with PB and yield inadequate BM tissue[3] ✓ Higher frequency of samples are of insufficient quality[3]	✓ May be affected by therapy (CD20 mAb, BCRi)[1,2] ✓ Results are not always accurate/conclusive – risk of false-negative due to difference in tumor clearance[1,2]

BCRi=B-cell Receptor Inhibitor. BM=Bone Marrow. LN=Lymph Node. mAb=Monoclonal Antibody. MRD=Minimal Residual Disease. PB=Peripheral Blood. 1. Hallek M, et al. Blood 2018; 131:2745–2760. 2. Rawstron AC, et al. Leukemia 2007; 21:956–964. 3. Lee SH, et al. Int J Lab Hematol 2008; 30:349–364. 4. FDA. Document # FDA-2018-D-3090. (January 2020). 5. Fürstenau M, et al. HemaSphere. 2019;3:5. Wierda WG et al. Leukemia 2021; 35.3059-3072. 6. Al Sawaf et al. Clin Adv Hematol Oncol 2022; 20:97-103.

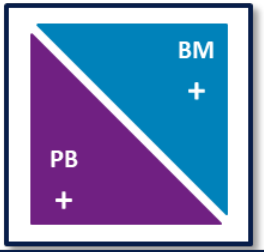
MRD Concordance in Bone Marrow and Peripheral Blood

Several studies have shown high concordance between levels of MRD in samples of PB and BM:

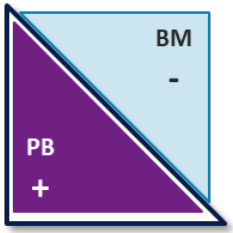
If PB is positive, BM is most likely also positive

If PB is negative, BM may be positive as BM tumor cells are more difficult for therapy to reach (and clear)

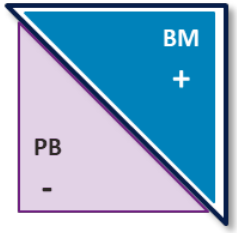
MRD detectable in PB and BM
In the presence of PB MRD, it is certain that the respective BM sample will be positive



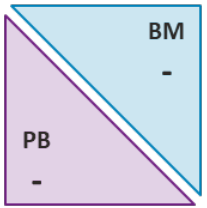
MRD only detectable in PB
Uncommon. May occur during regenerative phase after treatment



MRD only detectable in BM
May be due to temporary effect of targeted therapy



No detectable ("confirmed") MRD in PB or BM



It is recommended that patients are screened for CLL eradication in the PB first. If MRD is not detectable in PB, it may be important to confirm MRD status in the BM.[1,2]

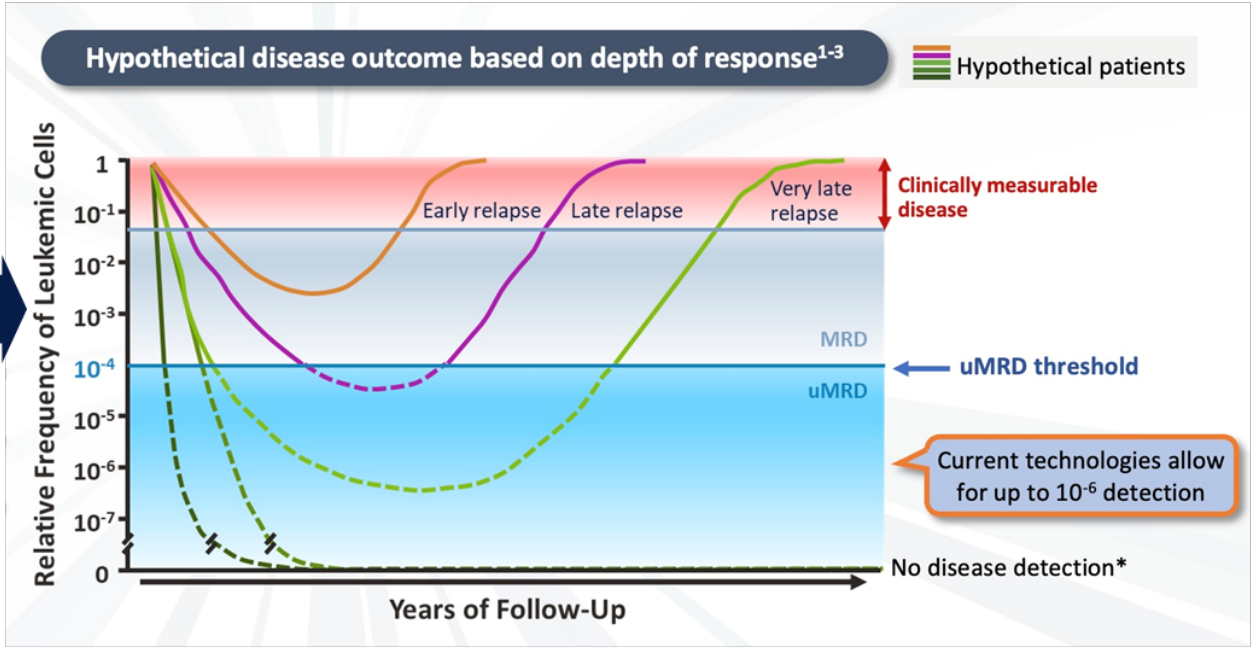
BM=Bone Marrow. CLL=Chronic Lymphocytic Leukemia. MRD=Minimal Residual Disease. PB=Peripheral Blood. 1. Hallek M, et al. Blood 2018; 131:2745–2760. 2. EMA. Document #EMA/CHMP/703715/2012 Rev. 2. 3. Wierda WG et al. Leukemia 2021; 35.3059-3072. 4. Al Sawaf et al. Clin Adv Hematol Oncol 2022; 20:97-103.

MRD Is Potentially Associated with Long-Term Outcomes

MRD is ultimately responsible for clinical relapse, and accurate quantification of post treatment burden in CLL is prognostically relevant

The ***timing of relapse depends on the quantity of residual disease*** and the kinetics of leukemic cell division

Similar MRD levels predicts **better outcome in patients with a CR compared with patients who have a partial remission (PR).**



MRD quantification usually allows for improved prediction of long-term outcomes for patients with CLL.[3]

* No limit of detection has been established to be indicative of a cure. CLL=Chronic Lymphocytic Leukemia. MRD=Minimal Residual Disease.

1. Szczepański T, et al. Lancet Oncol 2001; 2:409–417. 2. Böttcher S, et al. J Clin Oncol 2012; 30:980–988. 3. Böttcher S, et al. Hematol Oncol Clin North Am 2013; 27:267–288. 4. Hallek M, et al. Blood 2018; 131:2745–2760. 5. Thompson PA and Wierda WG. Blood 2016; 127:279–286. 6. Buckley SA, et al. Bone Marrow Transplant. 2013;48(5):630-641. 7. Wierda WG et al. Leukemia 2021; 35.3059-3072. 8. Al Sawaf et al. Clin Adv Hematol Oncol 2022; 20:97-103.

