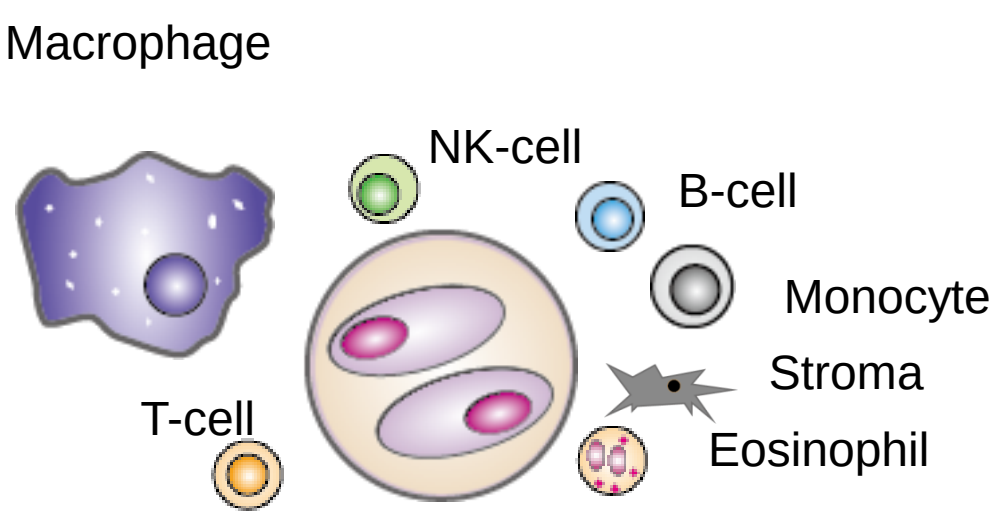
Immunogy Q&A session

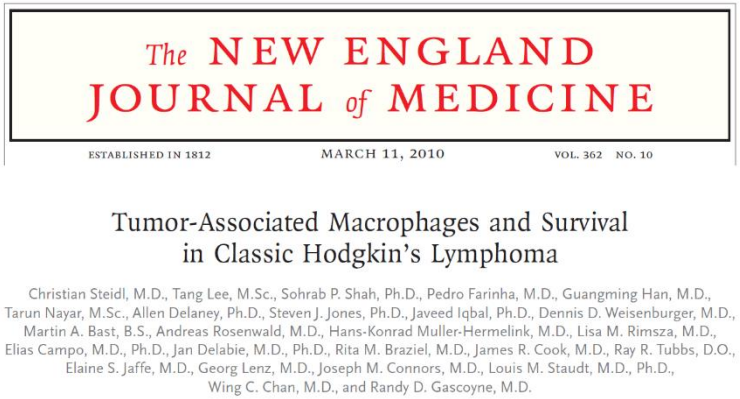
Tomohiro Aoki, Feb 8, 2021

Background

- The Tumor-microenvironment (TME) is composed mainly of various types of immune cells, including T cells, B cells, natural killer (NK) cells, and macrophages; stromal elements such as fibroblasts and vasculature are also present, but they are currently not as comprehensively studied.

- Many studies have consistently demonstrated that the extent and composition of the immune cells in the TME are associated with clinical outcome using standard therapies but also with therapies integrating novel immunotherapeutic approaches raising hope for effective development of predictive biomarkers.

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Tumor-Associated Macrophages and Survival in Classic Hodgkin's Lymphoma

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Background

- Current pathogenesis models postulate that the immune cells in the TME are educated by signals from lymphoma cells including cytokines/chemokines and by altered expression of cell surface molecules.

- These changes are linked in part to genetic alterations that contribute to the development of lymphoma-specific cellular ecosystems that allow the escape of the malignant cells from the host immune system.

