A Somewhat Different View of the Genetic Portrait of Chronic Lymphocytic Leukemia

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Acknowledgments

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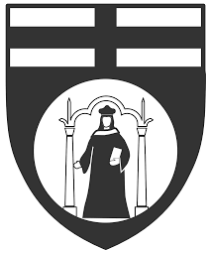

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Tenets

- In general, the human genome is evolutionarily designed to correct somatic mutations that might occur
- Cancer is a disease in which these protective mechanisms ultimately fail, resulting in genetic derangements of specific, biologically important genes
- CLL is the same in some ways and different in others.
- B lymphocytes, the cells that become leukemic in CLL, need to deal with a different evolutionarily design, i.e., the desired accumulation of mutations in the genes coding for the antigen-binding regions of an IG molecule by activation-induced deaminase (AID)

IG V-region gene mutations in CLL

- Somatic mutations of IGHV genes divide CLL clones/ patients into two categories: U-CLL and M-CLL
- U-CLL and M-CLL patients differ in their clinical courses
- IGHV mutation status remains an important and reliable indicator of clinical course and survival in CLL

- Almost invariably “IGHV mutation status” is fixed.
  - Very rarely does a clone “switch” from U-CLL to M-CLL, although this can occur
- However, Sanger sequencing studies indicate that the *IGHV-D-J* rearrangement of CLL clones can undergo intraclonal heterogeneity, even U-CLL cells

  (**Gurrieri et al. 2002; Bagnara et al. 2006; Volkheimer et al. 2007; Sutton et al. 2015)**
- AID, the gene responsible for IGHV-D-J mutations, is functionally active in CLL.
- If intentionally upregulated, this enzyme will mutate CLL V regions, even in U-CLL cells.

Questions:

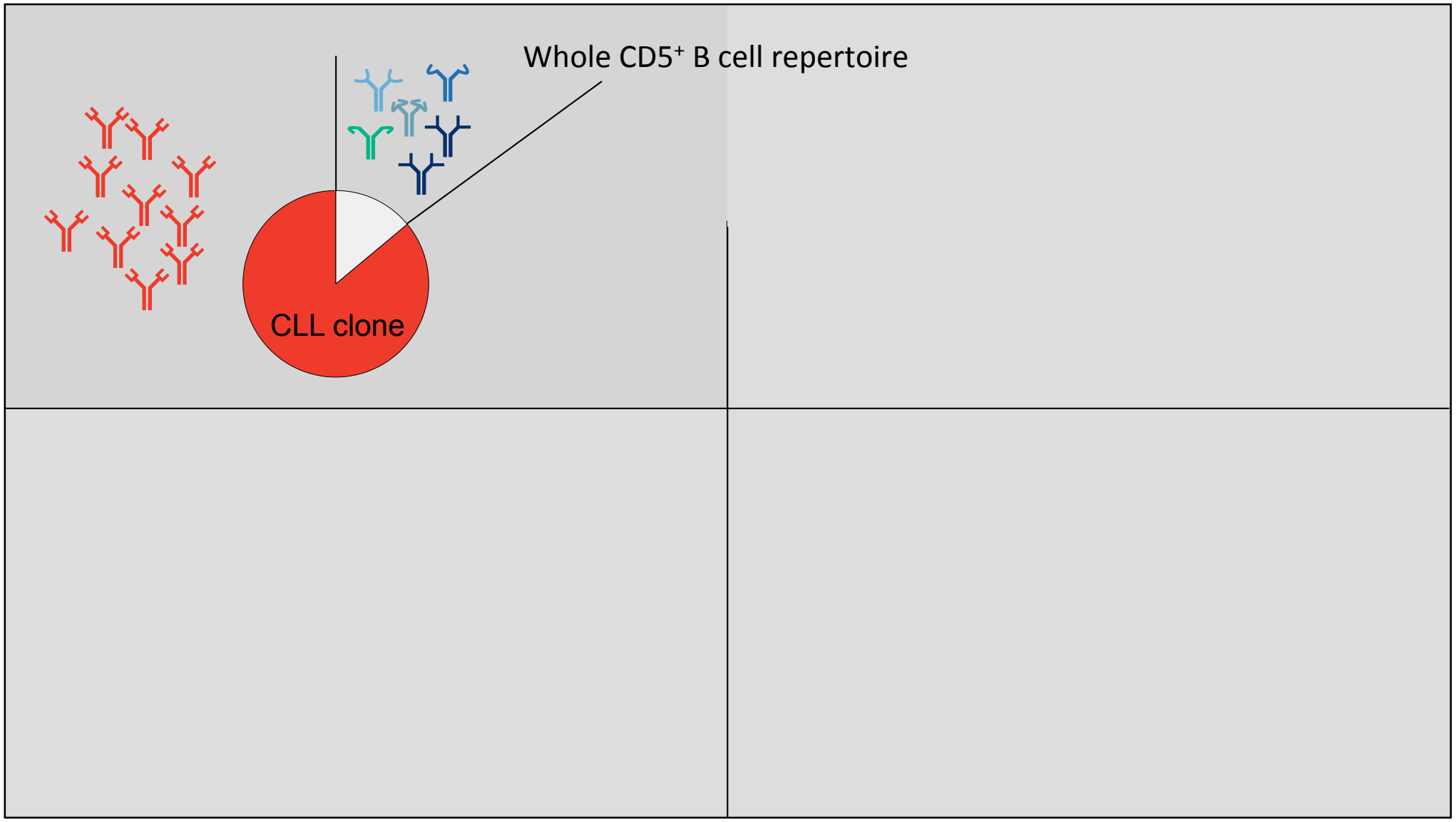
- How extensive is the ongoing IGHV-D-J mutation process in CLL?

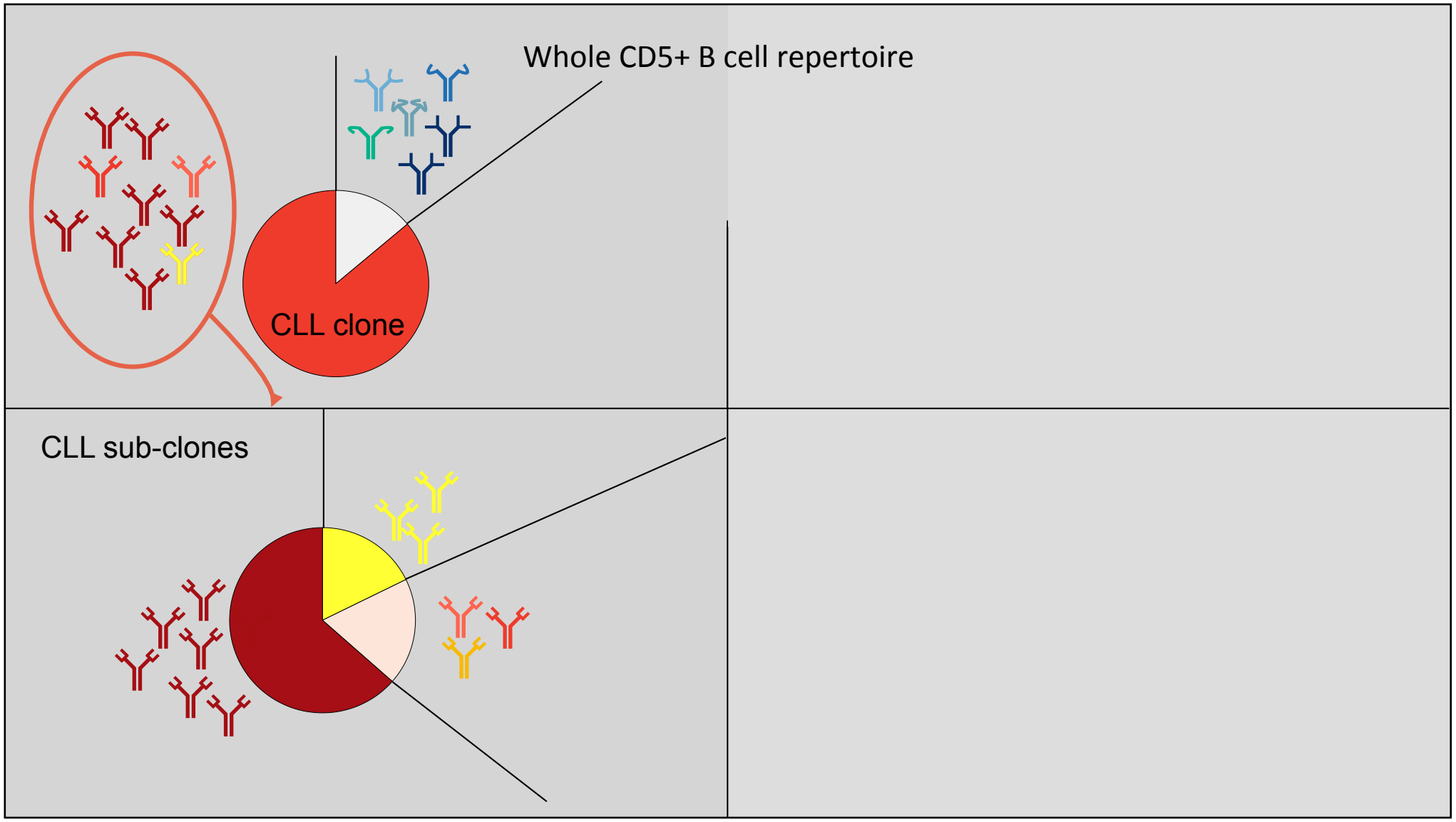
- Can this ongoing process be used to predict the likelihood that mutations, at least those caused by AID, will occur at sites throughout the genome and thereby possibly lead to clinical progression?