Summary of MRD inclusion in the trial design

Trial	Arms	Phase	Treatment naive (TN) or relapsed/ refractory (RR)	Fixed duration for non-CIT arms	Total participants	MRD as a primary outcome	MRD as a secondary outcome	MRD stopping rules
MURANO	BR *vs.* VR	3	RR	Yes	389	No	Yes	No
CLARITY	IV single arm	2	RR	No	54	Yes	No	Yes
ELEVATE-TN	A *vs.* AO *vs.* ClbO	3	TN	No	535	No	No[a]	No
CLL14	ClbO *vs.* VO	3	TN	Yes	432	No	Yes	No
CAPTIVATE MRD	IV then I or placebo if U-MRD4	2	TN	Yes	54	No	Yes, for the ibrutinib *vs.* placebo cohort	No[b]
CAPTIVATE FD	IV	2	TN	Yes	159	No	Yes	No
GLOW	IV *vs.* ClbO	3	TN	Yes	211	No	Yes, for both arms	No
FLAIR	FCR, IR, I, IV	3	TN	No	1,516	Yes, for IV *vs.* I or IR	Yes, for FCR *vs.* IR	Yes, for the ibrutinib-containing arms
CLL13	CIT *vs.* VR *vs.* VO *vs.* VIO	3	TN	Yes	926	Yes	No	No[c]
GALACTIC	O consolidation	2	TN or RR	Yes	48	Yes	No	No

A, acalabrutinib; B, bendamustine; CIT, chemoimmunotherapy; Clb, chlorambucil; FCR, fludarabine, cyclophosphamide, and rituximab; I, ibrutinib; O, obinutuzumab (also known as "G" in CLL13 and CLL14); R, rituximab; V, venetoclax.

[a]ELEVATE-TN and RR have MRD as an exploratory outcome.

[b]Randomized depending on U-MRD.

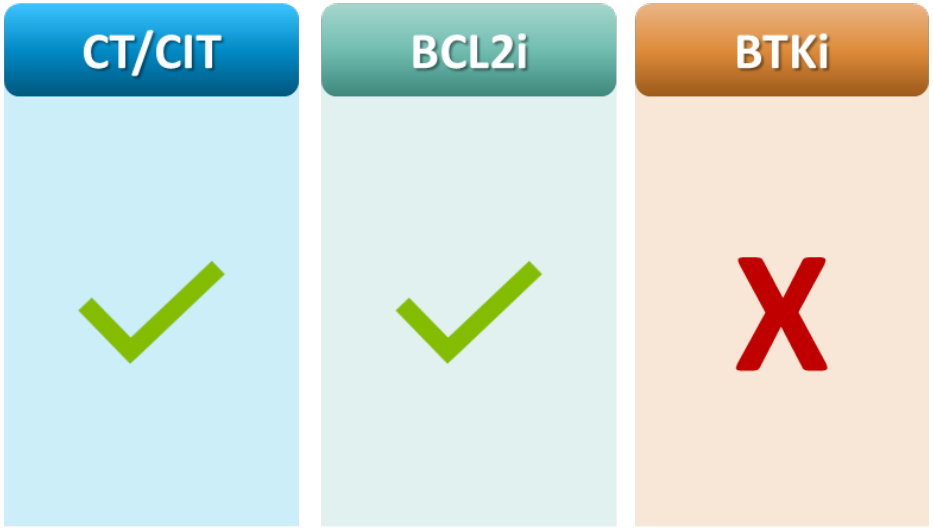
[c]Option to extend from 12 to 36 cycle duration if MRD positive.

Fisher A, et al. (2023) The evolving use of measurable residual disease in chronic lymphocytic leukemia clinicaltrials. Front. Oncol. 13:1130617. doi: 10.3389/fonc.2023.1130617

✓ = supported

X = not supported

Is uMRD always predictive of long-term outcomes?



BCL2i=B Cell Lymphoma 2 Inhibitor. BTKi=Bruton's Tyrosine Kinase Inhibitor. CIT=Chemoimmunotherapy. CT=Chemotherapy. MRD=Minimal Residual Disease. u=Undetectable. 1. Kramer I, et al. Blood 2017;130:1477-1480.

✓ = supported

X = not supported

Is uMRD always predictive of long-term outcomes?

CT/CIT
✓

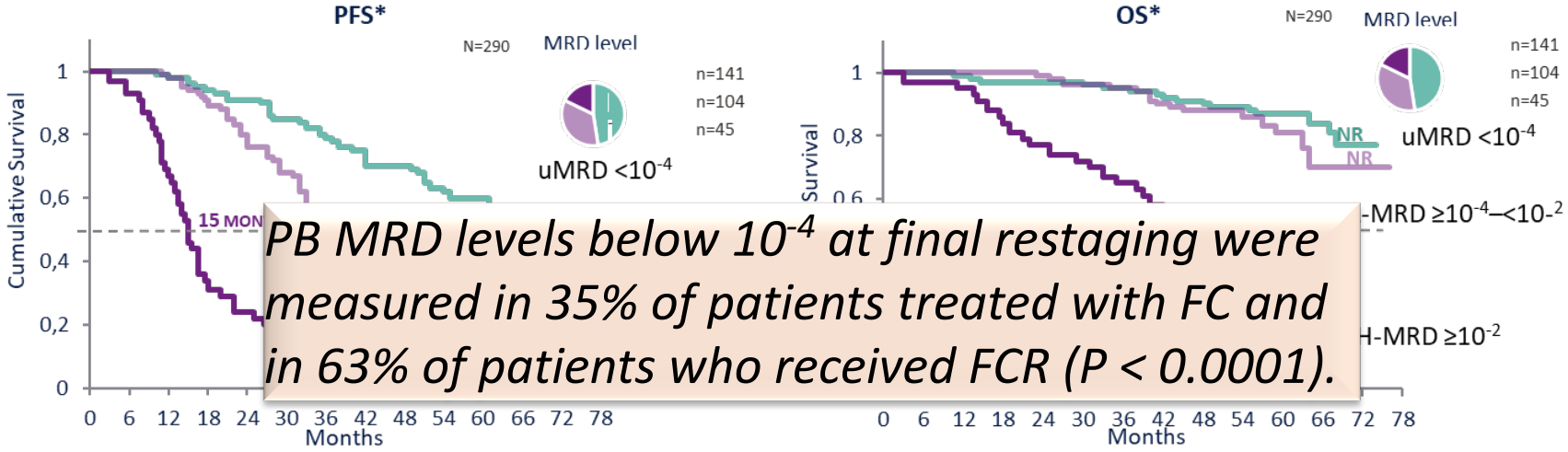
CIT=Chemoimmunotherapy. CT=Chemotherapy uMRD=undetectable Minimal Residual Disease.

uMRD is a predictor of improved long-term outcomes with CT/CIT

CLL8: PFS and OS by PB MRD Level at Final Restaging (3 months after last treatment cycle)[1,2]

Open-label, randomized, phase 3 trial comparing FC and FCR in 817 treatment-naive physically fit patients with CLL (median follow-up of 52.4 months)

Treatment with both FC and FCR significantly reduced MRD levels, but more profound reductions of MRD were observed in patients who received FCR



PB MRD levels below 10^{-4} at final restaging were measured in 35% of patients treated with FC and in 63% of patients who received FCR ($P < 0.0001$).

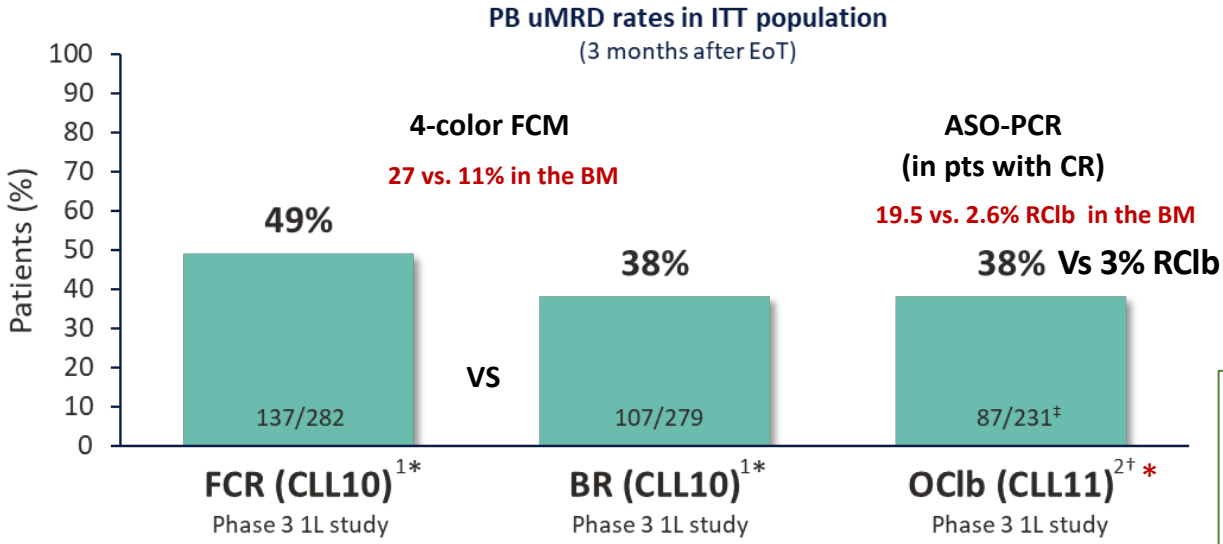
- **Each increase in PB MRD level was associated with significantly shorter PFS (p<0.0001).**
- **Highest MRD level (≥10^{-2}) was associated with significantly shorter OS when compared with the 2 lower MRD levels (p<0.0001).**