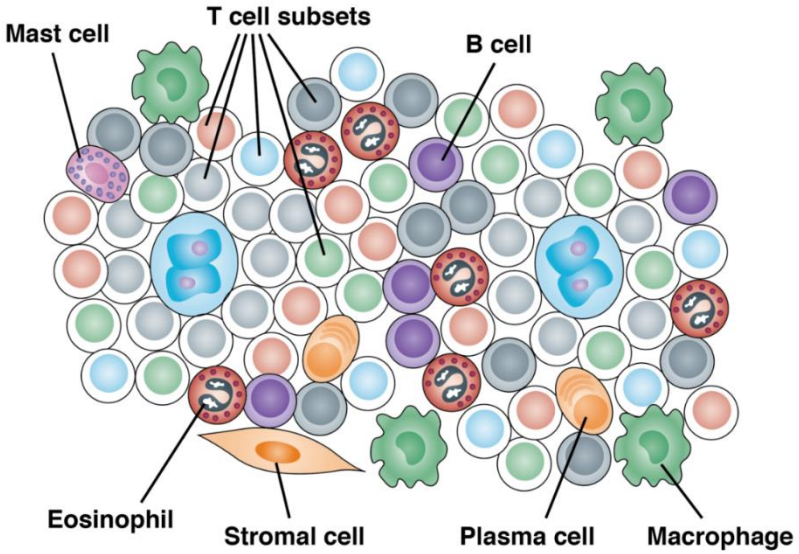
- This “acquired immune privilege” is best exemplified in Hodgkin lymphoma (HL), where an extensively predominant TME component is recruited by rare (approximately 1%) clonal malignant Hodgkin and Reed- Sternberg (HRS) cells.

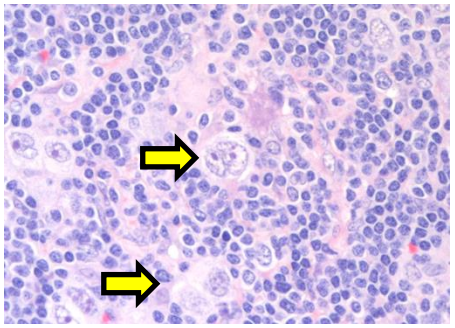
- The most abundant cells in the TME of both HL and non-Hodgkin lymphoma (NHL) are T cells, highlighting the importance of malignant B-cell crosstalk with T cells and macrophages/dendritic cells in the context of tumor-derived neoantigen presentation by major histocompatibility complex (MHC).

- In particular, this crosstalk can elicit immune checkpoint inhibitory responses, cellular interactions can be viewed as important **therapeutic targets**.

Background

- Malignant HRS cells represent a very rare population within the tumor bulk of Hodgkin lymphoma tumors
- Regardless of lack of B cell receptor (BCR) expression and of numerous other B-cell markers, HRS cells are thought to be mainly derived from GCB cell because of IgH/V gene rearrangement
- HRS cells are difficult to isolate from whole tumors, so have not been well characterized including their heterogeneity and gene alteration that could explain loss of B cell gene expression (no published WGS data)





Heterogeneous?

