IGHV Next-Generation Sequencing in CLL: Integrating Mutational Status and Clonotype Tracking for MRD Assessment

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INTRODUCTION

Measurable residual disease (MRD) is a key biomarker for assessing treatment response and prognosis in chronic lymphocytic leukemia (CLL). Flow cytometry (FC), the standard MRD method, offers rapid immunophenotyping with sensitivity around 10^{-4} and established prognostic value, especially for undetectable MRD (uMRD). However, its limited sensitivity and inability to track clonal heterogeneity restrict its use. Next-generation sequencing (NGS) of the immunoglobulin heavy chain variable region (IGHV) advances MRD detection by providing higher sensitivity (~10^{-6}) without patient-specific primers. It also allows simultaneous determination of IGHV mutational status and detailed longitudinal clonal tracking within a polyclonal background. This study evaluates IGHV analysis by NGS for mutational status and dynamic MRD monitoring to improve prognostic accuracy and treatment guidance in CLL.

PATIENTS AND METHODS

A retrospective observational study included 58 untreated patients diagnosed with CLL/CLL-like monoclonal B-cell lymphocytosis (MBL) from 1999 to 2024. IGHV mutational status was determined by Sanger sequencing and compared to an automated method using shorter reads (Illumina 2 × 150 bp), and the subsequent analysis with in-house pipeline (https://github.com/afuentri/B347 MyRepCLL). Clonotype monitoring by NGS was performed on 127 sequential samples from 23 patients treated with chemoimmunotherapy (CIT; 40%) and 35 patients treated with ibrutinib (60%). Flow cytometry was conducted on peripheral blood after erythrocyte lysis using the ERIC 8-color panel following EuroFlow guidelines. Data acquisition was performed on a FACSCanto II (BD) and analyzed with Infinicyt™ (Cytognos). MRD was expressed as the fraction of CLL cells among nucleated cells, with negativity defined as <1×10^{-4}.

RESULTS

- The mean number of sequences analyzed per patient was comparable between groups—112,885 for CIT (range: 1,471–1,427,745) and 118,308 for ibrutinib (range: 1,000–585,659). The average dominant clonotype count was higher in CIT-treated patients (104,823; range: 0–1,427,019) than in those receiving ibrutinib (48,288; range: 0–488,194). V-gene mapping efficiency was lower in CIT (mean: 25.85%) compared to ibrutinib (mean: 80.46%).
- In the CIT subgroup, the primary IGVH rearrangement was undetectable in 34% of samples (19/56). Among these, 100% (19/19) of patients achieved complete remission (CR). In contrast, 81.3% (13/16) of patients with an NGS IGVH ratio >10% relapsed (P < 0.001). In 5% of cases (3/56), the clonotype read ratio was <1%, while in 29% (16/56) it remained >18%.
- Among ibrutinib-treated patients, the initial clonotype was undetectable in only 3% of samples (2/80), both from the same individual. After excluding 28 samples (35%) with persistent lymphocytosis, 62% (50/80) of the remaining samples showed an NGS IGVH ratio >14%, with a mean ALC of 2.95 × 10^9/L (range: 0.90–4.98).
- A separate comparative analysis of MRD detection by NGS and flow cytometry (FC) was conducted in 42 CIT-treated patients (67 follow-up samples). At baseline, 81% had a single dominant clonotype, while 19% had dual IGVH rearrangements. The median number of sequences per sample was 148,381 (range: 1,897–1,845,191), with a mean dominant clonotype count of 111,219 and median V-gene mapping of 48.68%. NGS demonstrated >90% concordance with FC at the <0.01% (<10^{-4}) threshold, with two discordant cases in which NGS detected low-level MRD (0.4% and 0.9%) not identified by FC. Both cases converted to MRD-negative in subsequent samples. In samples with MRD between <10^{-4} and 10^{-4}, polyclonal patterns were observed (mean maximum inter-rearrangement difference: –1.25×).
- In dual-rearranged cases, MRD was undetectable by FC in 11 of 16 samples. NGS showed full concordance (11/11) when tracking only the productive clonotype; non-productive rearrangements yielded detectable MRD down to 0.020%.