- Can the IGHV-D-J sequences of the remaining non-CLL CD5+ B lymphocytes provide clues to the development of CLL from normal B cells?

Approach:

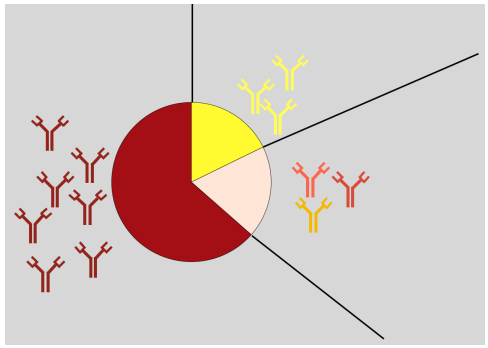
- Sort all $CD5^+$ cells from CLL patient's blood. This should yield the CLL clone, its intraclonal variants, and any normal CD5+ B cells left in the blood
- Perform high-throughput "deep" sequencing of the IGHV-D-J repertoire of FACS sorted $CD19^+CD5^+$ from 39 untreated CLL patients
- Analyze data using appropriate bioinformatic tools

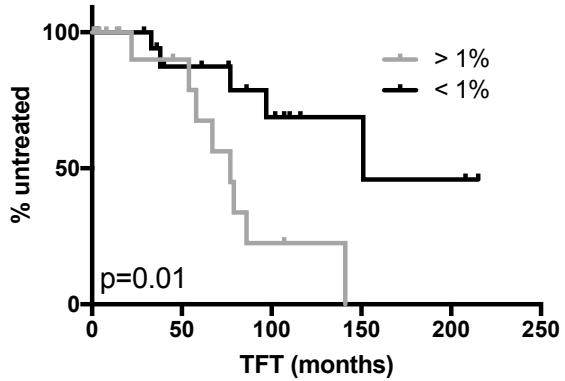

Whole CD5+ B cell repertoire
CLL clone


Whole CD5+ B cell repertoire
CLL clone
CLL sub-clones

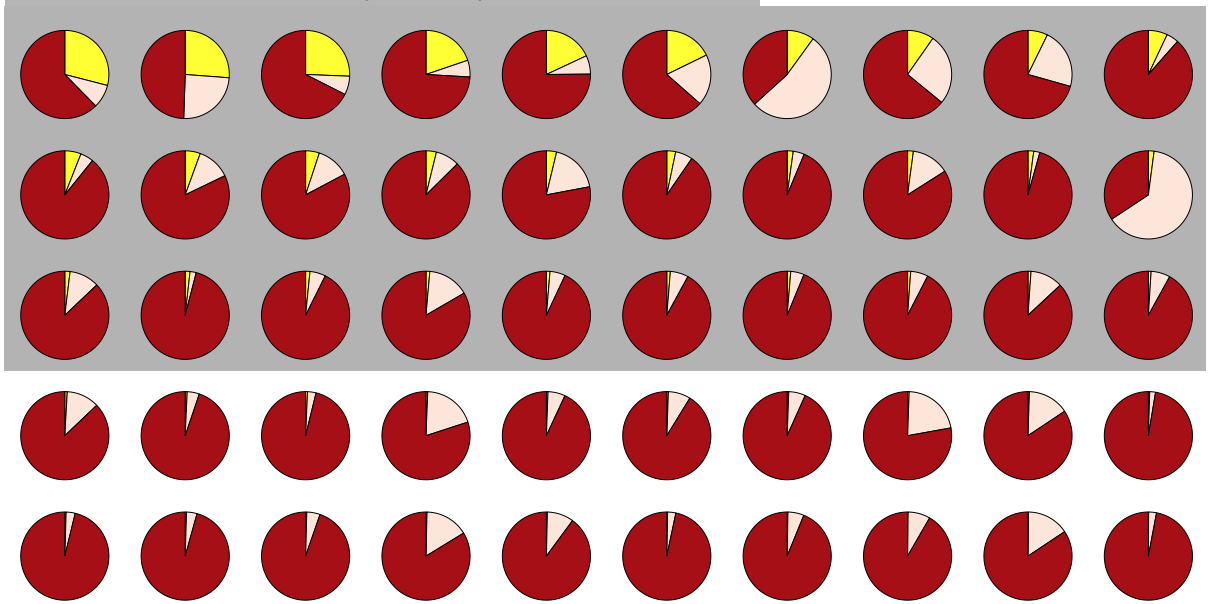
CLL sub-clones developing through intraclonal diversification can be significantly expanded

