CLL cell dynamics

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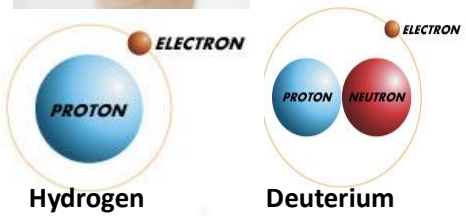
Outline of Presentation

1. Overview of published information gleaned from patients who drank deuterated “heavy” water (^2H_2O)

2. Explanation of why we should be interested and concerned with the proliferative fraction of a CLL clone

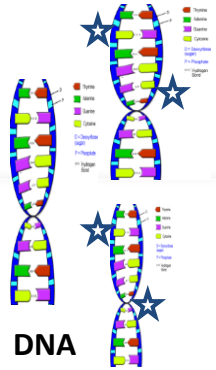
3. Methods to preferentially target the proliferative fraction of a CLL clone

Methodology of deuterated “heavy” water use

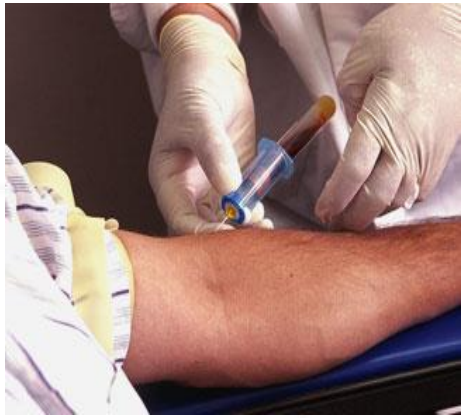


Hydrogen

Deuterium

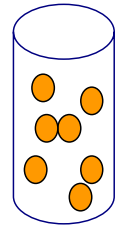


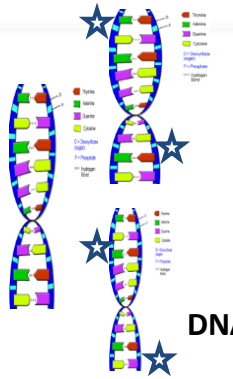
DNA



In vivo “pulse-chase” study

CLL cells





DNA

Gas chromatography/
Mass spectrometry

What can these studies tell us?

- Birth and death/elimination rates of CLL clones
- Means to indirectly identify cells that have most recently been born/divided in patients

Initial *in vivo* findings

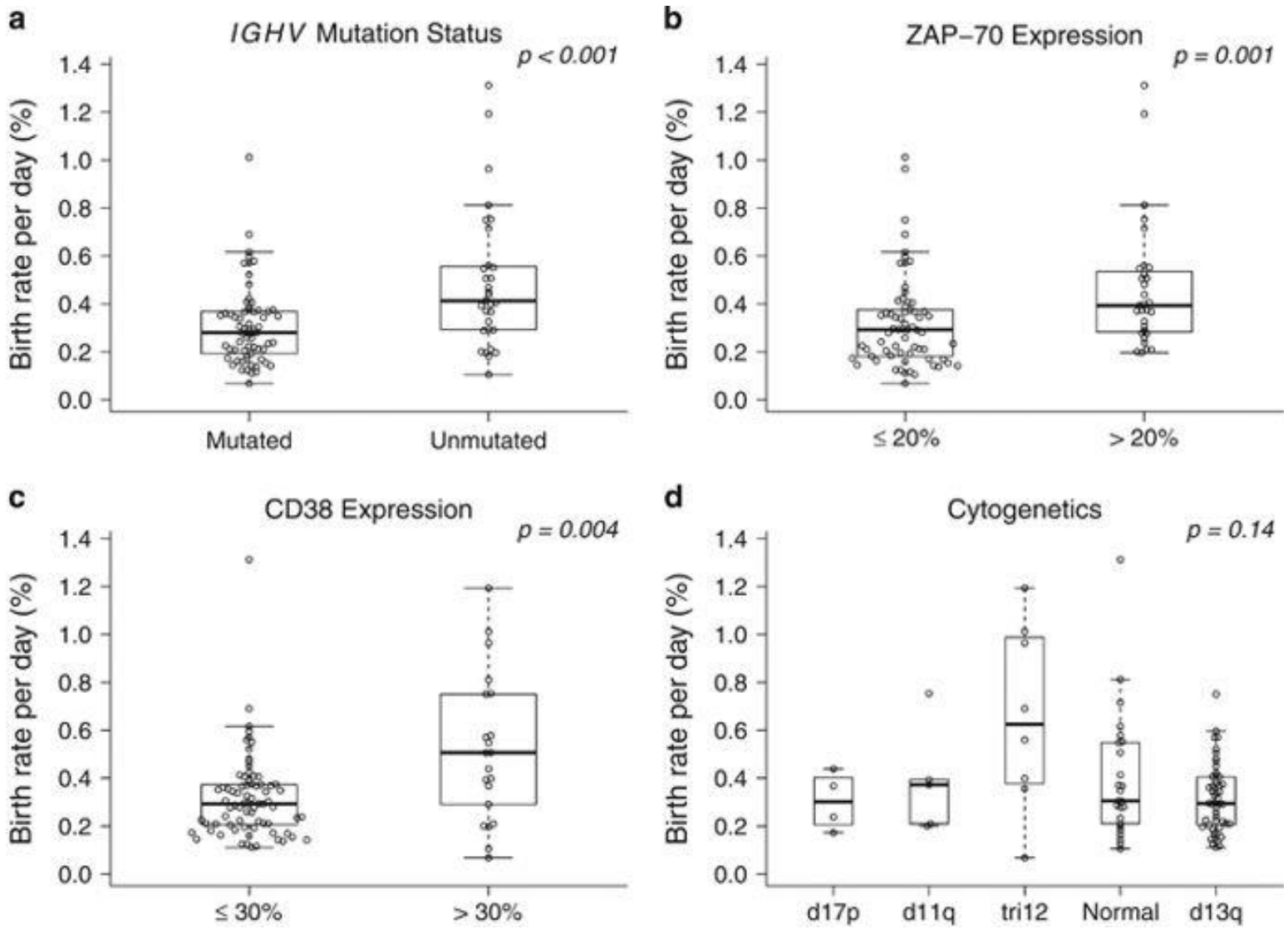
- CLL cells proliferate faster than originally appreciated
  ~0.1% - ~2% of the clone divides daily

- Higher birth rates correlated with disease activity,
  and therefore appear to be a key factor in disease outcome

- Calculated *in vivo* deaths rates are often comparable or only slightly unbalanced. Thus it is the rate of growth – **not necessarily the absolute lymphocyte change** – that is the crucial variable in clinical course.

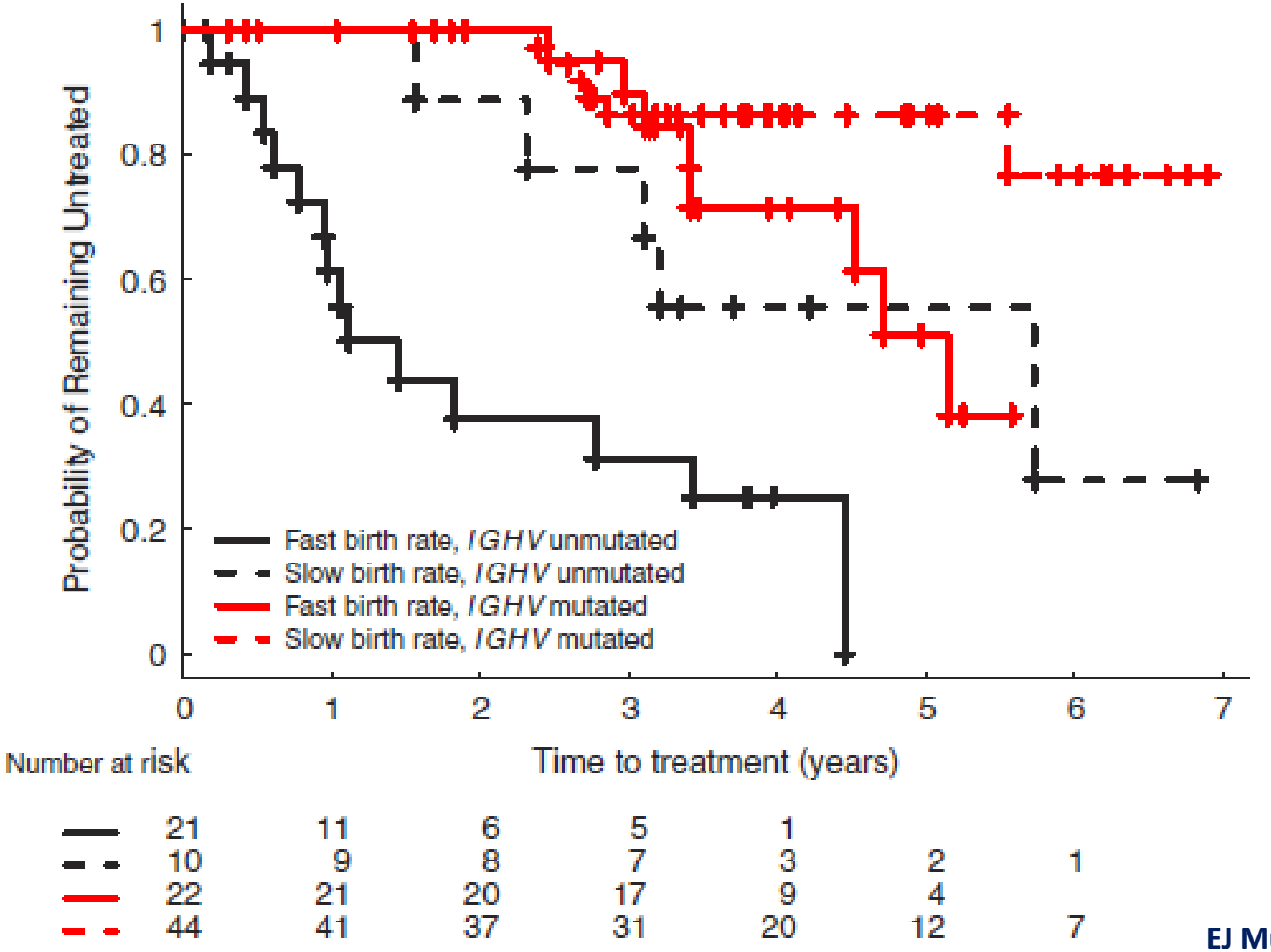
Messmer *et al*. J Clin Invest 115: 755, 2005
van Gent *et al*. Cancer Res 68: 10137, 2008
deFoiche *et al*. Br J Haematol: 143: 240, 2008

Faster birth rates correlate with markers predicting worse clinical outcomes



EJ Murphy *et al*. Leukemia 31, 1348, 2017

Kaplan-Meier curve of treatment-free survival stratified by *IGHV* mutation status and CLL-cell birth rate



EJ Murphy *et al*. Leukemia 31, 1348, 2017

What can these studies tell us?

- Birth and death/elimination rates of CLL clones

- Means to indirectly identify cells that have most recently been born/divided in patients

  - **Intraclonal cell fractions containing more cells with ^{2}H- labeled DNA are enriched for the most recently replicated/born cells**

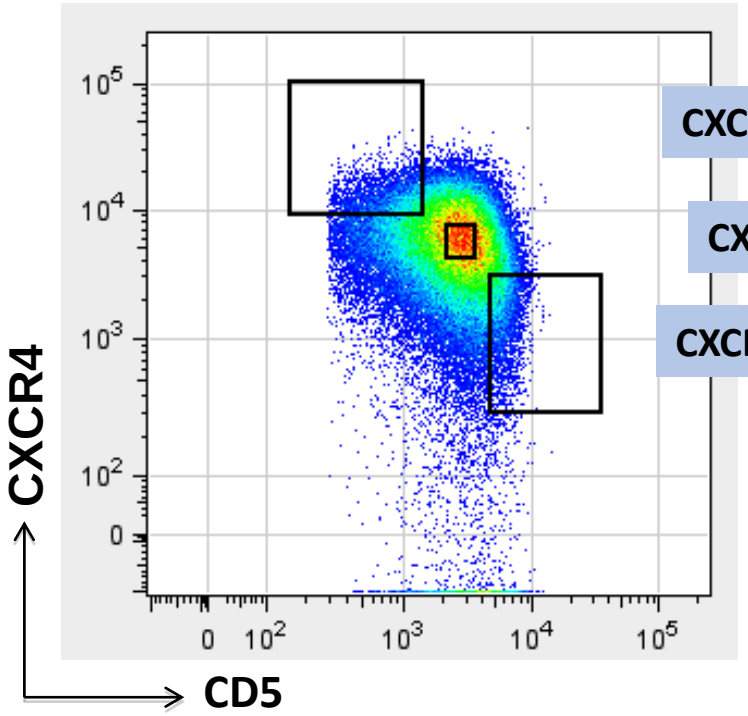
Hypotheses used to define the membrane phenotype of the "proliferative fraction (PF)"

1. Cells that were stimulated to divide will express "activation markers" on their cell surfaces.
   - CD5 is an activation antigen on human B lymphocytes

2. Cells from the blood that have recently left a solid lymphoid niche will have a chemokine display that supports emigration.
   - Low CXCR4 levels are on recent tissue emigrants

3. Therefore, the fraction of circulating CLL cells with a $CD5^{Bright}$ and a $CXCR4^{Dim}$ phenotype will be enriched in recently-divided and recently-emigrated cells.