*MRD was quantified by multiparametric flow cytometry (sensitivity of ≥10^{-4}). Out of 817 patients, 290 patient samples were evaluated at final restaging.
CIT=Chemoimmunotherapy. CLL=Chronic Lymphocytic Leukemia. CT=Chemotherapy. FC=Fludarabine and Cyclophosphamide.
FCR=Fludarabine, Cyclophosphamide, and Rituximab. MRD=Minimal Residual Disease. NR=Not Reached. OS=Overall Survival. PB=Peripheral Blood. PFS=Progression-Free Survival.

1. Böttcher S, et al. J Clin Oncol 2012; 30:980–988. 2. Hallek M, et al. Lancet 2010; 376:1164–1174.

Less than 50% of patients achieve uMRD with certain CIT regimens

uMRD during treatment with CIT in 1L CLL was evaluated in CLL10 and CLL11



* 6-courses of obinutuzumab-Chl (O-Chl) or R-Chl or Chl single agent in 781 previously untreated and unfit CLL patients.

*MRD was quantified by multiparametric flow cytometry (sensitivity of ≥10^{-4}). †MRD was quantified by ASO-PCR (sensitivity of ≥10^{-4}). ‡ITT was not reported.
ASO-PCR=Allele Specific Oligonucleotide Polymerase Chain Reaction. B=Bendamustine. CIT=Chemoimmunotherapy. Clb=Chlorambucil. CLL=Chronic Lymphocytic Leukemia. EoT=End of Treatment.
FCR=Fludarabine, Cyclophosphamide, and Rituximab. ITT=Intent-to-Treat. MRD=Minimal Residual Disease. O=Obinutuzumab. PB=Peripheral Blood. PD=Progressive Disease. R=Rituximab. u=Undetectable.
1. Eichhorst B, et al. Lancet Oncol. 2016;17:928-42. 2. Goede V, et al. N Engl J Med. 2014;370(12):1101-10.

✓ = supported

X = not supported

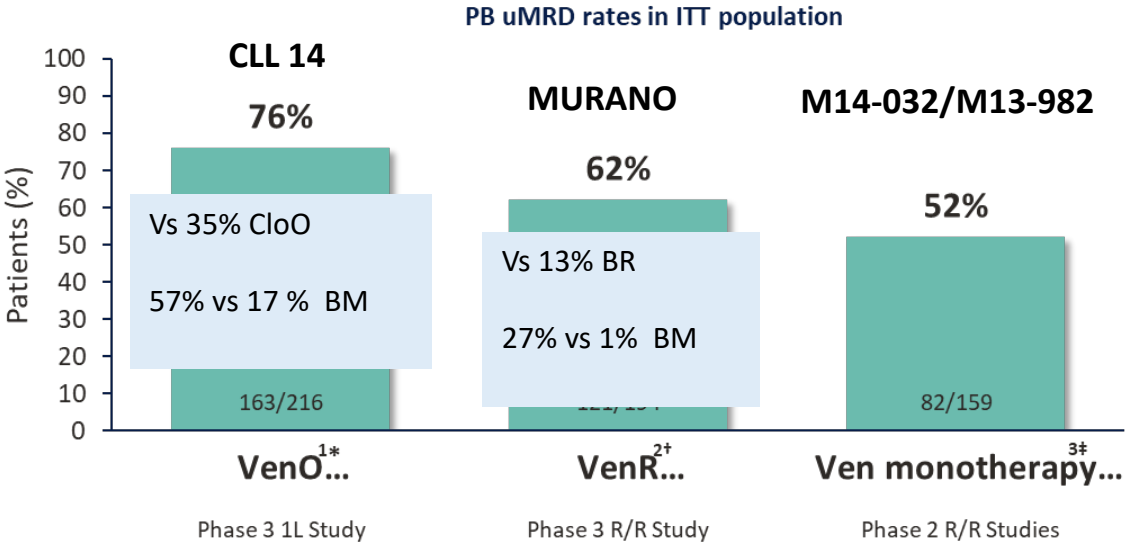
Is uMRD always predictive of long-term outcomes?

BCL2i
✓

. BCL2i=B Cell Lymphoma 2 Inhibitor. uMRD=Undetectable Minimal Residual Disease.

Majority of patients achieve uMRD with BCL2 inhibitors

Venetoclax regimens were evaluated in CLL14 in 1L CLL and in MURANO and monotherapy trials in R/R CLL



*MRD was quantified by ASO-PCR (sensitivity of ≥10^{-4}). †MRD was quantified by multiparametric flow cytometry or ASO-PCR (sensitivity of ≥10^{-4}). ‡MRD was quantified by multiparametric flow cytometry (sensitivity of ≥10^{-4}).
1L=First-Line. ASO-PCR=Allele Specific Oligonucleotide Polymerase Chain Reaction. BCL2=B-Cell Lymphoma 2. CLL=Chronic Lymphocytic Leukemia. EoCT=End of Combination Treatment. EoT=End of Treatment.
ITT=Intent-to-Treat. MRD=Minimal Residual Disease. O=Obinutuzumab. PB=Peripheral Blood. R=Rituximab. R/R=Relapsed/Refractory. u=Undetectable. Ven=Venetoclax

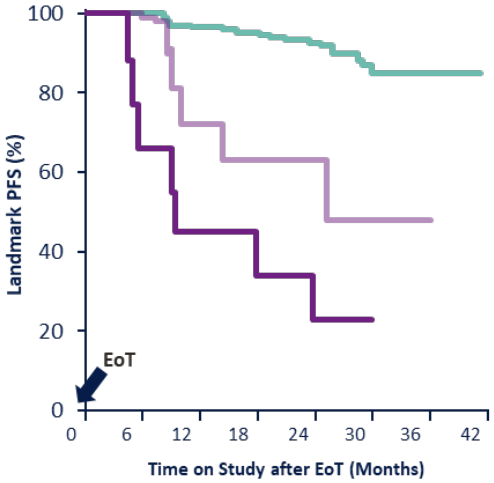
1. Fischer K, et al. N Engl J Med. 2019;380:2225-36. 2. Seymour JF, et al. N Engl J Med. 2018;378(12):1107-1120. 3. Weirda WG, et al. Poster #3134. 60th ASH Annual Meeting; December 1-4, 2018; San Diego, CA.

