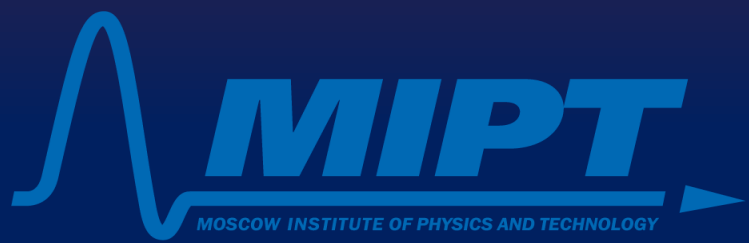


NGS APPROACH FOR IGHV REARRANGEMENT ANALYSIS IN COMPLICATED CLL CASES



Bella V. Biderman¹, Sergey Ya. Dumpis², Ekaterina B. Likold¹, Hunan L. Julhakyan¹, Andrey B. Sudarikov¹
¹National Research Center for Hematology, Moscow, Russian Federation
²Institute of Physics and Technology, Moscow, Russian Federation



Background

Mutational status of the IGHV genes, IGHV gene family usage, and B-cell receptor stereotype are the key prognostic factors for CLL. However, the routinely used Sanger sequencing technique may fail for the cases with an expansion of more than one tumor B-cell clones, especially with rearranged genes of the same IGHV family (up to 4% CLL cases – Figure 1a, 1b)