$CXCR4^{Dim}CD5^{Bright}$ faction is most enriched in ^{2}H-DNA labeled cells



$CXCR4^{bright}CD5^{dim}CD19^{+}$: Resting, RF

$CXCR4^{int}CD5^{int}CD19^{+}$: Intermediate, IF

$CXCR4^{dim}CD5^{bright}CD19^{+}$: Proliferative, PF

Calculated percentage of cells with ^{2}H-DNA

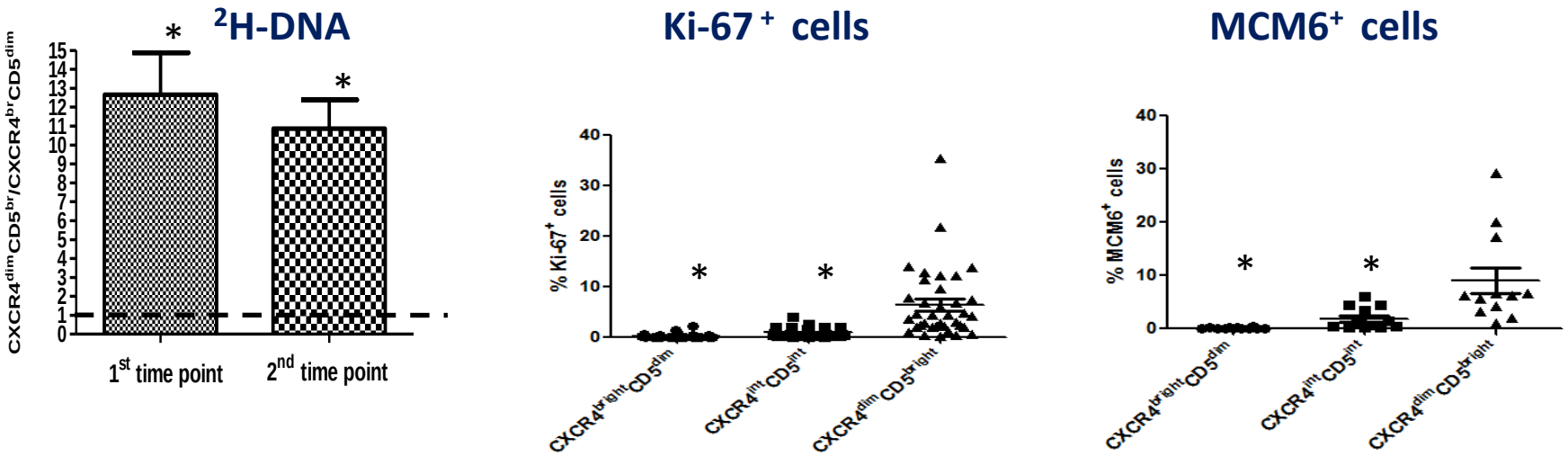
0%

1 – 10%

10 - 45%

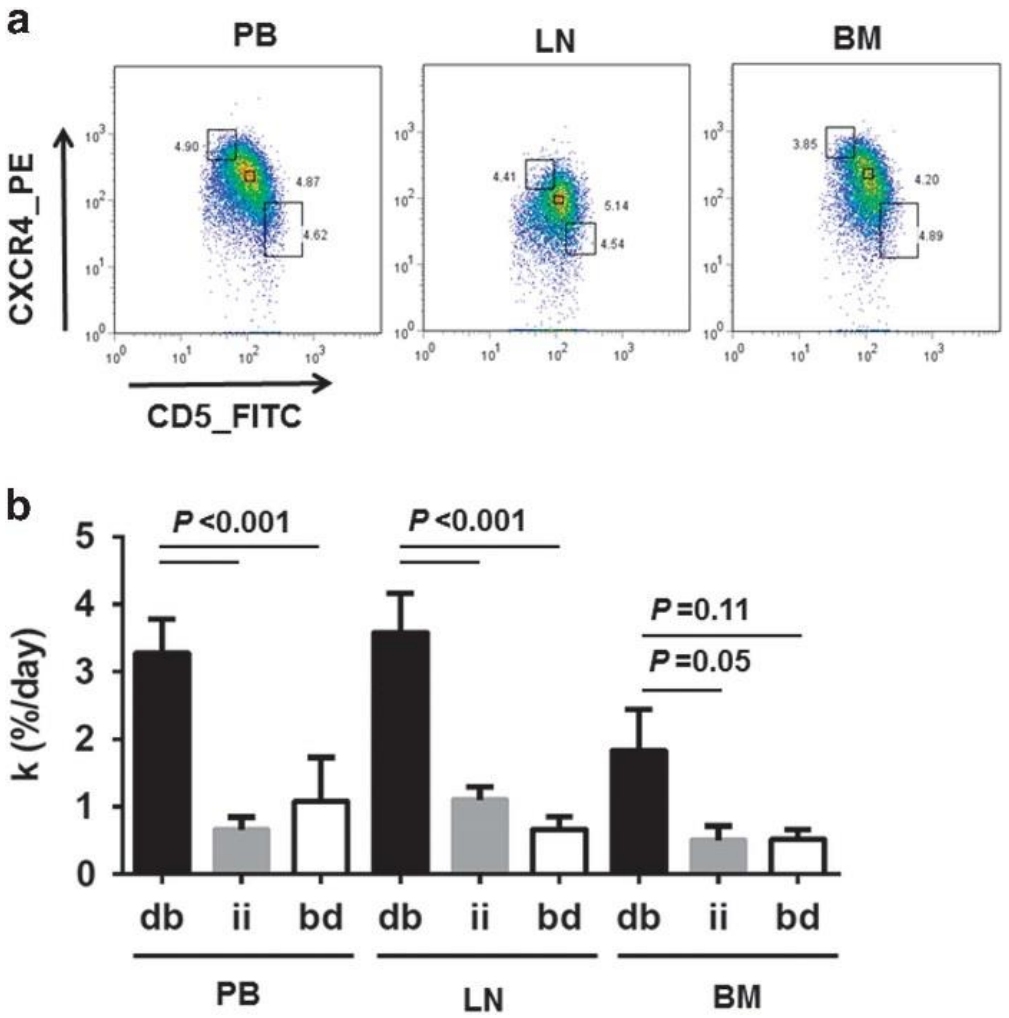
C Calissano *et al.* Mol Med 7: 1374, 2011

$CXCR4^{Dim}CD5^{Bright}$ fraction contains the majority of recently divided cells in CLL clones



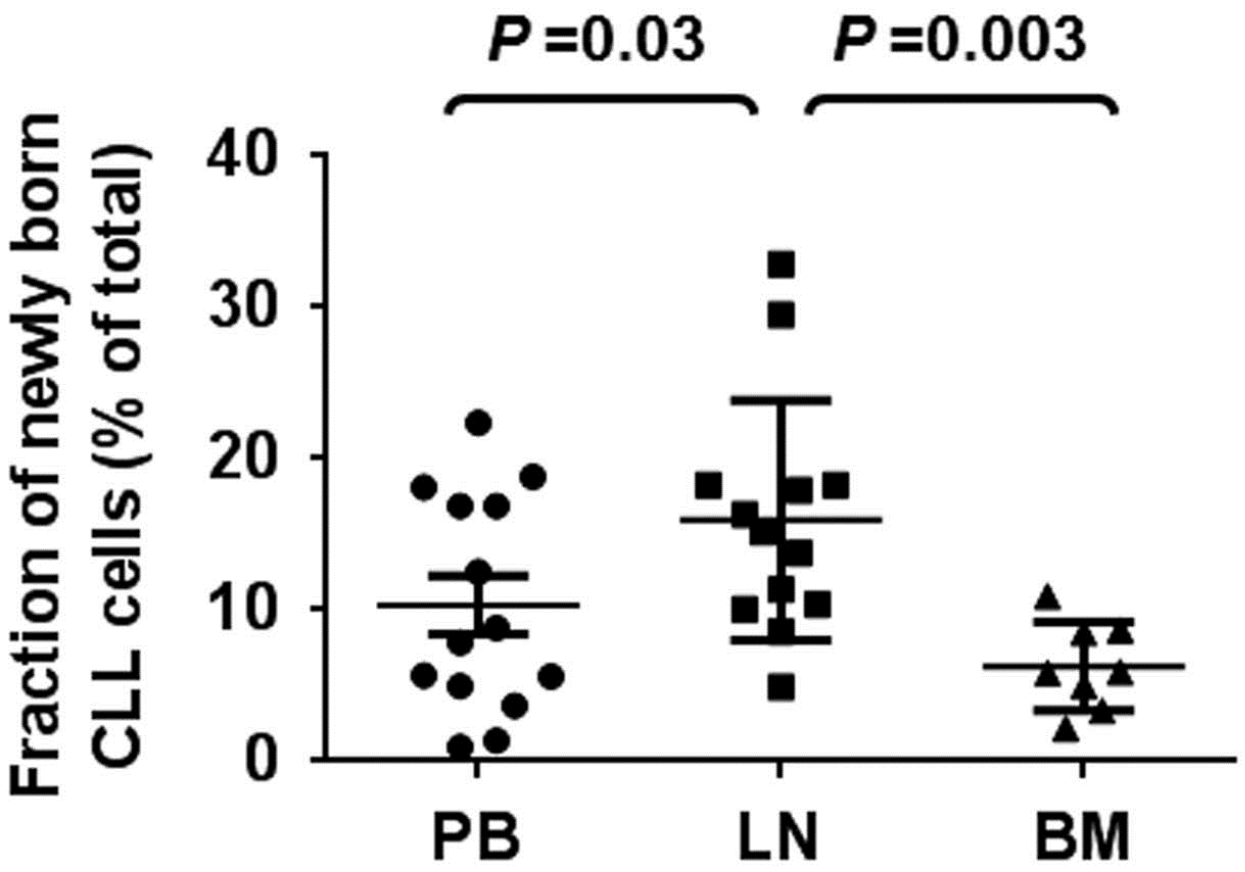
C Calissano *et al.* Mol Med 7: 1374, 2011

$CXCR4^{Dim}CD5^{Bright}$ defines the proliferative fraction in blood, lymph node, and bone marrow



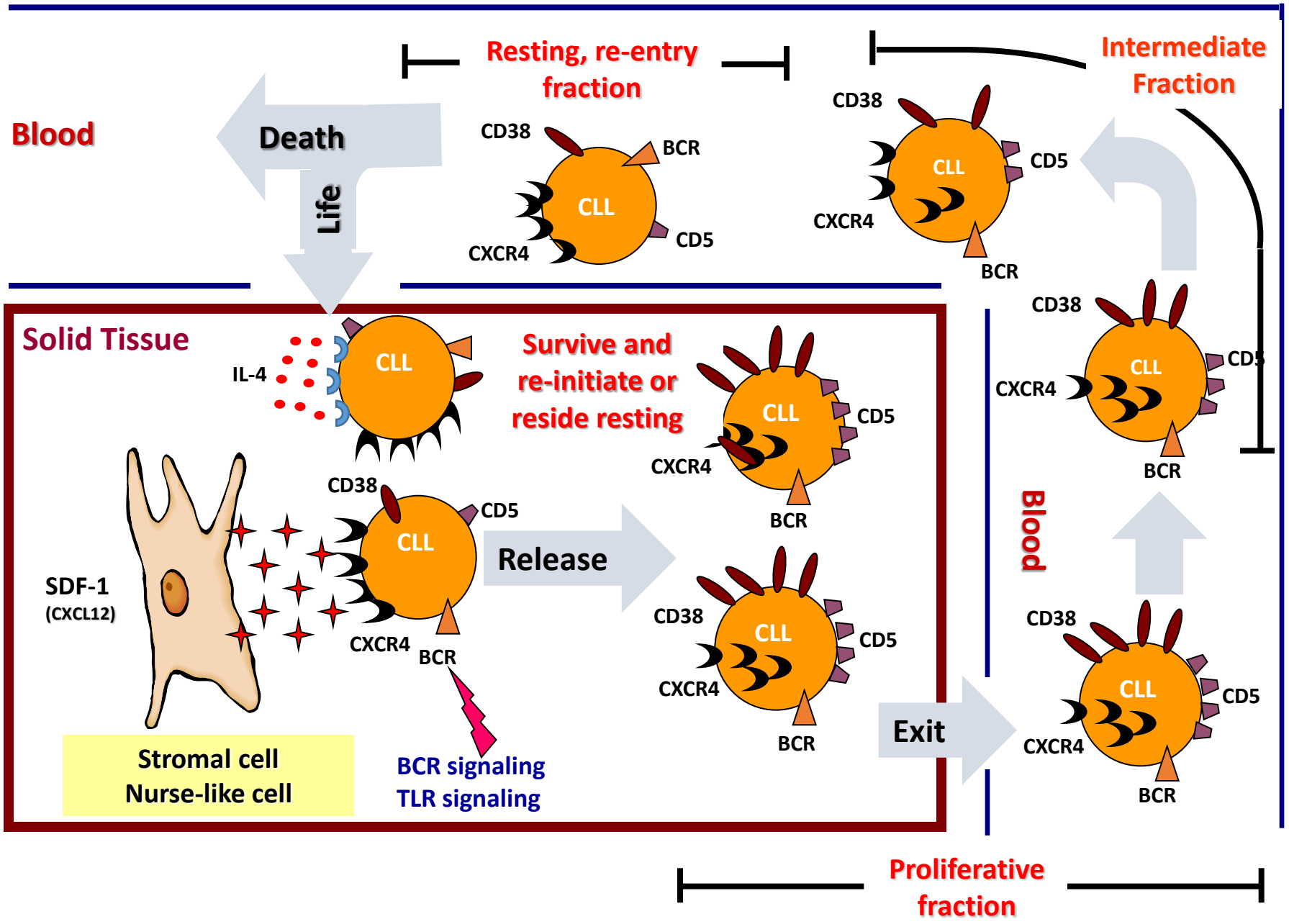
TM Herndon *et al*. Leukemia 31, 1340, 2017

Fraction of newly-born CLL cells is highest in the lymph node



TM Herndon *et al*. Leukemia 31, 1340, 2017

Life cycle of a CLL cell



Important concepts and questions:

The $CXCR4^{Dim}CD5^{Bright}$ “Proliferative Fraction” represents, also exclusively, post-mitotic/recently divided cells – NOT dividing cells

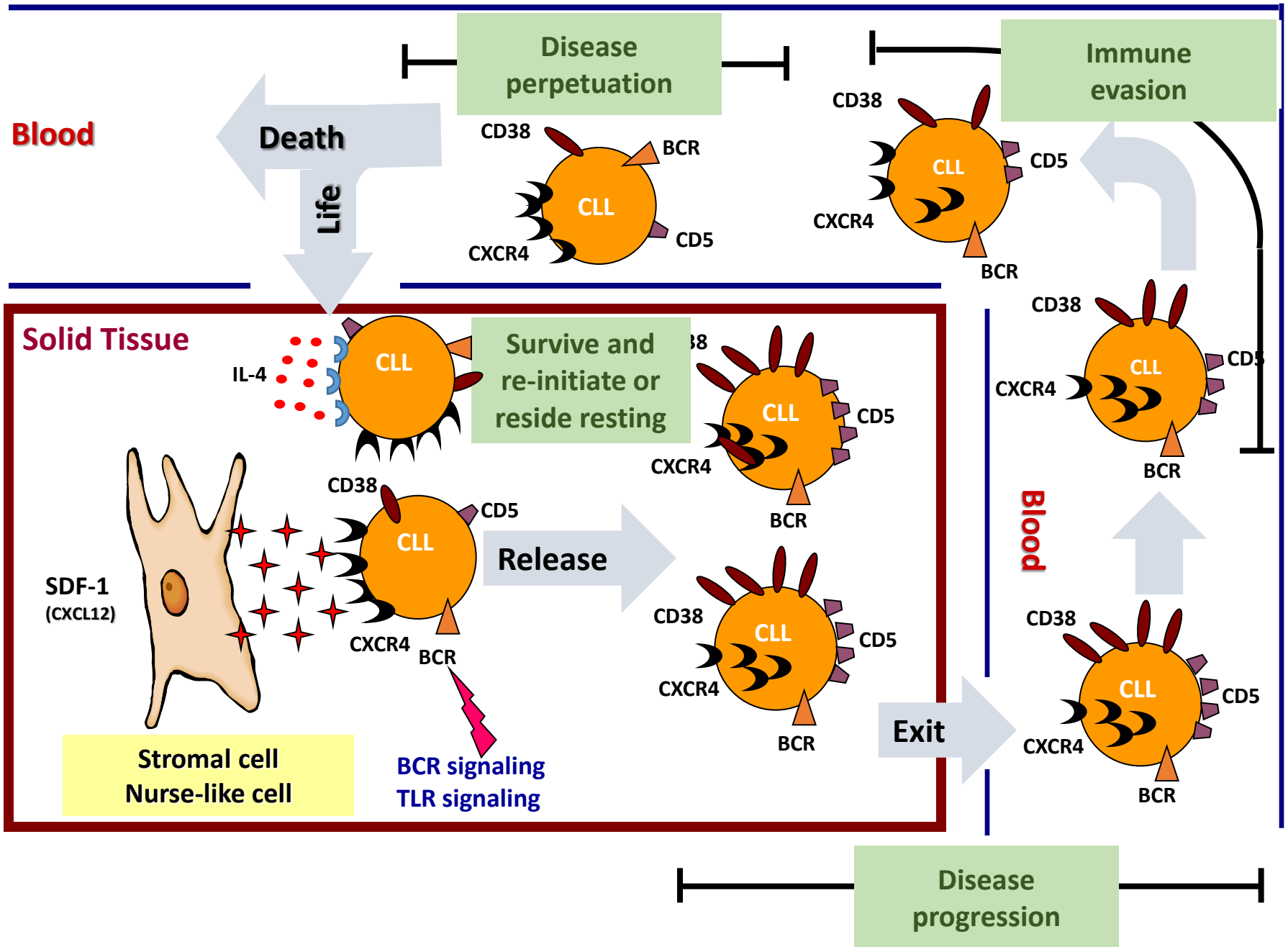
This fraction resembles and differs in some respects from the cycling fraction in lymph nodes, e.g., not a dominant BCR-related gene expression pattern but more of a trafficking, post-replicative fraction

Therefore, more work is needed to define more precisely in “Cycling Fraction” by isolating and characterizing the $CXCR4^{Dim}CD5^{Bright}$ fraction of lymph nodes

The fraction of dividing cells is ~ 0.1 – 4.0% of the clonal load per day

Why should we be concerned with such a small fraction of the clone?