CLL sub-clones

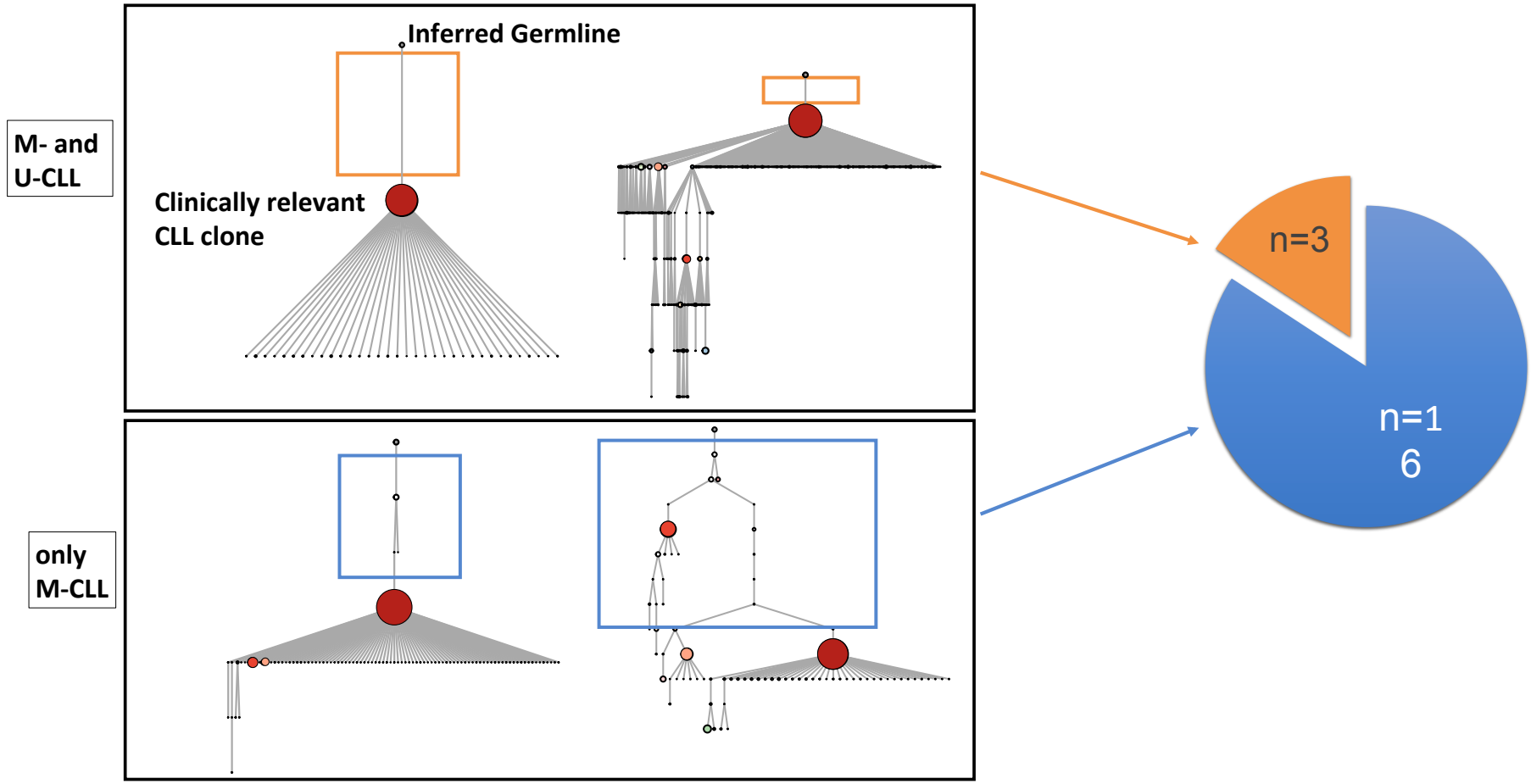


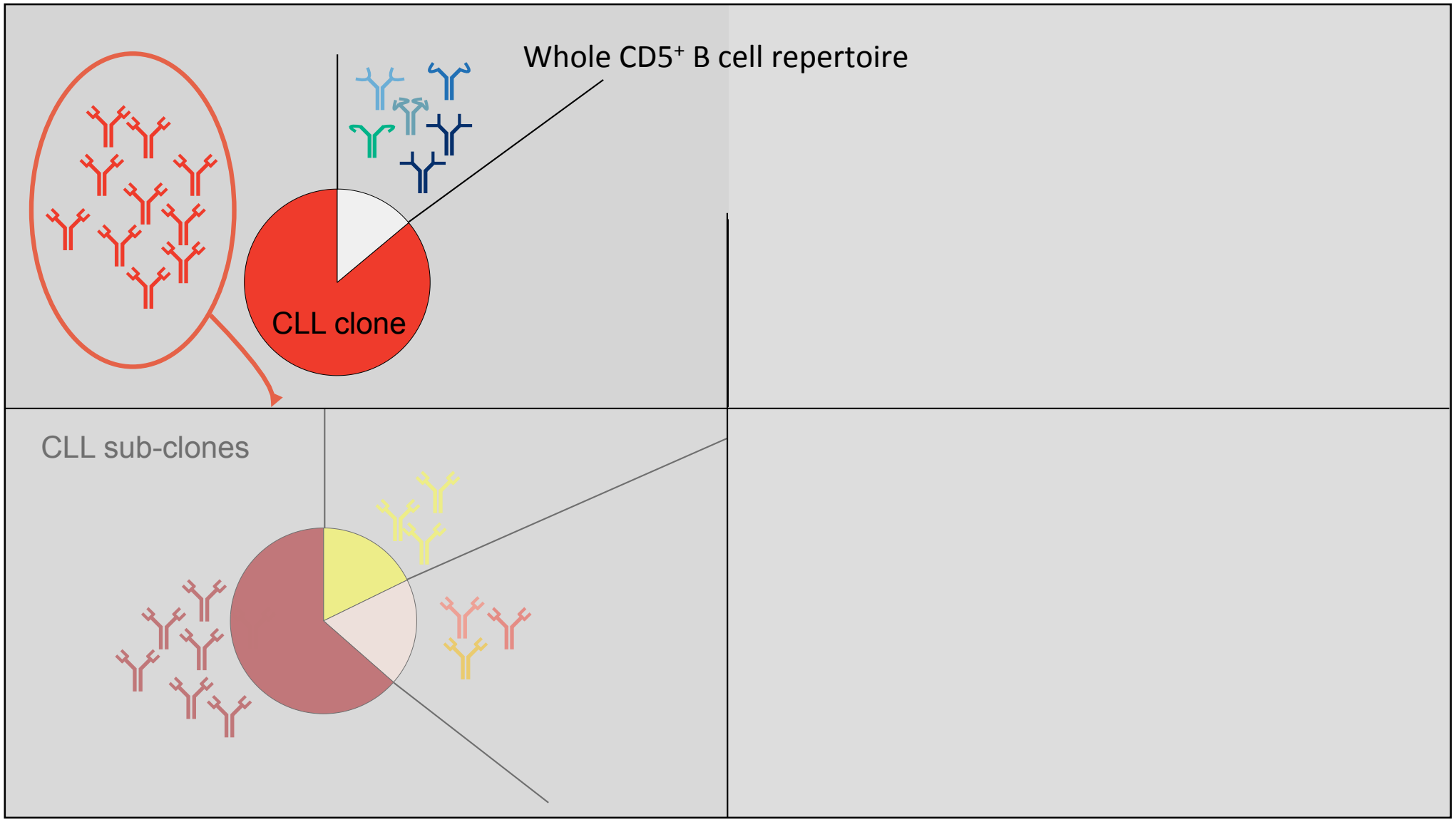


CLL with most expanded sub-clone at > 1%
60% (n = 30)

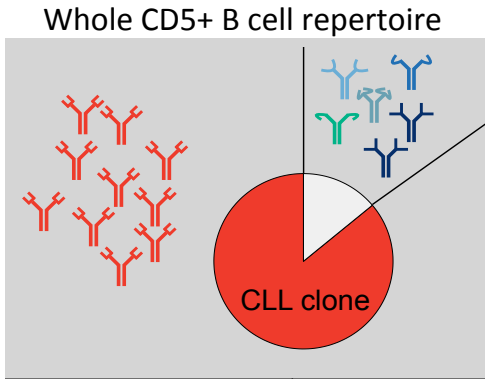


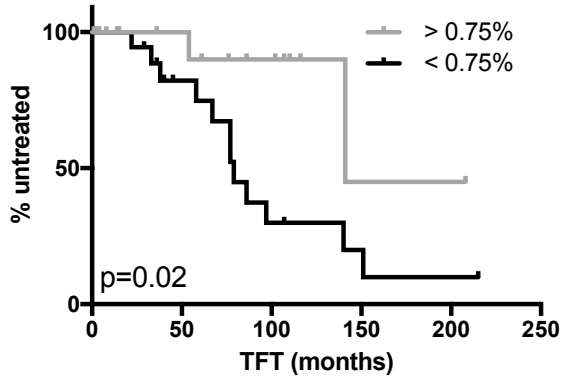
CLL clones have evolved from precursors differing in IGHV mutational load

