Poster #: 1007

Clonality assessment in multiclonal chronic lymphocytic leukemia using Sanger and next-generation sequencing approaches

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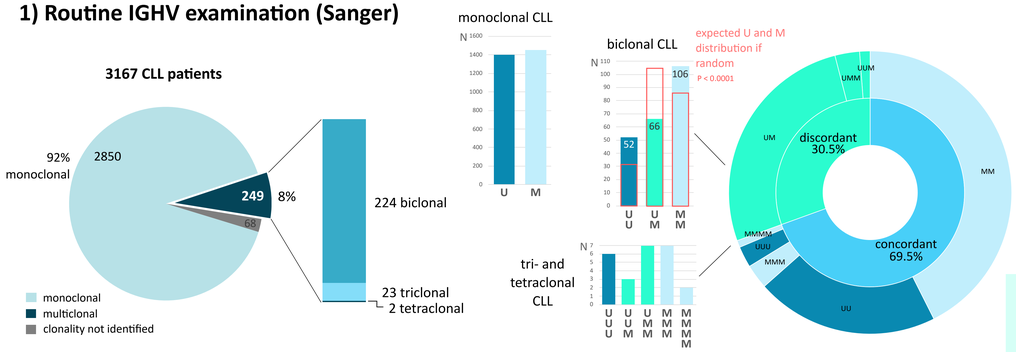
BACKGROUND

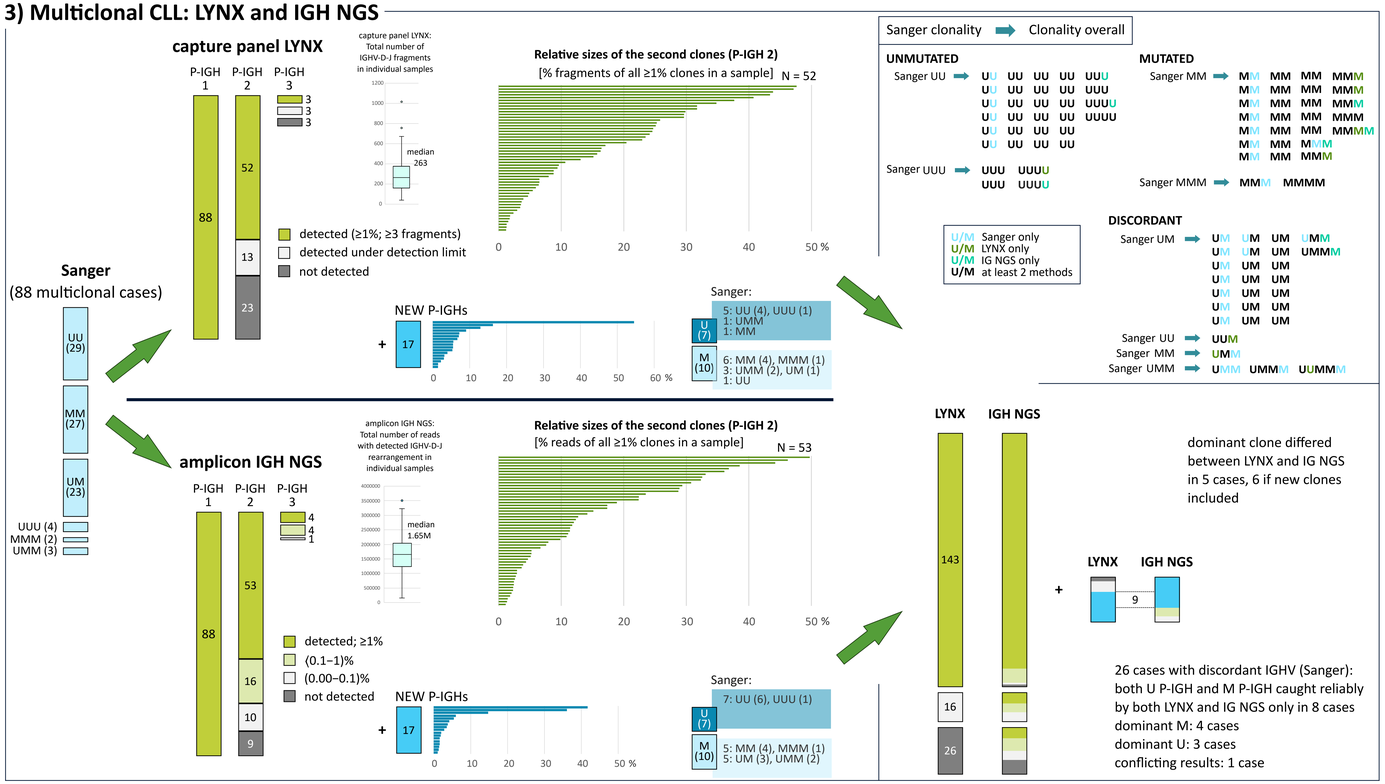
Chronic lymphocytic leukemia (CLL) is typically characterized by the presence of a dominant B-cell clone with a unique immunoglobulin heavy-chain (IGH) gene rearrangement. However, a subset of patients exhibits multiple clonal IGH rearrangements, indicating underlying multiclonality. At the single cell level, we have demonstrated that each productive IGH gene rearrangement (P-IGH) represents a distinct B-cell clone [1]. Shifts in clonal ratios have been observed [2] highlighting the importance of their monitoring. Understanding of clonal architecture and its evolution over time is essential for insights into disease biology and progression. Here we employ capture-based and amplicon NGS approaches and compare their results with traditional Sanger sequencing.