Scott and Gascoyne *Nat Rev Cancer* 2014

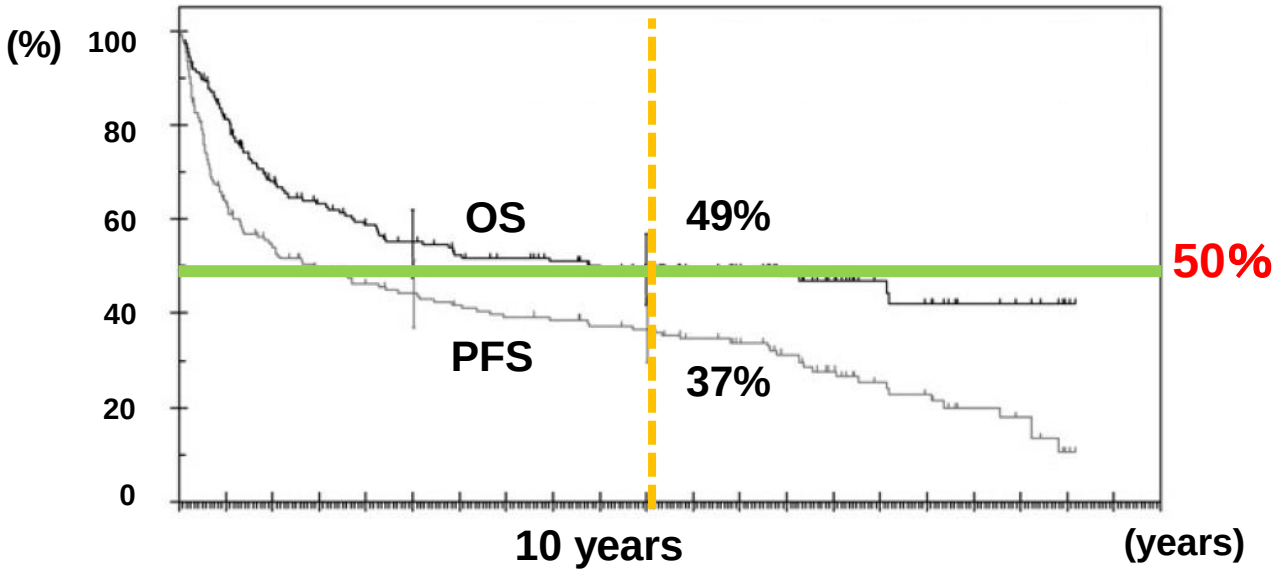
Prognosis of cHL

- Advances in the management of cHL have led to achieve high cure rates exceeding 70%

- About 30% of cHL patients suffer from refractory disease and a significant proportion of these patients eventually succumb to their disease.

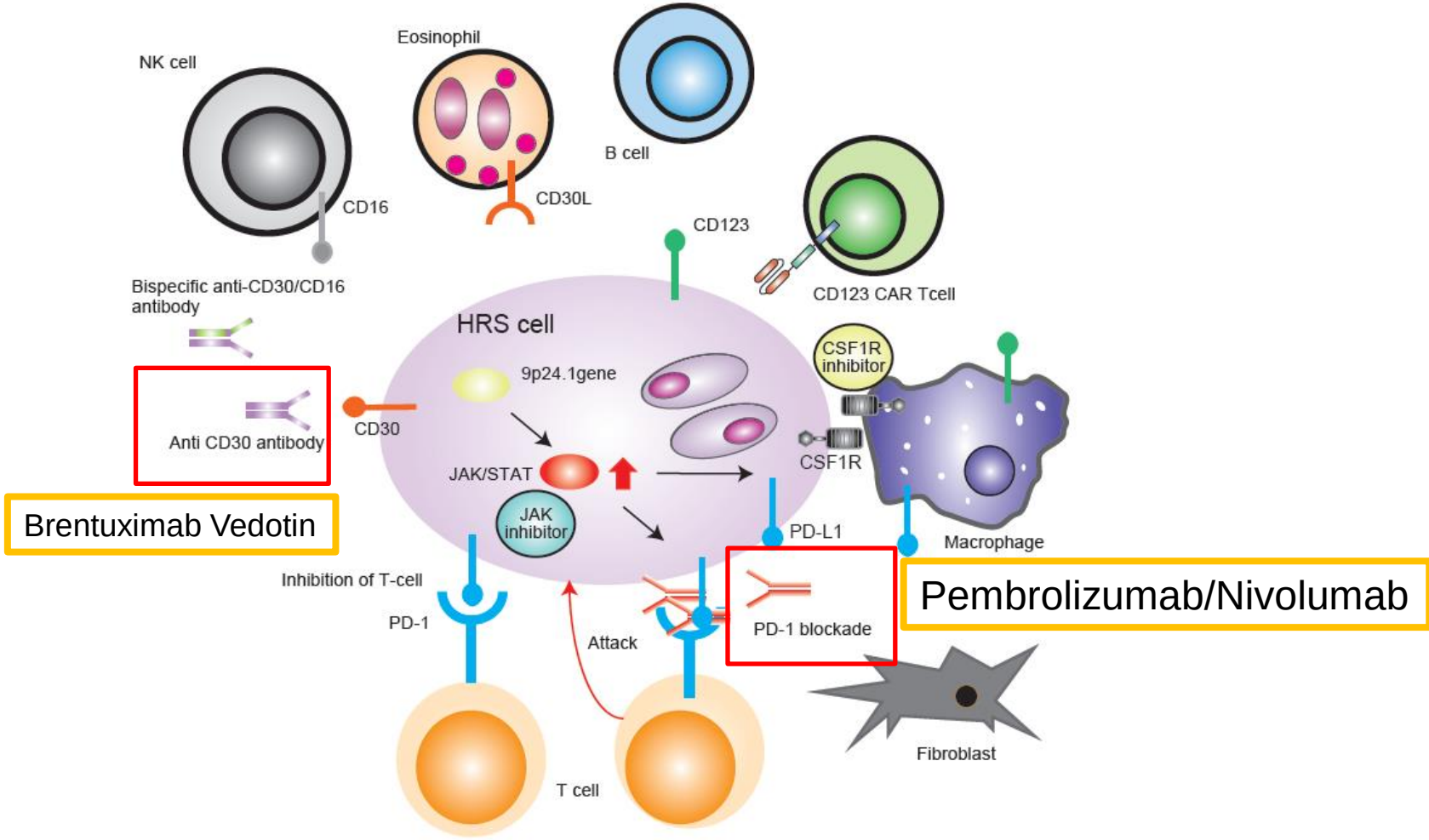
- Immune checkpoint inhibitors, such as the programmed death 1 (PD-1) inhibitors nivolumab and pembrolizumab, have shown dramatic efficacy in relapsed or refractory cHL with an overall response rate (ORR) of 65-87%

