XVIII IWCLL

International Workshop on CLL

20-23 SEPTEMBER 2019 EDINBURGH

Linking the Microenvironment with CLL Models

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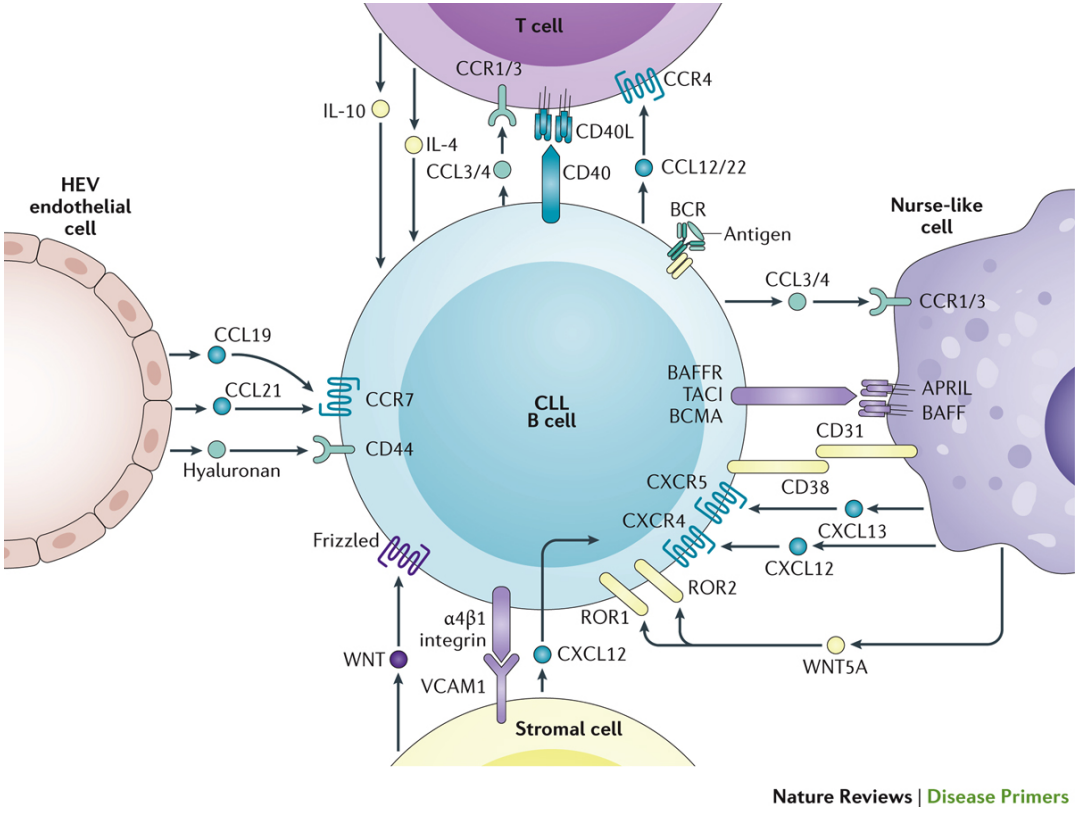
CLL B cell - microenvironment interactions



TJ Kipps *et al.* (2017) *Nat Rev Dis Primers* doi:10.1038/nrdp.2016.96

Acknowledgments

The Feinstein Institute

Davide Bagnara

Piers Patten

Shih-Shih Chen

Gerardo Ferrer

Rukhsana Aslam

Rita Simone

Joy Yan

Barbara A. Sherry

Northwell Health

Kanti R. Rai

Jacqueline C. Barrientos

Steven L. Allen

Jonathan E. Kolitz

Kings College London

Alan G. Ramsay

Nikolaos Ioannou

Why xenograft human CLL cells?

- CLL is a complex disease that appears to progress through multiple steps starting in some patients as early as hematopoietic stem cells and involving at least one compulsory pre-leukemic stage, MBL.
- Progression to more virulent and lethal disease involves development of more genetic abnormalities. However, the specific abnormalities that develop are not all currently known and are not the same for each person.
- Primary mouse models will only be complete disease mimics when all of these factors are precisely defined and will need to be tailored to subsets of patients based on these specific abnormalities.

SS Chen, N Chiorazzi Semin Hematol. 2014

Advantages and disadvantages

- Advantages:
  - Can use an individual patient's leukemic B cells that contain specific genetic and epigenetic differences unique to that patient.
  - Can transfer other non-leukemic cells from the same patient that could be responsible, directly or indirectly, for biologic actions of the leukemic cells. These actions may also be diverse, either supporting or inhibiting the leukemia and its evolution, depending on the different types and numbers of non-leukemic cells contained in the innocula.
- Disadvantage:
  - Although these parameters might more accurately reflect the biologic milieu that CLL cells find themselves, they add considerably more intricacy, complexity, and heterogeneity to the experimental system.

Factors important for xenografting human CLL cells

- These relate to the **recipient/host animal** and to the **donor cells** to be transferred into the host.
- **For the recipient animal:**
  - Histocompatibility barriers and responsiveness of the recipient and of the donor to these genetic differences
  - Ability of the recipient's tissue microenvironment to provide supportive "factors" that foster survival and expansion of the donor cells.
- **For the donor cells:**
  - Types and numbers of cells available
  - Extent that the cells need to reside and grow in the same anatomic site at which they dwelled in the donor
  - Abilities of the donor cells to respond to the cues provided by the recipient in those anatomic areas.

Recipient/host considerations

- **Histocompatibility barriers:**
  - Xenoantigens that define the phylogenetic hierarchy
  - Recognition and response of human T cells to murine xenoantigens is not robust and in our hands GvH rarely develops when only one human sample is transferred into various strains of alymphoid mice.

Recipient/host considerations

- **Recipient's response to xenogeneic (human) differences:**
  - Combining spontaneous and introduced mutations in key genes affecting the development of lymphoid cells (T, B, and NK), several hosts for human xenografts are currently available and are relatively easy to use.

