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Linking the Microenvironment with CLL Models

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Disclosures of NAME SURNAME

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Murine genetic models of CLL

TCL-1 Tg mice: aggressive disease

DLEU2/miR15a/16-1 cluster-deleted mice: indolent disease
miR15b/16-2 -deleted mice: indolent disease + NHL
miR15a/16-1 + miR15b/16-2 DKO: CLL/NHL + Acute Myeloid Leukemia
IgH-Eμ-miR-29 Tg mice: indolent disease

BCL-2:Traf2DN double Tg mice: refractory disease
c-Myc:BAFF Tg mice: : male more virulent disease than female mice

$IRF^{-/-}$VH11 mice: indolent and aggressive disease

Advantages and disadvantages of murine genetic models of CLL

Advantages

1. Can precisely evaluate the effects of specific genomic aberrations in a controlled setting to understand the resultant biology in vivo
2. Can repetitively ask questions based on the knowledge that has emerged from previous studies
3. Can serve as models for novel therapeutics addressed at a defined genetic defect

Advantages and disadvantages of murine genetic models of CLL

Disadvantages.

1. Because of their precision, do not, at this point, reflect the broad genetic that humans with CLL exhibit
2. When that is possible, will need many distinct models due to the complexity of genetic abnormalities in patients

Advantages and disadvantages of xenografting primary CLL cells into alymphoid recipients

Advantages

1. Can use an individual patient's leukemic B cells, which contain specific genetic and epigenetic differences unique to that patient. Information directly applicable
2. Can transfer other non-leukemic cells from the same patient that could be responsible, directly or indirectly, for biologic actions of the leukemic cells.

Advantages and disadvantages of murine genetic models of CLL

Disadvantages.

1. Because of the unique (genetic) features of each patient, the results from studying one patient might not relate to others
2. Although these parameters may more accurately reflect the biologic features of CLL, they add considerably more complexity, and heterogeneity to the experimental system
3. Requires recipient animals with various genetic manipulations that allow xenografting and growth. Some murine cytokines and chemokines are not effective on human cells.

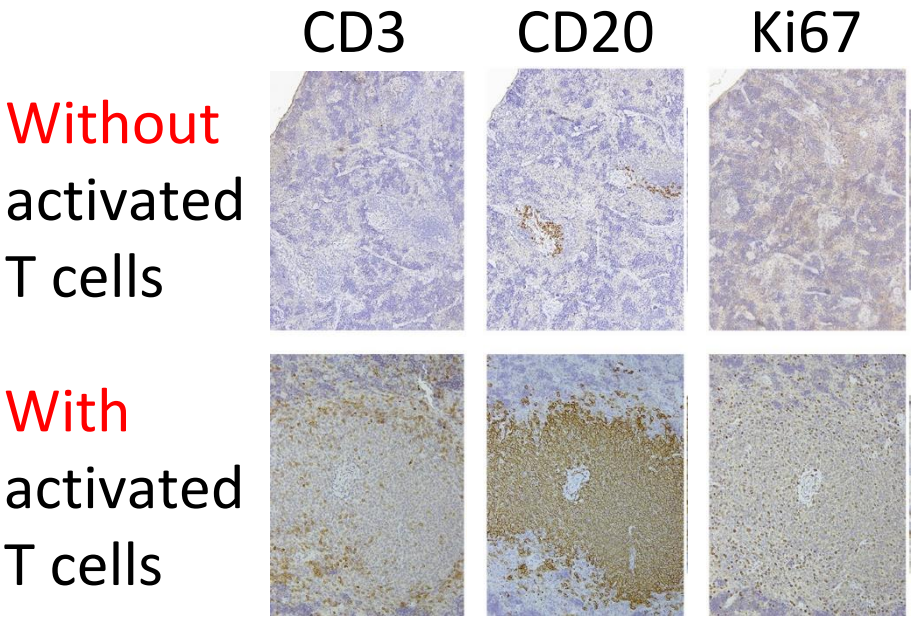
Xenografting sacrifices the robust mechanistic specificity of murine genetic models to hopefully discover the natural biology of disease in patients with CLL.

Today, we will discuss the interactions of primary, patient leukemic B cells with the tumor microenvironment, focusing on normal hematopoietic and non-hematopoietic cells.