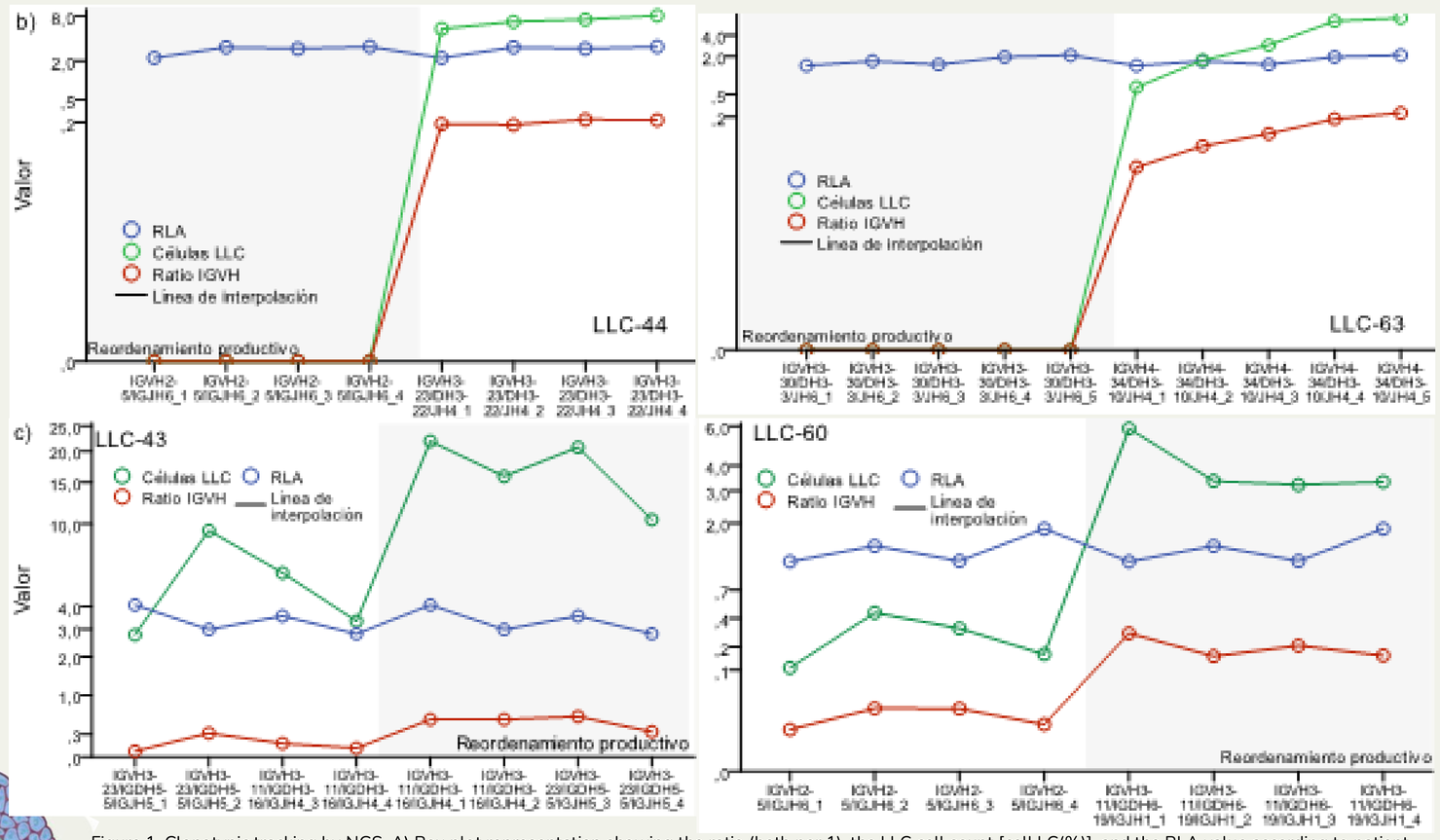

Figure 1. Clonotypic tracking by NGS. A) Box plot representation showing the ratio (both per 1), the LLC cell count [celLLC(%)], and the RLA value according to patient subgroups with IGVH undetectable, IGVH<1%, or >10%. The differences were significant between the value of each subgroup (P<0.001). B) Scatter plots showing the dynamics of RLA, celLLC(%), and the ratio (%), based on each of the rearrangements detected in the sequential samples from cases LLC-44 and LLC-63 treated with IQT c) and from cases LLC-43 and LLC-60 treated with TD. (RLA: absolute lymphocyte count (×10^9/L)).

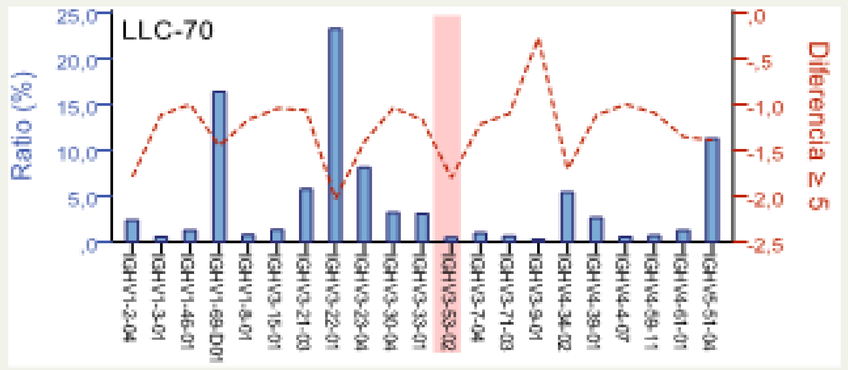

Figure 2. Clonal distribution of the different IGVH rearrangements when evaluating the MRD by NGS. Bar graph LLC-70 and LLC-79 with MRD >10-4 by NGS, but with polyclonal distribution (red dashed line, right axis) where the initial clonotype (shaded in red) does not exceed 0.4% and 0.9% (left axis) of the total mapped reads in the IGH region.

CONCLUSIONS

- NGS-based MRD monitoring offers high sensitivity and strong clinical correlation in CLL. Undetectable clonotypes post-chemoimmunotherapy were associated with complete remission, while persistent clonotypes predicted relapse. In ibrutinib-treated patients, clonotypes often remained detectable, reflecting distinct clonal dynamics. NGS showed high concordance with flow cytometry, particularly when tracking productive rearrangements. These results support NGS as a reliable tool for MRD assessment and risk stratification in CLL.