Life cycle of a CLL cell



Life cycle of a CLL cell

Cells in the Proliferative Fraction (PF) lead to **disease progression** because of the ability to:

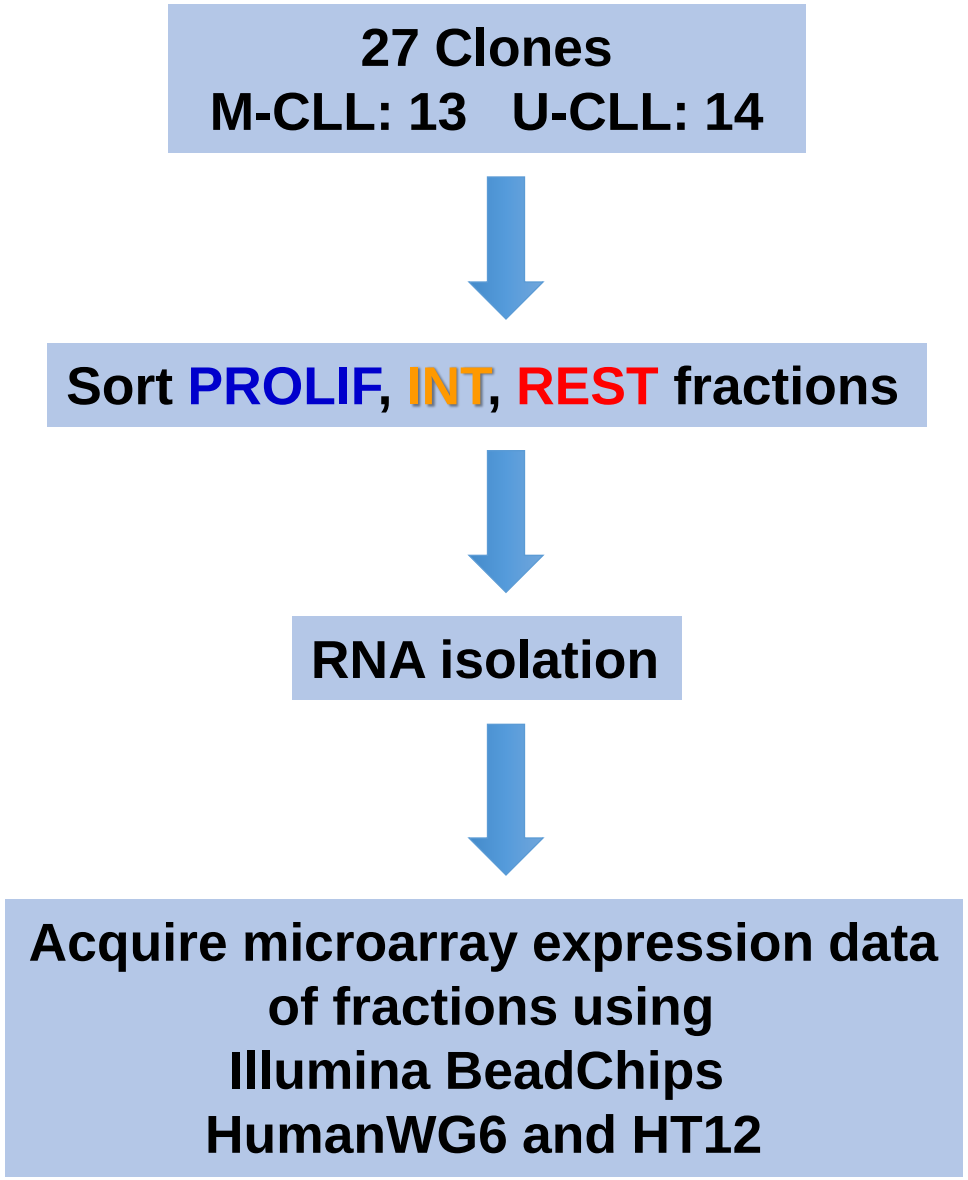
- interact with and activate T cells
- cause a Th2 polarization bias, resulting in not only survival signals but also in dampening of anti-tumor cytolytic responses
- produce activation-induced cytidine deaminase (AID) and reactive oxygen species (ROS) that can each cause DNA mutations and deletions and thereby lead to new mutations throughout the genome

Life cycle of a CLL cell

Cells in the Proliferative Fraction lead to disease progression because of the ability to:

- interact with and activate T cells

Gene expression comparisons of the Proliferative (PF) vs. Resting (RF) fractions

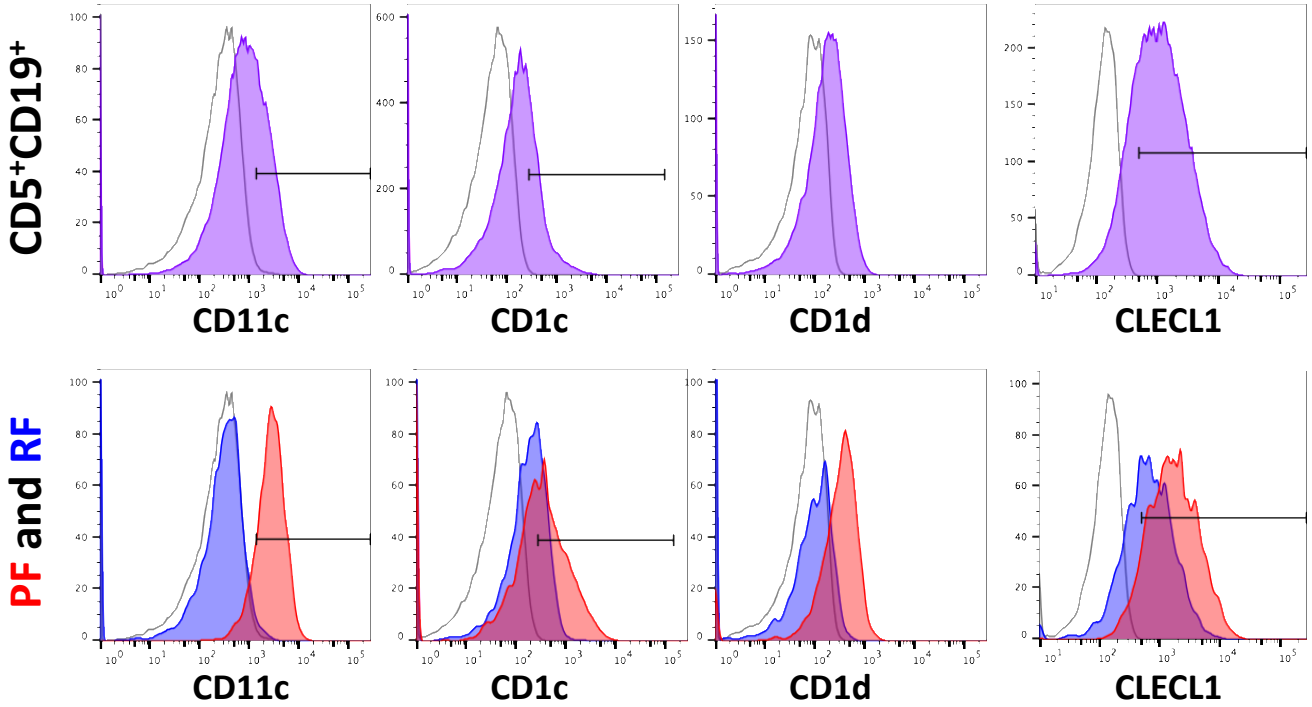


Gene Set Enrichment Analysis indicates that the PF most resembles myeloid cells and activated B cells

GSEA GENE SET	PF SIGNATURE	RF SIGNATURE	*P* VALUE
B Cell vs. Myeloid Dendritic Cell	Myeloid Dendritic Cells	B Cell	<0.0001
B Cell vs. Monocyte	Monocyte	Naïve B Cell	0.0019
IgD Positive vs. IgD Negative Blood B Cells	IgD Negative B Cell (GC B Cell)	IgD Positive B Cell (Pro-GC B Cell)	<0.0001
Naïve vs. IgM Memory B Cell	IgM Memory B Cell	Naïve B Cell	<0.0001

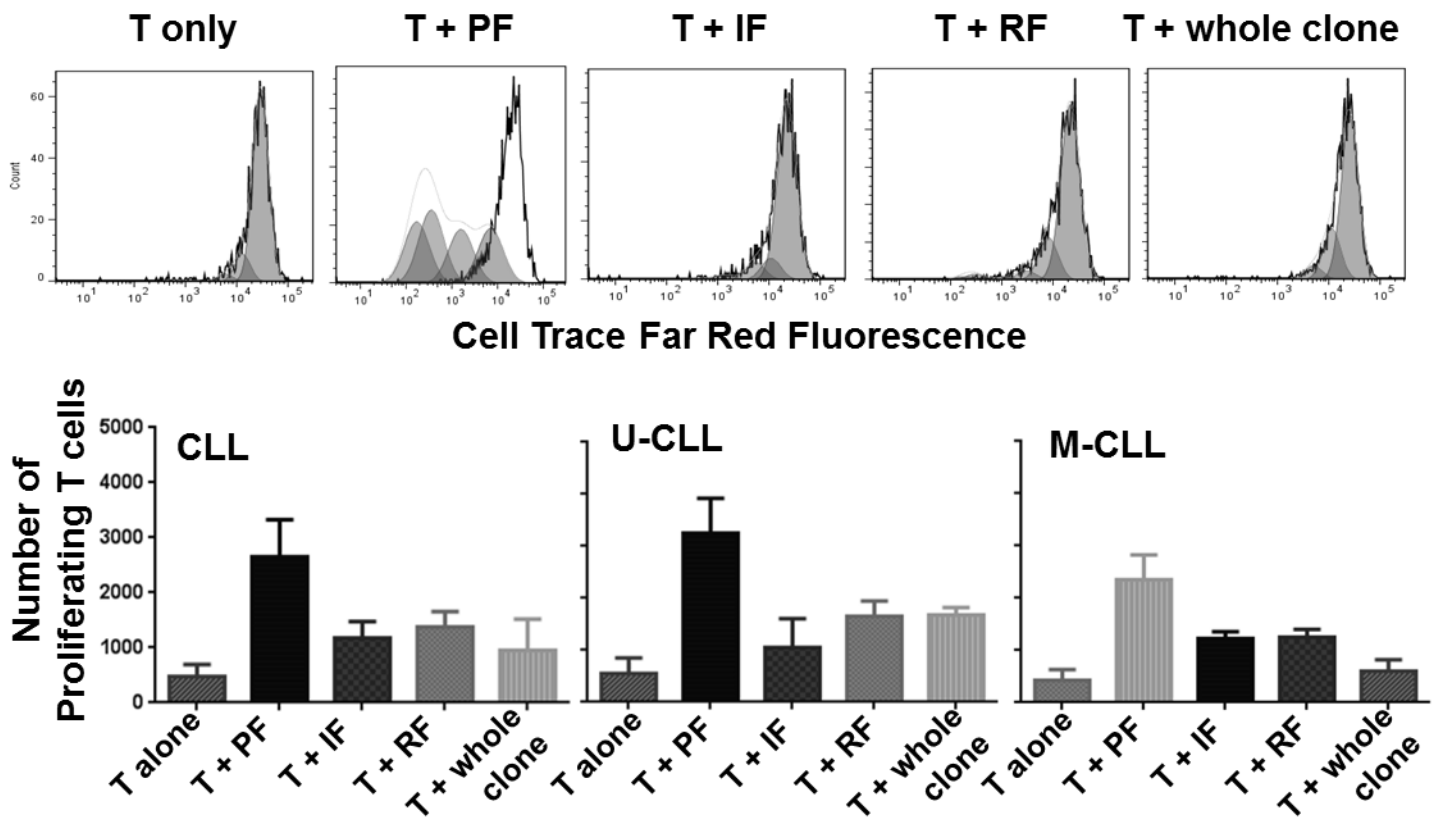
PF displays active B-cell and myeloid cell-signatures and may play a role as antigen-presenting cells in vivo

Flow cytometry confirms that the PF displays a myeloid cell phenotype



PF is the most effective intraclonal fraction for antigen presentation: *in vitro* evidence

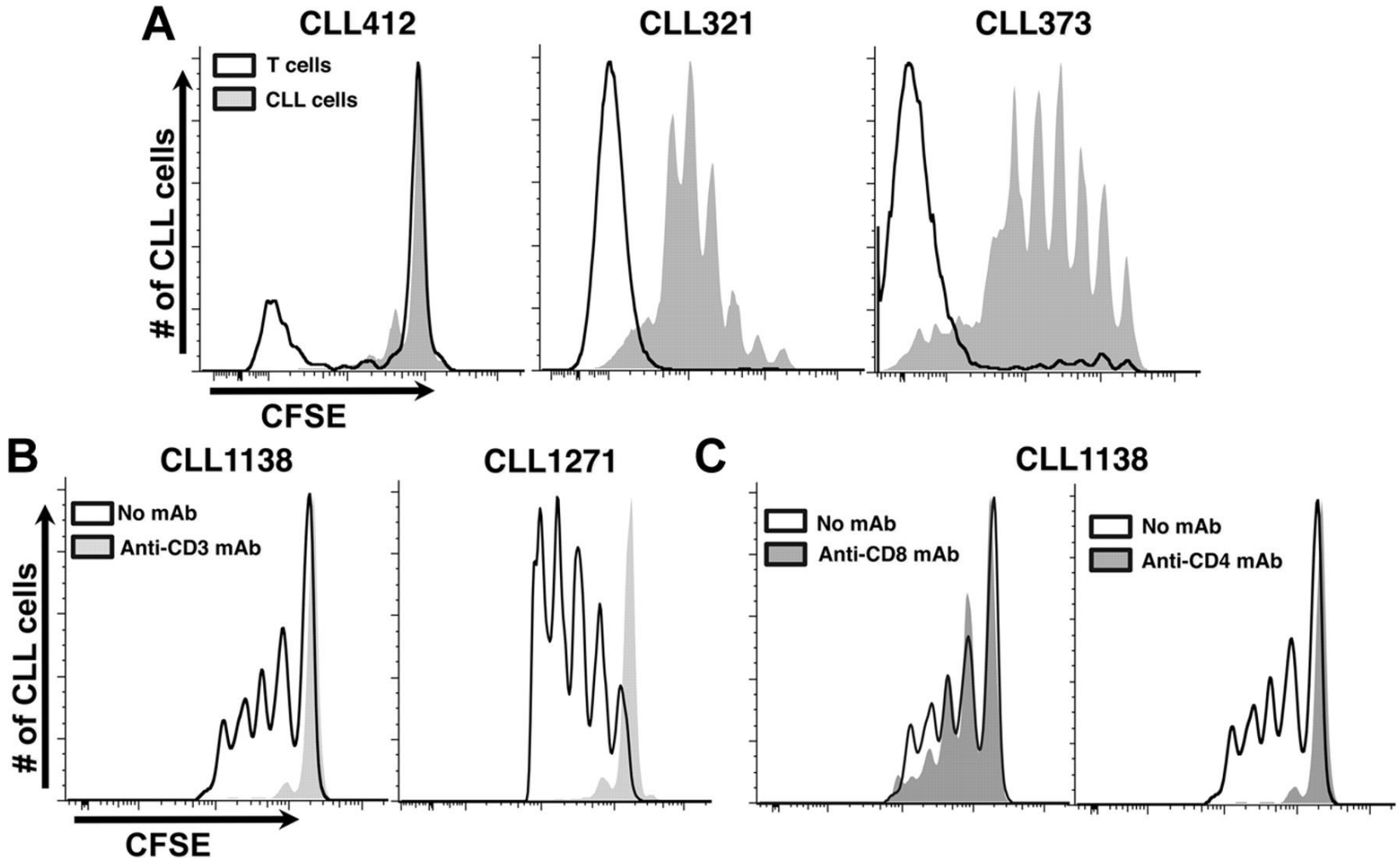
1. Allo-mixed lymphocyte reaction



PF is the most effective intraclonal fraction for antigen presentation: *in vivo* evidence