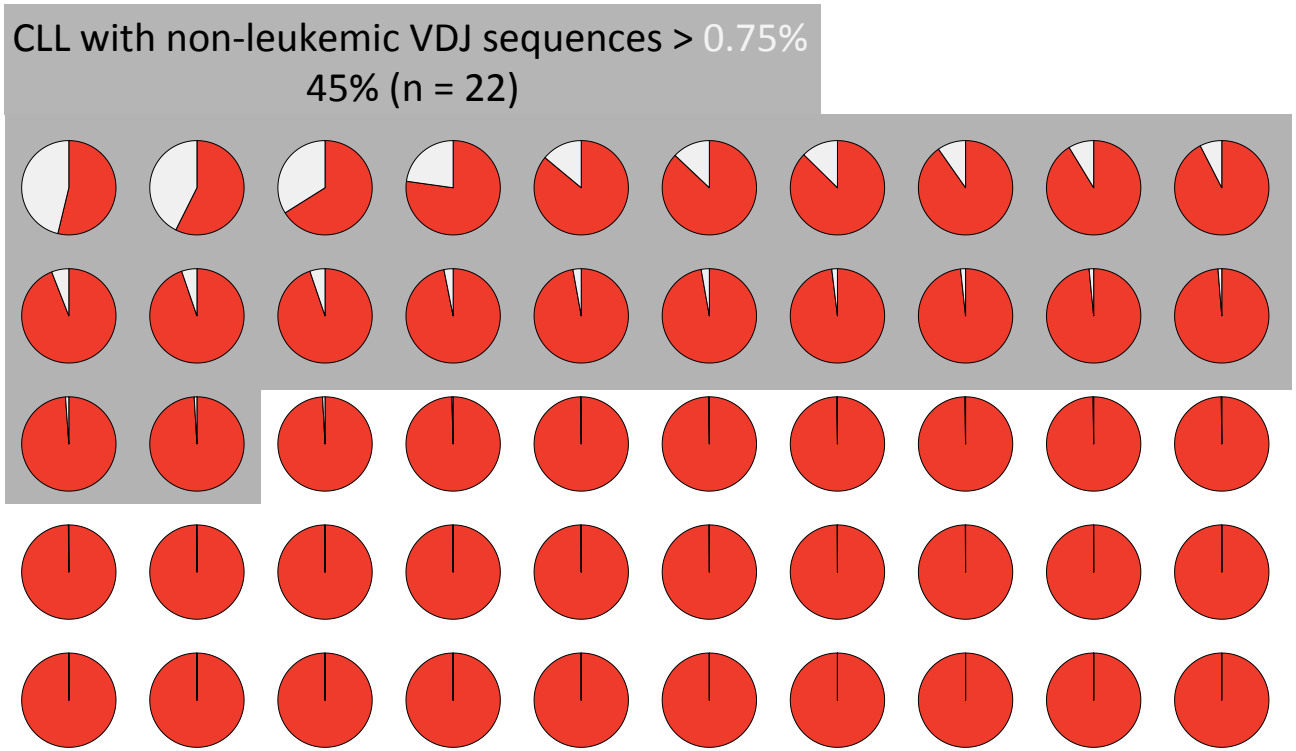


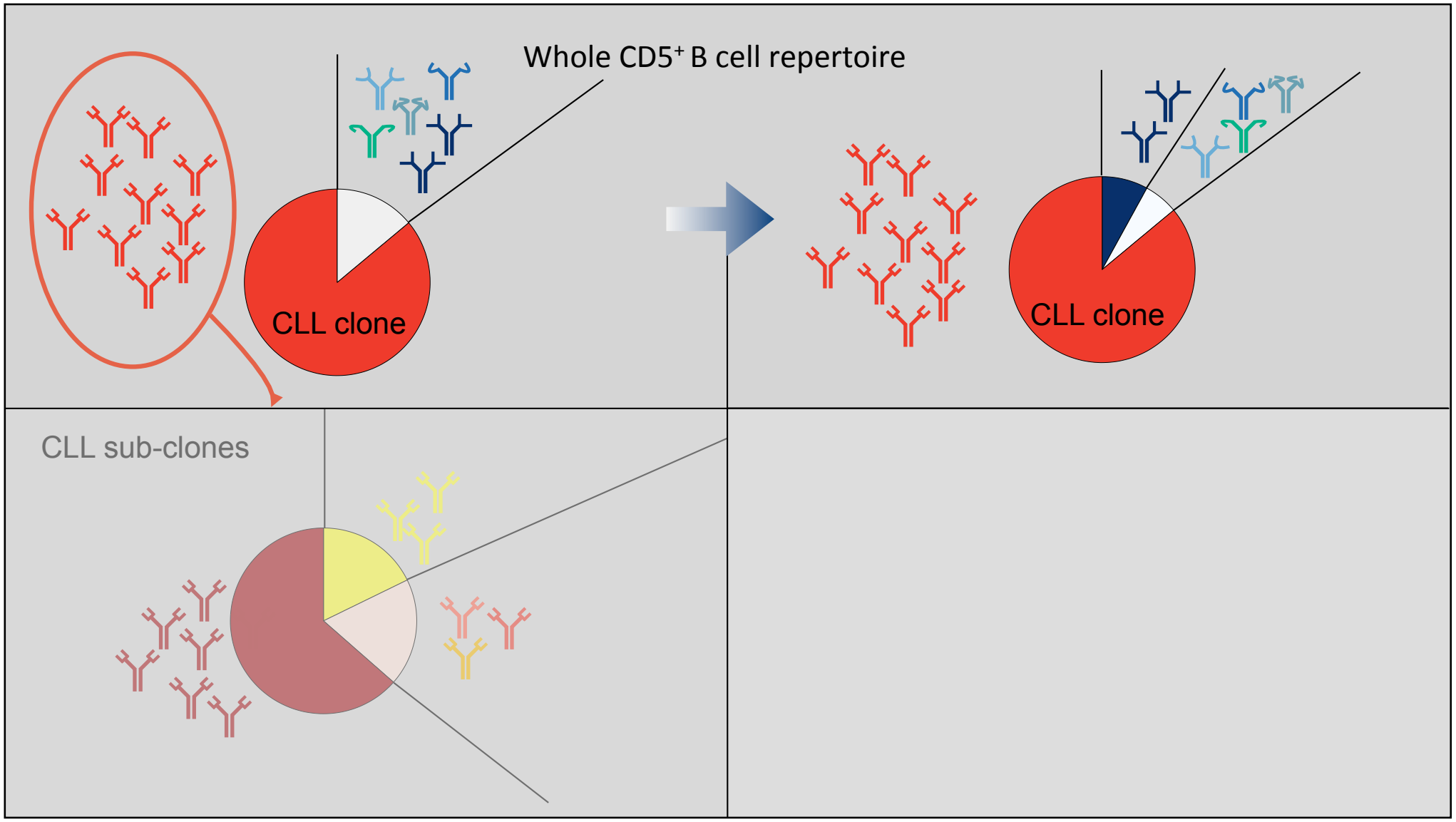

Whole CD5⁺ B cell repertoire
CLL clone
CLL sub-clones

The CD19^{+}CD5^{+} compartment in CLL patients contains leukemic and non-leukemic B-cell clones



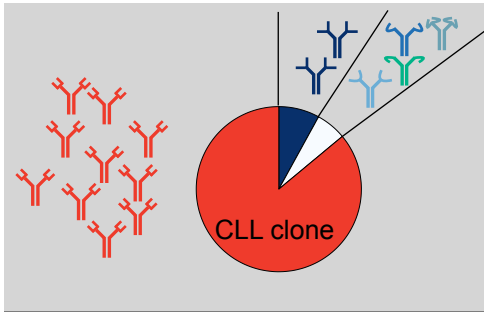


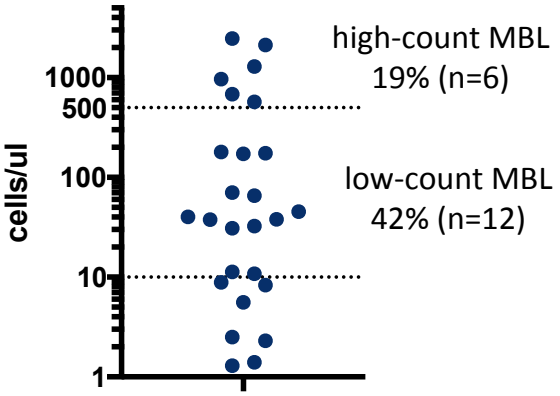



Whole CD5⁺ B cell repertoire
CLL clone
CLL clone
CLL sub-clones

$CD19^{+}CD5^{+}$ B-cell population can present an a second MBL-like clonal expansion