Long-term outcome after auto-HSCT in relapse/refractory cHL



Connors JM. Blood 2015; 125(11): 1693-702.
Annals of Oncology, 2008; 19: 1312–1319.

Cellular interactions and treatment targets in cHL



Interactions between HRS cells and numerous nonmalignant immune cells in the TME are shown. The complex cross-talk provides immune privilege and growth advantage to the HRS cells. Multiple pathways and proteins are targetable in cHL.

Cluster of differentiation (CD)

Question Are the co-expression patterns of the cluster of differential(CD) antigens important in influencing the proportion of TME? When two or more CD antigens are present on the same cell type, are there any interactions between them?

What is CD?

The CD is a protocol used for the identification and investigation of cell surface molecules providing targets for immunophenotyping of cells.

CD3 T cell
CD19 B cell
CD68 Macrophage
CD56 NK cell
CD30 HRS cell

In terms of physiology, CD molecules can act in numerous ways, often acting as receptors or ligands.

CD279 PD1 (receptor)
CD274 PD-L1 (ligand)

Answer: Yes for both, but has not been fully understood

Science

RESEARCH ARTICLES

Cite as: D. Sugiura *et al.*, *Science*
10.1126/science.aav7062 (2019).

Restriction of PD-1 function by *cis*-PD-L1/CD80 interactions is required for optimal T cell responses