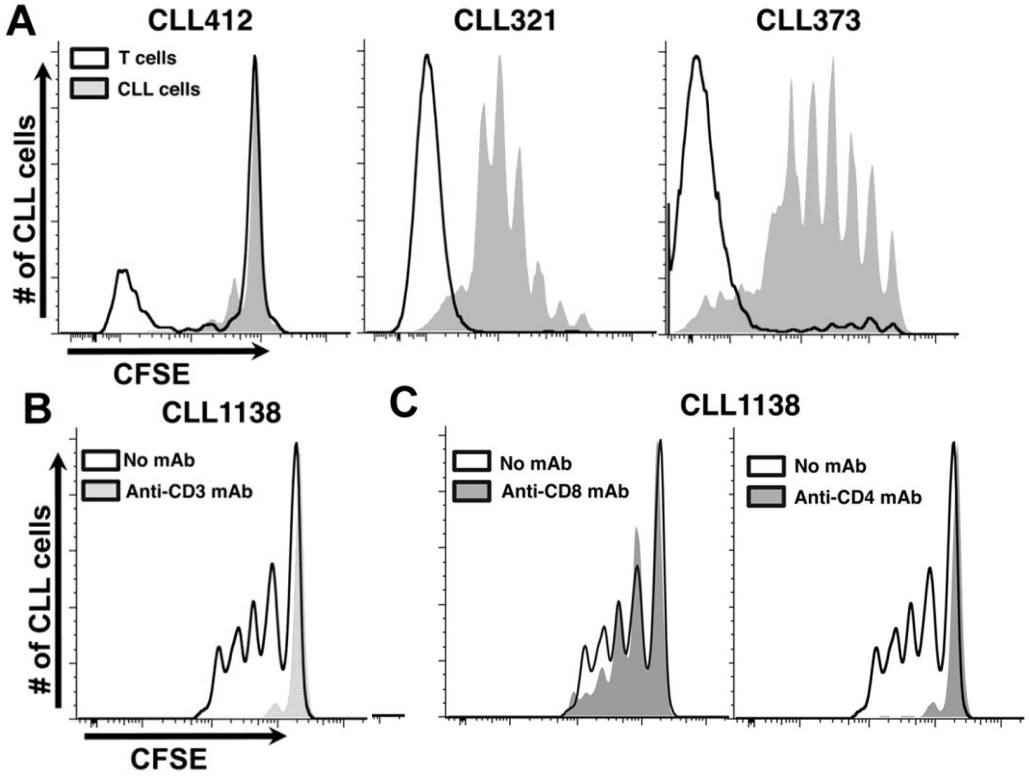
Interaction of CLL cells with T cells into alymphoid mice

- CLL cells require the help of activated T lymphocytes to engraft and grow in alymphoid NSG mice

Interaction of CLL cells with activated T cells allows growth in recipient alymphoid mice

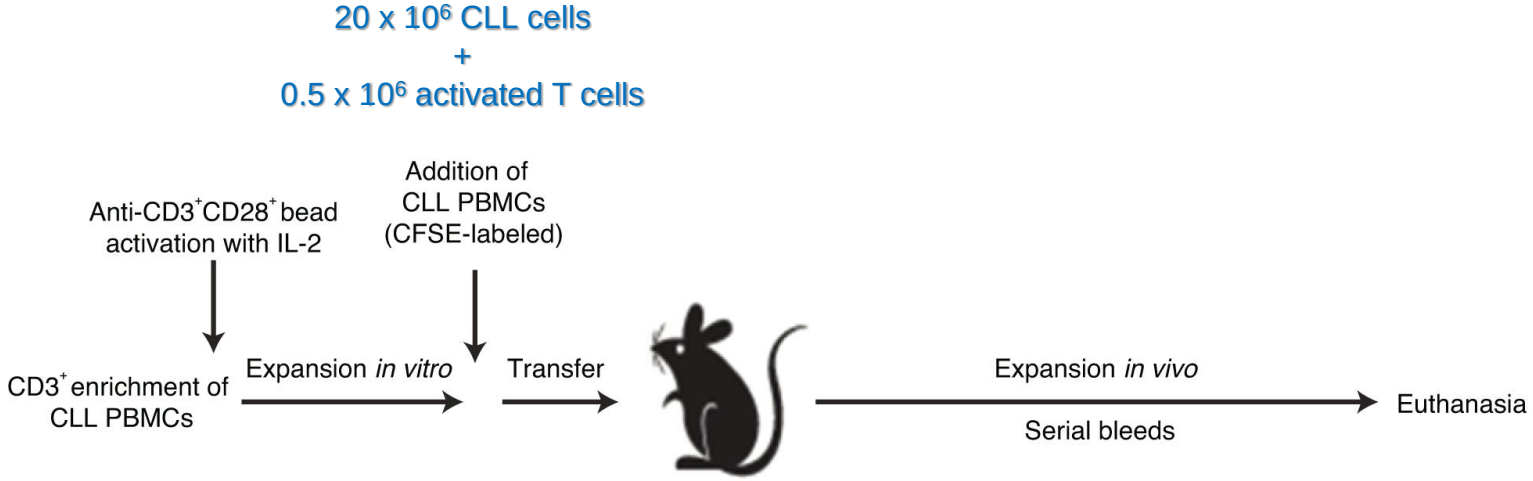


Growth of CLL cells in NSG mice is CD4-cell dependent



D Bagnara *et al*.
Blood 2011

Xenograft system employing CLL cells + activated T cells transferred into alymphoid mice