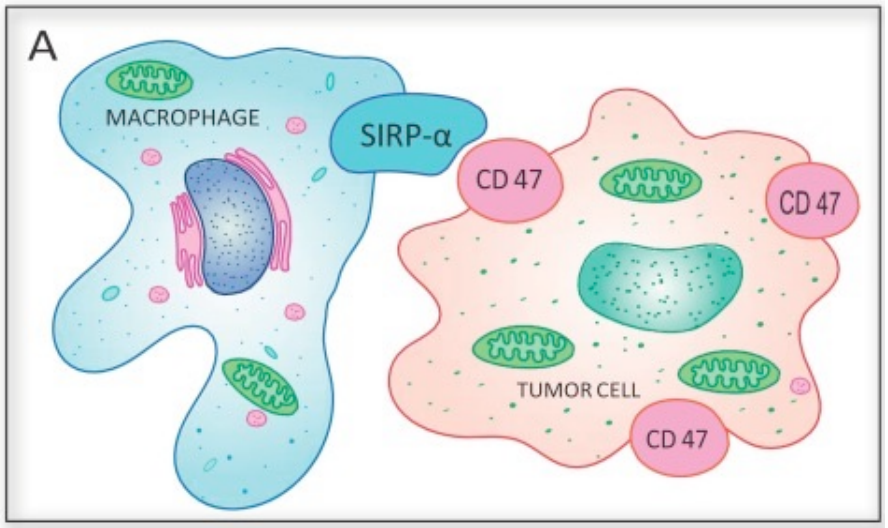
    Readily available strains are

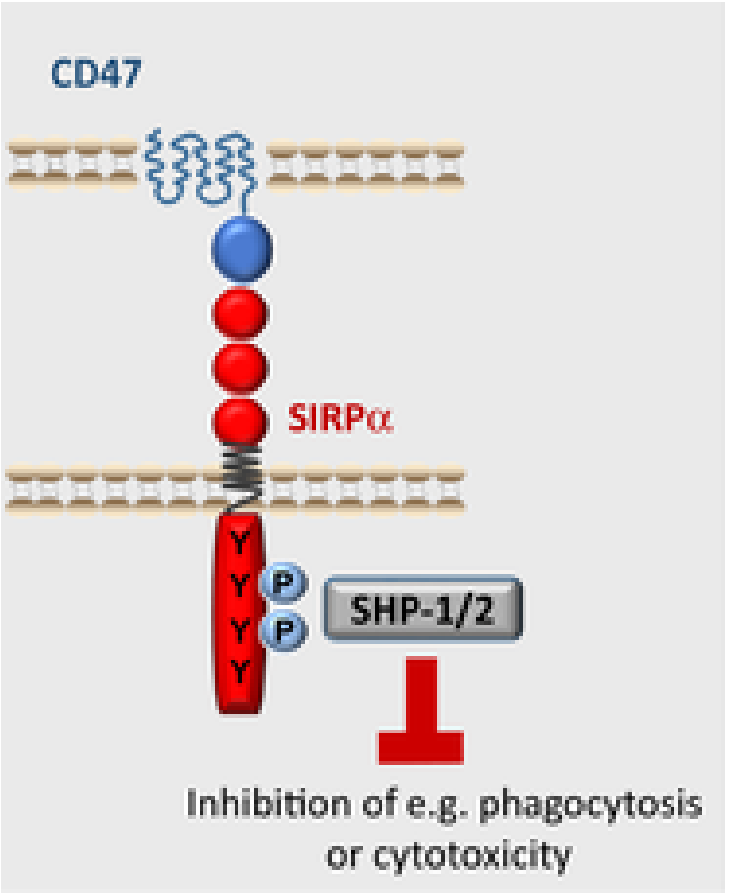
    1. NOD/Shi-SCID IL2Rc$\gamma^{-/-}$ (NSG or NOG)
    2. BALB/c-RAG2$^{-/-}$IL2Rcγ^{-}

Recipient/host considerations

- **Recipient's response to human cells through "eat me/don't eat me" signals**
  - Myelomonocytic lineage cells can recognize, engulf, and eliminate xenogeneic (and allogeneic) cells based on the similarity in structure of specific ligand-receptor pairs. A key pair is to be the SIRPα-CD47 dyad that is involved in blocking phagocytic function via a "don't eat me" signal.
  - Specifically, strains of mice exhibit polymorphic structural differences in the SIRPα gene, and by using murine strains whose SIRPα gene more closely resemble that of humans, animals with a reduced ability to recognize and "EAT" human cells have been created. This then improves the number of donor cells that survive the translocation across the blood vessel wall on their way to solid lymphoid tissues.

“Don’t eat me” signal delivered by CD47 – SIRPα interaction





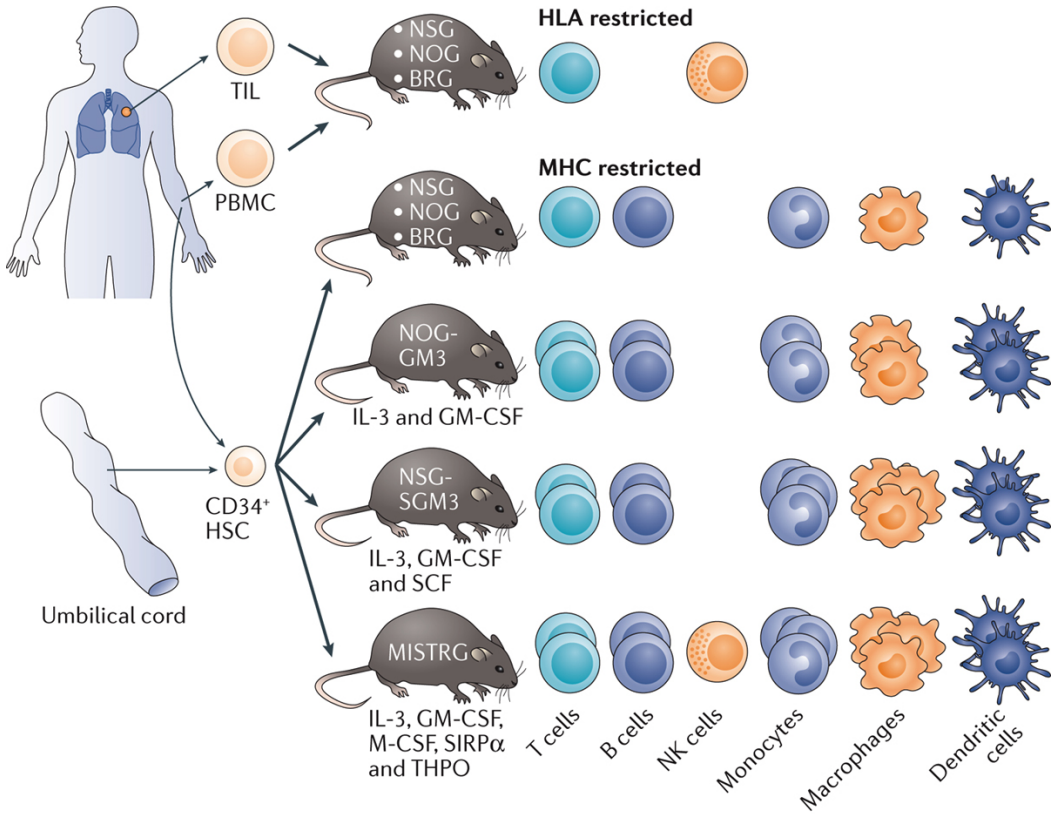
Recipient/host considerations

- Of the three readily available alymphoid strains mentioned previously, the NOD/Shi-SCID IL2Rc$\gamma^{-/-}$ (NSG or NOG) mouse is more resistant to “eating” because there is a greater structural similarity between CD47 and SIRPα.