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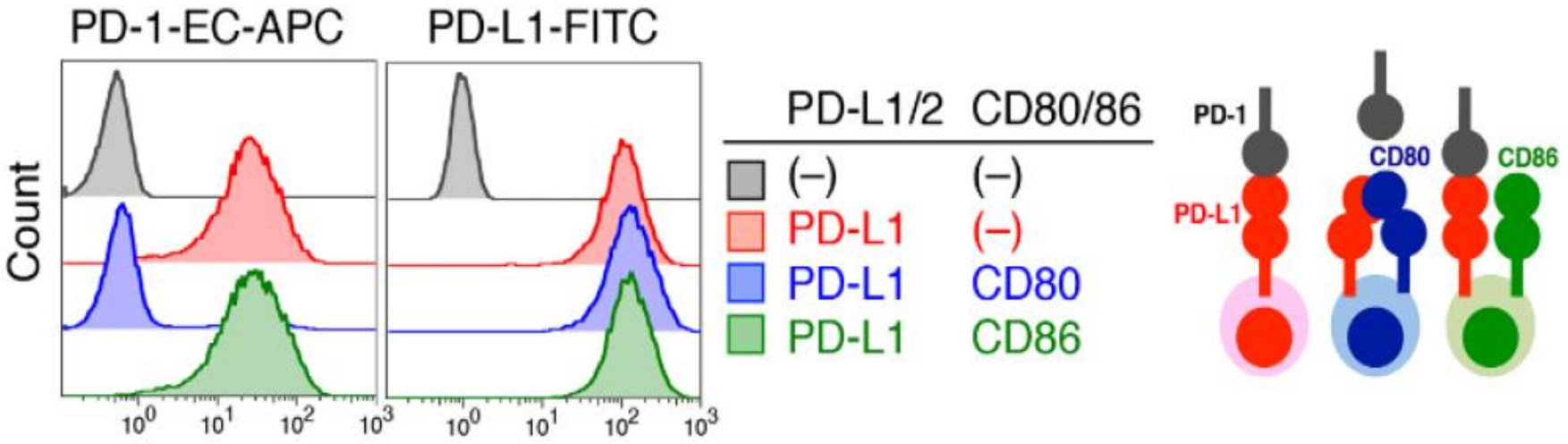


CD80 interacts with PD-L1 (CD279) in cis on antigen presenting cells (APCs) to disrupt PD-L1/PD-1 binding.

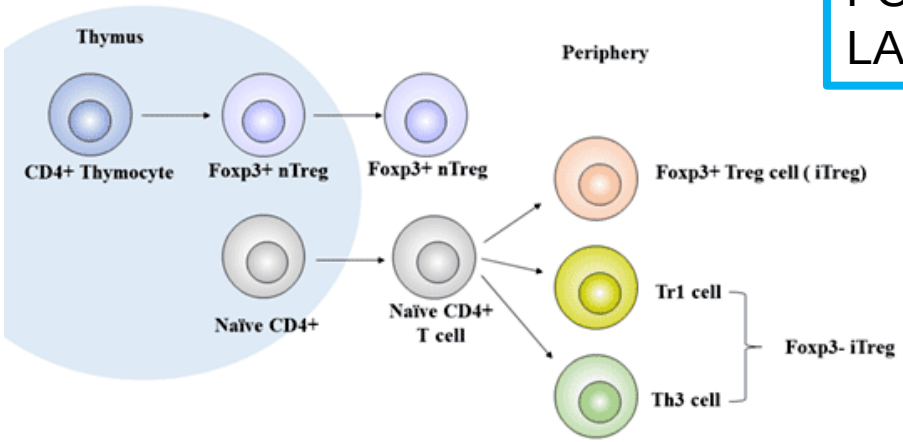
Subsequently, PD-L1 cannot engage PD-1 to inhibit T cell activation when APCs express substantial amounts of CD80.

T reg differentiation

Question How do LAG3+ and FOXP3+ Tregs differ in terms of functionality and origin?

