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Immunogenetics And Antigen Reactivity Profiling Contribute To Unravelling The Ontogeny of CLL Stereotyped Subset #4

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Introduction & Aim

Background

Stereotyped subset #4 is the largest subset in IGHV-mutated chronic lymphocytic leukemia (M-CLL) and a prototype of clinically indolent disease[1]. The clonotypic B cell receptor immunoglobulin (BcR IG) is IgG switched and characterized by the expression of IGHV4-34/IGKV2-30 genes and a distinctive SHM profile.

Open issue

The immunogenetic characterization of subset #4 has relied on low-throughput sequencing approaches that are inherently limited, precluding comprehensive assessment of antigenic impact on (sub)clonal composition.

Aim

To explore the immune trajectory and clonal dynamics of CLL subset #4, particularly focused on the role of SHM.

Methods

Longitudinal samples from 6 subset #4 and 6 non-subset #4 IGHV4-34-expressing M-CLL cases

- RT-PCR amplification with IGHV- and IGHJ-specific primers
- Paired-end NGS (Illumina MiSeq platform)

- Quality filtering (in-house, purpose-built pipeline)

- Annotation with IMGT/HighV-QUEST
- Sequence alignment with tripr[2]
- Junction analysis with custom R scripts

Expression of the dominant clonotypes as well as sub-clonotypes of subset #4 cases as recombinant monoclonal Abs (rmAbs) and reactivity profiling experiments with flow cytometry and ELISA against viable cells and (auto)antigens

Results

1. Different patterns of subclonal architecture are observed in CLL subset #4

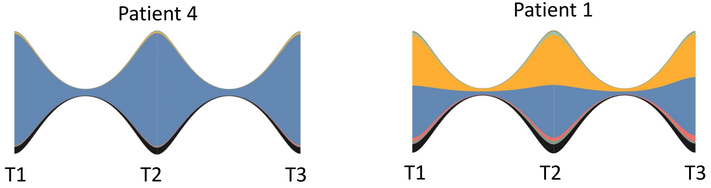

Fig. 1. Representative streamgraphs of the subclonal architecture in the IGHV4-34 gene rearrangements of subset #4 cases longitudinally.
Patient 4 was characterized by the presence of a single dominant clonotype overtime whereas patient 1 by the co-existence of two expanded clonotypes with comparable relative frequencies.

2. Clonal drift can occur in CLL subset #4

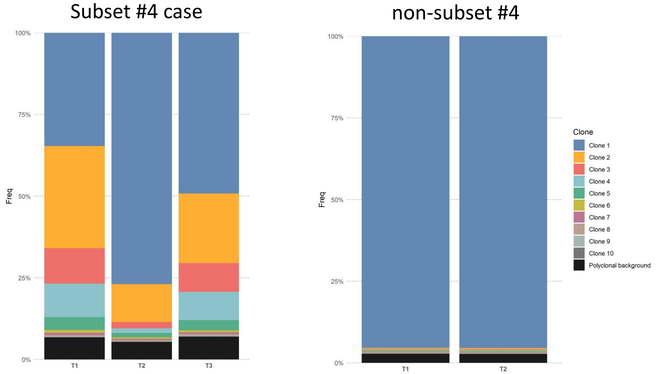

Fig. 2. Stacked bar charts presenting the occurrence of clonal drift in CLL subset #4. Patient 3 displayed significant clonal drift overtime compared to non-subset #4 IGHV4-34-utilizing M-CLL cases.

3. NGS analysis revealed the existence of 'truly unmutated' sub-clonotypes (rearrangements with 100% IG gene identity to the closest germline gene and allele) with the sub-clonotypic rmAb showing strong antigen reactivity compared to the authentic, clonotypic rmAb.

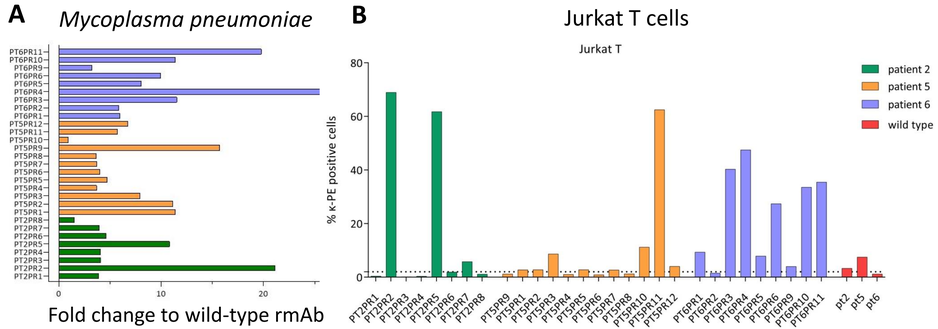

Fig. 3. Comparison of the reactivity profile against (A) *M. pneumoniae* and (B) viable Jurkat T cells between the 'truly unmutated' sub-clonotypic and the respective clonotypic rmAb.

Conclusions

- SHM in CLL subset #4 is driven by ongoing selection by (auto)antigens, ultimately leading to significant shifts in antigen reactivity.
- The identification of truly unmutated clonotypic gene transcripts likely reflects a complex trajectory of clonal evolution, offering hints about the precise timing of SHM in relation to class switch recombination in the natural history of CLL subset #4.

References

1. Sutton LA, et al. *Blood*. 2009
2. Kotouza M, et al. *BMC Bioinformatics*. 2020

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