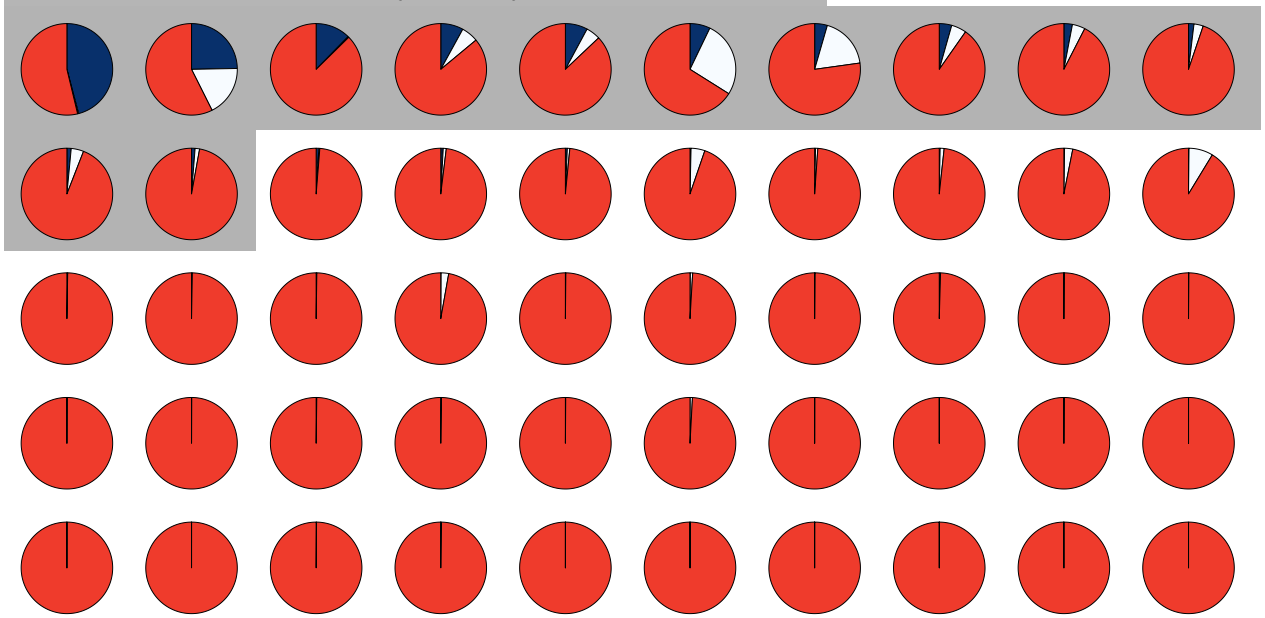
Whole CD5+ B cell repertoire



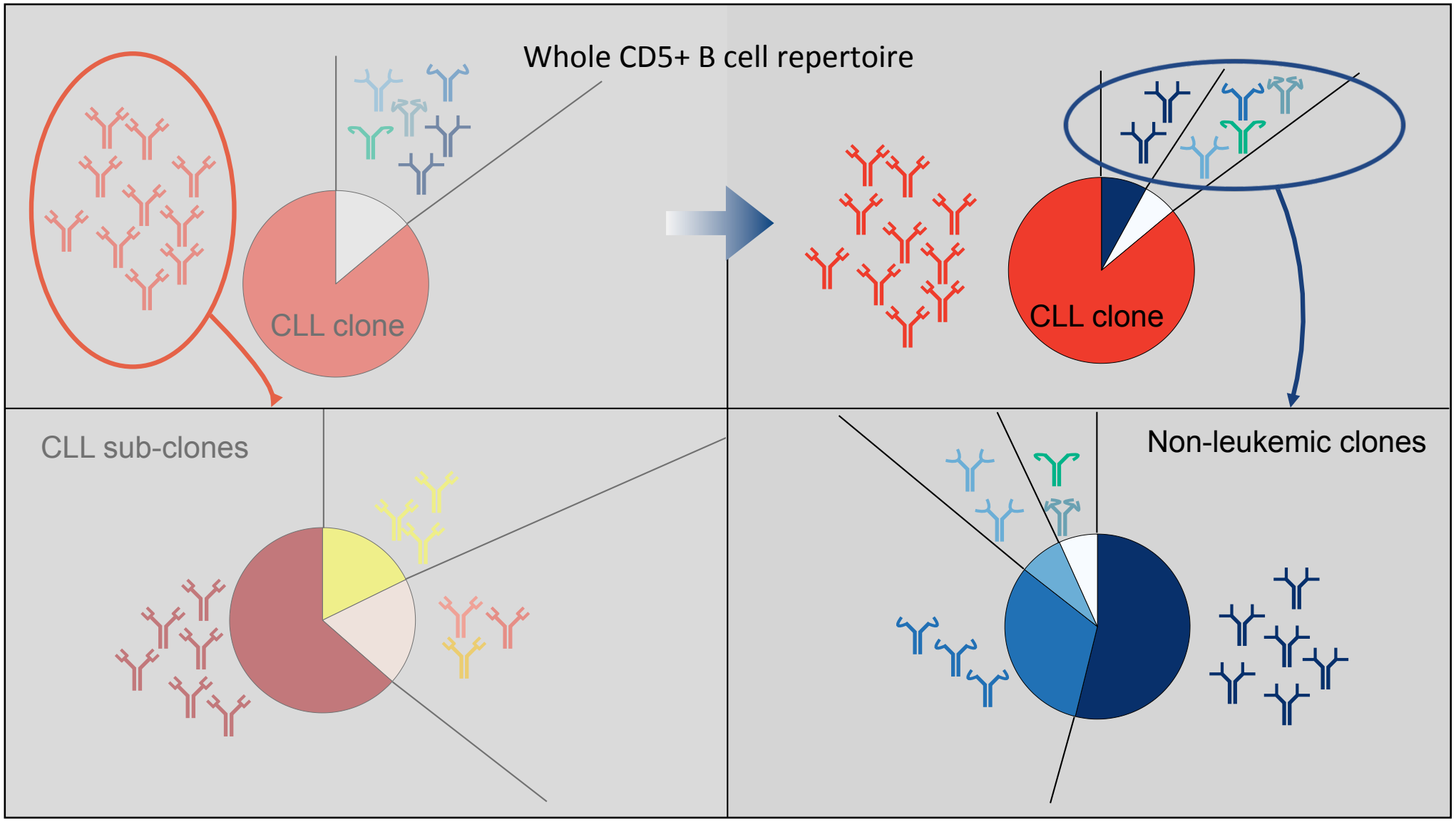


Absolute fitness

CLL with most expanded non-CLL clone at > 1%
24% (n = 12)