- The BALB/c-RAG2$^{-/-}$IL2Rcγ^{-} strain has a less avid CD47 – SIRPα interaction ans therefore has a greater loss of cells at transfer.

- This can be / has been overcome by increasing the affinity of the CD47 – SIRPα interaction, e.g., MISTRG mice

Alymphoid strains and their derivatives available for xenografting studies



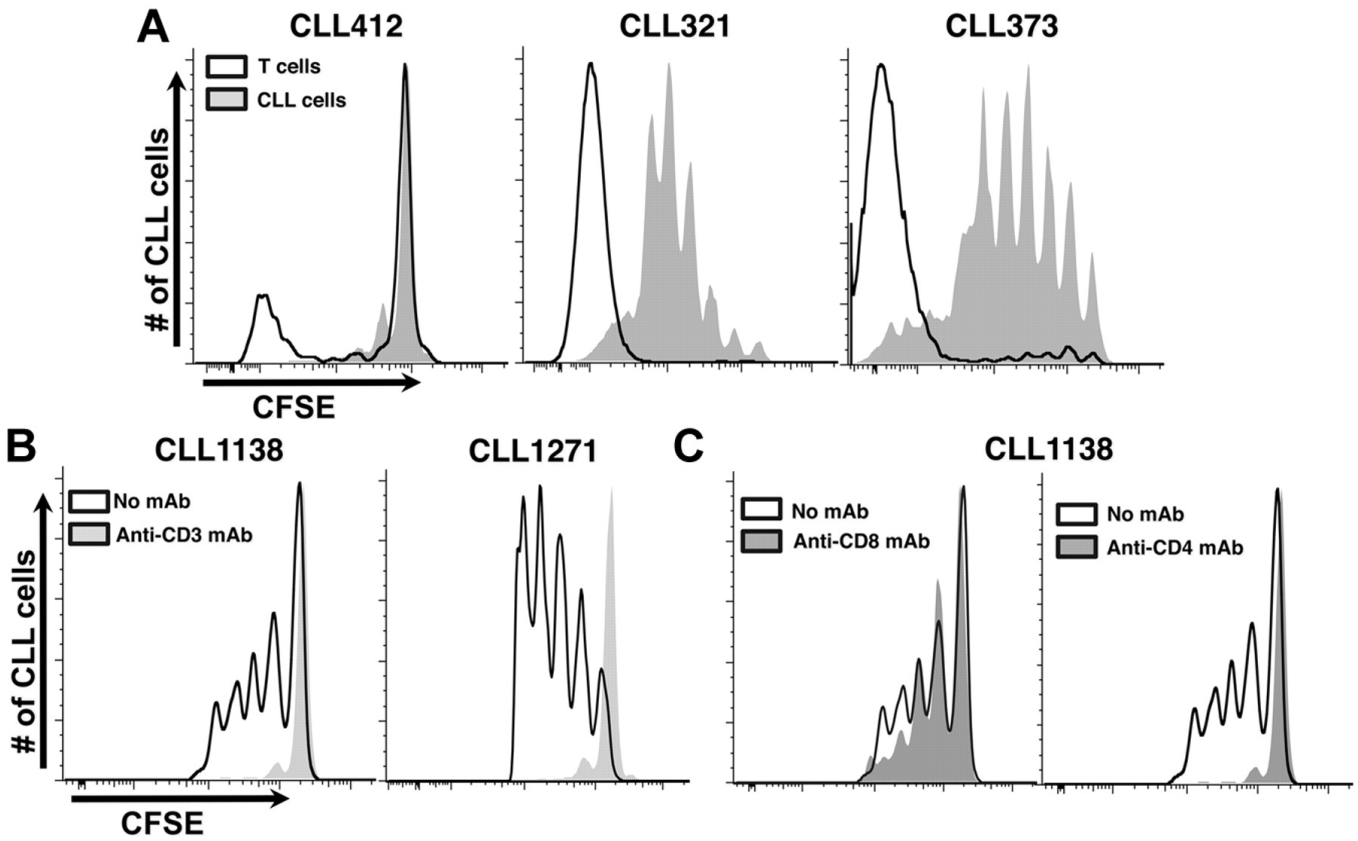
Outcome dependent on state of maturation of the transferred cells and their ability to interact with the murine microenvironment

AT Byrne et al. Nat Rev Cancer. 2017 Sep 15;17(10):632. doi: 10.1038/nrc.2017.85.

Transfer of mature CLL cells into alymphoid mice

- A model using CLL PBMCs and cells from the PBMC fraction in NSG mice

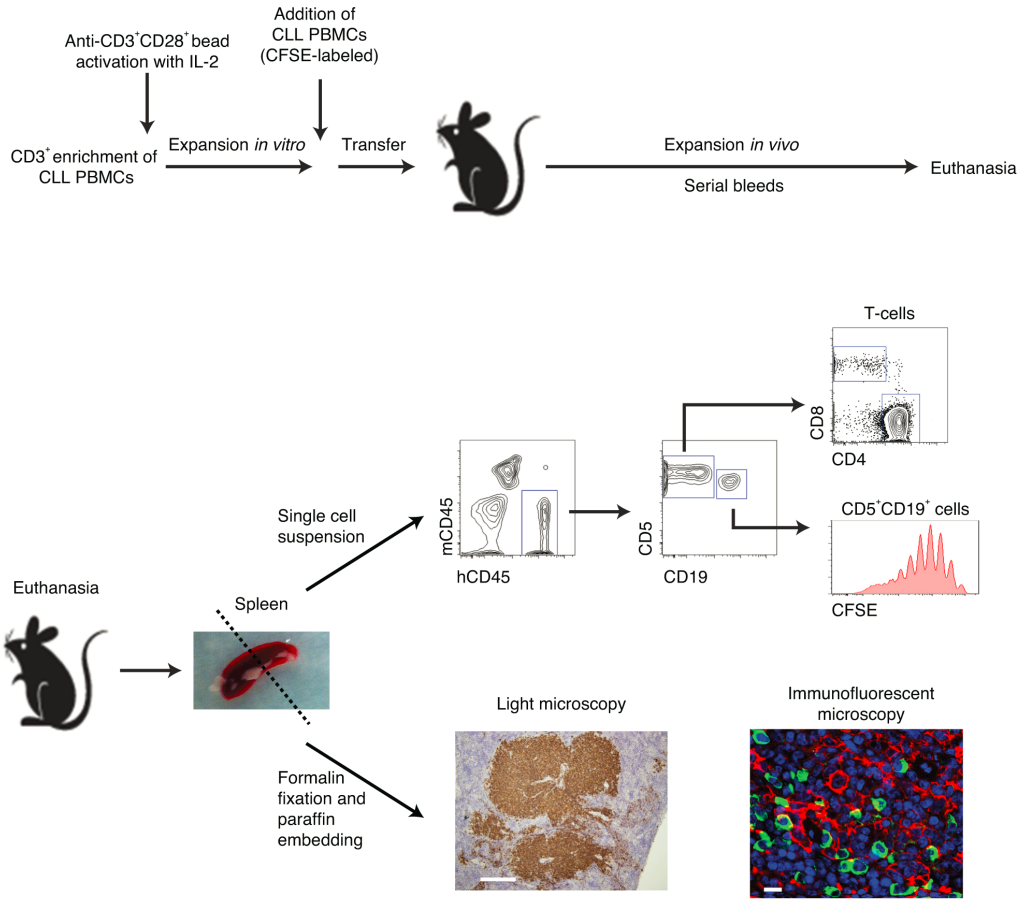
Growth of CLL cells in NSG mice is $CD4^+$ T-cell dependent