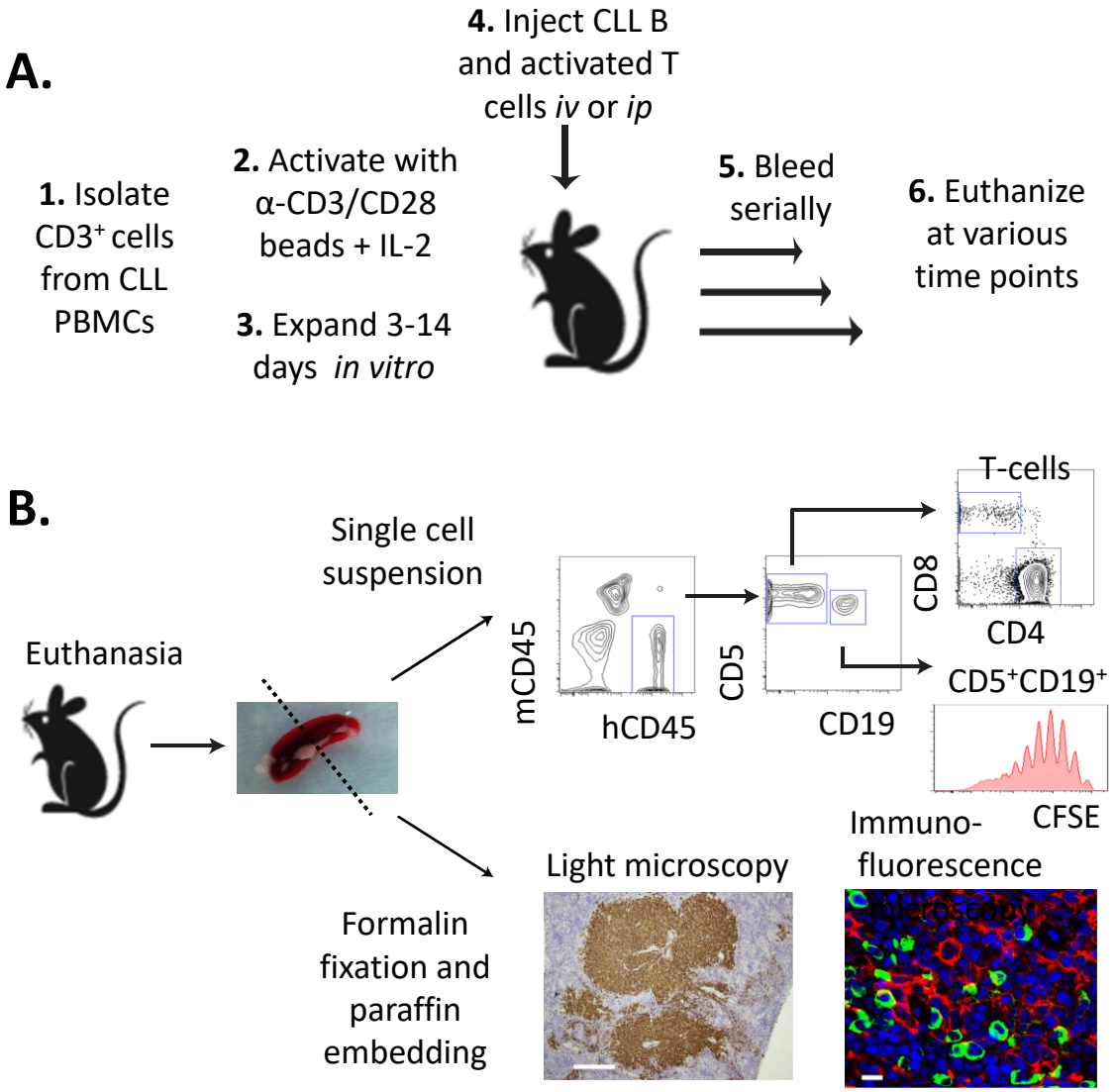
2. Xenografting CLL B and T cells into alymphoid mice

Growth of CLL cells in alymphoid NSG mice is T-cell dependent

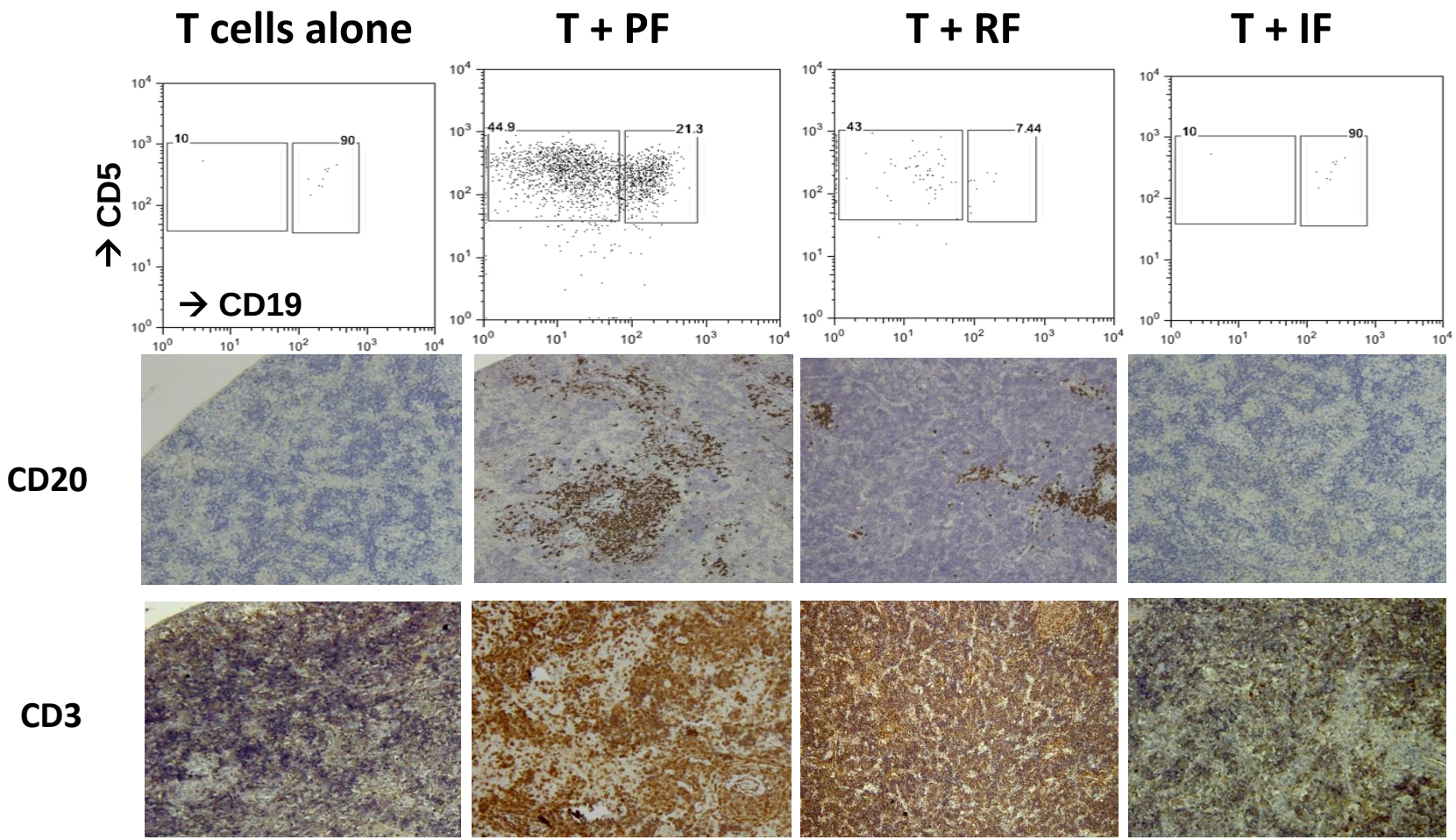


Bagnara *et al*. Blood 117: 5463-5472, 2011

Growth of primary CLL B and T cells in alymphoid (NSG) mice



PF stimulates resting T cells to divide and grow in alymphoid mice

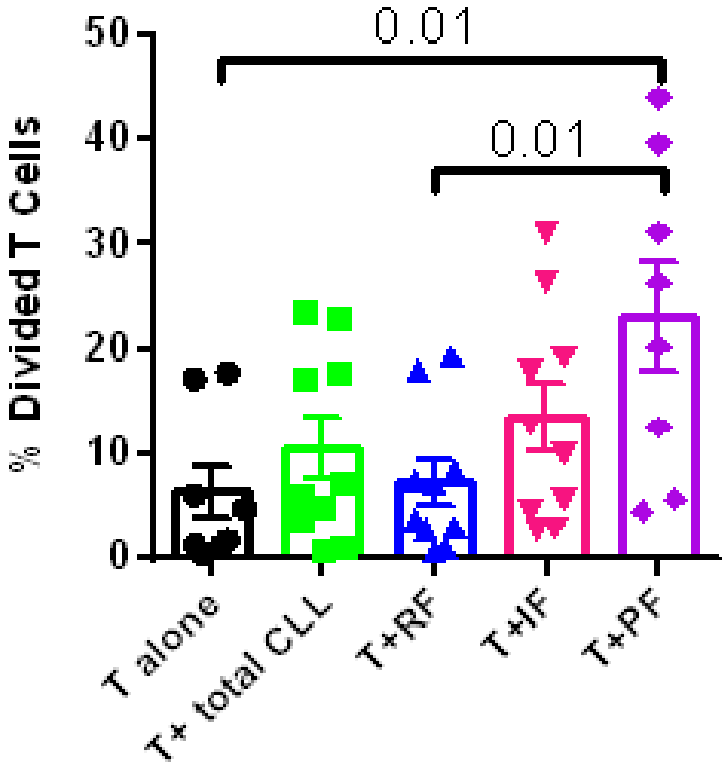


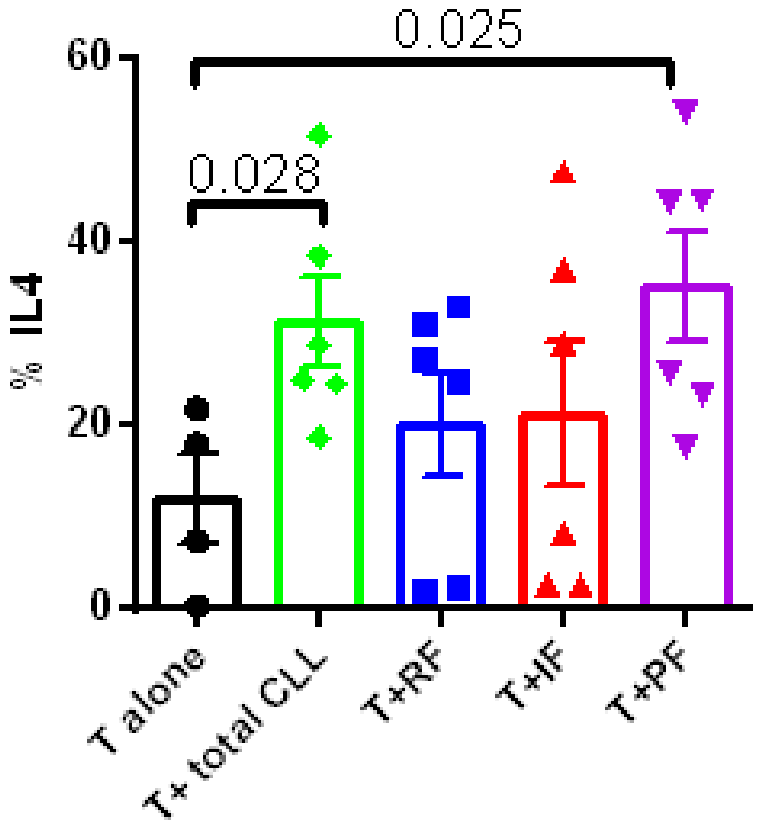
Life cycle of a CLL cell

Cells in the Proliferative Fraction lead to disease progression because of:

- T-B interaction leads to a Th2 polarization bias, resulting not only in survival signals but also in dampening of anti-tumor cytolytic responses

The $CXCR4^{Dim}CD5^{Bright}$ proliferative fraction preferentially induces IL-4 production by naïve T cells



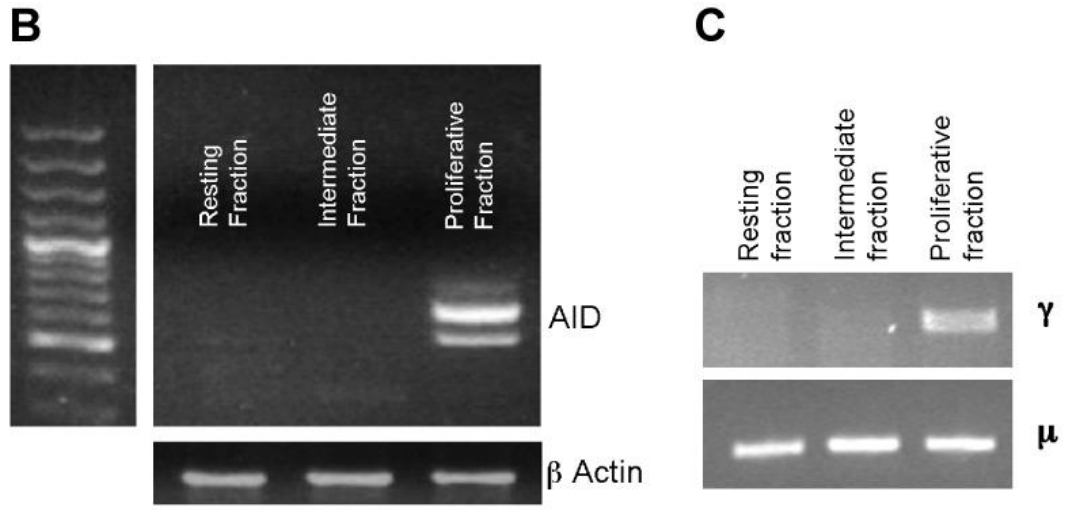


Life cycle of a CLL cell

Cells in the Proliferative Fraction lead to disease progression because of:

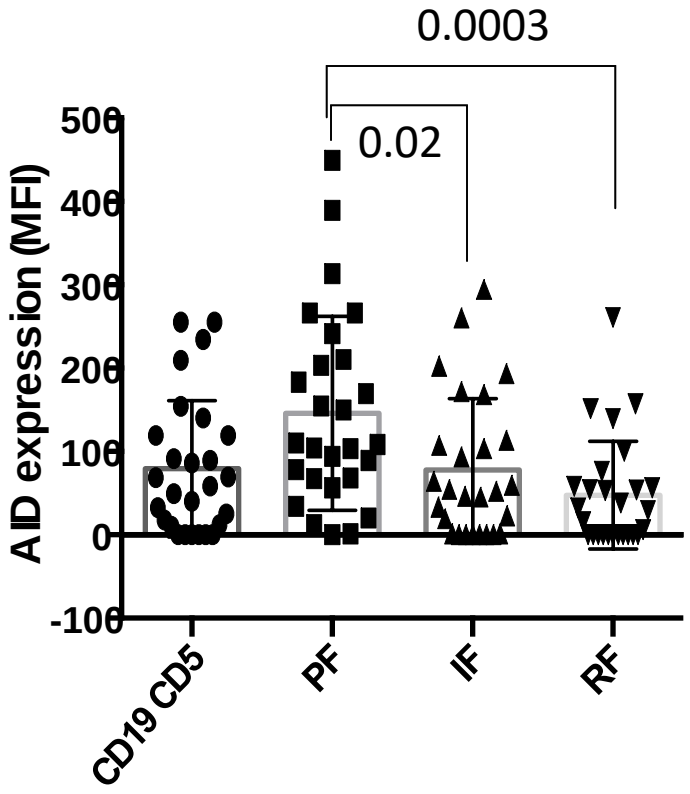
- production of activation-induced cytidine deaminase (AID) and reactive oxygen species (ROS) that can cause mutations and DNA deletion and thereby lead to new mutations throughout the genome

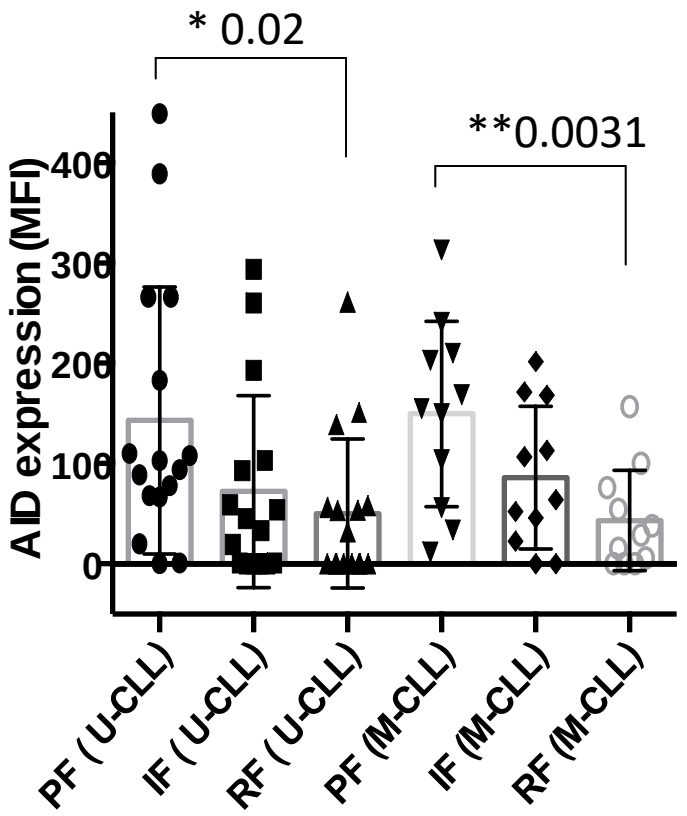
AID mRNA is most expressed in the CXCR4^{Dim}CD5^{Bright} proliferative fraction



P Patten *et al*. Blood 120: 4802-4811, 2012

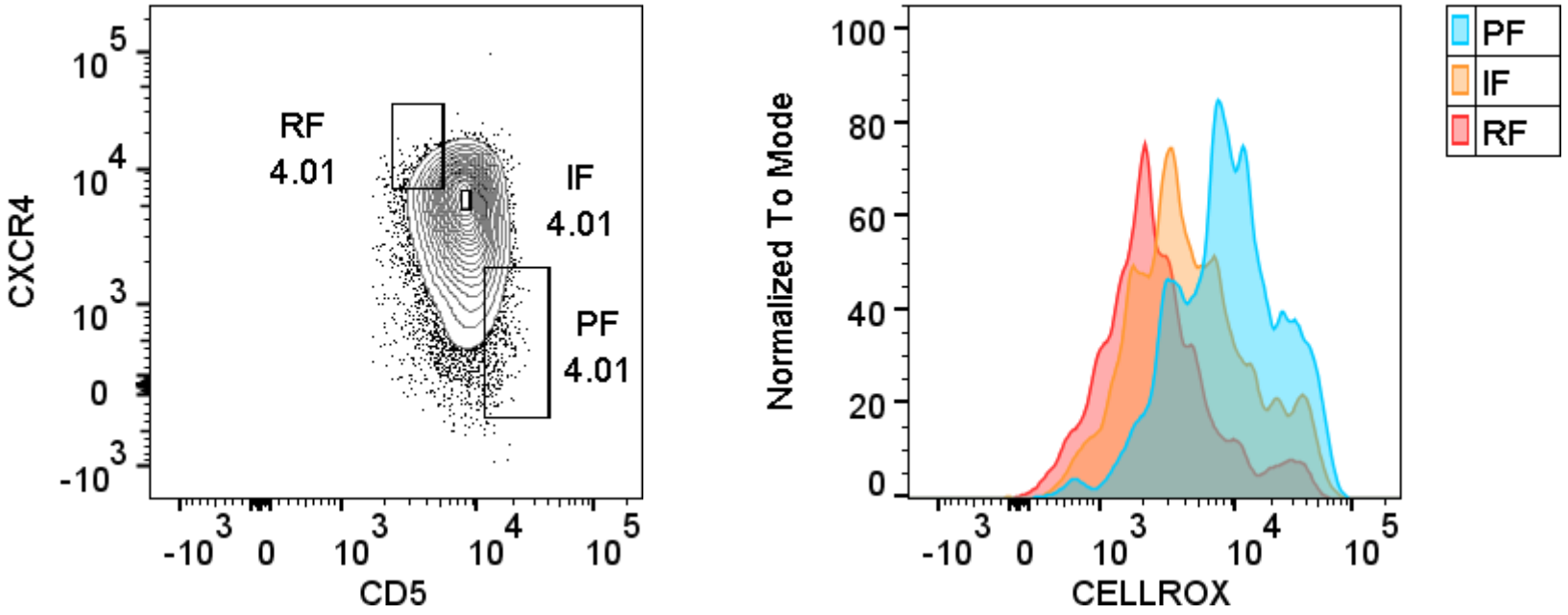
AID protein is most expressed in the CXCR4^{Dim}CD5^{Bright} proliferative fraction





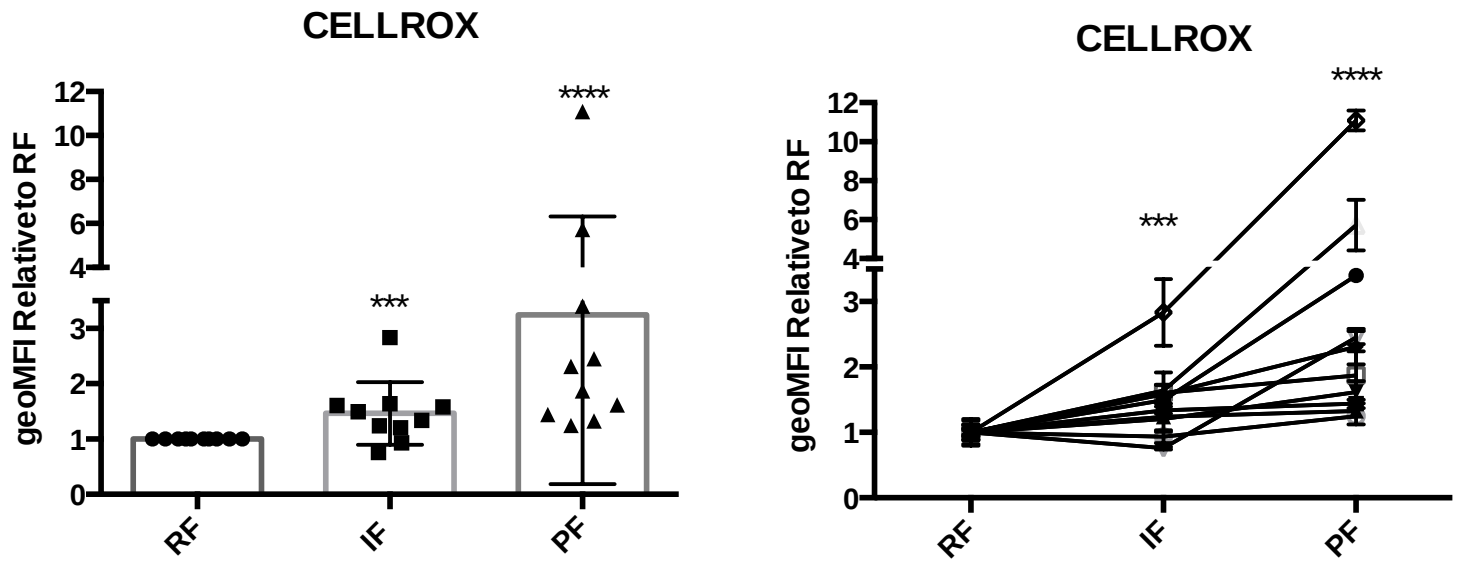
F Palacios *et al*. unpublished

Reactive oxygen species are most abundant in the CXCR4^{Dim}CD5^{Bright} proliferative fraction



E Sturgill *et al*. unpublished

Reactive oxygen species are most abundant in the CXCR4^{Dim}CD5^{Bright} proliferative fraction



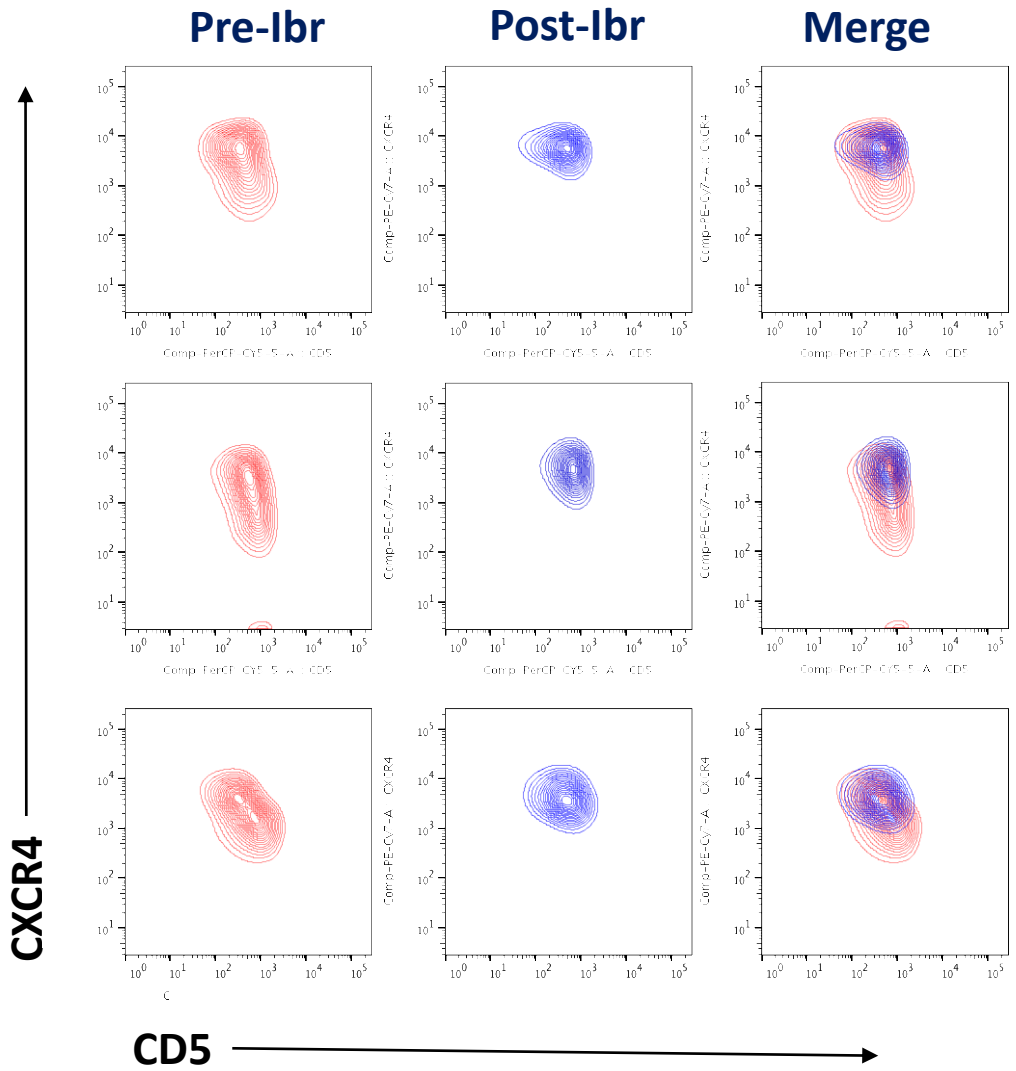
E Sturgill *et al*. unpublished

Unanswered questions

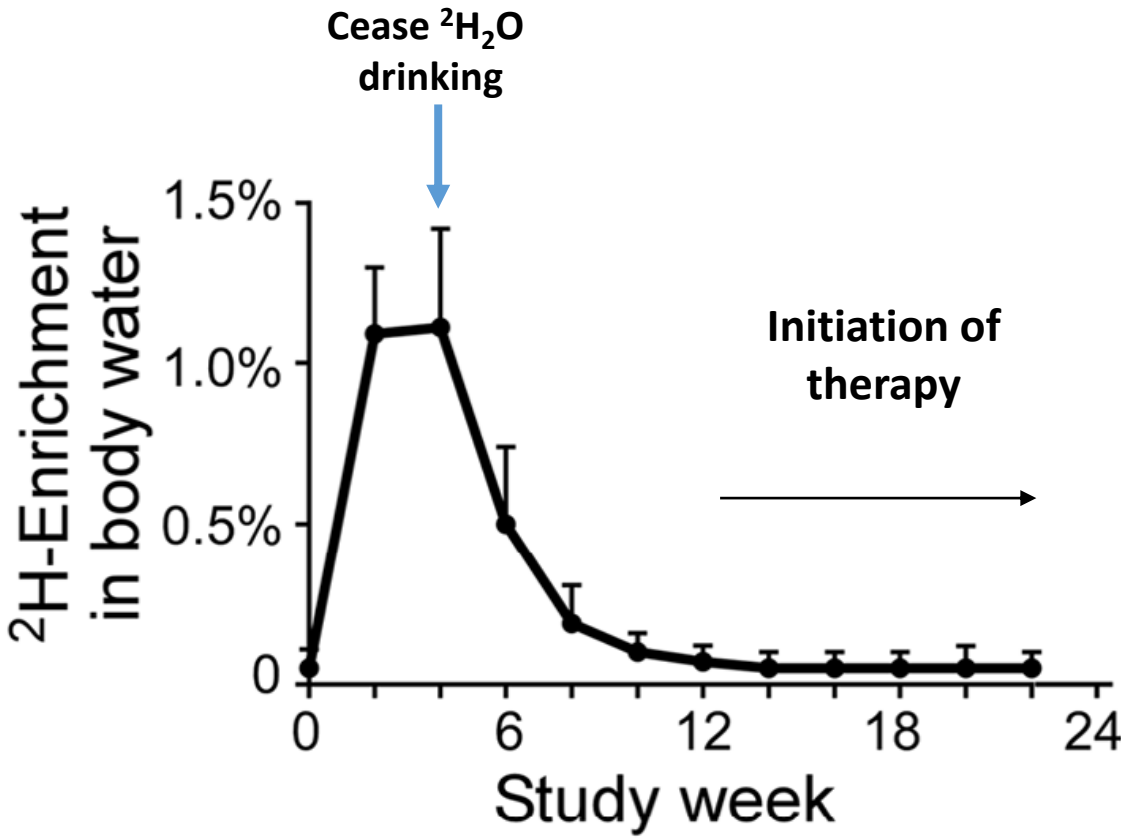
1. Will elimination of the $CXCR4^{Dim}CD5^{Bright}$ Proliferative Fraction in patients impact on clonal evolution?