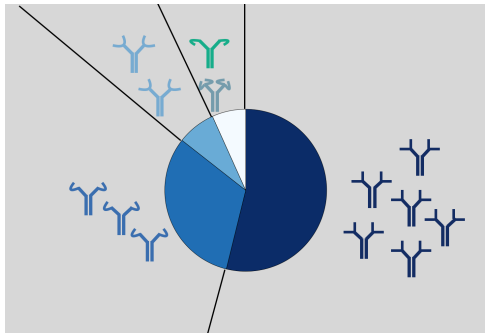


Relative fitness

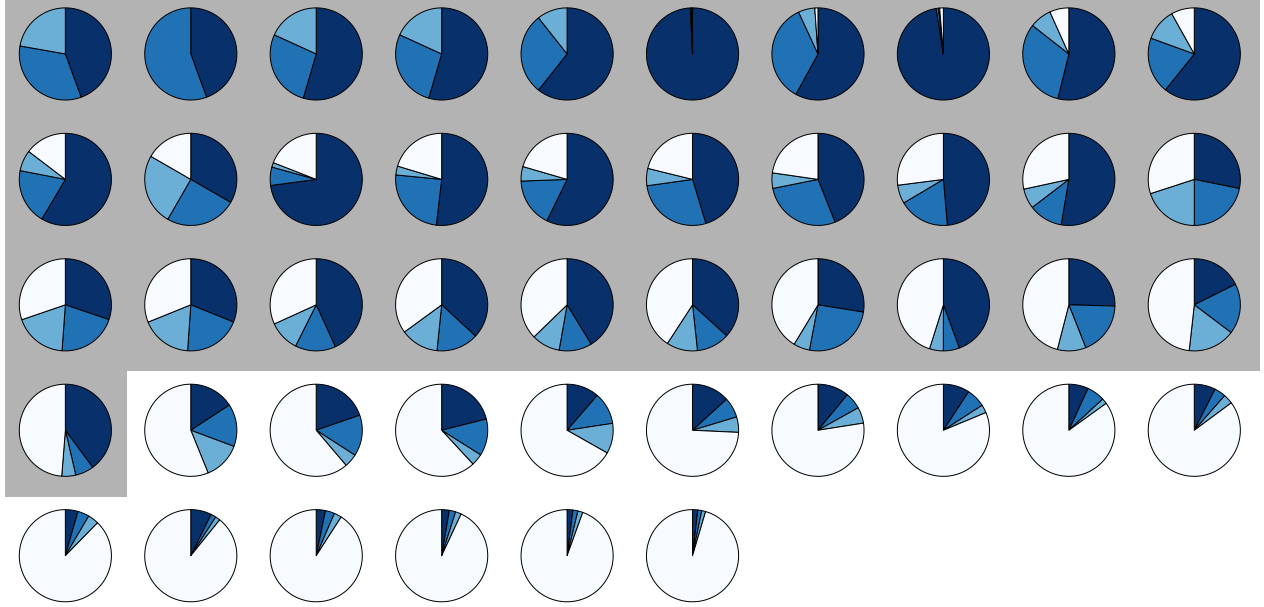

Whole CD5+ B cell repertoire
CLL clone
CLL clone
CLL sub-clones
Non-leukemic clones

$CD19^{+}CD5^{+}$ non-leukemic B-cell population can be oligoclonally expanded

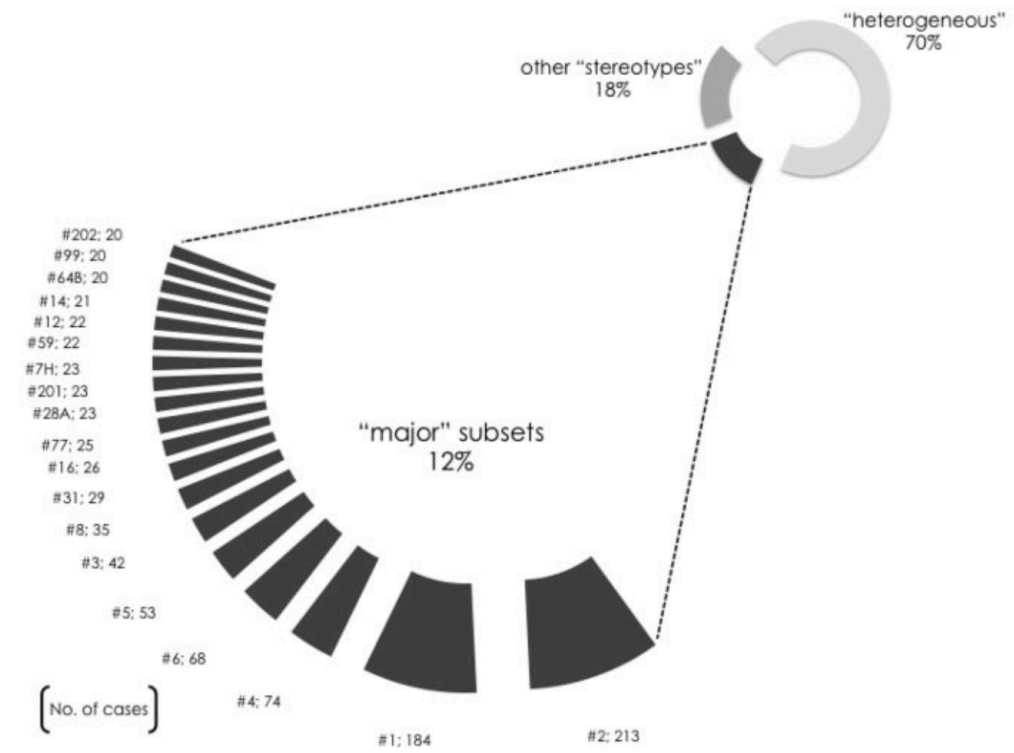
Non-leukemic clones



CLL with 3 clones constituting > 50% of the non-leukemic sequences
67% (n = 31)

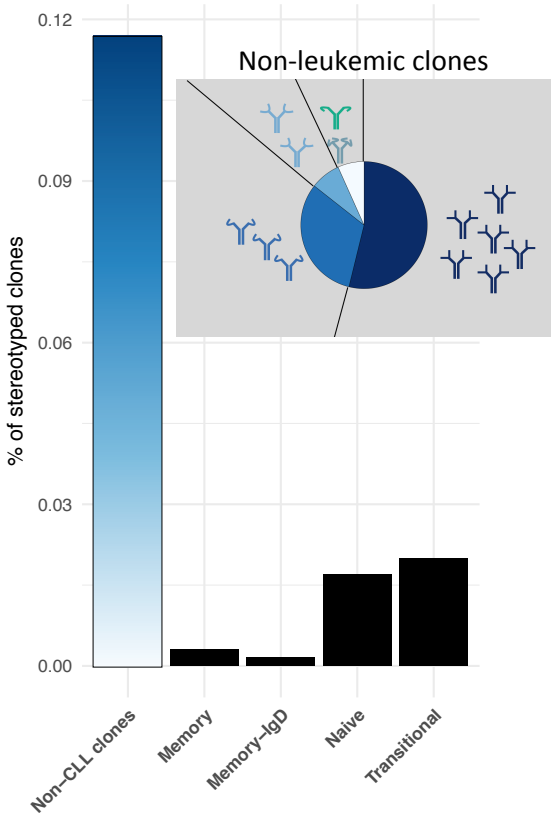


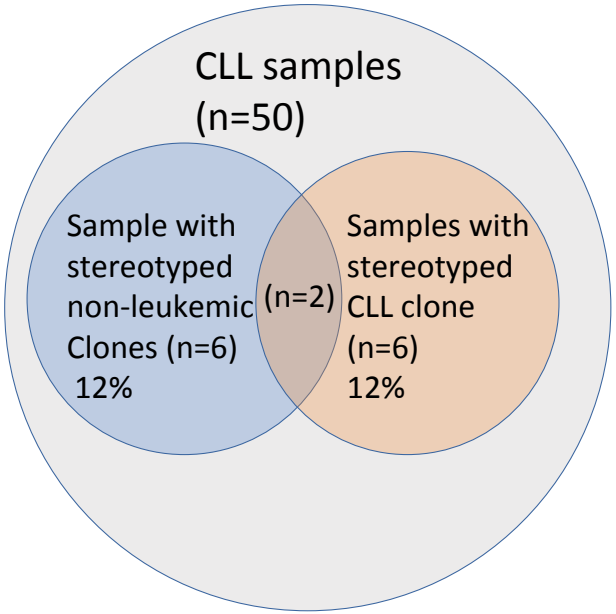
CLL present stereotyped IGHV-D-J sequences



(Agathangelidis et al. Blood, 2012)

CLL-like stereotyped IGHV-D-J sequences can often be found among the non-leukemic clones in the CD19^{+}CD5^{+} compartment





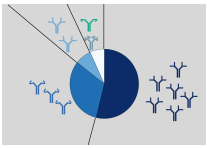
CLL	Leukemic clone	Non leukemic clones
	CLL major subsets	
1	**1**	
2	**1**	
3	**1**	
4	**201**	
5	**2**	**2**
6	**1**	**2** (x7), **3**, **5**, **14** (x2), **64B** (x2)
7		**2**
8		**64B**
9		**1** (x5), **8** (IgG, UM)
10		**4** (IgG, M), **64B**

