CLONAL SPACE ASSESSMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA USING AN UNBIASED NGS APPROACH

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INTRODUCTION

Chronic Lymphocytic Leukemia (CLL) is characterized by the presence of clonal B-cells in peripheral blood. Leukemic B-cells express a unique VDJ rearrangement, and the somatic hypermutation status and V gene usages serve as prognostic markers.
Flow cytometry is the standard method for the identification of clonal B-cells by light chain restriction in the presence of an aberrant immunophenotype. Advances in sequencing technologies enables the detection of the total set of VDJ rearrangement in each sample at a given time point. In this work we evaluate a relatively simple and cost-effective method to obtain information on clonal composition in CLL samples based on immunoglobulin heavy variable genes (IGHV) parallel sequencing.

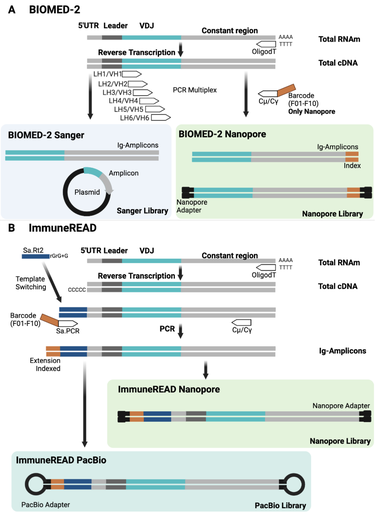
AIM

We propose a novel approach that combines balanced amplification and long-read sequencing technology to display the entire B-cell repertoire and distinguish tumoral clonal reads from non-clonal B-cell repertoire reads, thereby defining the clonal space (CS), along with the somatic hypermutation load and characterization of non-clonal B-cell repertoire.

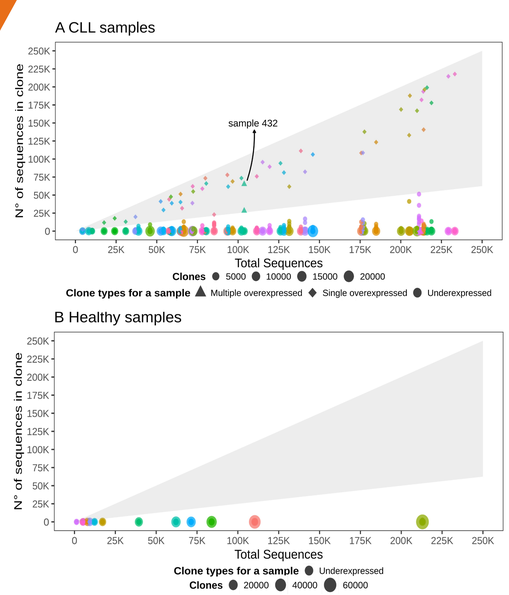
METHOD

A total of 47 CLL patients and 13 healthy donors (HD) were processed using by immunoglobulin specific Anchored-PCR and Nanopore sequencing. We developed an error-correction sequencing bioinformatic clustering system to group overexpressed reads. The clonal space was determined by the ratio of overexpressed reads to the non-clonal B-cell repertoire reads. The somatic hypermutation status was assessed in the overexpressed reads to characterize as mutated or unmutated cases. The rearrangement obtained was validated using BIOMED-2 PCR and sanger sequencing, which is considered the gold standard method for determining the IGVH mutational status.

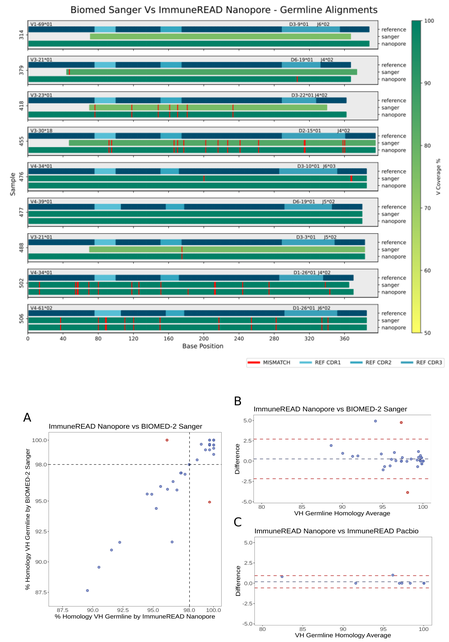
RESULTS



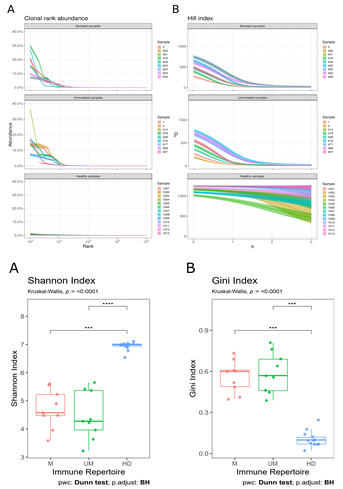
Two different PCR methods were used to amplify the IGHV rearrangement in this study. The first method, BIOMED-2 PCR, utilized primers that annealed to the leader or V region and Cmu or Cgamma of IGHC were developed by our group previously. The second method, ImmuneREAD PCR, utilized anchored-IGHV primers annealing to the Template Switched-cDNA and Cmu or Cgamma constant region. Sequencing methods employed in this study were Sanger, PacBio and Nanopore technology.



The method allowed for the detection of overexpressed clones in all CLL samples. The average of IGVH reads detected was 123,170, with an average of 96,065 overexpressed clonal reads. Using IGVH data from healthy donors (HD) and clonality data from flow cytometry we established a cut-off value of 0.25 for defining a clonal expansion. The clonal space (CS) had a mean value of 0.68 for CLL samples, with a CS of 0.70 for mutated-CLL (M-CLL) (95% CI 0.58-0.83) and 0.67 for unmutated-CLL (UM-CLL) (95% CI 0.58-0.75). Additionally, biclonality was identified in one sample (VH1-69, CS: 0.63, IGHV5-51, CS: 0.27).



The mutational status obtained for each predominant rearrangement had a sensitivity of 91%, specificity of 100% and correlation of 0.8 (p< 0.005) compared to the gold standard method. No overexpressed sequences were detected in HD.



We analyzed the remaining non clonal B-cell repertoire and found several alterations compared to the immune repertoire of HD; significant abundance of clones as measured by the number of reads composing each clone (reads per clone mean; range: 2.05-10.5), relative expansion of individual rearrangements (5-40% of the repertoire), lower repertoire richness as measured by Hill's index; (1. 178 vs 554 in M-CLL and 651 in UM-CLL), lower repertoire diversity measured by Shannon index (4.48 in M-CLL cases, 4.75 in UM-CLL cases, and 6. 94 in HD), greater inequality measured by Gini´s index (0.55 In mutated cases, 0.48 in unmutated cases, and 0.48 in HD), along with altered homogeneity composing predominant rearrangement. We also characterized the CDR3s of the repertoire finding differences in CDR3 sizes (18.2 aa in mutated cases, 19.6 aa in unmutated cases, and 15.8 aa in HD) and in the electrical charge they present (0.75 in mutated, 0.58 in unmutated, and 0.15 in HD).

CONCLUSIONS

These findings indicate the feasibility of using IGVH high throughput sequencing for identifying clonal B cells in peripheral blood. The proposed method allows the characterization of the clonal architecture of CLL and highlight the importance of accurate CS assessment. Our approach provides data about the proportion of tumoral reads and intraclonal diversity overcoming economic and technical limitations of assessing clonal architecture and intraclonal diversity by other conventional methods.

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