D Bagnara *et al*. Blood. 2011 May 19;117(20):5463-72. doi: 10.1182/blood-2010-12-324210.

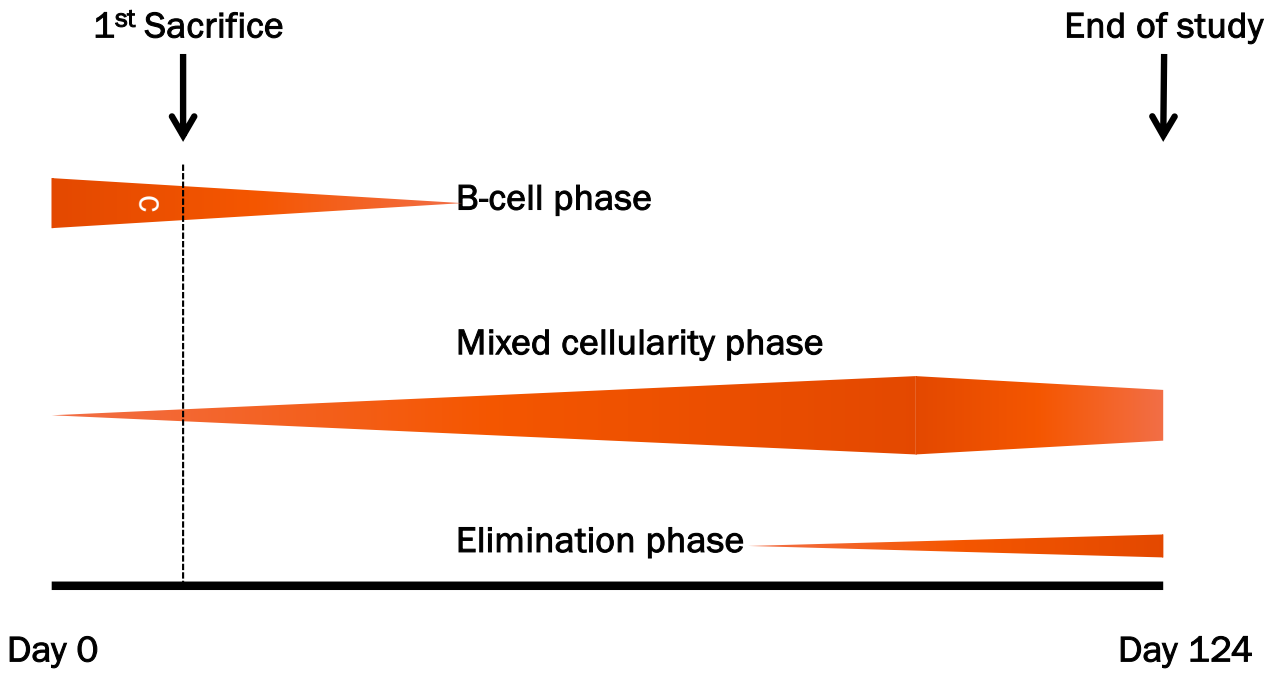
CLL-derived xenograft system employing NSG mice activated autologous T cells and primary CLL cells

PEM Patten *et al.*
Poster # 2031



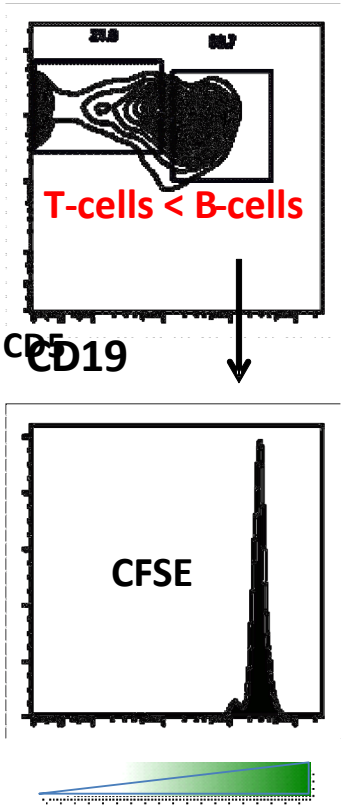
P Patten JCI Insight 2016

Different phases of CLL patient-derived xenografts

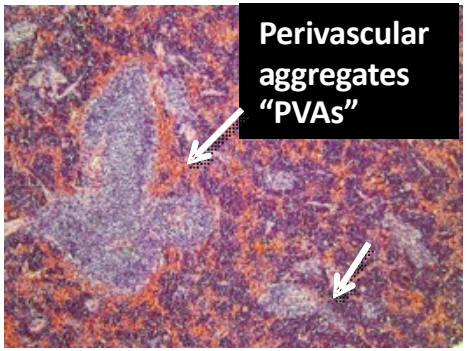


“B-cell phase”