CT/CIT | BCL2i | BTKi

uMRD is associated with improved long-term outcomes across BCL2i-based regimens

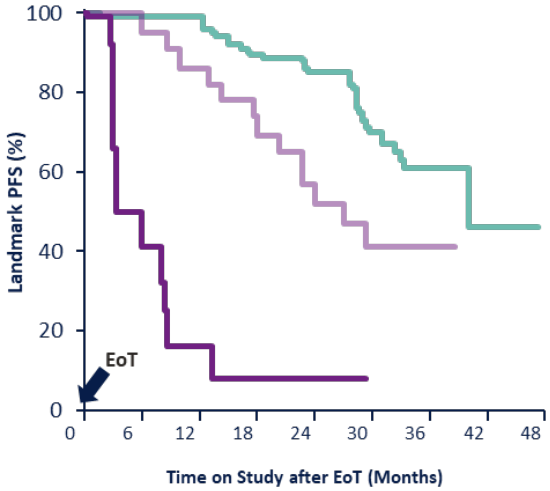
CLL14: VenO 1L CLL[1]
(median follow-up of 39.6 months)

PFS by MRD status in PB at EoT*



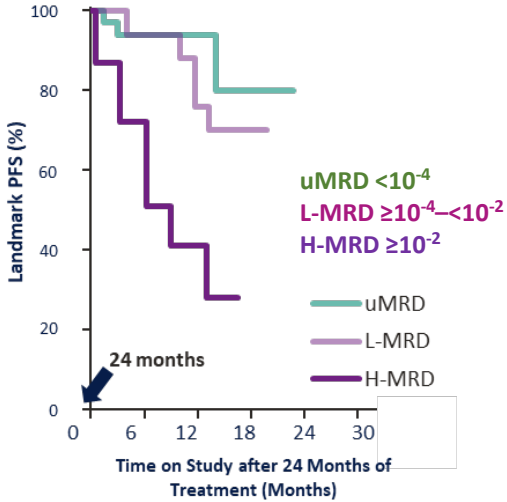
MURANO: VenR R/R CLL[2]
(median follow-up of 59 months)

PFS by MRD status in PB at EoT†



M14-032/M13-982: Ven mono R/R CLL[3]
(median follow-up of 28.8 months)

PFS by PB MRD Status at 24 Months‡



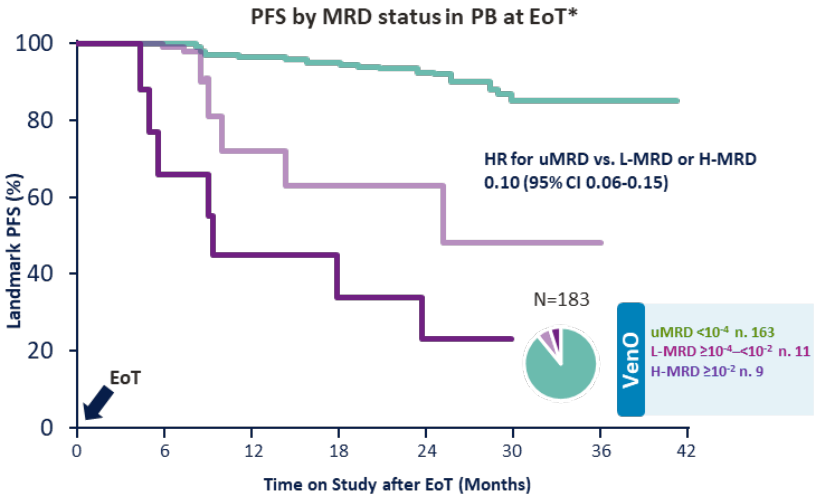
*MRD was quantified by ASO-PCR (sensitivity of ≥10^{-4}). †MRD was quantified by multiparametric flow cytometry or ASO-PCR (sensitivity of ≥10^{-4}). ‡MRD was quantified by multiparametric flow cytometry (sensitivity of ≥10^{-4}). uMRD <10^{-4}; L-MRD ≥10^{-4}–<10^{-2}; H-MRD ≥10^{-2}. 1L=First-Line. BCL2i=B-Cell Lymphoma 2 Inhibitor. CLL=Chronic Lymphocytic Leukemia. EoT=End of Treatment. H=High. L=Low. MRD=Minimal Residual Disease. O=Obinutuzumab. PB=Peripheral Blood. PFS=Progression-Free Survival. R=Rituximab. R/R=Relapsed/Refractory. u=Undetectable. Ven=Venetoclax.

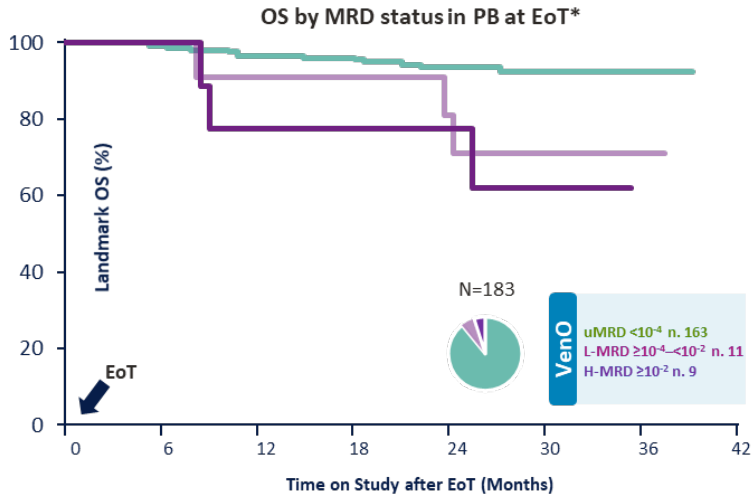
1. Al-Sawaf O, et al. Lancet Oncol 2020;21:1188–1200.
2. Kater A, et al. Oral #125. 62nd ASH Annual Meeting. December 6-10, 2020. Virtual. 3. Weirda WG, et al. Poster #3134. 60th ASH Annual Meeting; December 1-4, 2018; San Diego, CA.

uMRD is associated with improved long-term outcomes

CLL14: PFS and OS by MRD status in PB at EoT*

Phase 3 randomized study of VenO vs OClb in patients with 1L CLL with coexisting conditions (median follow-up of 39.6 months)





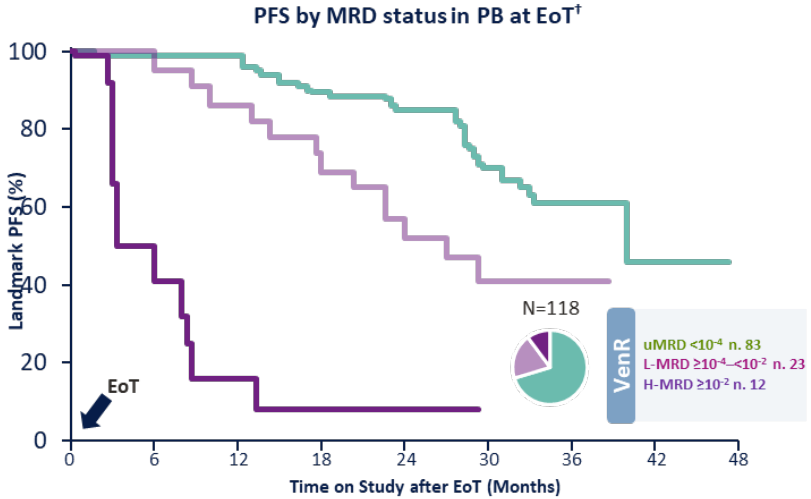
*3 months after treatment completion. MRD was analyzed by quantitative ASO-PCR (sensitivity of $\geq 10^{-4}$). uMRD $<10^{-4}$; L-MRD $\geq 10^{-4}$–$<10^{-2}$; H-MRD $\geq 10^{-2}$.
1L=First Line. ASO-PCR=Allele Specific Oligonucleotide Polymerase Chain Reaction. BCL2i=B-Cell Lymphoma 2 Inhibitor. CI=Confidence Interval. Clb=Chlorambucil. CLL=Chronic Lymphocytic Leukemia. EoT=End Of Treatment. H=High. HR=Hazard Ratio. L=Low. MRD=Minimal Residual Disease. O=Obinutuzumab. OS=Overall Survival. PB=Peripheral Blood. PFS=Progression-Free Survival. u=Undetectable. Ven=Venetoclax.