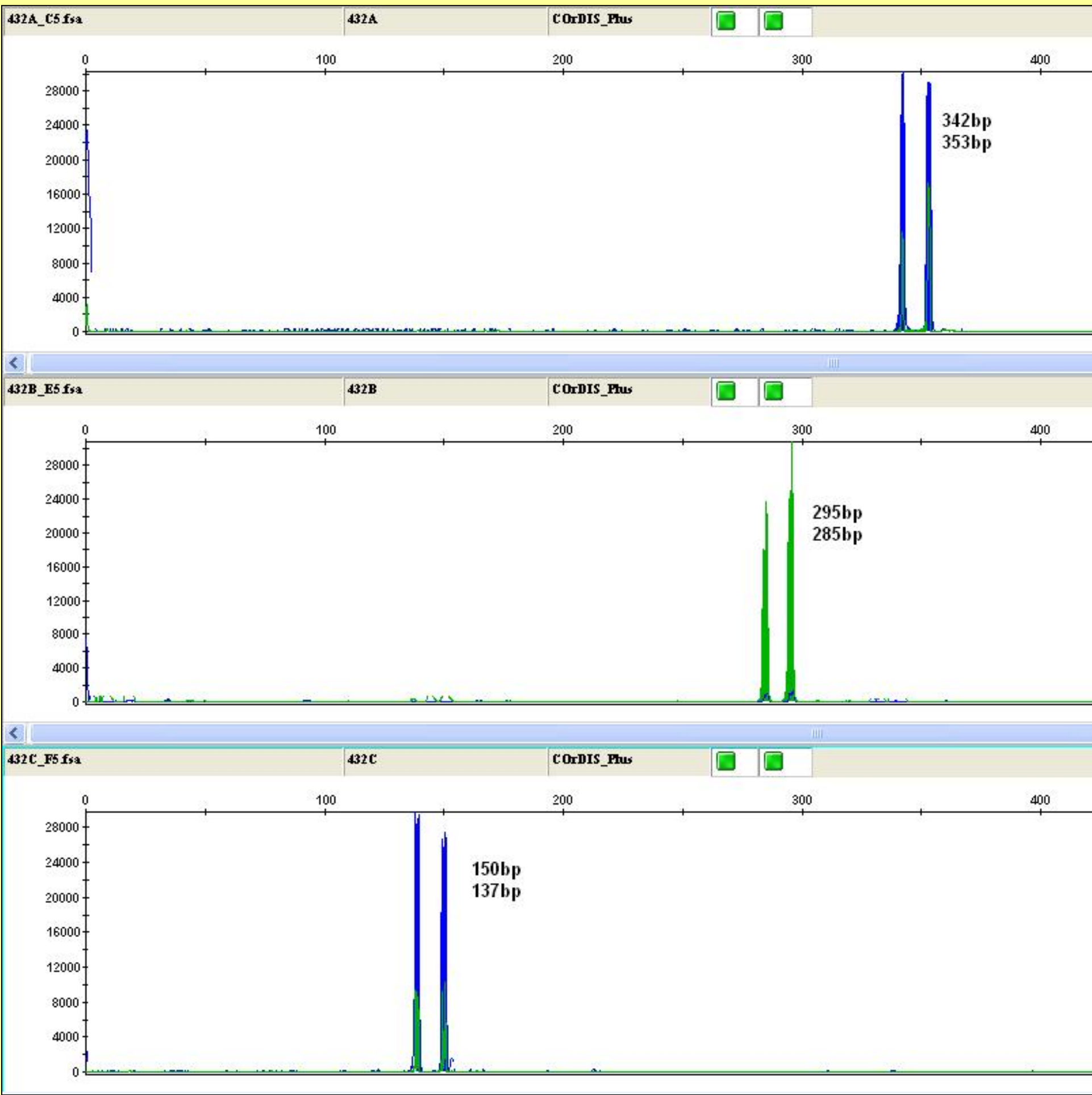


Fig 1a. Fragment analysis data of a CLL case with two clonal rearrangements in the same IGHV family.

Aim

To evaluate the robustness of NGS technology for IGHV analysis in complicated CLL cases.

Materials & Methods

DNA samples from CLL patients (N=95) for whom we previously failed to obtain IGHV rearrangements data by Sanger sequencing included in the study. IGHV loci were PCR amplified as described in BIOMED-2 protocol and by Campbell et al [1,2]. Sequencing libraries were prepared with Nextera XT kit (Illumina, USA) according to the manufacturer’s recommendations and were analyzed with MiSeq Genome Analyzer (Illumina, USA). Sequencing data were processed via Mixcr software [3]. The mutational status and receptor stereotype were identified using IMGT and ARRest databases according to ERIC recommendations [4].