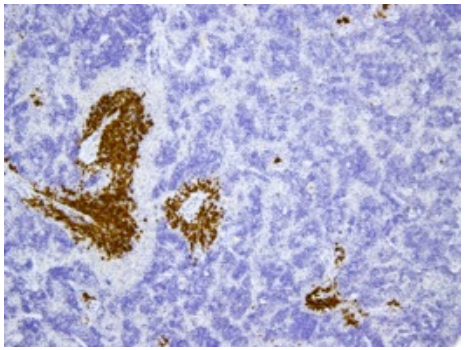
Flow Cytometry: Spleen



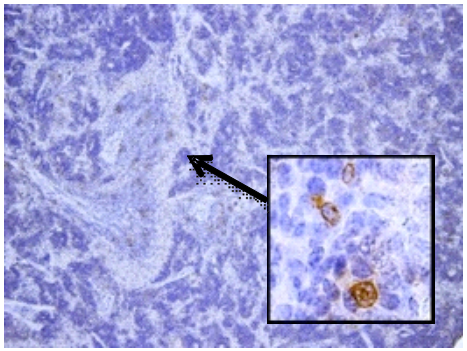
Splenic Histology: x10 images



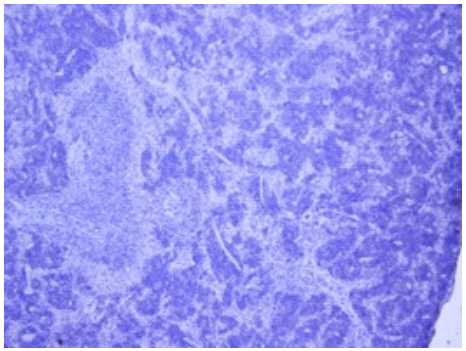
H&E



hCD20



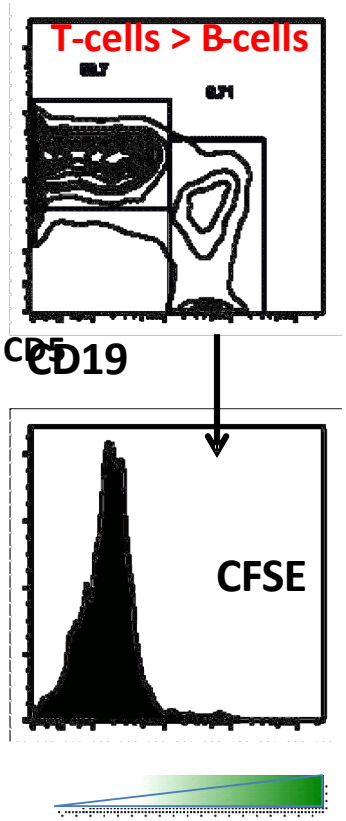
hCD3



hCD38

“Mixed cellularity phase”

Flow Cytometry: Spleen



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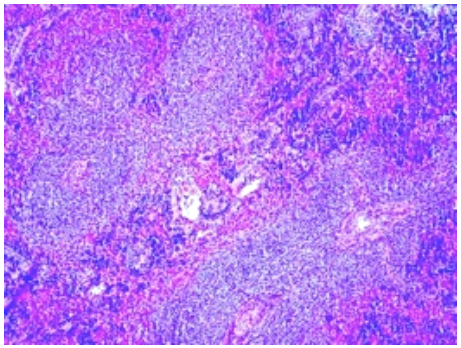

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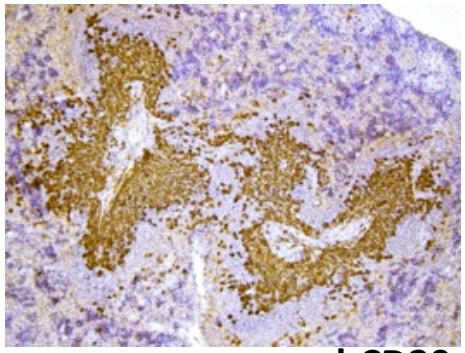

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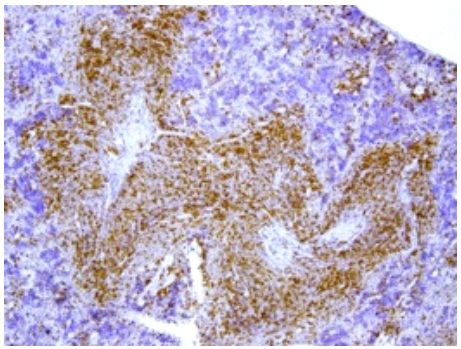

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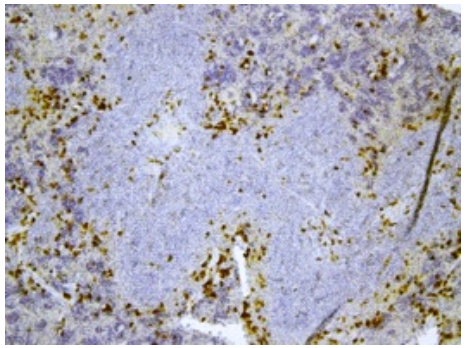

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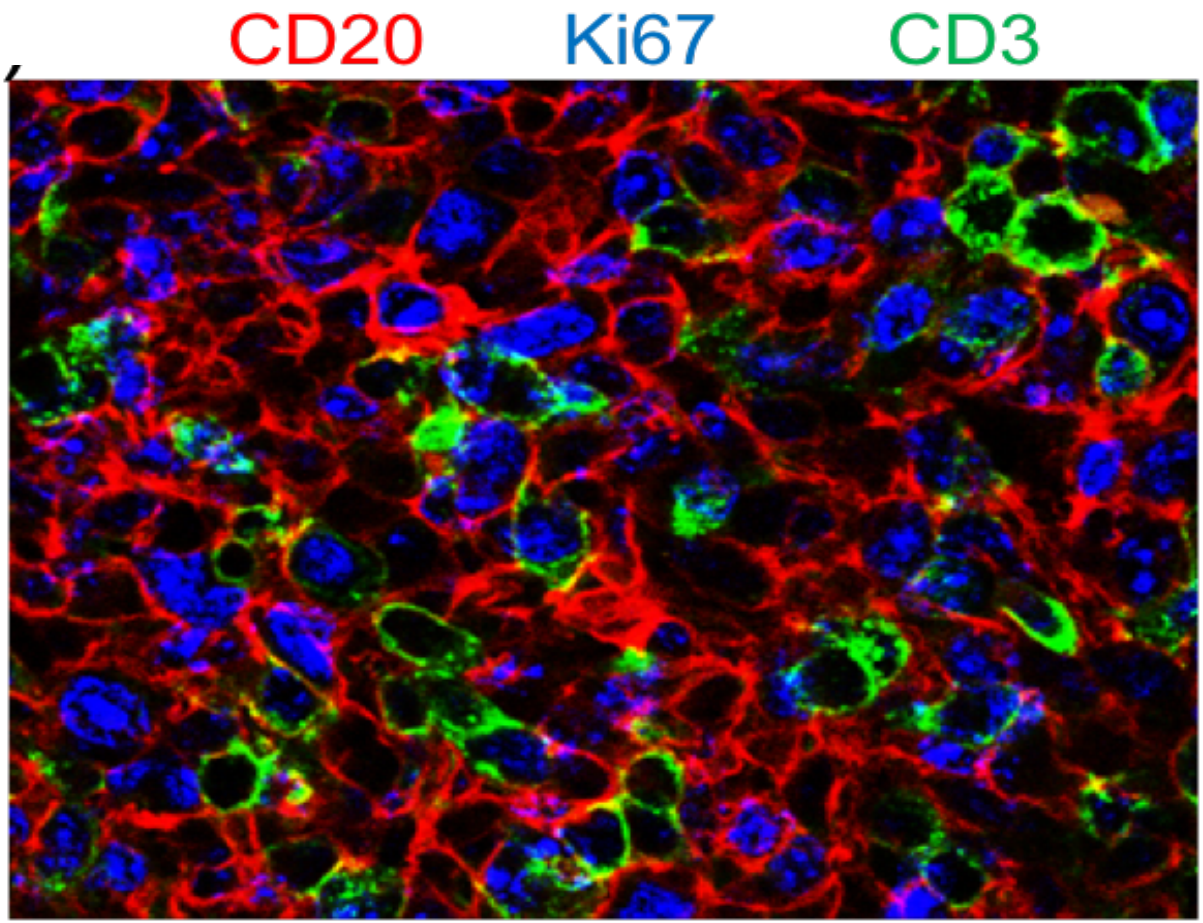
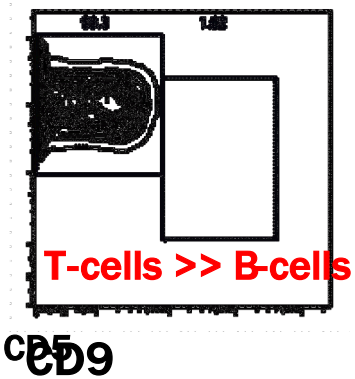