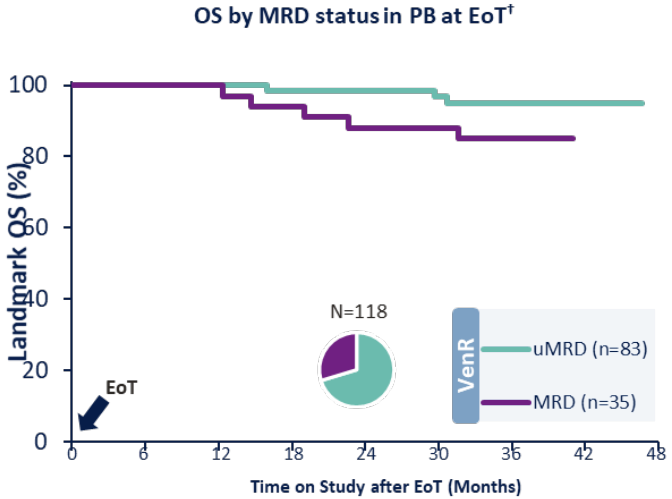
1. Al-Sawaf O, et al. Lancet Oncol 2020;21:1188–1200.

uMRD is associated with improved long-term outcomes irrespective of line of therapy with BCL2i

MURANO: PFS by MRD status in PB at EoT†

Phase 3 randomized study of VenR vs BR in patients with R/R CLL (median follow-up of 59 months)





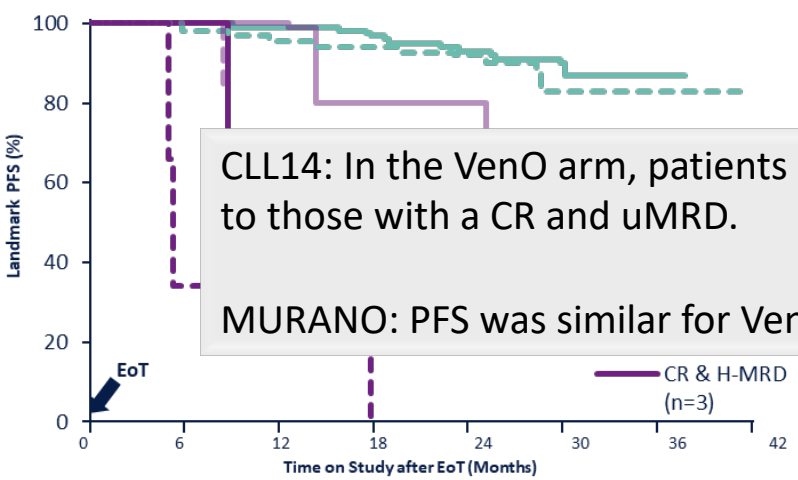
†MRD status was assessed centrally in PB with the use of both ASO-PCR and flow cytometry (sensitivity of $\geq 10^{-4}$). Only 118 samples were available for follow-up assessment. uMRD $<10^{-4}$; L-MRD $\geq 10^{-4}$–$<10^{-2}$; H-MRD $\geq 10^{-2}$.
ASO-PCR=Allele-Specific Oligonucleotide-Polymerase Chain Reaction. BCL2i=B-Cell Lymphoma 2 Inhibitor. CLL=Chronic Lymphocytic Leukemia.
EoT=End of Treatment. H=High. L=Low. MRD=Minimal Residual Disease. OS=Overall Survival. PB=Peripheral Blood. PFS=Progression-Free Survival. R=Rituximab.
R/R=Relapsed/Refractory. u=Undetectable. Ven=Venetoclax. Kater A, et al. Oral #125. 62nd ASH Annual Meeting. December 6-10, 2020. Virtual.

uMRD is associated with improved long-term outcomes regardless of clinical response with BCL2i

uMRD <10^{-4}; L-MRD ≥10^{-4}–<10^{-2}; H-MRD ≥10^{-2}

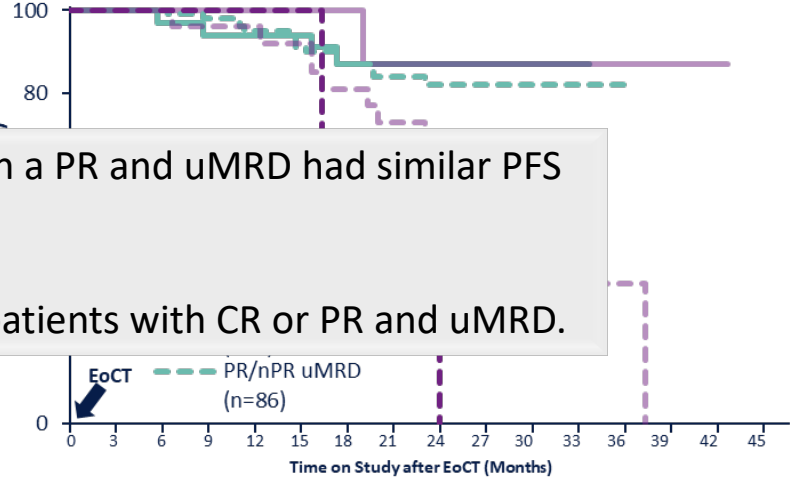
CLL14: VenO

PFS by response status and MRD status in PB at EoT*
(median follow-up of 39.6 months)



MURANO: VenR

PFS by response status and by MRD status in PB at EoCT†
(median follow-up of 36 months)



CLL14: In the VenO arm, patients with a PR and uMRD had similar PFS to those with a CR and uMRD.

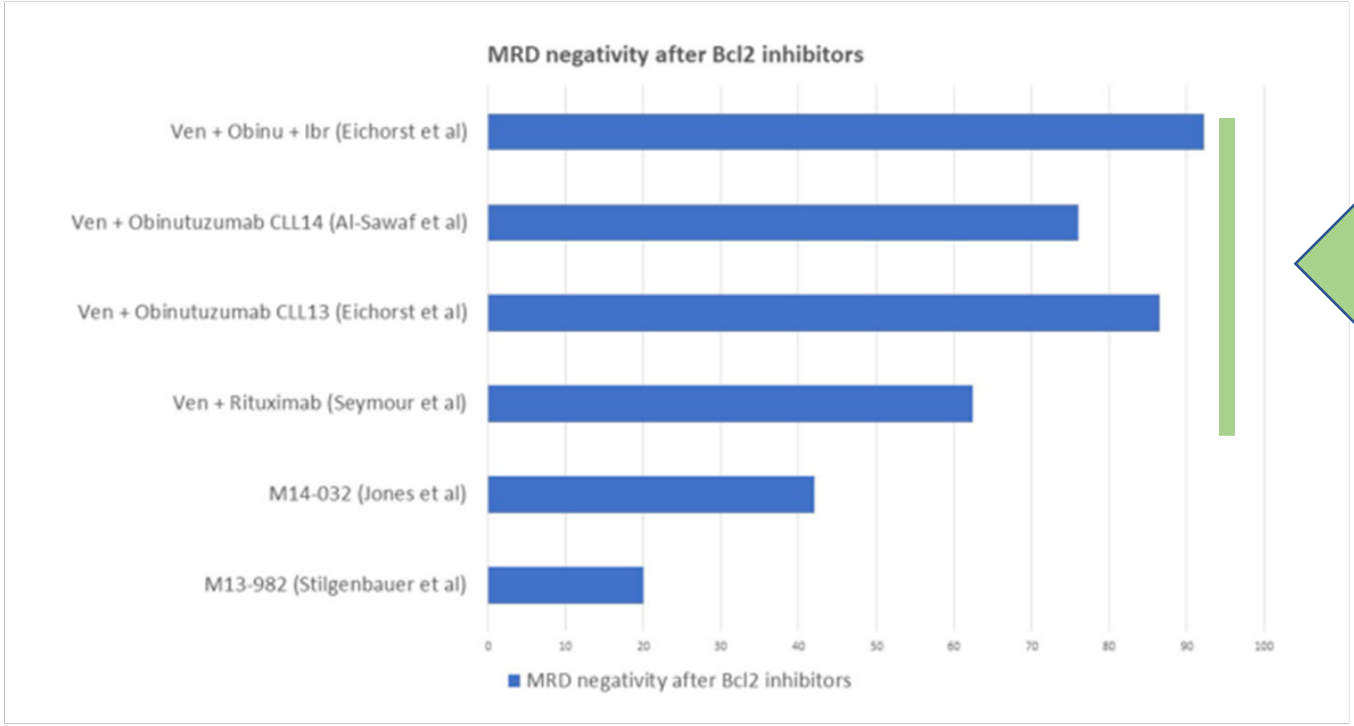
MURANO: PFS was similar for VenR patients with CR or PR and uMRD.