Implications

In > 80% of M-CLL patients, IGHV-D-J sequence with lower mutation load can be detected

The leukemogenic process might start in a cell with less *IGHV* somatic mutations

Implications



- Non-leukemic $CD5^+$ B cells can display MBL-like oligoclonal expansion and CLL stereotypes

  - MBL-like oligoclonal origin of CLL
  - Genomic mutational events can occur at two stages of B-cell maturation:
    - **prior to IGHV-D-J recombination:** initiating mutation. (contribution of inherited factors?)
    - **after IGHV-D-J recombination:** final leukemogenic mutation

- Do not recapitulate typical CLL VH genes usage and HCDR3 length distribution

  - The final leukemogenic hint involve a further BCR selection

Implications

IGHV-D-J repertoire of $CD5^+$ B cells can be clinically relevant:

- Expanded CLL subclones might be a proxy of AID off-target mutations activity and thereby indicate the potential generation of aggressive sub-clonal variants
- Abundance of $CD5^+$ non-leukemic sequences directly correlates with better outcome

Acknowledgments

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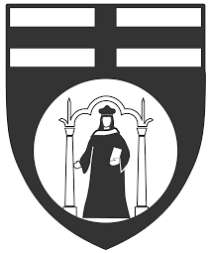

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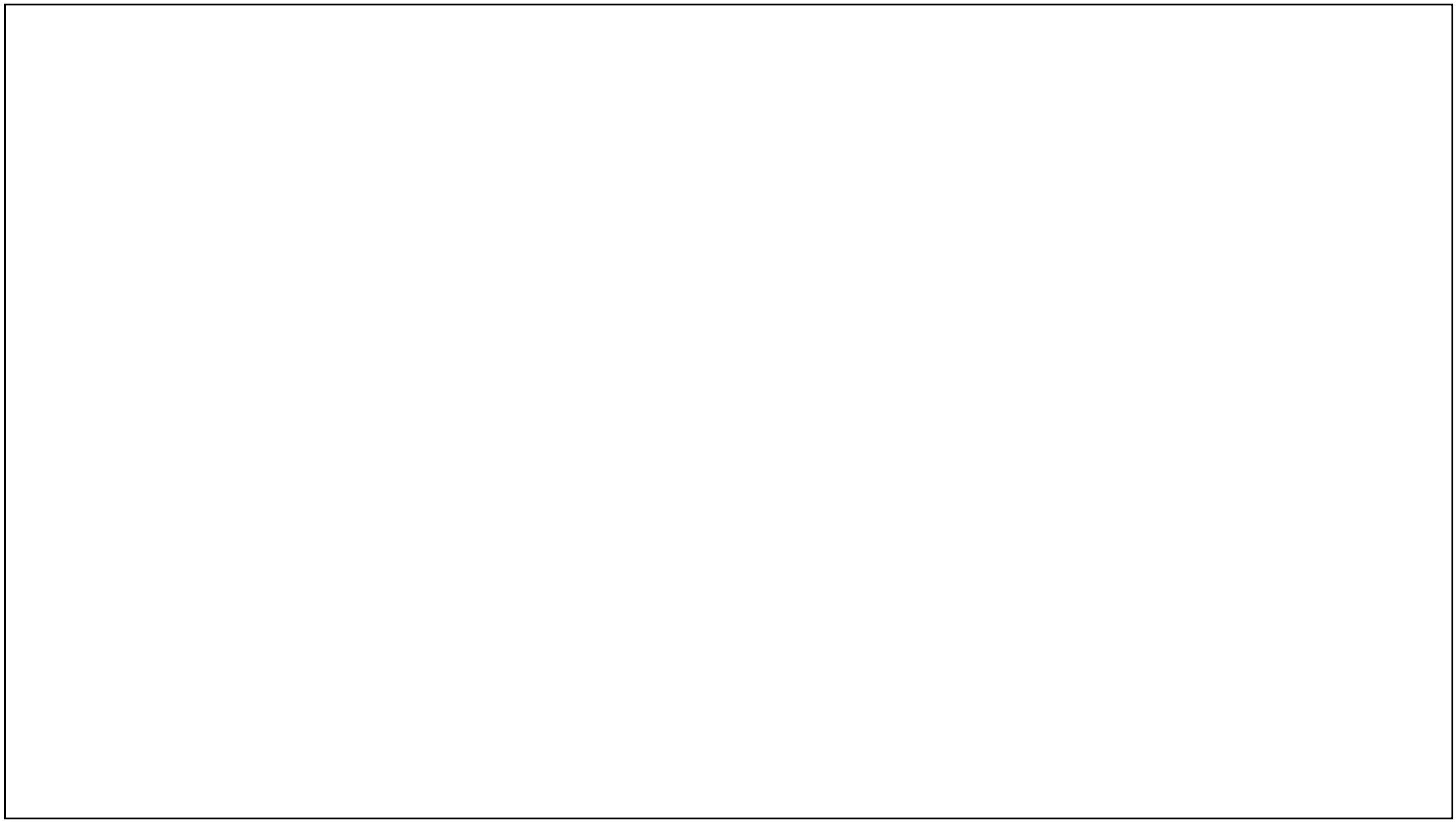
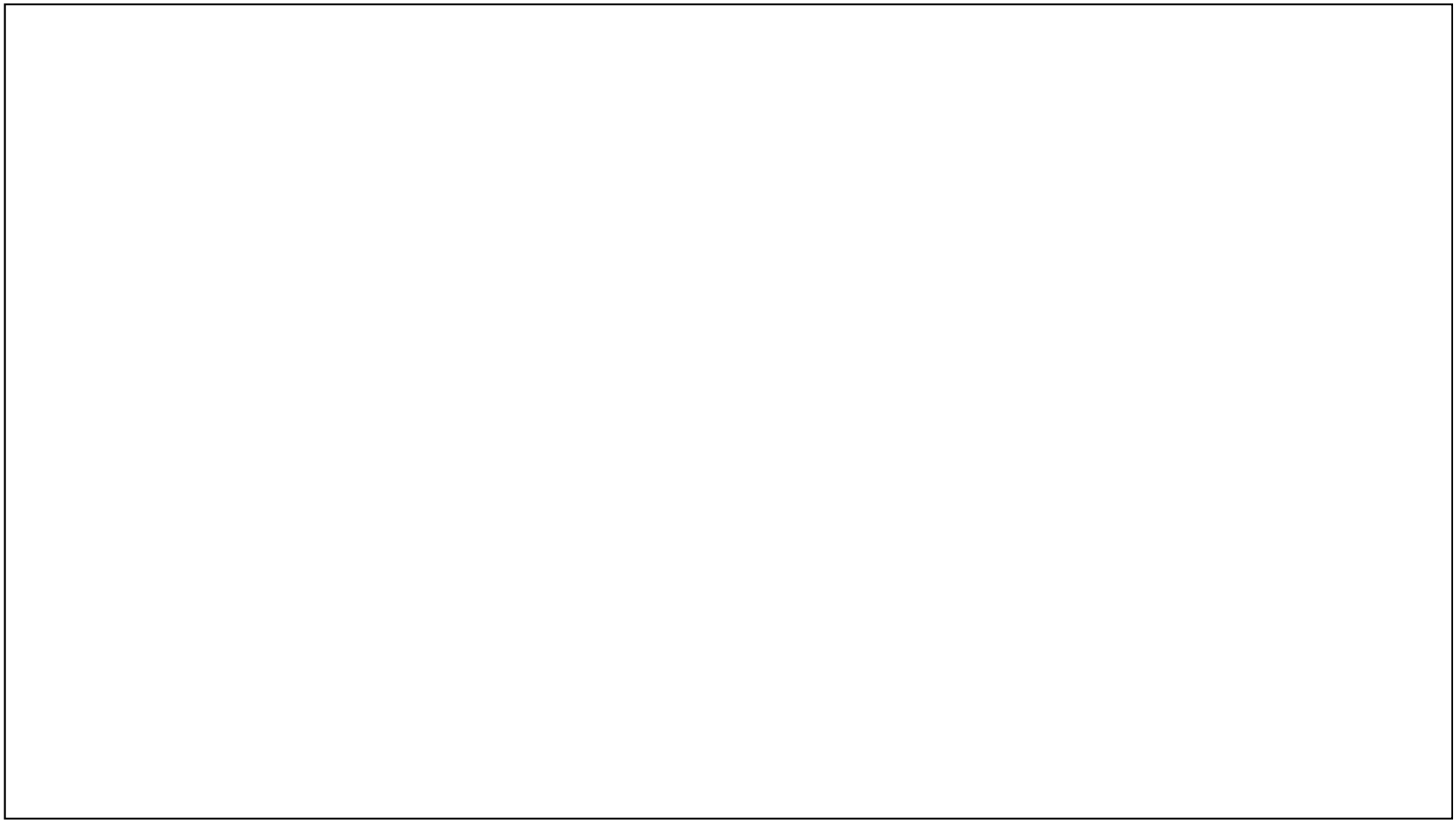
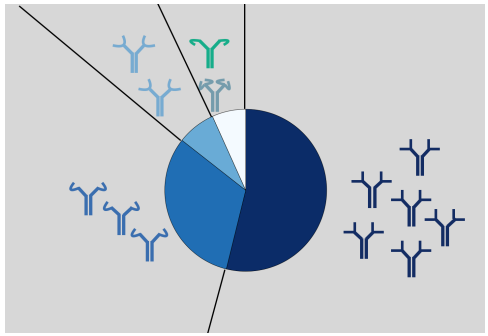
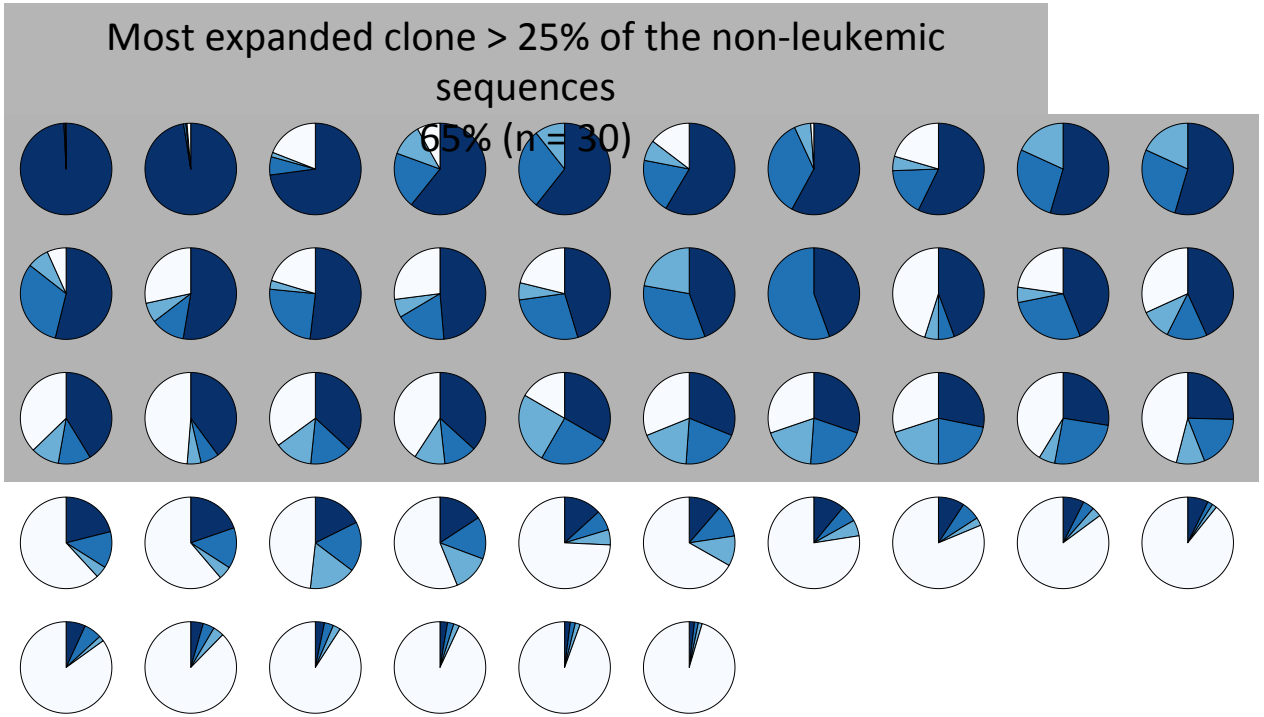