CD20
Ki67
CD3

“Elimination phase”

Flow Cytometry: Spleen



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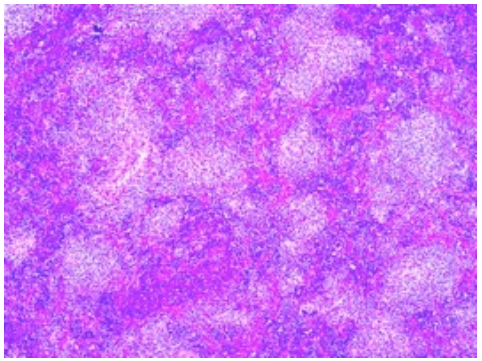

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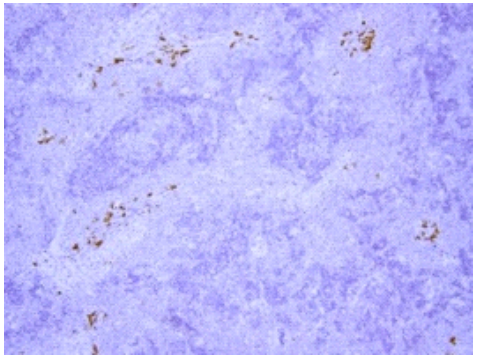

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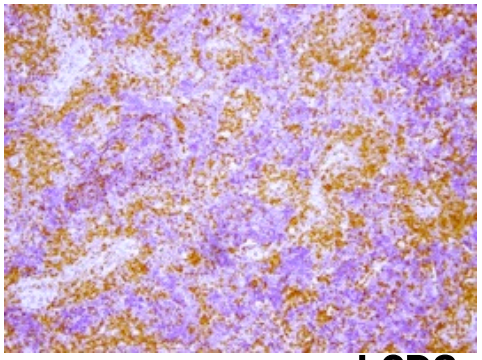

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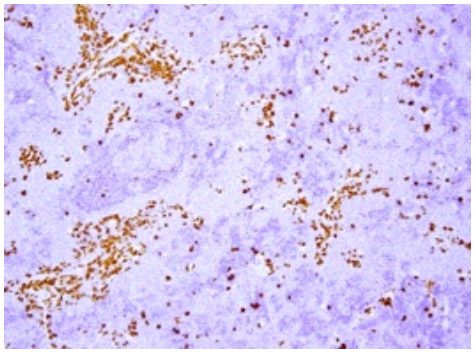
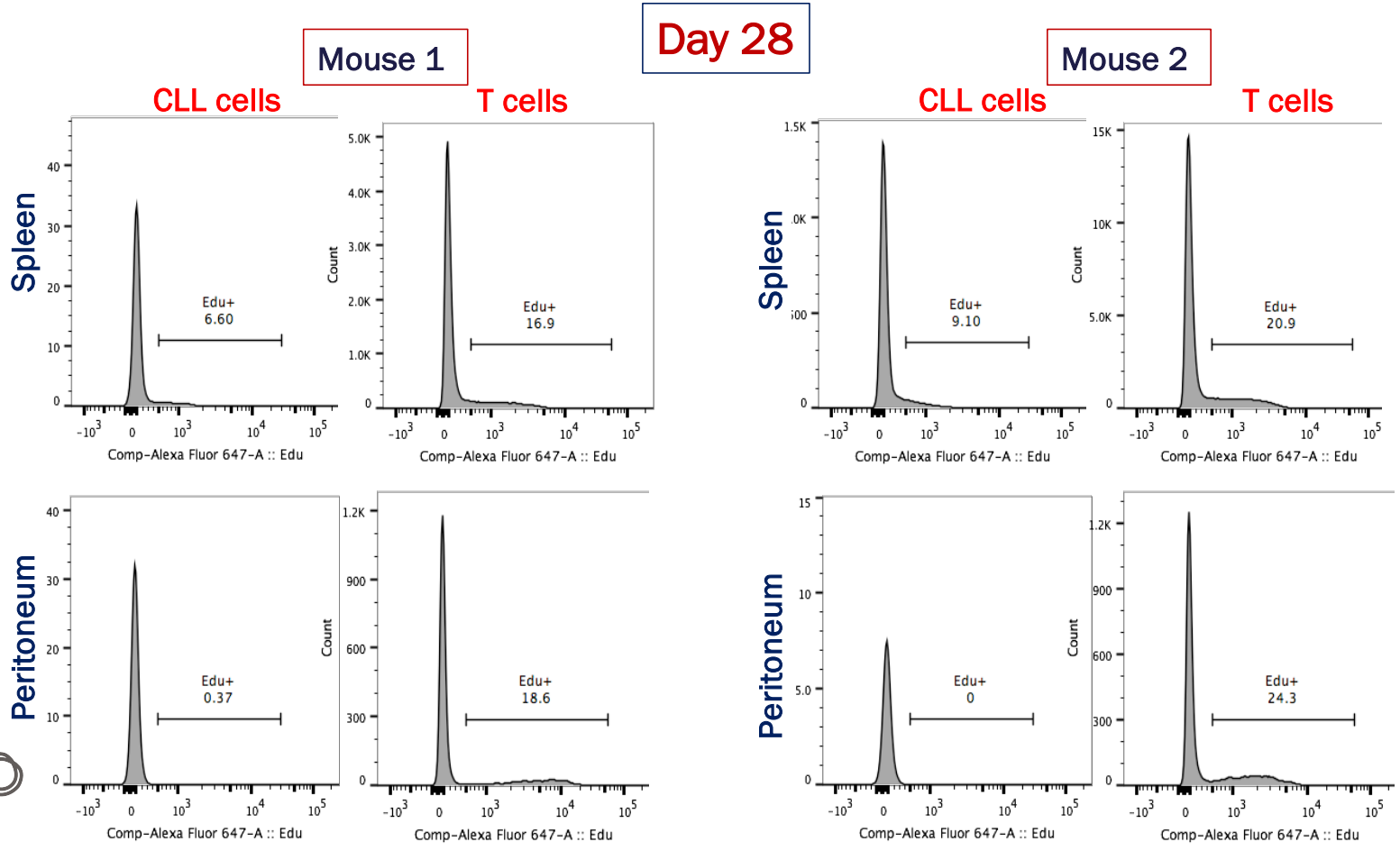