*3 months after treatment completion. MRD was quantified by ASO-PCR (sensitivity of ≥10^{-4}).

†2-3 months after end of combination therapy. MRD was quantified by multiparametric flow cytometry or ASO-PCR (sensitivity of ≥10^{-4}).

1L=First Line. ASO-PCR=Allele Specific Oligonucleotide Polymerase Chain Reaction. BCL2i=B-Cell Lymphoma 2 Inhibitor. CLL=Chronic Lymphocytic Leukemia. CR=Complete Response. CRi=CR with Incomplete Marrow Recovery. EoCT=End of Combination Treatment. EoT=End of Treatment. H=High. L=Low. MRD=Minimal Residual Disease. nPR=Nodular PR. O=Obinutuzumab. PB=Peripheral Blood. PFS=Progression-Free Survival. PR=Partial Response. R=Rituximab. u=Undetectable. Ven=Venetoclax. 1. Al-Sawaf O, et al. Lancet Oncol 2020; 21:1188–1200). 2. Kater AP, et al. Oral #695. 60th ASH Annual Meeting. Dec 1-4, 2018. San Diego, CA.

Rate of MRD negativity obtained by venetoclax monotherapy and venetoclax combined with anti CD20 monoclonal antibodies



Benintende G, Pozzo F, Innocenti I, Autore F, Fresa A, D'Arena G, Gattei V and Laurenti L (2023) Measurable residual disease in chronic lymphocytic leukemia. Front. Oncol. 13:1112616. doi: 10.3389/fonc.2023.1112616

✓ = supported

X = not supported

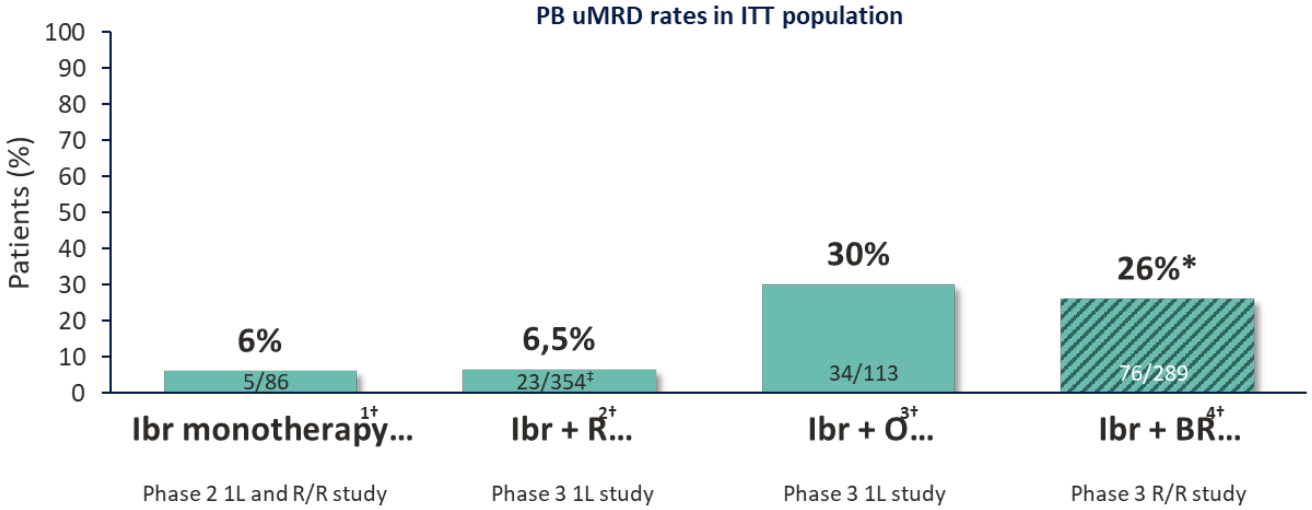
Is uMRD always predictive of long-term outcomes?

BTKi
X

BTKi=Bruton's Tyrosine Kinase Inhibitor. uMRD=UndetectableMinimal Residual Disease..

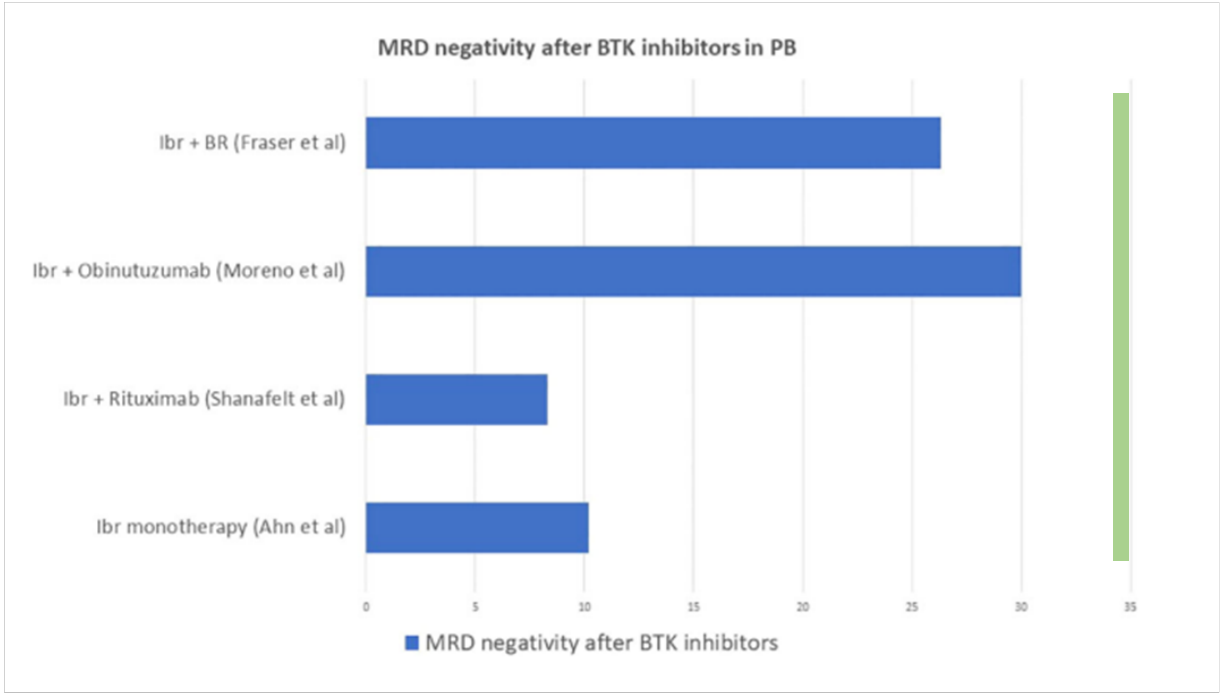
Minority of patients achieve uMRD with BTKi monotherapy

uMRD during treatment with ibrutinib monotherapy and ibrutinib regimens was evaluated in 1L and R/R CLL



*PB and BM combined. †MRD was quantified by multiparametric flow cytometry (sensitivity of $\geq 10^{-4}$).
‡ITT not reported in publication; MRD in ITT population reported in email communication from Shanafelt TD, 7 Jan 2021.
1L=First-Line. B=Bendamustine. BM=Bone Marrow. BTKi=Bruton's Tyrosine Kinase Inhibitor. C=Cycle. CLL=Chronic Lymphocytic Leukemia. Ibr=Ibrutinib.
ITT=Intent-to-Treat. mo=Month. MRD=Minimal Residual Disease. O=Obinutuzumab. PB=Peripheral Blood. R=Rituximab. R/R=Relapsed/Refractory. u=Undetectable. y=year.
1. Ahn IE, et al. Blood 2018;131(21):2357-2366. 2. Shanafelt TD, et al. N Engl J Med 2019;381:432–443. 3. Moreno C, et al. Lancet Oncol 2019;20:43–56. 4. Fraser G, et al. Leukemia 2019;33:969–980.

