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Assessing measurable residual disease (MRD) by cell-free DNA (cfDNA): results from a real-world series

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OBJECTIVES

- To define the role of cell-free DNA (cfDNA) as a tool to monitor measurable residual disease (MRD) in CLL, in comparison with other techniques like flow cytometry (FC) and genomic DNA (gDNA).

METHODS

- Pre- and post-treatment peripheral blood samples were collected for patients with CLL that required therapy (either treatment-naïve or relapsed/refractory).

CONCLUSIONS

- cfDNA MRD correlated well with both FC (r=0.48, p<0.01) and gDNA (r=0.92, p<0.0001).
- cfDNA MRD did not increase sensitivity or specificity over gDNA nor FC.

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INTRODUCTION

Measurable residual disease (MRD) correlates with outcomes in chronic lymphocytic leukemia (CLL). The techniques most frequently employed to detect MRD are flow cytometry (FC) and molecular approaches including the detection of Ig rearrangement by high-throughput sequencing (HTS) or real-time quantitative PCR (RQ-PCR). While cell-free DNA (cfDNA) is widely used in lymphomas, in CLL there is little information on whether cfDNA could be more representative of the tumor burden than other methods. Against this background, we aimed to define the role of cfDNA, tentatively compared to other techniques, to assess MRD in patients with CLL.

METHODS

This is a non-randomized, prospective, and observational study in which patients with CLL that required therapy (either treatment-naïve or relapsed/refractory) according to iwCLL guidelines were included.

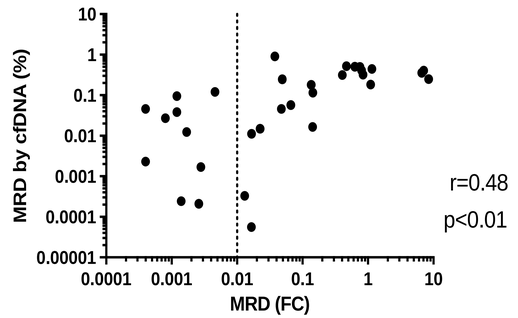
Peripheral blood samples were prospectively collected before treatment initiation, at the time of response, and every 3 months thereafter up to two years. Bone marrow samples were collected at the time of response whenever possible.

MRD was assessed by multiparameter FC (by 6-colour, CD19, CD43, CD20, CD81, CD79b, and CD5) and clonoSEQ®. For the detection of the rearranged IGHV sequence, genomic DNA was extracted from PMN cells and sent to Adaptive alongside with frozen plasma samples. The cut-off used for MRD positivity by HTS was above 10^{-6} (LOD) and 10^{-4} in flow MRD samples.

RESULTS

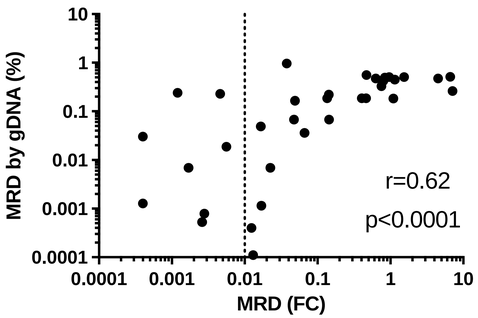
- **Patient characteristics**: between 2020 and 2024, fifty-five patients with CLL who required therapy were enrolled. Here we show the preliminary data from the first 49 patients. Ten patients were excluded due to progressive disease (n=7), premature dead (n=2), and screening failure (n=1). Among responders (n=39), median age was 69 (range 42-86) and 41% were male. Twenty patients were treated with BTKi (19 ibrutinib, 1 acalabrutinib), 15 with venetoclax-based regimens (4 with obinutuzumab, 4 with rituximab, 3 with ibrutinib, and 4 in monotherapy), and 5 with FCR; 28 were treatment-naïve and 11 relapsed/refractory. Twenty patients (51.3%) achieved complete response and 19 (48.7%) partial response.
- **MRD results**: MRD follow-up was available for 26 patients. At the first post-treatment evaluation, 9/26 (34.6%) achieved undetectable (uMRD) by FC, 10/21 (47.6%) by cfDNA and 9/26 (34.6%) by gDNA. The median number of MRD determinations was 2 (range 1-5).
- **Correlation studies (Fig. 1, 2, and 3)**: both cfDNA (56 samples from 22 patients) and gDNA (68 samples from 26 patients) results from peripheral blood samples were moderately correlated with FC independent of type of therapy. In addition, the correlation between cfDNA and gDNA determinations was very high.
- **Predictive values: Tables 1-3.**
- **Role of cfDNA and lymph nodes** (**Fig. 4**): we further explored the assessment of MRD using cfDNA in patients who had detectable lymph nodes by CT scan vs those who did not. The cfDNA was positive in 9/12 (75%) patients with lymph nodes vs 3/10 (30%) without them [OR 6.31 (95% CI 0.80-68.43, p=0.08)]. Of note, in this setting cfDNA did not show an increased sensitivity compared to either gDNA or FC MRD detection.