Ig k chains

CLL B cells residing in the peritoneum do not divide appreciably despite the presence of activated T cells

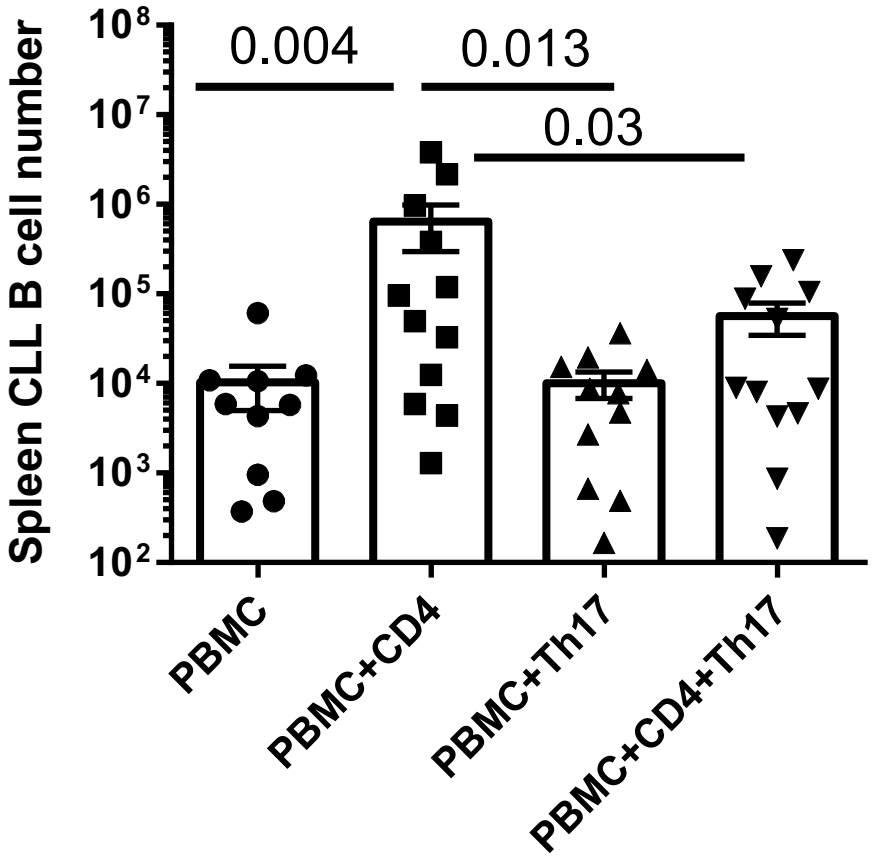


Disadvantage

- T cells that evolve are primarily Th1 (IFNγ-secretors).

- Can other distinct Th cells subsets be added to the mix, if wanted?

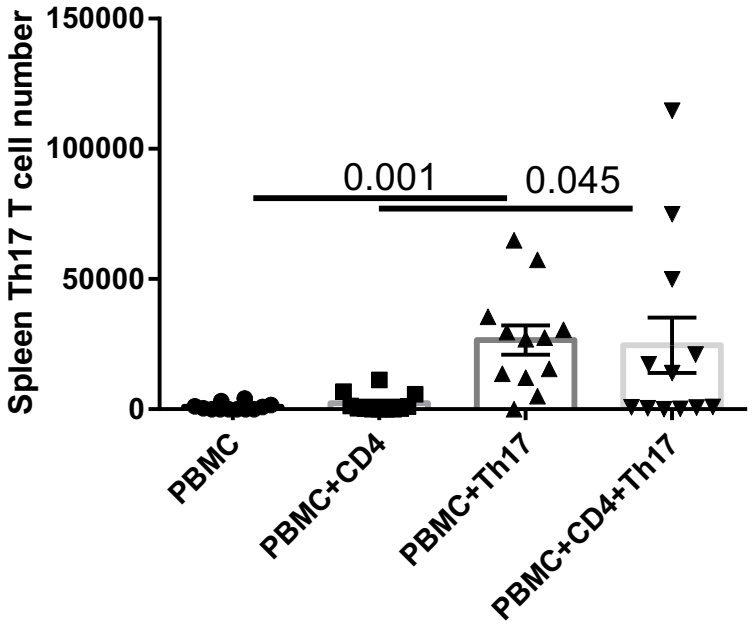
Defined Th cell subsets (Th17) can engraft and function in the xenograft setting

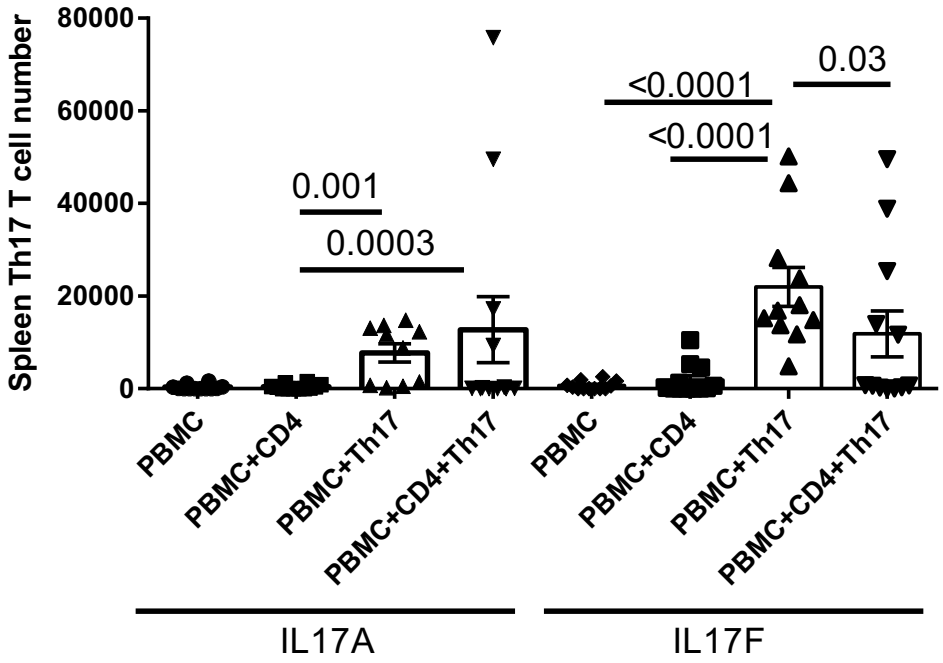


CD4 cells: $CD4^+$ - Th17 depleted

Th17 cells: $CD4^+$ - Th17 enriched

Defined Th cell subset (Th17) maintain phenotype and expand in the xenograft setting