Rate of MRD negativity obtained by ibrutinib monotherapy, ibrutinib combined with anti CD20 monoclonal antibodies or with BR

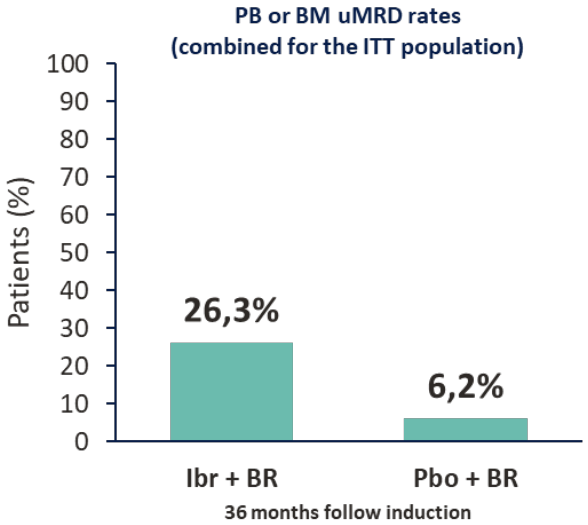


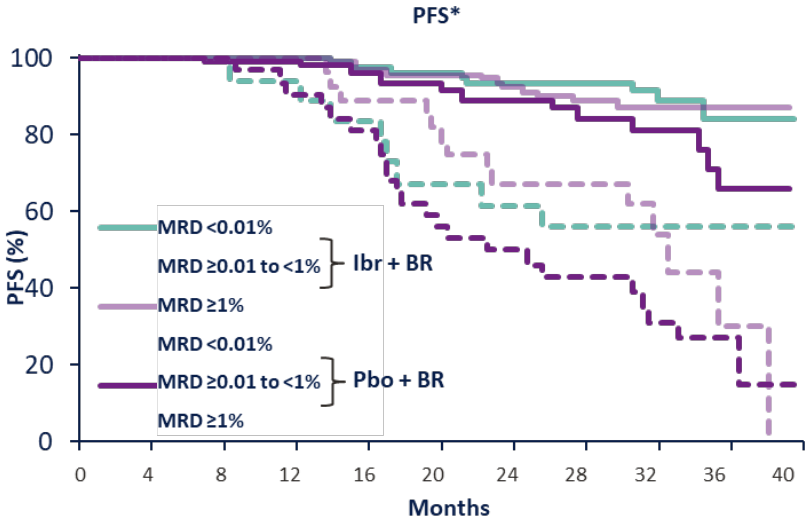
Benintende G, Pozzo F, Innocenti I, Autore F, Fresa A, D'Arena G, Gattei V and Laurenti L (2023) Measurable residual disease in chronic lymphocytic leukemia. Front. Oncol. 13:1112616. doi: 10.3389/fonc.2023.1112616

MRD status achieved with BTKi + CIT was not associated with improved long-term outcomes

HELIOS: PB or BM uMRD and PFS

Phase 3 study: Ibrutinib + BR vs. BR in previously treated CLL patients (median follow-up: 34.8 months)





*Investigator-Assessed PFS. MRD measured by flow cytometry (sensitivity of $\geq 10^{-4}$).
BM=Bone Marrow. BR=Bendamustine + Rituximab. BTKi=Bruton's Tyrosine Kinase Inhibitor. CIT=Chemoimmunotherapy. CLL=Chronic Lymphocytic Leukemia. Ibr=Ibrutinib. ITT=Intent-to-Treat. MRD=Minimal Residual Disease. PB=Peripheral Blood. Pbo=Placebo. PFS=Progression-Free Survival. u=Undetectable. 1. Fraser G, et al. Leukemia 2019; 33:969–980.

Treatment-naive fixed-duration traditional trial outcomes

Trial	Median age/ years	Regimen	Number recruited	ORR	CR/ CRi rate	PFS	OS	U-MRD4 rate PB	U-MRD4 rate BM
CLL14	72	ClbO	216	NR	NR	Median PFS 35.6 m, 49.5% at 39.6 m	87% at 39.6 m	35% at 3 m post-treatment completion, 7% at 18 m post-treatment completion	56.9% at 3 m post-treatment completion
		VO	216	NR	NR	Median PFS not reached, 81% at 39.6 m	87% at 39.6 m	76% at 3 m post-treatment completion, 47% at 18 m post-treatment completion	17.1% at 3 m post-treatment completion
CAPTIVATE FD	60	IV	159	96%	56%	95% at 24 m	98% at 24 m	77% TP53-WT 81% TP53-D	62% TP53-WT 41% TP53-D
GLOW	71	IV	106	86.8%	38.7%	84.4% at 24 m, 80.5% at 30 m	90% at 27.7 m	54.7%	51.9%
		ClbO	105	84.8%	11.4%	44.1% at 24 m, 35.8% at 30 m	88.6% at 27.7 m	39.0%	17.1%
CLL13	61	CIT (FCR or BR)	229	NR	NR	NR	NR	52% at 15 m	37.1% at 15 m
		VR	237	NR	NR	NR	NR	57% at 15 m	43% at 15 m
		VO	229	NR	NR	NR	NR	86.5% at 15 m	72.5% at 15 m
		VIO	231	NR	NR	NR	NR	92.2% at 15 m	77.9% at 15 m

PFS and OS reporting statistics are reported after the duration stated on treatment.
ClbO, chlorambucil–obinutuzumab; VO, venetoclax–obinutuzumab; IV, ibrutinib–venetoclax; CIT, chemoimmunotherapy; FCR, fludarabine–cyclophosphamide–rituximab; BR, bendamustine–rituximab; VR, venetoclax–rituximab; VIO, venetoclax–ibrutinib–obinutuzumab; m, months.

Fisher A, et al. (2023) The evolving use of measurable residual disease in chronic lymphocytic leukemia clinical trials. Front. Oncol. 13:1130617. doi: 10.3389/fonc.2023.1130617

Treatment-naive continuous therapy trial outcomes

Trial	Median age/years	Regimen/ arm	Number recruited	ORR	CR/CRi rate	PFS	OS	U-MRD4 rate PB	U-MRD4 rate BM
ELEVATE-TN	70	A	179	86%	1% at 24 m, 11.2% at 48 m	87% at 24 m	95% at 24 m, 87.6% at 48 m	7%[a] at CR/CRi, 10% at 48 m	0%[a]
		AO	179	94%	13% at 24 m, 30.7% at 48 m	93% at 24 m	95% at 24 m, 92.9% at 48 m	49%[a] at CR/CRi, 38% at 48 m	61%[a]
		ClbO	177	79%	5%	47% at 24 m	92% at 24 m	61%[a] at CR/CRi, 9% at 48 m	10.9%[a]
CAPTIVATE MRD	58	IV	164	97%	46%	≥95% at 30 m	NR	75% at 15 m	68% at 15 m
FLAIR	70	FCR	385	NR	NR	67 m	92% at 52.7 m	NR	NR
	NR	IR	386	NR	NR	Not reached	92% at 52.7 m	NR	NR
		I	138	NR	8% at 9 m	NR	NR	0%	0%
		IV	136	NR	59.6% at 9 m, 93.4% at 24 m	NR	NR	71.3% at 24 m	65.4% at 24 m

PFS and OS reporting statistics are reported after the duration stated on treatment.
A, acalabrutinib; AO, acalabrutinib–obinutuzumab; ClbO, chlorambucil-obinutuzumab; IV, ibrutinib–venetoclax; FCR, fludarabine–cyclophosphamide–rituximab; IR, ibrutinib–rituximab; I, ibrutinib; m, months.
[a]In ELEVATE-TN, MRD was measured in participants with CR/CRi, and the percentage reflects the CR/CRi cohort only.