Figure 1. Correlation between cfDNA and FC MRD



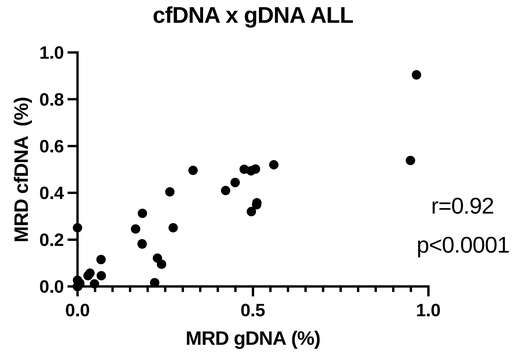
Scale is logarithmic. MRD, measurable residual disease; cfDNA, cell-free DNA; FC, flow cytometry

Figure 2. Correlation between gDNA and FC MRD



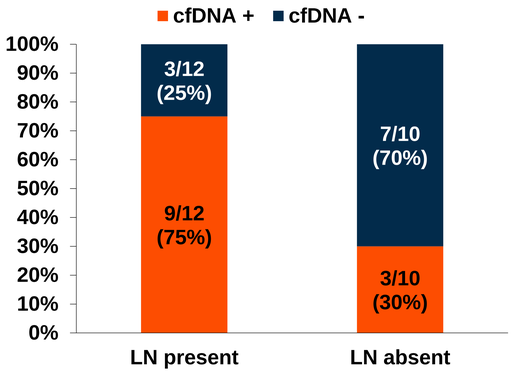
Scale is logarithmic. MRD, measurable residual disease; gDNA, genomic DNA; FC, flow cytometry

Figure 3. Correlation between cfDNA and gDNA MRD



MRD, measurable residual disease; cfDNA, cell-free DNA; gDNA, genomic DNA

Figure 4. cfDNA MRD relationship with lymph nodes



cfDNA, cell-free DNA; LN, lymph nodes

Tables 1/2. Cross tables cfDNA/gDNA vs FC MRD
Table 3. Sensitivity, specificity, PPV, and NPV

Table 1	cfDNA +	cfDNA -
FC +	24/56 (42.9%)	11/56 (19.6%)
FC -	5/56 (8.9%)	16/56 (28.6%)

Table 2	gDNA +	gDNA -
FC +	34/68 (50%)	9/68 (13.2%)
FC -	10/68 (14.7%)	15/68 (22.1%)

Table 3	Sensitivity	Specificity	PPV	NPV
cfDNA	68.6%	76.2%	82.8%	59.3%
gDNA	79.1%	60%	77.3%	62.5%

cfDNA, cell-free DNA; gDNA, genomic DNA; PPV, positive predictive value; NPV, negative predictive value

CONCLUSIONS

- cfDNA HTS did not increase sensitivity or specificity over genomic DNA nor multiparametric FC MRD assessment including patients with lymph nodes.
- Collectively, there was a strong correlation between cfDNA and gDNA, suggesting that cfDNA MRD monitoring in CLL is feasible.

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DISCLOSURES

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CM is presently employed by AstraZeneca