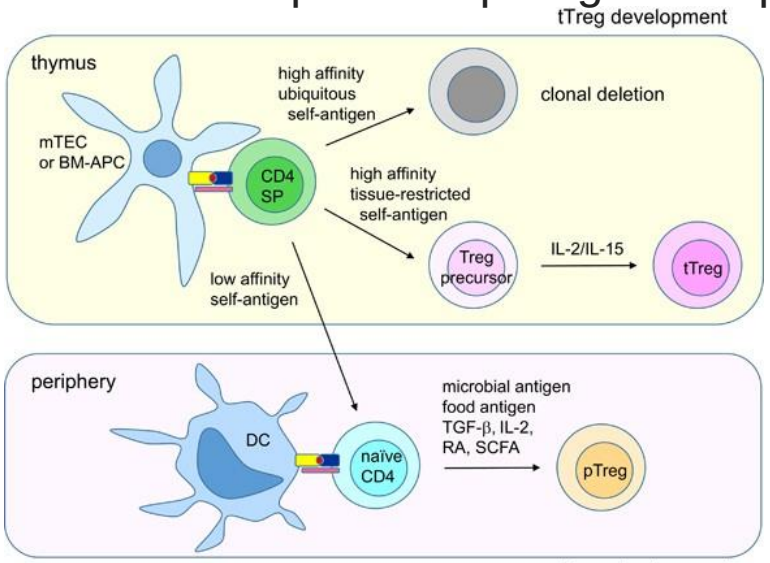
FOXP3: tTreg
LAG3:p(i)Treg

Both imuunosuppressive
LAG3+ could be more

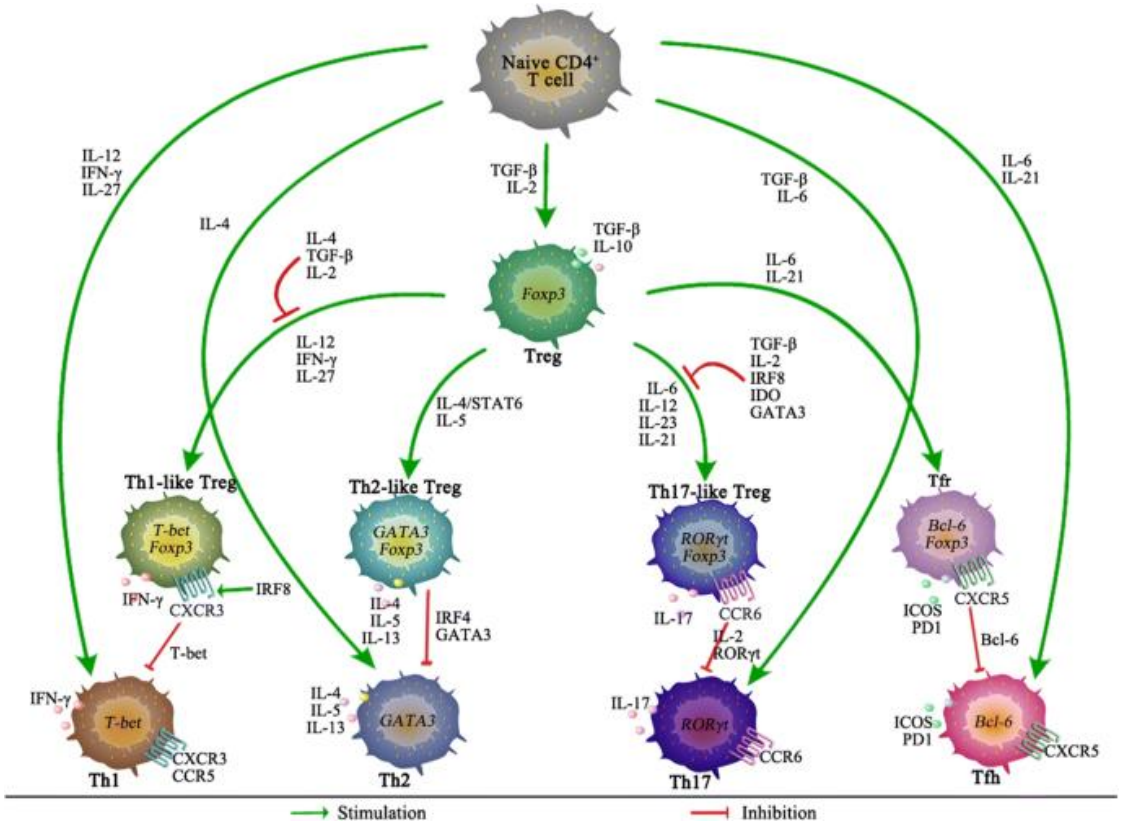


pTreg(inducible) cells develop in the periphery by antigens such as microbial antigens presented by mucosal tissue-resident dendritic cells (DCs). CYtokinens/(TGF-β,IL-6) retinoic acids (RAs) and short chain fatty acids (SCFAs) produced in the immunosuppressive environments promote pTreg development.

tTreg cells develop in the **thymus** by two-step process. First, high-affinity tissue-restricted self-antigens presented by medullary thymic epithelial cells (mTECs) or bone-marrow derived antigen-presenting cells (BM-APCs) derive single-positive (SP) T cells into Treg pathway. Second, cytokine IL-2 or IL-15 derives the precursor cells into fully committed tTreg cells.