Fisher A, et al. (2023) The evolving use of measurable residual disease in chronic lymphocytic leukemia clinical trials. Front. Oncol. 13:1130617. doi: 10.3389/fonc.2023.1130617

MRD to Guide Treatment Decisions is Still Under Study in Clinical Trials

Potential implications of MRD in guiding treatment in routine clinical practice in the future:

Guiding Treatment Duration

- Data has shown that **MRD-guided stopping** of FCR after 3 cycles is feasible without affecting long term survival and possibly sparing treatment-related toxicities.[2]
- MRD as a tool to ***limit the duration of chemo-free combinations*** is under evaluation, with a relevant impact on compliance, clonal selection/resistance, toxicity, and costs.[3]

Monitoring for Relapse

- Longitudinal monitoring of MRD to study CLL disease kinetics and the increase/reappearance of MRD could ***guide the rechallenge of therapy after treatment discontinuation***.[3]

Screening for Resistance

- Interim MRD data to screen for emerging mutations conferring resistance to a given drug (ex. BTK, PLCγ2, BCL2 mutations), could guide the ***switching to a non cross-resistant agent or combinations of agents***, in order to anticipate the clinical relapse.[3]

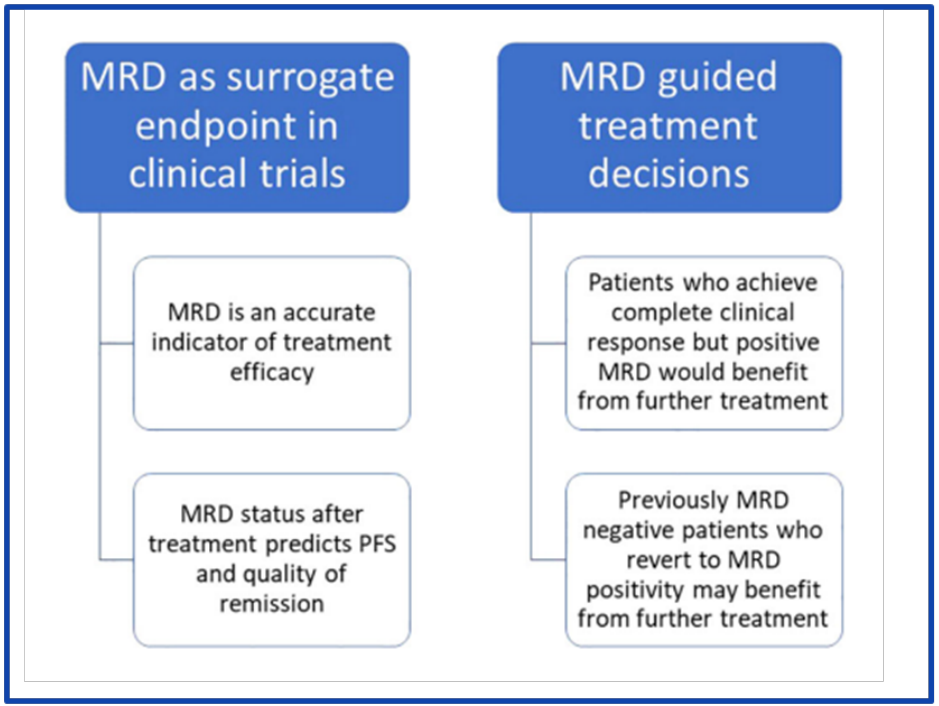
An ideal MRD-guided treatment strategy in CLL would have to be simple, affordable, use widely available methods and improve treatment outcomes in terms of safety and/or efficacy.[2]

At present it is not known whether long term outcome can be improved if MRD assessment is used to guide therapy, either to improve the quality of response through consolidation/maintenance therapy or to prevent relapse by therapies based on reappearance of MRD.[1]

CLL=Chronic Lymphocytic Leukemia. MRD=Minimal Residual Disease. PFS=Progression-Free Survival. 1. EMA. Document #EMA/CHMP/703715/2012 Rev. 2 (2014/2015). 2. Fürstenau M, et al. HemaSphere 2019; 3: e287. 3. Del Giudice, et al. Front Oncol. 2019;9:689. 4. Wierda WG et al. Leukemia 2021; 35.3059-3072. 5. Al Sawaf et al. Clin Adv Hematol Oncol 2022; 20:97-103.

Role of MRD in the clinical practice and potential use in routine management of CLL

The timing of MRD assessment can vary depending on the duration of the treatment and on the use of continuous or fixed time regiments, for which MRD is usually measured at the end of the treatment. The figure summarizes the role of MRD in the clinical practice.



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Given the clinical evidence, guidelines and regulatory agencies recognize uMRD as a predictor of outcomes, but only recommend MRD assessment as an endpoint in clinical trials (not in clinical practice)

MRD is a predictor of long-term outcomes

iwCLL[1]

*"Prospective clinical trials have provided substantial evidence that **therapies that are able to eradicate MRD** usually result in an **improved clinical outcome**"*

ESMO[2]

*"Patients who are **MRD-negative after therapy** show a **longer response duration and survival**"*

NCCN[3]

*"Evidence from clinical trials suggests that **undetectable MRD** in the peripheral blood after the end of treatment is an important **predictor of treatment efficacy**"*

FDA[4]

*"Literature suggests there is an **association between MRD negativity and OS** in patients with CLL treated with **CIT**. The therapeutic paradigm with **small molecule inhibitors of the BCR signaling pathway and other novel products continue to rapidly evolve**"*

EMA[5]

*"Available data has shown that **undetectable MRD** at the end of induction treatment is a **strong predictor of PFS and OS** irrespective of type & line of treatment and known poor pre-treatment risk factors"*

MRD assessment recommended in clinical trials, but not in clinical practice

iwCLL[1]

*"In **clinical trials** aimed at maximizing the depth of remission, the presence of **MRD after therapy should be assessed**."* Measurement of MRD is ***"not generally indicated"*** **in clinical practice**

ESMO[2]

MRD analysis has been approved by the EMA as a **surrogate endpoint to assess treatment efficacy in randomized clinical trials** designed to show superiority in terms of PFS, however *"MRD assessment **is not generally recommended for monitoring post therapy outside clinical studies"***

FDA/EMA[4,5]

Both agencies have provided guidance documents on the use of **MRD as an endpoint in clinical studies**

BCR=B Cell Receptor.
CIT=Chemoimmunotherapy. CLL=Chronic Lymphocytic Leukemia. MRD=Minimal Residual Disease. OS=Overall Survival. PFS=Progression-Free Survival. u=Undetectable.

1. Hallek M, et al. Blood 2018; 131:2745–2760.
2. Eichhorst B, et al. Ann Oncol 2015; 26(Suppl 5):v78–84. 3. NCCN Guidelines. CLL/SLL. V1.2023. 4. FDA. Document #FDA-2018-D-3090. 5. EMA. Document #EMA/CHMP/703715/2012 Rev. 2