P Patten et al. JCI Insight 2016

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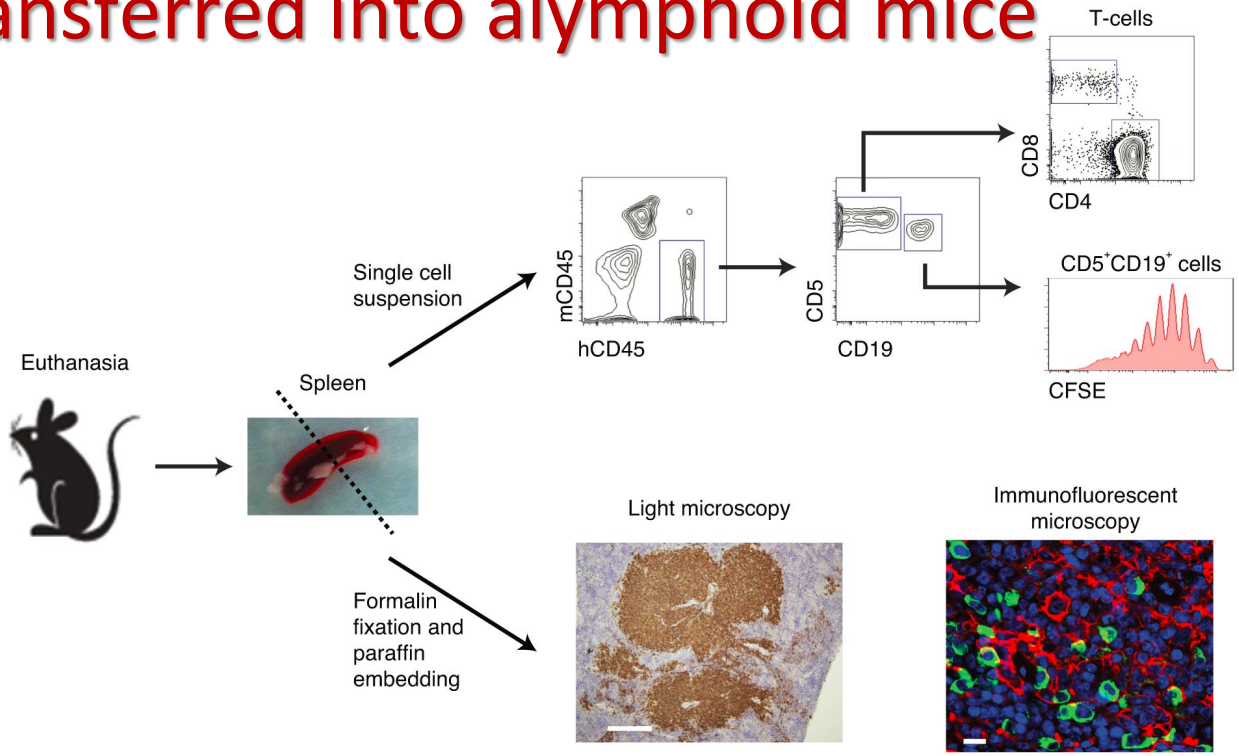
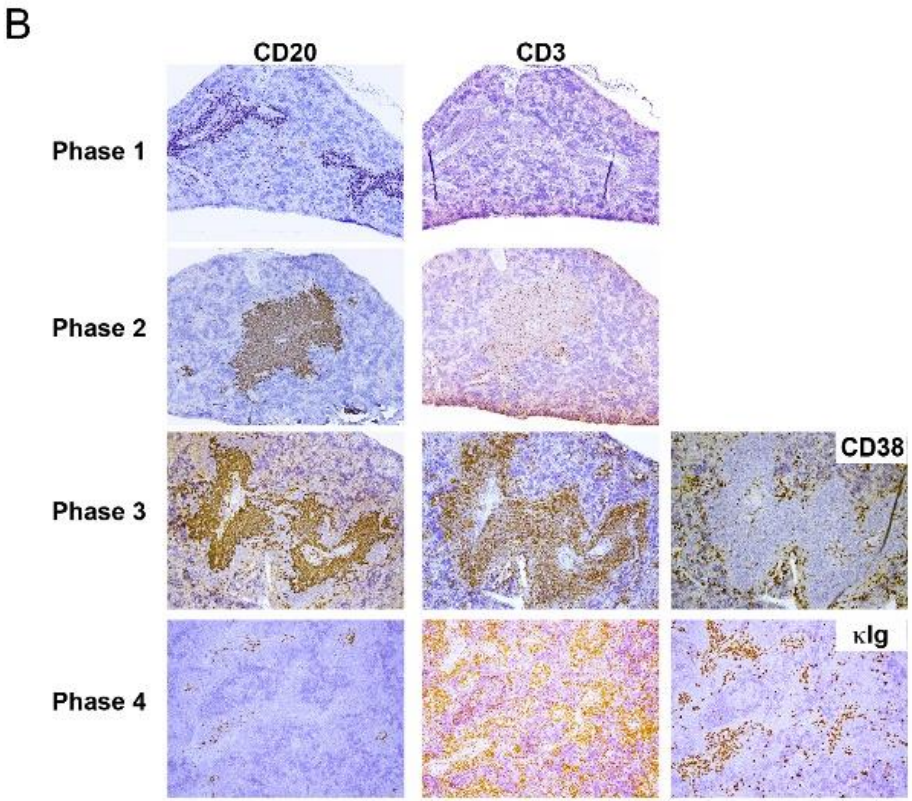
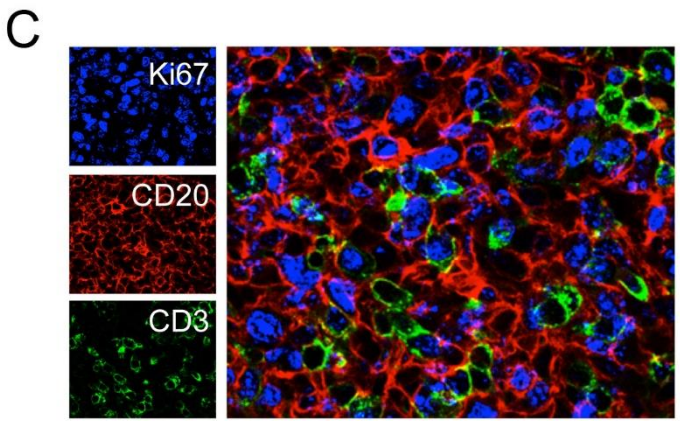