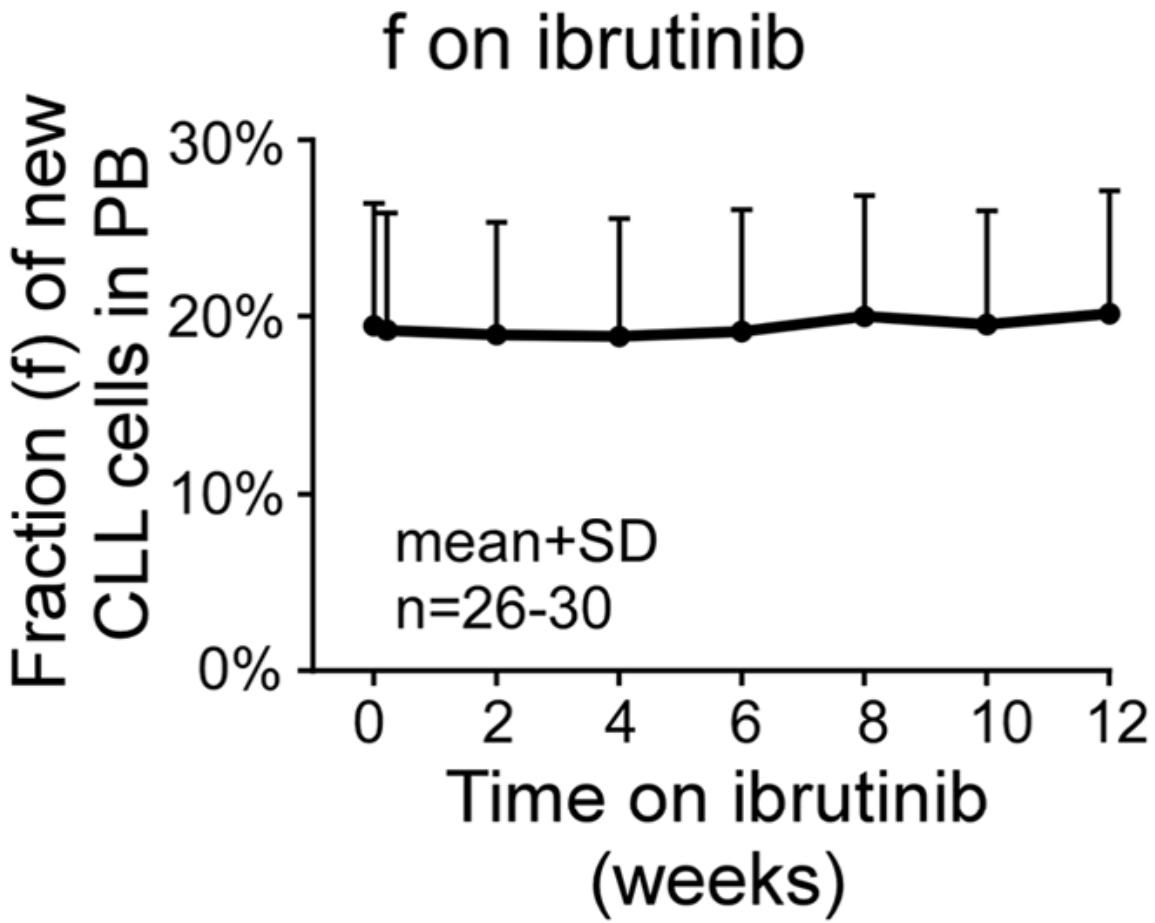
What are the effects of current and novel therapies on the $CXCR4^{Dim}CD5^{Bright}$ Proliferative Fraction?

Ibrutinib preferentially eliminates the $CXCR4^{Dim}CD5^{Bright}$ Proliferative Fraction

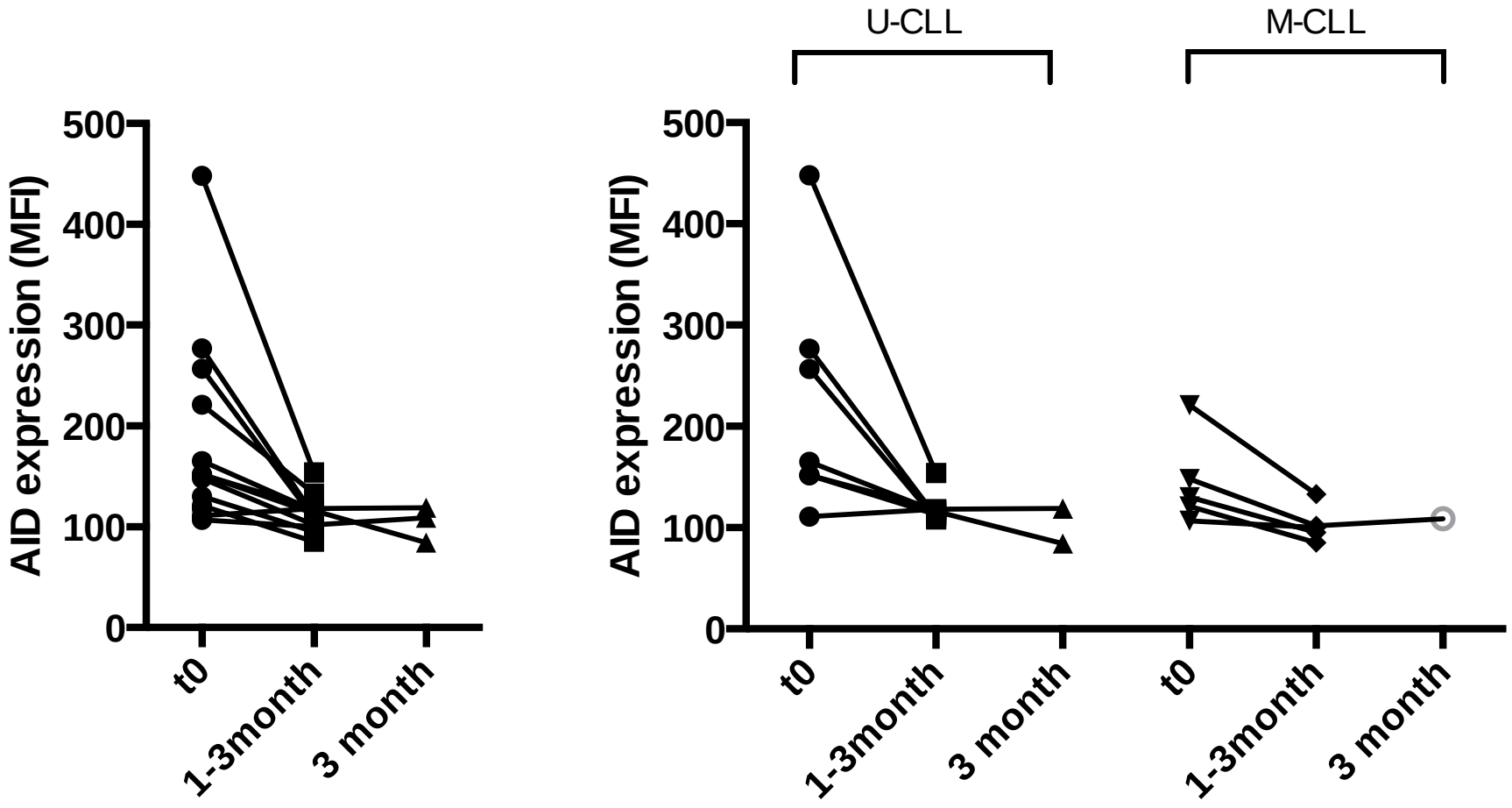


Ibrutinib rapidly prevents new cell growth as evidenced by the stability of the fraction of ^{2}H-DNA-labeled cells in the blood



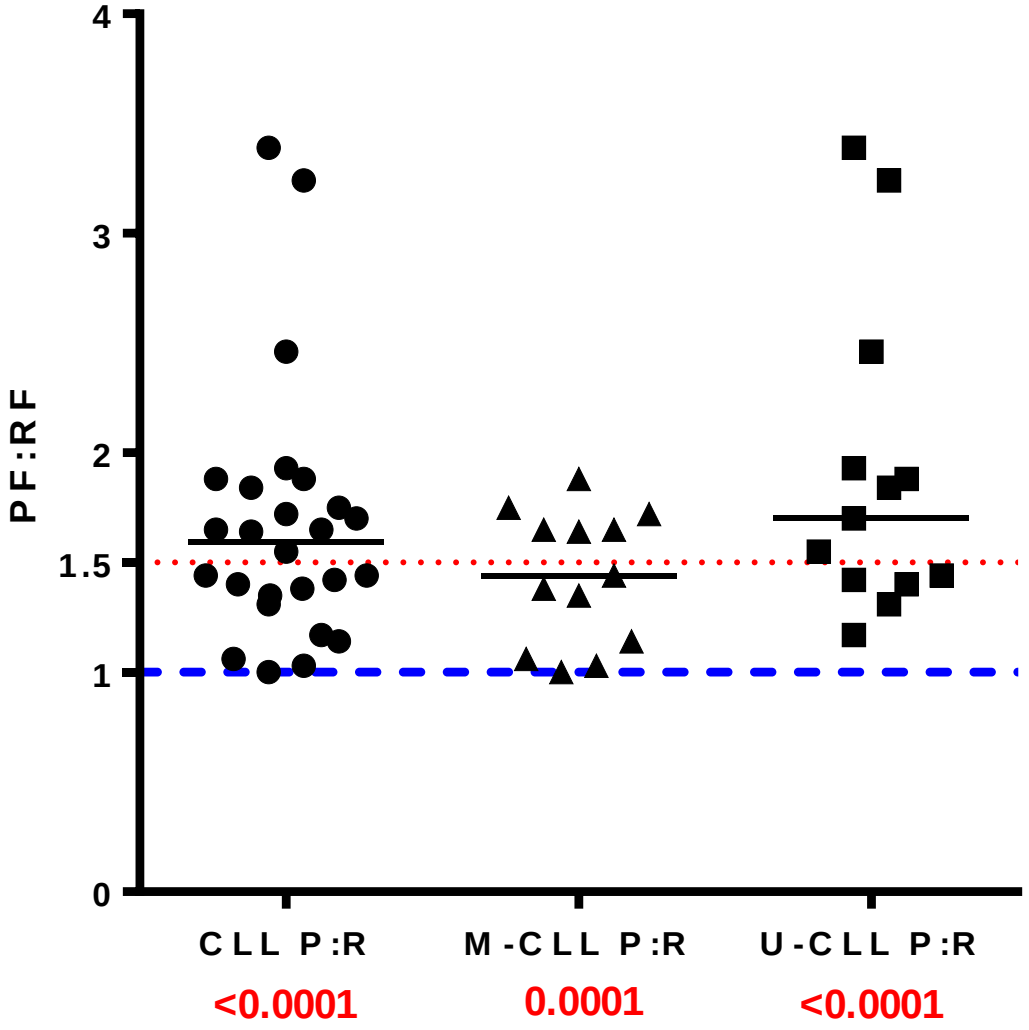


Ibrutinib inhibits AID expression

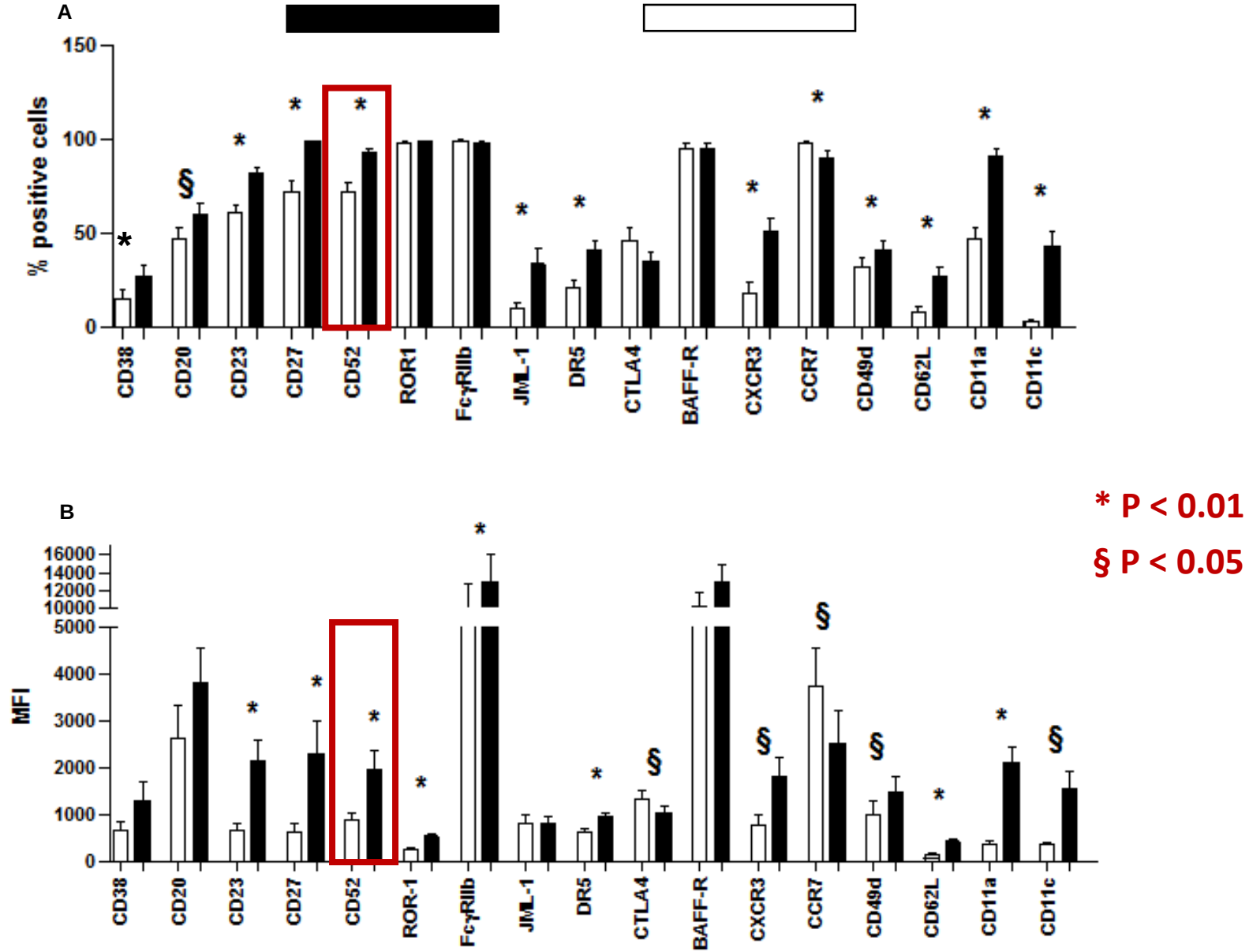


Novel approach to targeting the Proliferative Fraction

The CD52 gene is over-expressed in the proliferative vs. resting fraction



CD52 surface membrane levels are greater between proliferative and resting fractions



* P < 0.01

§ P < 0.05

Anti-CD52: Alemtuzumab/Campath

Highly effective at eliminating CLL B cells, even in the setting of disease relapse or therapeutic unresponsiveness

Also eliminates other, non-leukemic cells expressing CD52 necessary for immune function, leading to severe immune deficiency and infections

Removed from the CLL market because of these life-threatening side effects

Could the action and effectiveness of Alemtuzumab/Campath be persevered and its broad reactivity restricted to provide an efficient and safe therapy?