Disadvantage

- Mature human myeloid cells do not engraft well into alymphoid mice

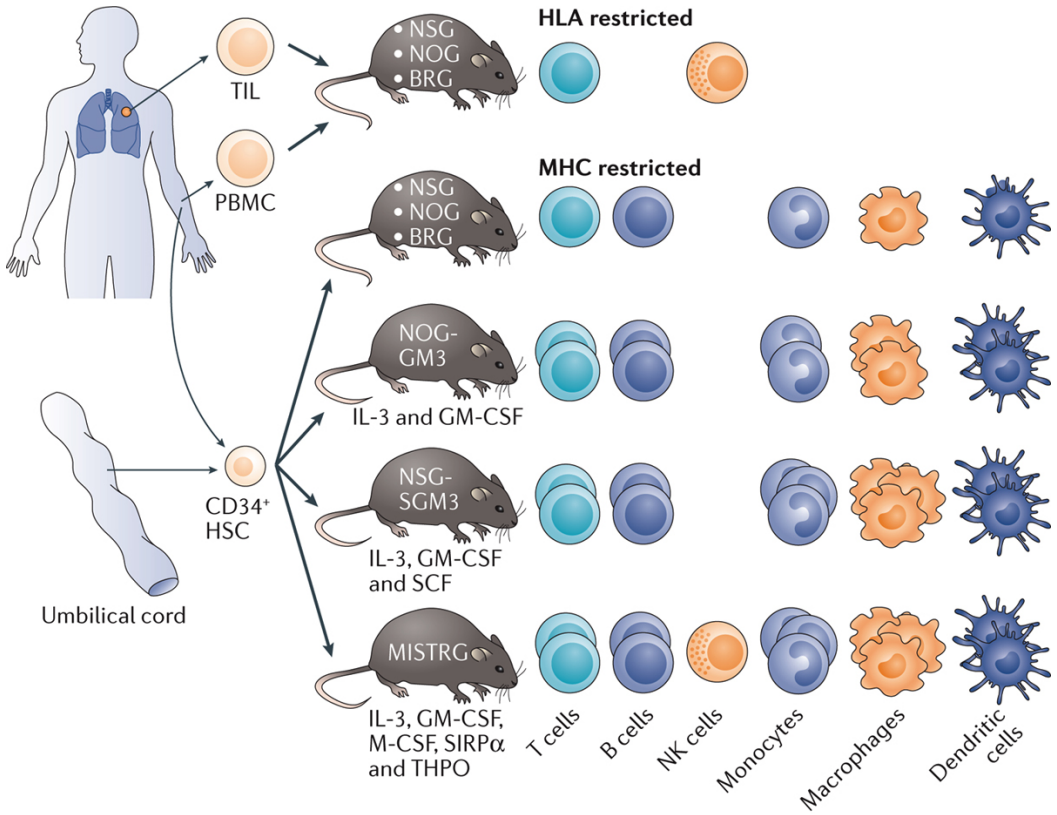
And

- NSG and NOG are reported to have a functionally reduced myeloid compartment

Production of human myelomonocytic cells in NSG mice

- NSG-SGM3 mice are engineered to produce human IL-3, GM-CSF and SCF/KIT ligand.
- Transfer of $CD34^+$ cells allows the development of a humanized hematopoietic system and the maturation of human myeloid cells.

Alymphoid strains and their derivatives available for xenografting studies



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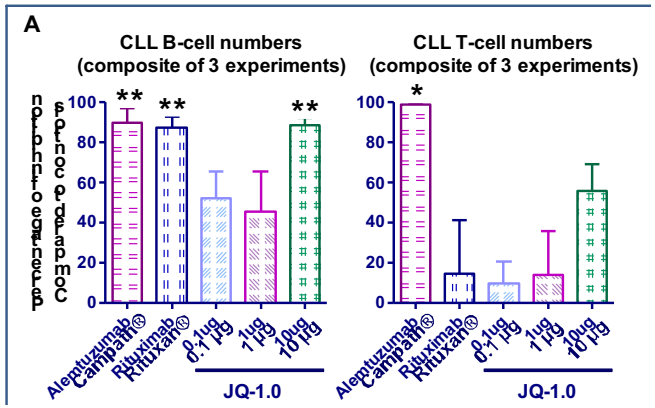
Use of NSG-SGM3 mice to demonstrate selected cytotoxicity of (CD52xCD20) biAb for B cells while sparing T cells and myeloid cells

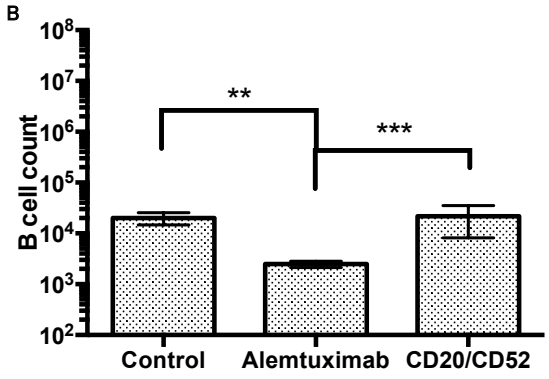
Using cells from CLL peripheral blood



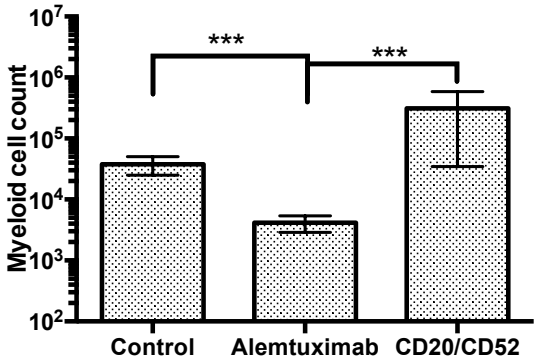
CLL B cells T cells

(J Qi et al. Methods 2019)

Using normal CD34+ cells from cord blood



CLL B cells



Myeloid cells

Conclusions

- Mature human CLL cells will engraft and grow in alymphoid mice if activated T cells are present.
- If cells are injected IP then one can study CLL cells that different in proliferation at two distinct microenvironments (spleen and peritoneum)
- Specific T-cell subsets can be transferred and these maintoian phenotype and are functionally active.
- Using NSG-SGM3 mice, human CD34+ cells differentiation in vivo and reconstitute a myeloid compartment that is amenable to study

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