P Patten et al.
JCI Insight 2016

Phases of CLL B- and T-cell engraftment and growth





Advantages and disadvantages of this approach

Advantages:

- Simple and reproducible for a given sample
- Allows study of the role of T cells in CLL B cell growth *in vivo*
- Can serve as a model to study:
  - Clonal evolution in vivo (NJ Davies *et al*. Oncotarget 2017)
  - Requirements for and influences of other cells and cytokines on growth
  - Various novel therapies (SE Herman et al. Leukemia 2013)

Advantages and disadvantages of this approach

Disadvantages:

- CLL B cells are eventually lost due to:
  - Exhaustion since rapidly dividing
  - Differentiation to plasma cells (P Patten et al. JCI Insight 2016)
  - Overgrowth of Th1 cells (P Patten et al. JCI Insight 2016)
- Growth characteristics do not reflect biologic features of CLL patient cells in that a high percentage of CLL cells are dividing
- Does not reflect the anatomic differences seen in patients where most cell division occurs in secondary lymphoid tissue and much less at other sites

Conclusions (1)

CLL B cells appear to require “help’ from activated T cells to expand In an alymphoid microenvironment.

The T cells are T helper cells of the CD4 type, and make predominantly IFNγ.

This help is so “strong” that it induces CLL cell division in a large fraction of the cells that have taken up residence in the mouse spleen.

This system appears to replicate at higher level the actions taking Place in proliferation centers in lymph nodes of patients with CLL

Disadvantage: Most CLL B cells are dividing

- Only 0.1% - ~2-4% of a CLL clone divides daily
  (Messmer et al. J Clin Invest 2005)
- In CLL, most division occurs in lymph nodes
  (Herndon et al. Leukemia 2017)
- In this model, 15 – 40% of human CLL cells divide
  (Patten et al. JCI Insight 2016)
- Division is occurring in mouse spleen (which is analogous to a secondary lymphoid tissue)
  (Patten et al. xxxxx)

Advantages and disadvantages of this approach

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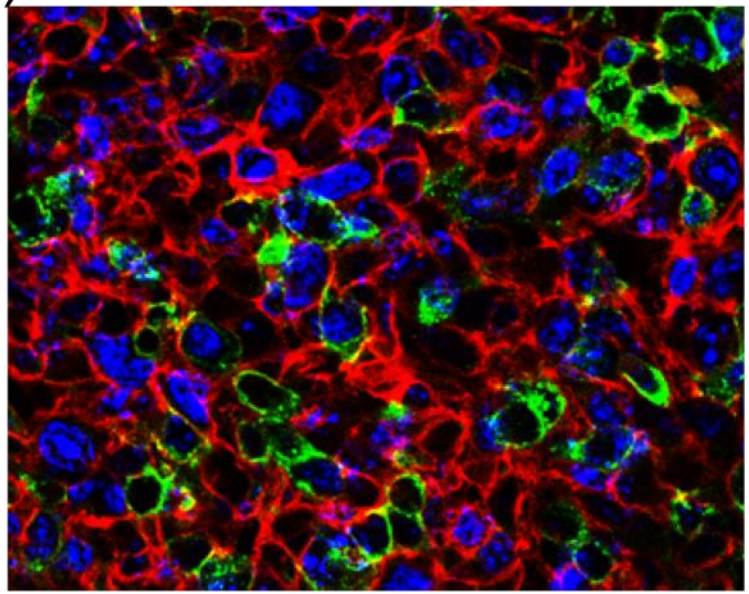
CLL cells grow differently at distinct anatomic sites