Could an effective and safe form of Alemtuzumab/Campath be used to eliminate those cells responsible for clonal evolution or disease relapse?

Question and approach

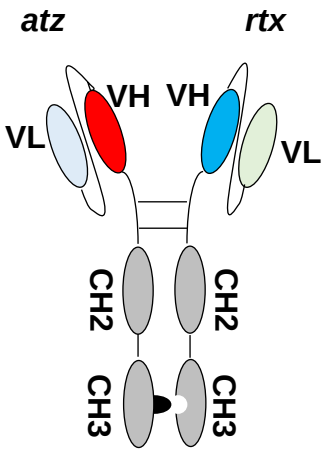
Could one engineer a therapeutic antibody with dual specificities for CD52 and for a B-cell restricted epitope (e.g., CD20) that would bind to and eliminate selectively B cells but not normal T cells and myeloid cells?

Would the B-cell reactivity be preferential for those cells responsible for clonal evolution and expanding during disease relapse?

CD52 (Alemtuzumab/Campath®) x CD20 (Rituximab/Rituxan®)

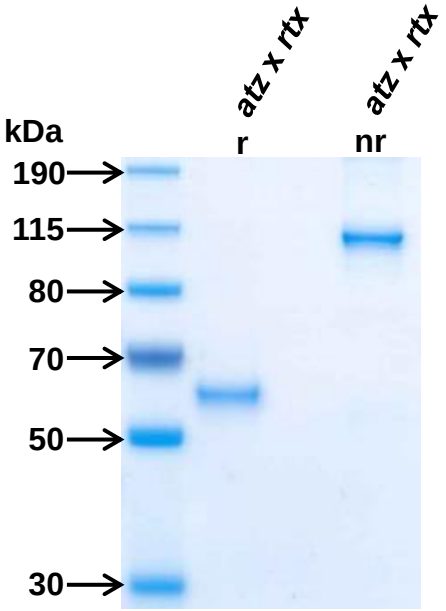
scFv-Fc IgG1-like “knob-in-hole” bispecific antibody

A.



IgG1

atz x rtx biAb



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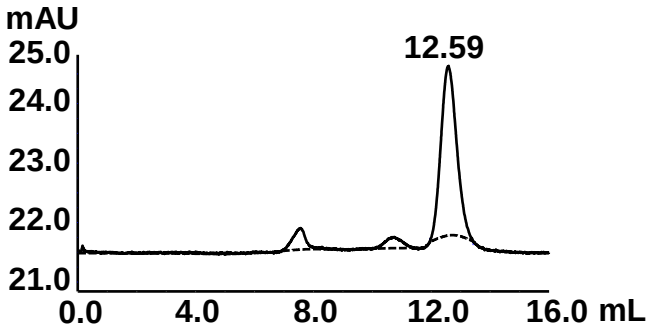
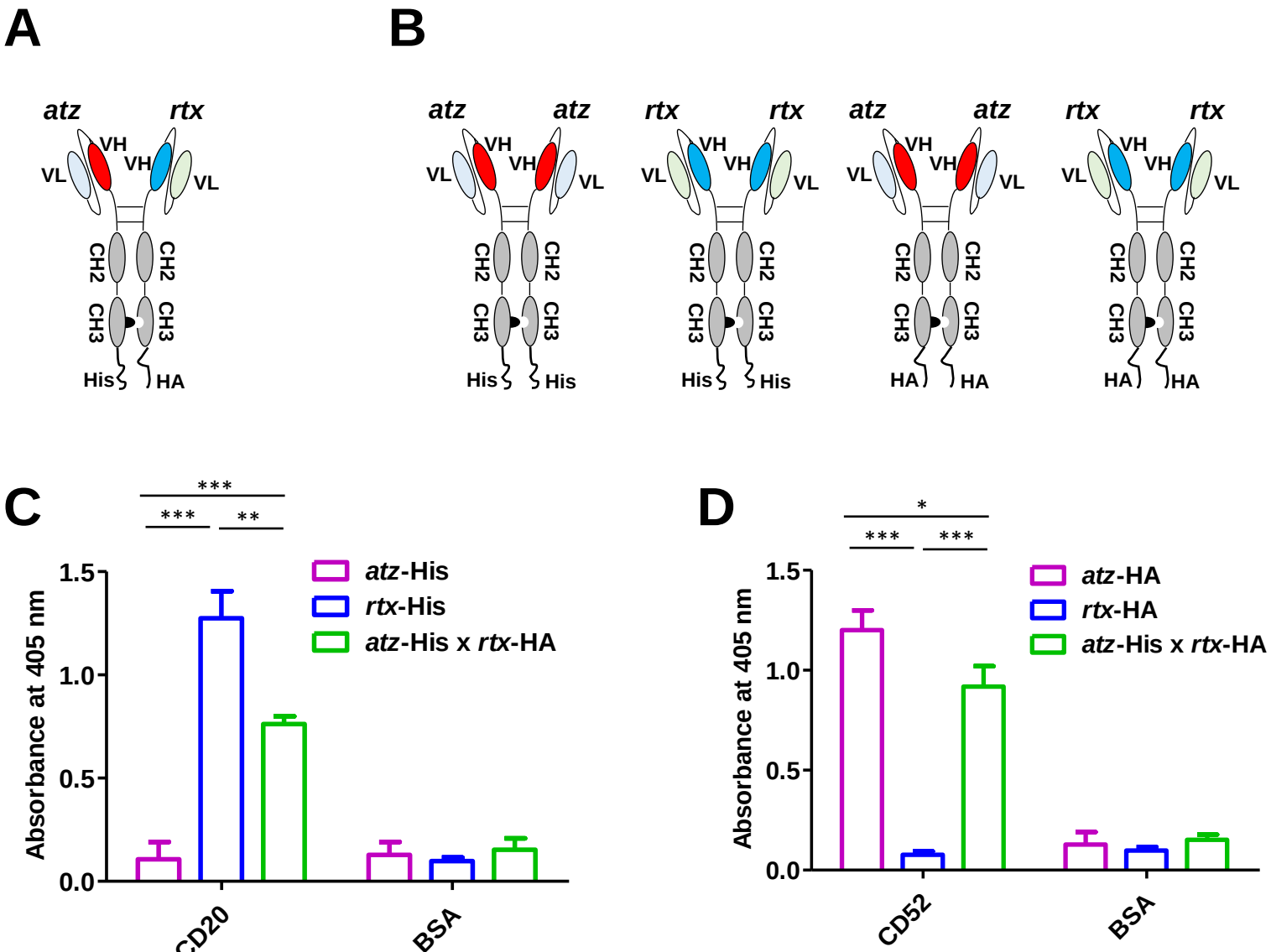


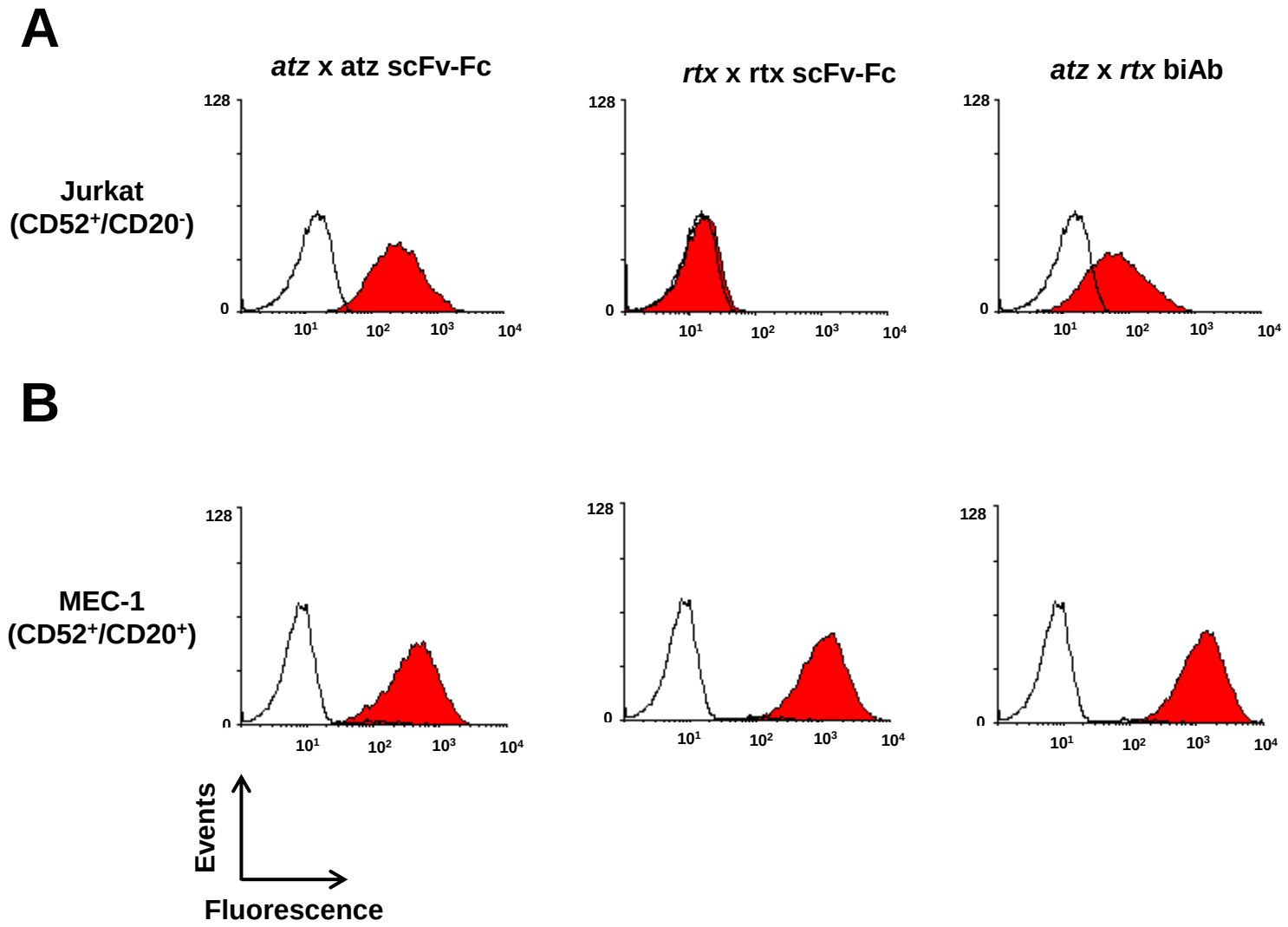
J Qi et al. Methods 2018 in press

Binding specificities of (atz x rtx), (atz x atz), (rtx x rtx) IgG1-like bispecific antibodies (ELISA)



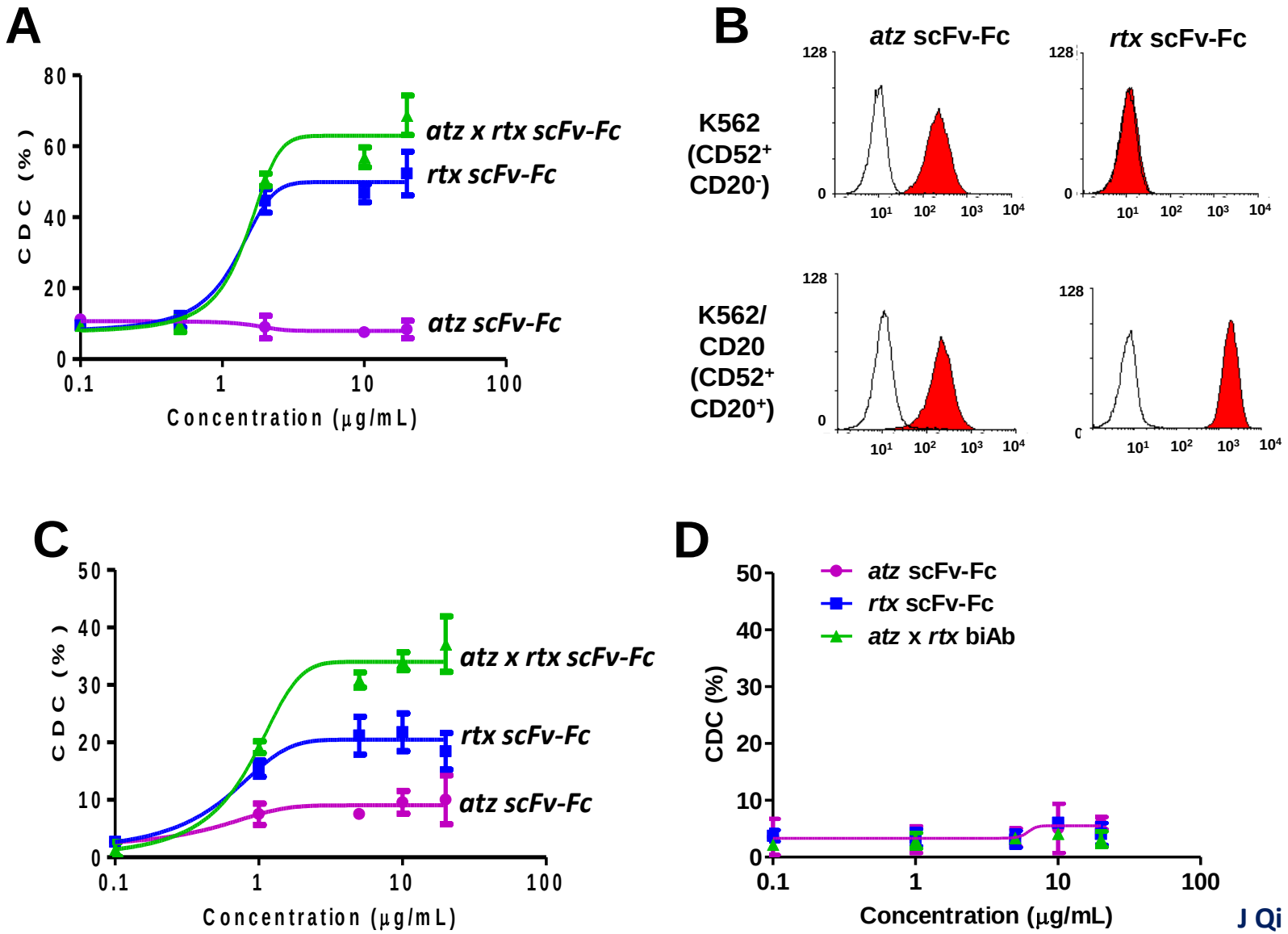
J Qi et al. Methods 2018 in press

Binding specificities of (atz x rtx), (atz x atz), (rtx x rtx) IgG1-like bispecific antibodies (flow cytometry)