$CD19^{+}CD5^{+}$ non-leukemic B-cell population can be oligoclonally expanded

Non-leukemic clones





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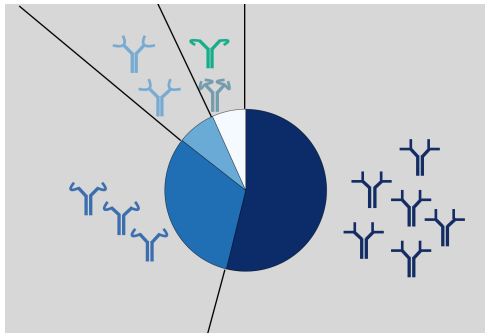
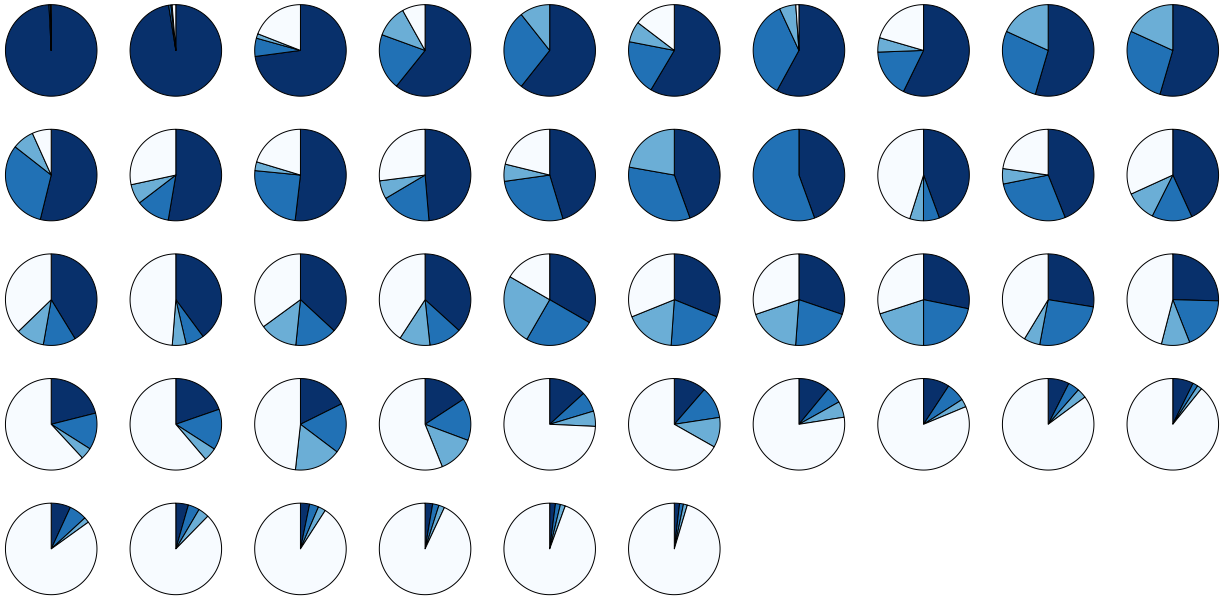
Non-leukemic clones





Non-leukemic clones in the CD19^{+}CD5^{+} compartment do not present VH genes usage and HCDR3 length distribution typical of CLL