Intravenous injection

Day 28

CD20 Ki67 CD3



Spleen

Peritoneal cavity

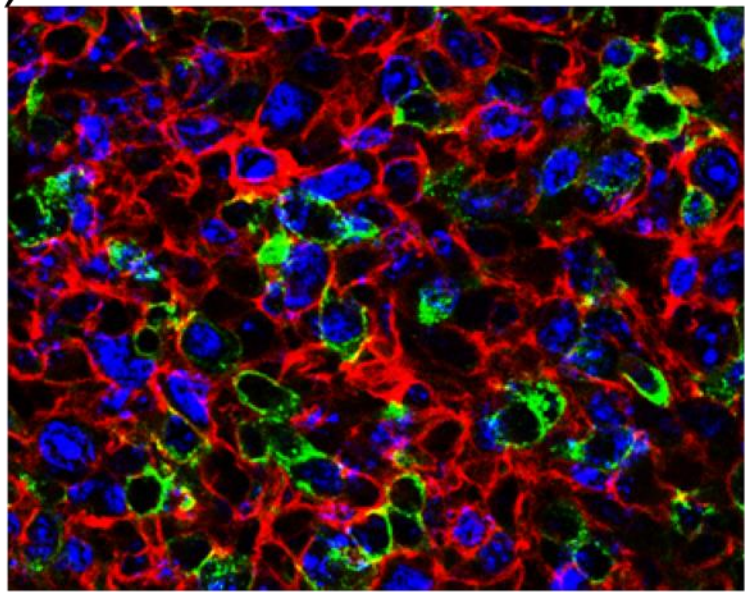
Virtually no cells

CLL cells grow differently at distinct anatomic sites

Intraperitoneal injection

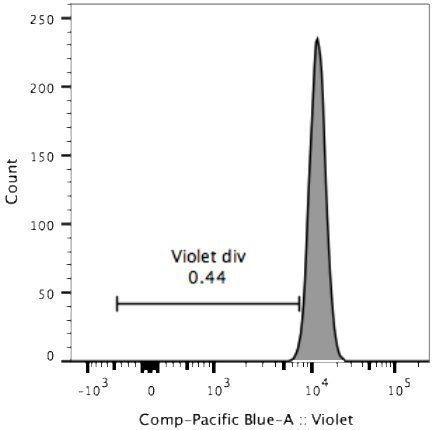
Day 28

CD20 Ki67 CD3

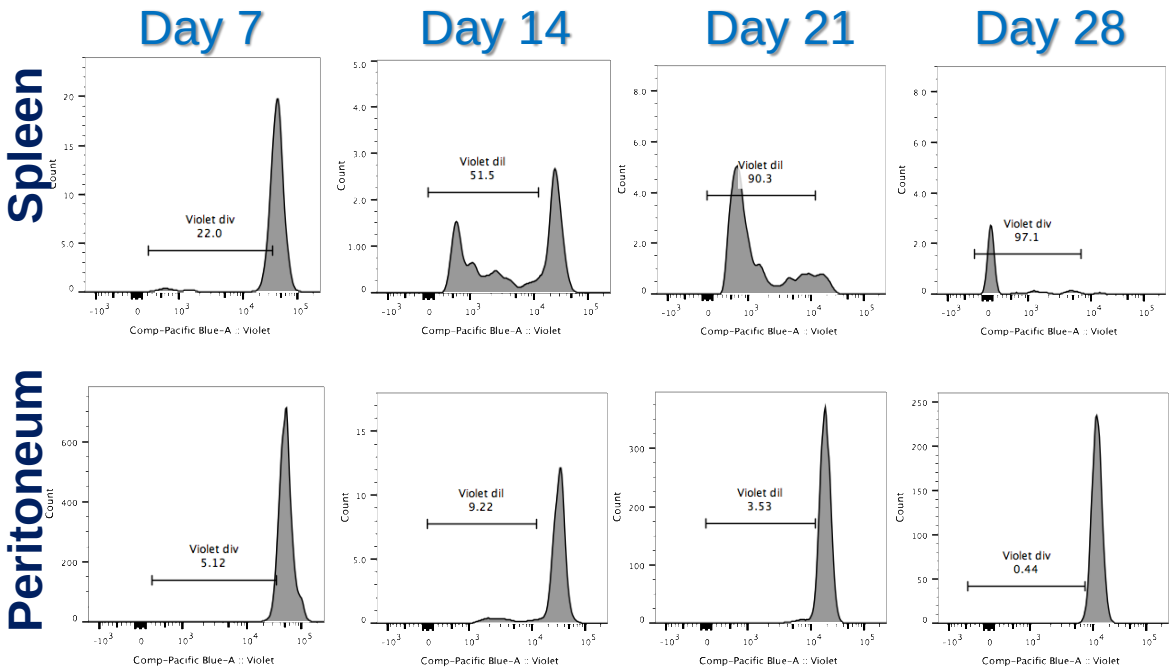


Spleen

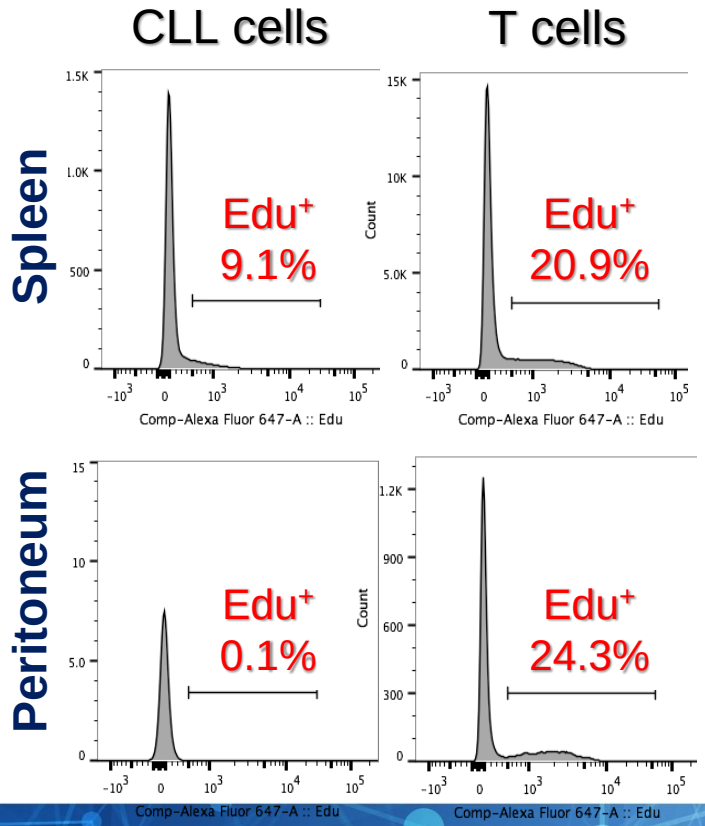
Peritoneal cavity



CLL cells divide differently at distinct anatomic sites



CLL cells do not divide in the peritoneum, even though T cells do



Conclusions (2)

There are very distinct signals being delivered to the CLL B cells depending on the anatomic site that the cells reside.

The spleen architecture/microenvironment supports CLL B cell as well as T cell growth.

The peritoneal cavity architecture/microenvironment does not support CLL B cell growth, although it allows T cell growth.

Is this analogous to leukemic cell division occurring much more extensively in the spleen than the bone marrow in CLL patients?

Advantages and disadvantages of this approach

Disadvantages:

- CLL B cells are eventually lost due to:
  - Exhaustion since rapidly dividing
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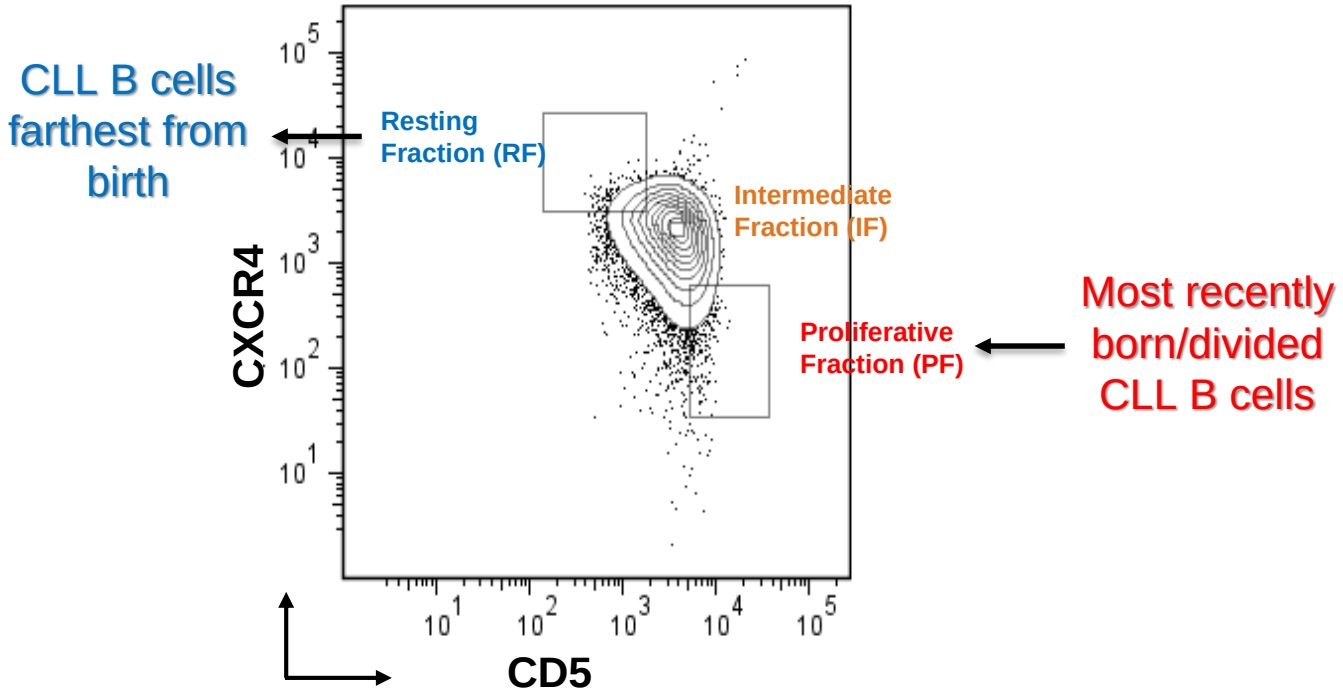
T helper (Th) subsets in CLL

1. Patients with CLL have more Th2 (IL-4 producing) cells than Th1 (IFNγ producing) cells
2. Th2/IL-4-producing cells are pro-tumor in CLL evidenced by:
   - IL-4 being a major survival signal for CLL cells (NE Kay et al. Leuk Lymphoma 2003)
   - treatment of CLL patients with IL-4 leading to disease progression (J Lundin et al. Br J Haematol 2001)
   - conversion of Th2 -> Th1 by ibrutinib being associated with disease regression (JA Dubovsky et al. Blood 2013)

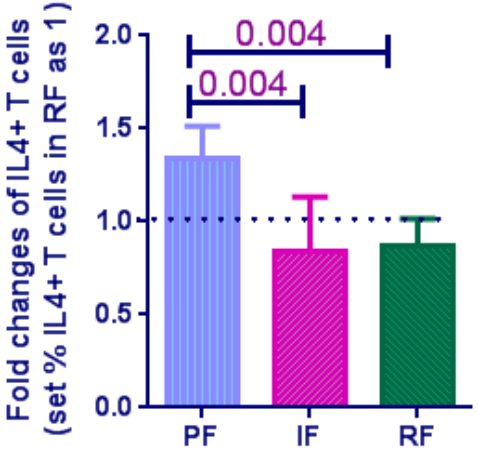
What CLL B - T cell interactions lead to Th2 cells

The most recently divided fraction of CLL cells can be identified by the reciprocal levels of CXCR4/CD5