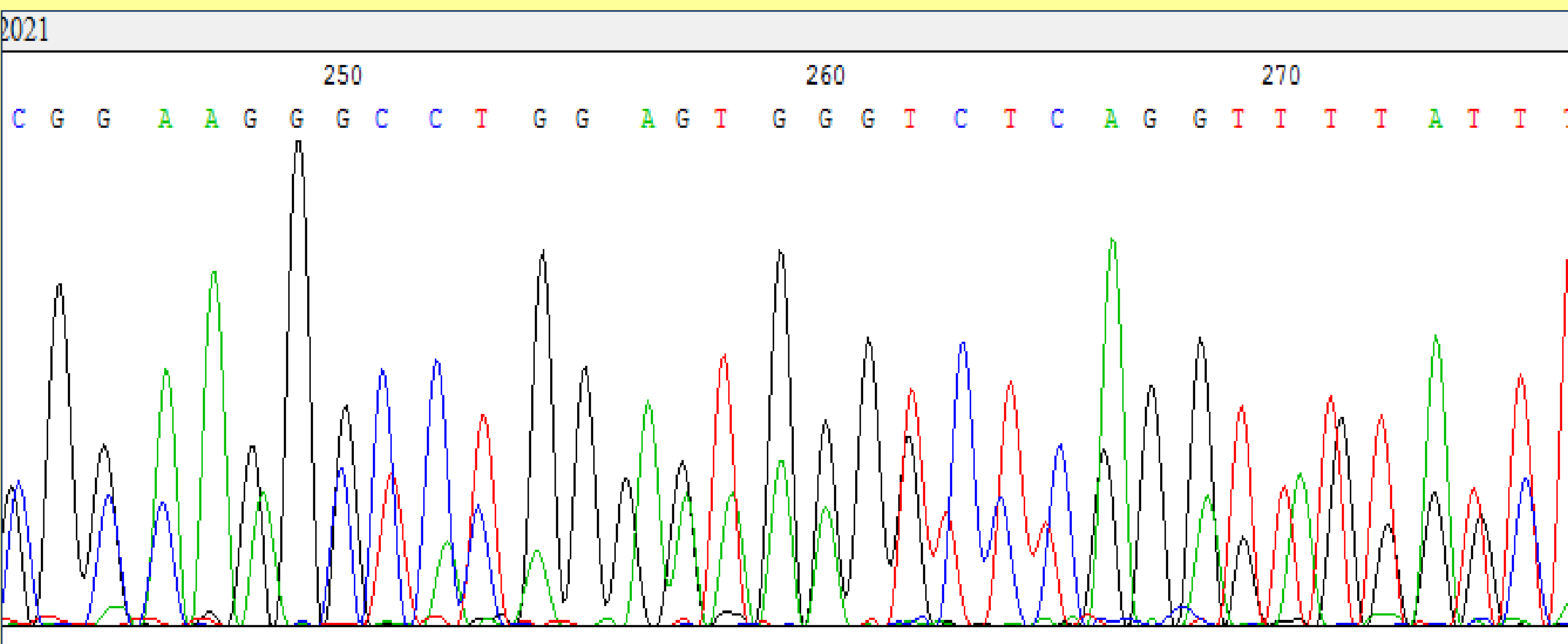


Fig 1b. Sanger sequence data of a CLL case with two clonal rearrangements in the same IGHV family.

Results

- In 64 cases two clonal rearrangements were identified (Table 1); in 60 cases IGHV genes of the same family were used in both clones (IGHV3 for 44 patients, IGHV1 for 11 and IGHV4 for 5). Two patients had IGHV1 and IGHV7 genes rearrangements (these two families are usually amplified with the same primer). Two more patients had combinations IGHV1-69+IGHV3-73 and IGHV3-15+IGHV4-59. In 63 cases only one rearrangement was productive, and one patient had two productive IGHV rearrangements. Herewith the mutational status did not differ within the rearrangement pairs.
- We determined U-CLL for 45 patients and M-CLL for 19 patients. Besides, 4 patients with stereotyped antigen receptor CLL#2 and 3 patients with CLL#1 were found (Table 1).
- 27 patients were found with only one productive clonal rearrangement. The heterogeneity of Sanger sequencing in these cases was presumably related to an insufficient amount of tumor cells in the sample comparing with a high polyclonal background.
- Four patients had three or more clonal rearrangements. In two cases all rearrangements were productive (IGHV3 and IGHV4 families) and highly mutated. One of these patients had two malignancies - CLL and mantle cell lymphoma. Both these two cases were classified as M-CLL. Other two patients had IGHV-rearrangements with different mutational status – this could be possible in the presence of additional lymphoproliferative disease.

Conclusions

Using NGS approach we have shown that in the cases with two IGHV gene rearrangements from the same VH gene family, variants with IGHV3 family are the most common (68,75%). U-CLL variants were also prevalent (70,3%). Though Sanger sequencing is still more suitable for routine IGHV gene analysis in CLL, a significant number of complicated cases might benefit from NGS technology that allow to obtain definite diagnosis and assign appropriate treatment.

	CLL with 2 clonal rearrangements	Russian CLL in general
U-CLL	70,3%	68%
M-CLL	29,7%	32%
IGHV3	68,75%	41,3%*
IGHV1	17,2%	34,3%*
IGHV4	7,8%	19,2%*
CLL#1	4,7%	3,3%
CLL#2	6,25%	1,4%*
	N = 64	N = 1800

Table 1. CLL types and IGHV families used in rearrangements in patients with 2 clonal rearrangements and in general. * marks the significant differences (p<0.05)

References

1. Campbell M.J., et al. Use of family specific leader region primers for PCR amplification of the human heavy chain variable region repertoire. Mol Immunol, 1992
2. van Dongen J.J. et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003
3. Bolotin D. et al. MiXCR: software for comprehensive adaptive immunity profiling. Nat. Methods, 2015
4. Rosenquist R et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. Leukemia. 2017