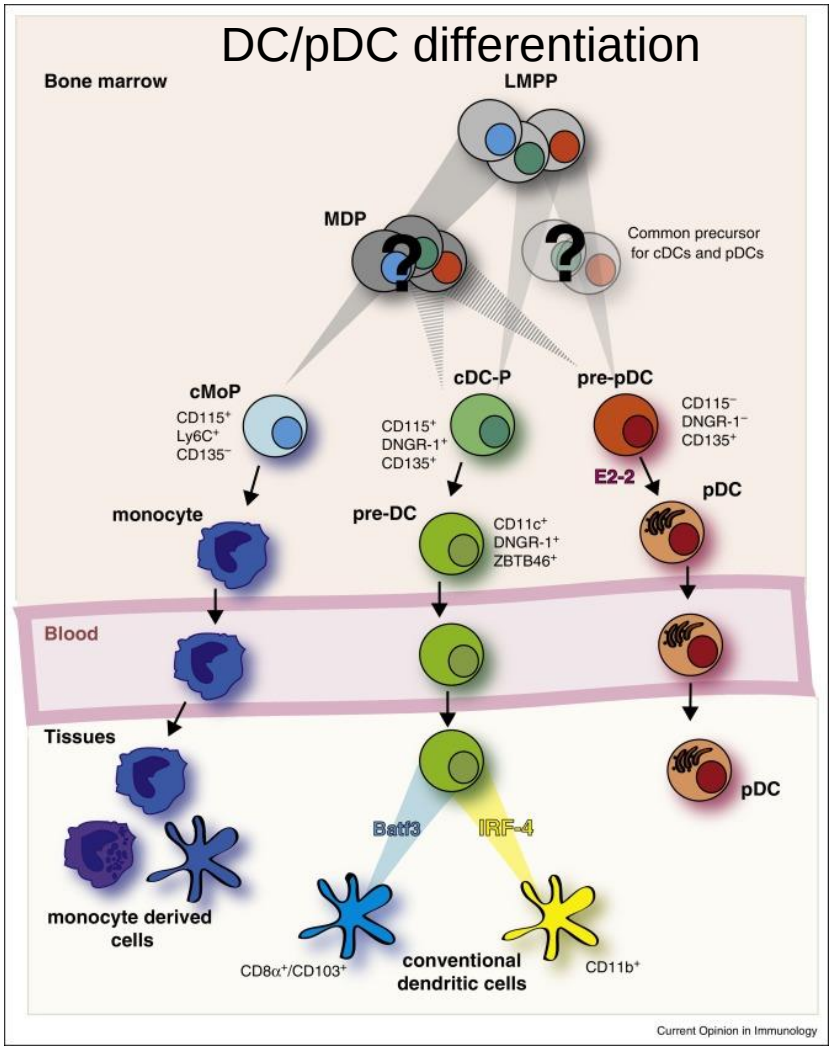
T reg Plasticity



In certain disease settings, Tregs demonstrate plastic differentiation. Plastic Tregs have some features of helper T (Th) cells, such as the secretion of Th-related cytokines and the expression of specific transcription factors in Th cells, but also still retain the expression of Foxp3, a feature of Tregs.

Question: What is the role of **plasmacytoid dendritic cells (pDCs)** in HL and are they the same as **DCs**?

Answers: Not yet investigated a lot.



Current Opinion in Immunology, 2015;32: 13-20

DCs play a basic role in pathogen sensing, antigen presentation to T lymphocytes and antigen-specific stimulation of CD4+ and CD8+ T cells.

pDCs exert potent antiviral activity by the production of Type-I interferon and contribute to anti-tumour responses through their synergistic interaction with mDCs

All DC subsets were lower in cHL patients than in healthy controls (PB). (especially, advanced stage/bulky)

Galati et al, BJH, 2019;184, 594–604

CD123+ pDCs were the most frequent DC type in the cHL microenvironment.