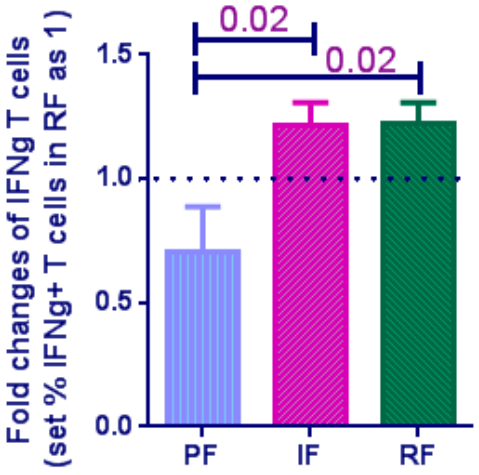


PF cells promote Th2 polarization *in vitro*

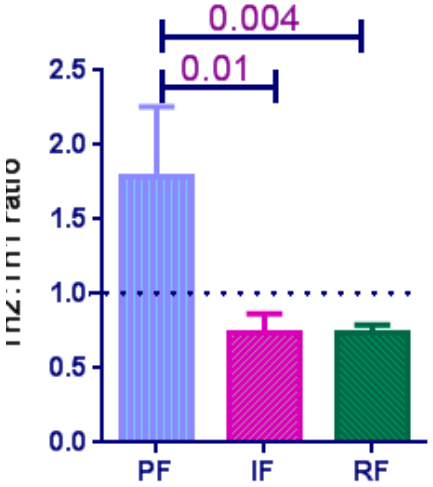
% IL-4$^+$ T cell



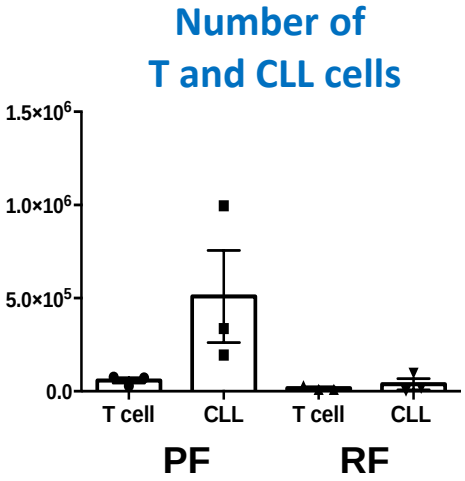
% IFNγ$^+$ T cell

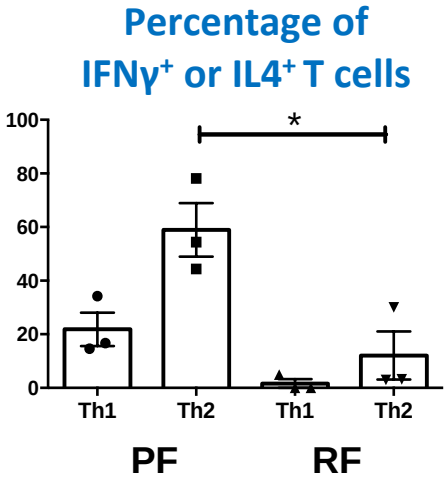


Th2:Th1 ratio

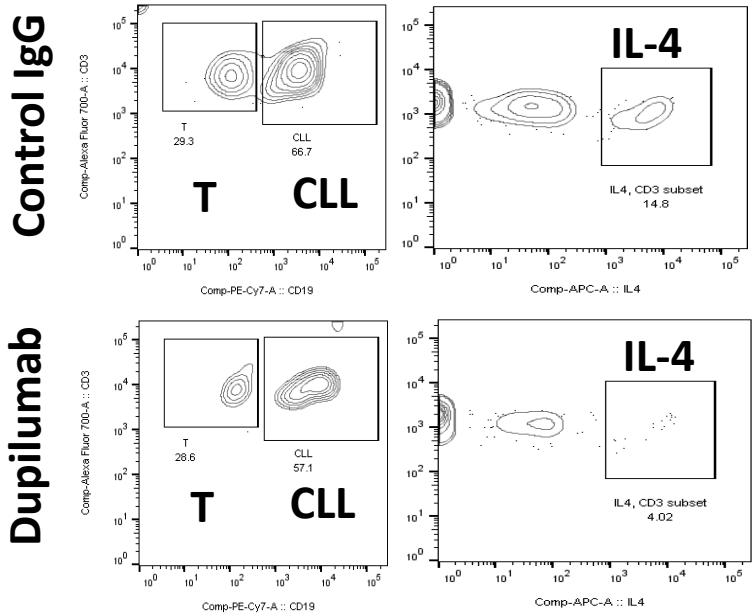


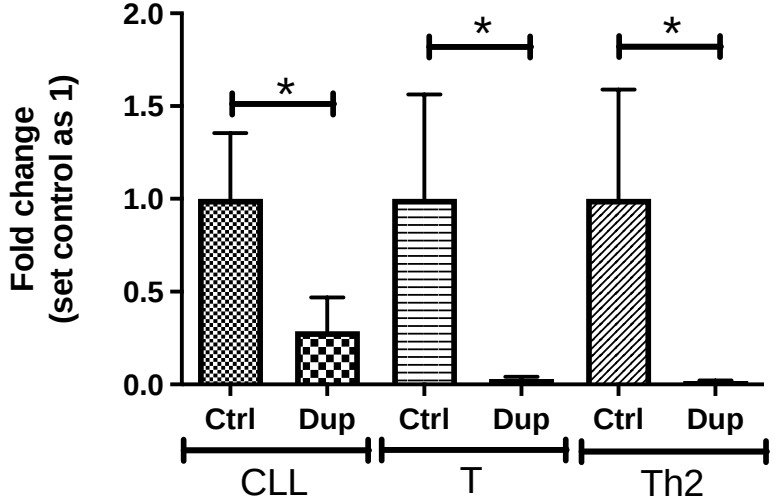
PF cells promote Th2 polarization, and these T cells, in turn, allow PF cells to grow in vivo





Blocking IL-4's actions as a novel therapeutic





Conclusions (3)

The recently divided fraction of CLL clones (PF) has the capacity to induce and expand IL-4-producing Th2 cells.

IL-4 then acts as a survival factor for CLL B cells, which preferentially Help the recently divided fraction (PF) to survive and expand. This Is a feed-forward action that allows the selected propagation of these Cells.

Interrupting this IL-4 axis with anti-IL-4R mAb might be a novel Therapeutic approach.

Acknowledgments

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