RESULTS

1) Routine IGHV examination (Sanger)



3) Multiclonal CLL: LYNX and IGH NGS



METHODS

PATIENTS

University Hospital Brno
2006–2025

3167 CLL patients → Sanger

- **88 multiclonal samples** (80 untreated patients, 8 treated) → Sanger; LYNX; IGH NGS
- **62 consecutive monoclonal samples** (62 patients at diagnosis) → Sanger; IGH NGS

SANGER SEQUENCING

- cDNA
- PCR amplification, fragment analysis, cloning if needed
- L–C primers; IGHV family singleplexes + multiplex

CAPTURE-BASED NGS PANEL LYNX [3]

lynx.ceitec.muni.cz

- DNA
- capture with probes, amplification with UMIs
- whole IGH sequence FR1–junction can be obtained
- analyzed with LYNX online tool
- typically hundreds of fragments (unique molecules) covering IGHV-D-J
- clonal productive IGH rearrangement (P-IGH) cut-off:
  1% of fragments covering IGHV-D-J; 3 fragments

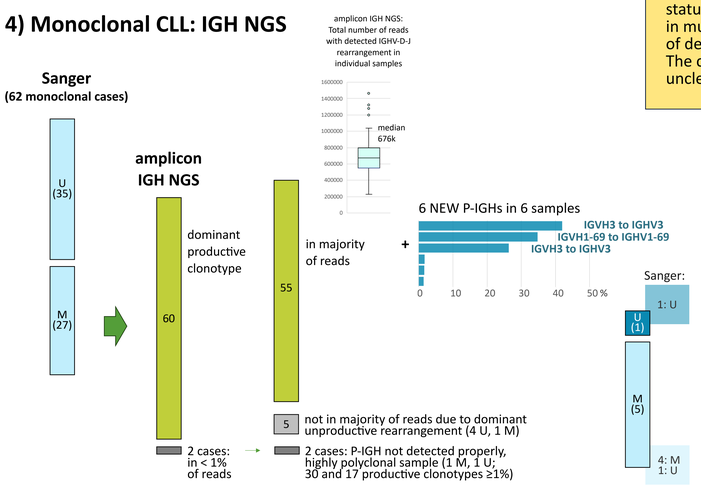
AMPLICON IGH NGS [4]

- DNA
- PCR amplification
- L–J primers; multiplex
- bioinformatics analysis: Interrogate amplicon mode
- typically millions of usable reads
- clonal P-IGH cut-off: 1% of reads
- if >5 clonotypes with size 0.2–1% identified, new clones were assigned above 5% cut-off

2) Multiclonal CLL: Evolution over time (Sanger)

- 100 multiclonal patients: IGHV examination performed repeatedly over time
  (first–last examination: median 3.4 years)
- one or more clones disappeared: 53/100 (53%) patients
  all 13 patients with discordant IGHV (UM, UUM) → unmutated IGHV (U, UU)
- new clone emerged later: 21/100 (21%) patients — 17/21 were monoclonal at dg
  5 patients: mutated IGHV (M, MM) → discordant/unmutated IGHV (UM, U)
- dominant clone replaced by another: 5 patients
  MM → U
  M1 → M2
  M1 → M2
  M1 → M1M2
  M1 → M1M2

4) Monoclonal CLL: IGH NGS



CONCLUSION

Our results provided a comprehensive assessment of clonality in the analyzed multiclonal CLL cases, with a notable prevalence of concordant IGHV mutational status among co-detected clones. Detecting minor clones in multiclonal CLL remains challenging, as the sensitivity of detection varies across different approaches. The clinicobiological significance of minor clones is still unclear and warrants further investigation.

REFERENCES

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[4] Langlois de Septenville, *Methods in Molecular Biology*, 2022

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ACKNOWLEDGEMENT
Funded by: MH CZ AZV NW24-03-00052 and RVO FNBr65269705; MEYS CZ MUNI/A/1685/2024 and NICR LX22NPO5102 - Funded by the European Union - Next Generation EU. Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by MEYS CZ