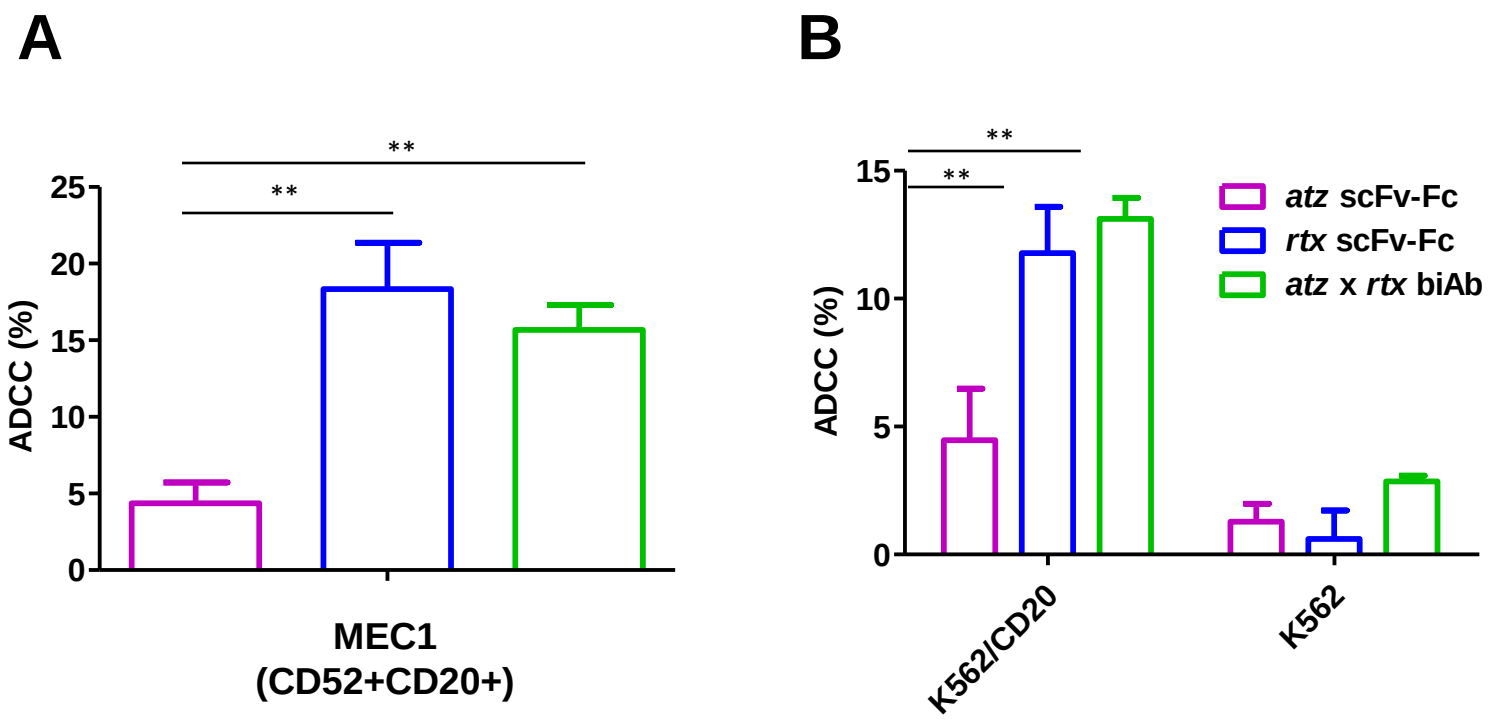
J Qi et al. Methods 2018 in press

Effector function – CDC - of (atz x rtx), (atz x atz), (rtx x rtx) IgG1-like bispecific antibodies



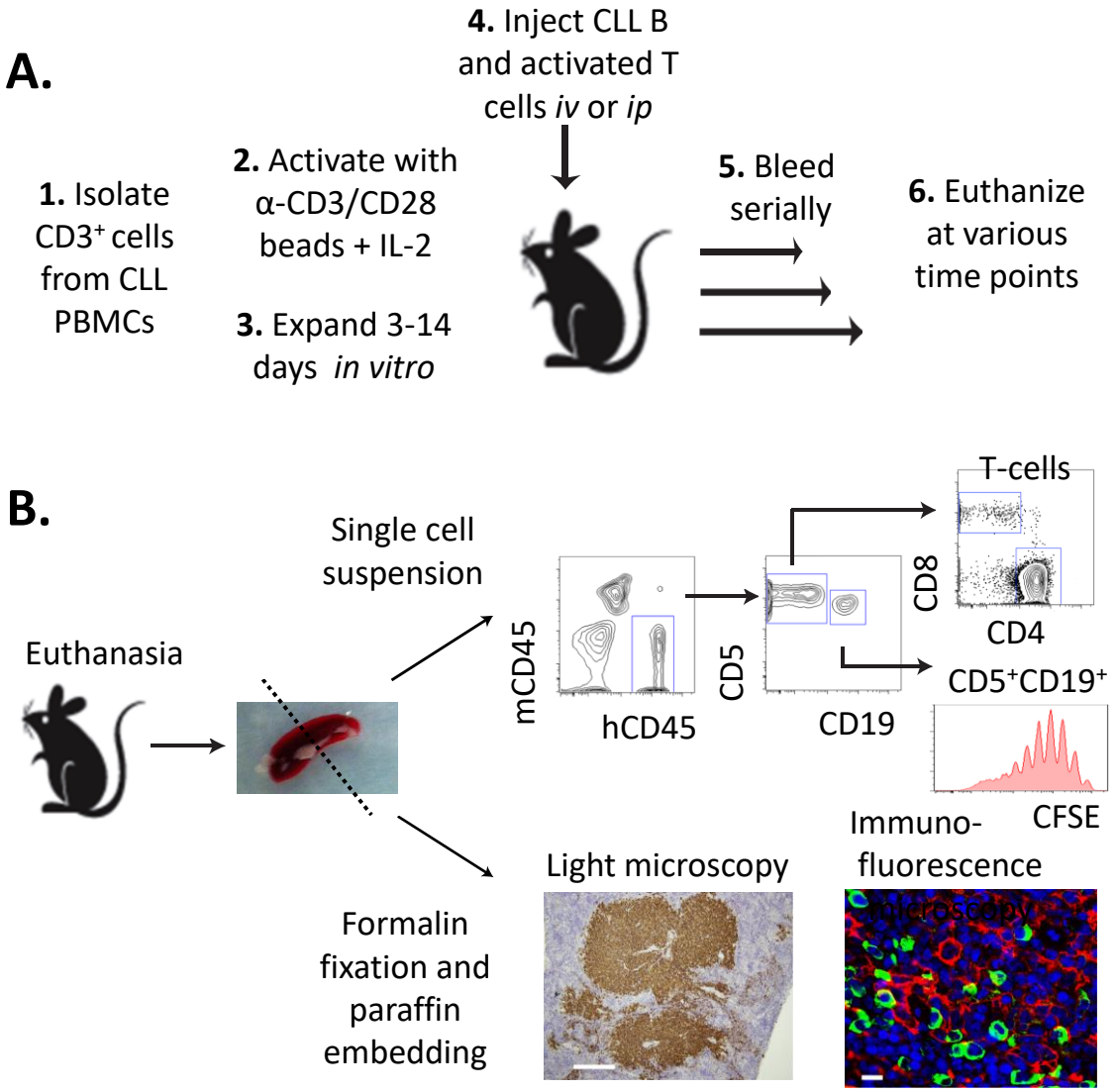
J Qi et al. Methods 2018 in press

Effector function - ADCC - of (atz x rtx), (atz x atz), (rtx x rtx) IgG1-like bispecific antibodies

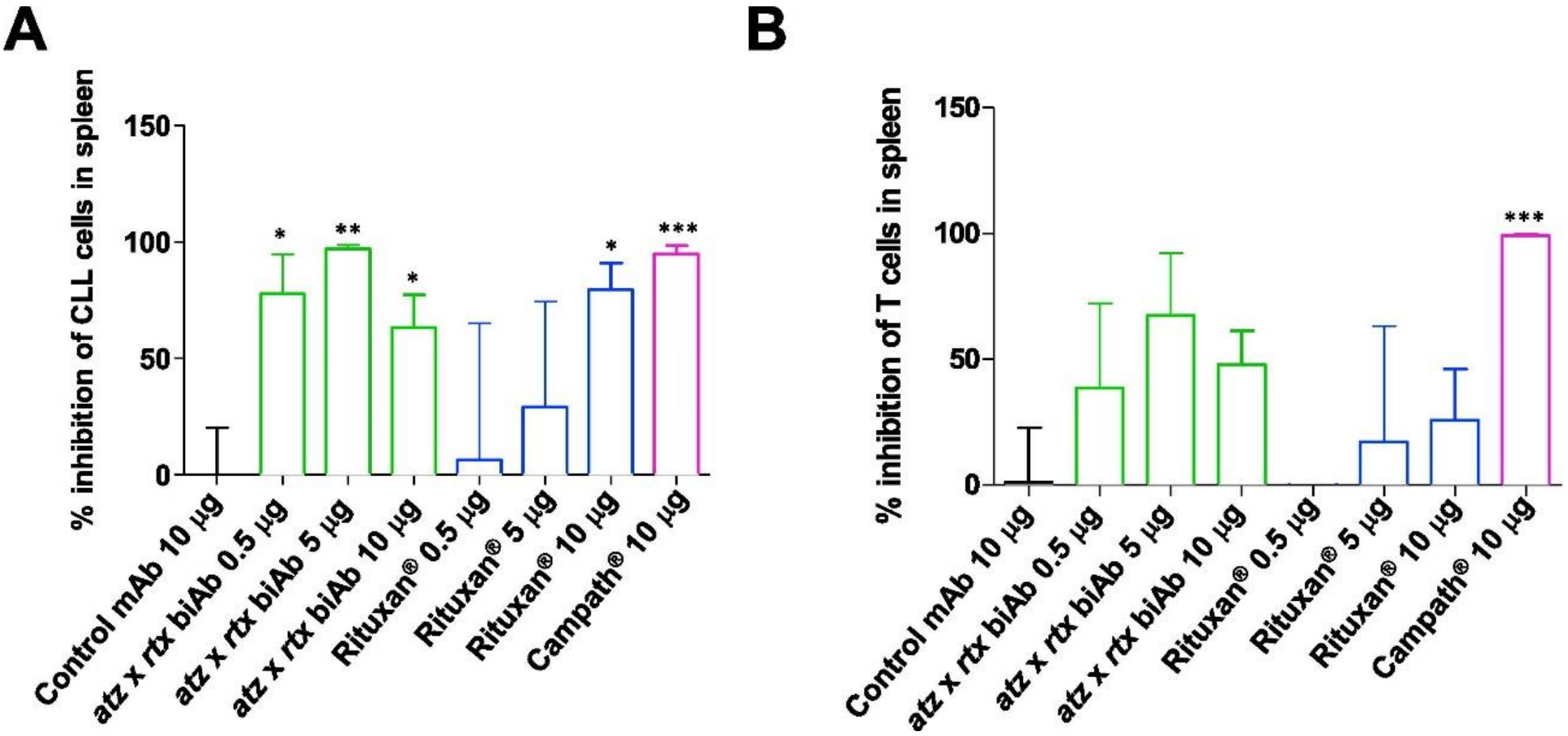


J Qi et al. Methods 2018 in press

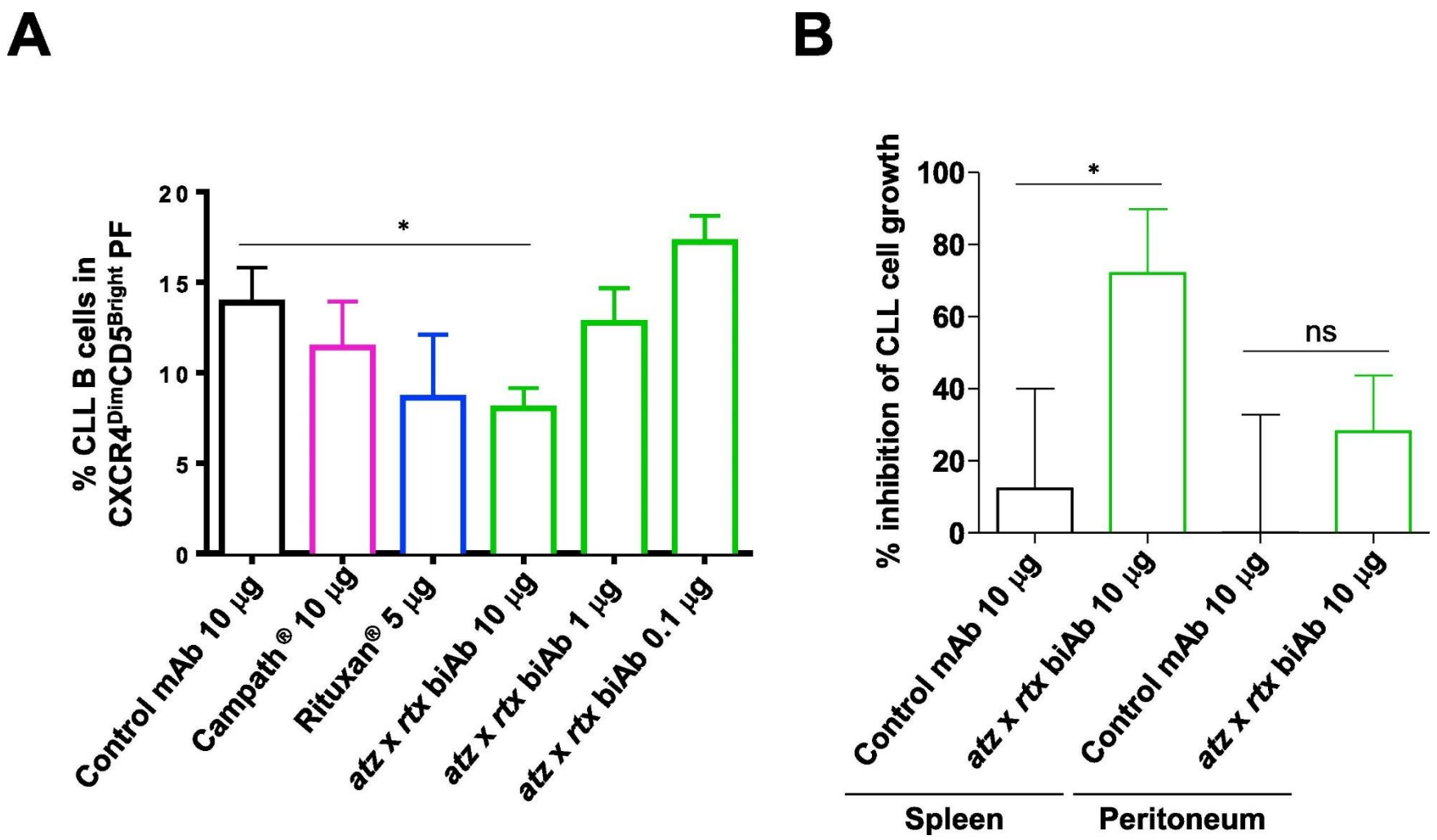
Growth of primary CLL B and T cells in alymphoid (NSG) mice



atz x rtx bispecific antibody selectively eliminates CLL B cells but not T cells



atz x rtx bispecific antibody preferentially eliminates the $CXCR4^{Dim}CD5^{Bright}$ proliferative fraction



Summary

A CD52 x CD20 bispecific antibody (biAb) based on alemtuzumab and rituximab:

1. preferentially bound $CD52^{+}CD20^{+}$ B cells and not $CD52^{+}CD20^{-}$ T cells

2. mediated potent CDC and ADCC *in vitro*

3. selectively eliminated leukemic B cells and preferentially eliminated the proliferative fraction of malignant B cells, in a patient-derived xenograft model

Major unanswered question

Would repetitive pulse therapies targeting dividing and recently divided cells be able to eliminate/markedly decrease the proliferative fraction and cycling fraction and thereby prevent clonal progression?

Would a combination of therapeutic approaches targeting each of the three CXCR4/CD5 fractions by valuable and cost effective?

Thank you

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