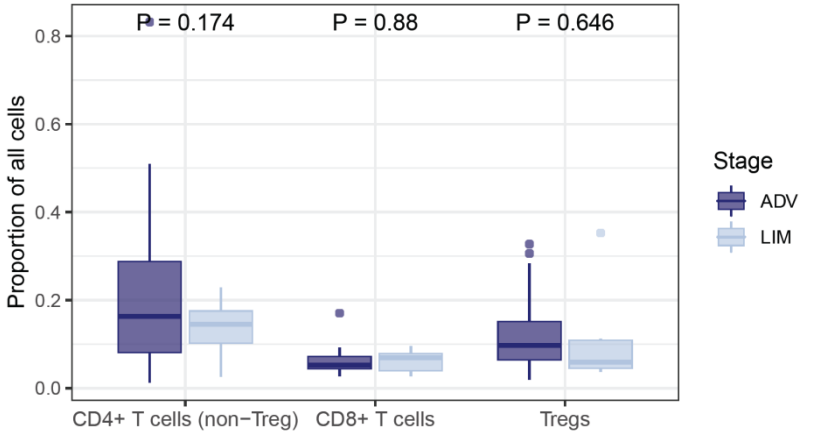
High numbers of CD83+ mDC and low numbers of CD163+ MW were associated with improved disease specific survival.

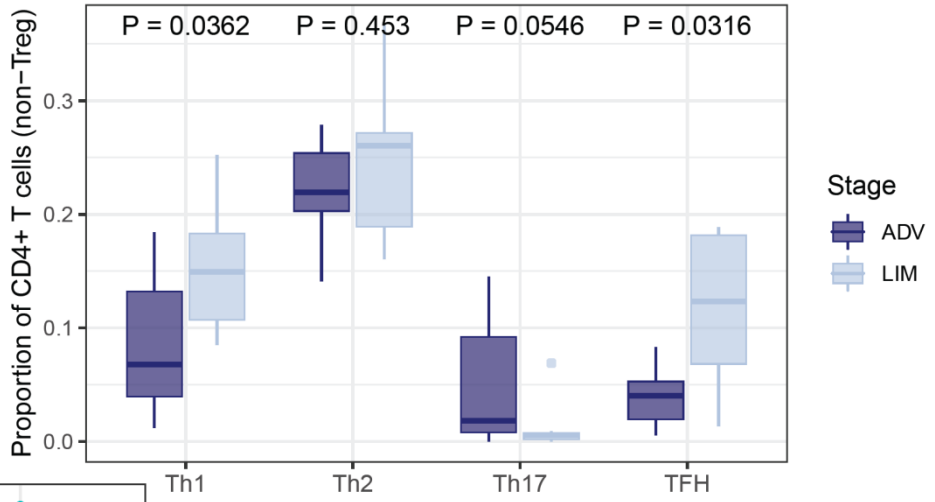
Relative frequencies of CD1a+,
CD68+ and CD123+ cells in tumour tissues had no apparent association with patients' outcome

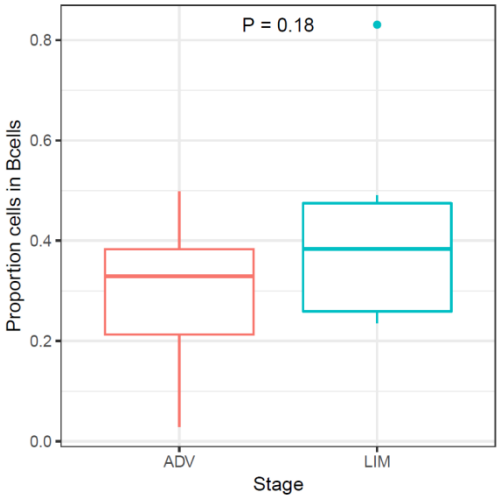
Tudor et al, PLoS One, 2014;e114345

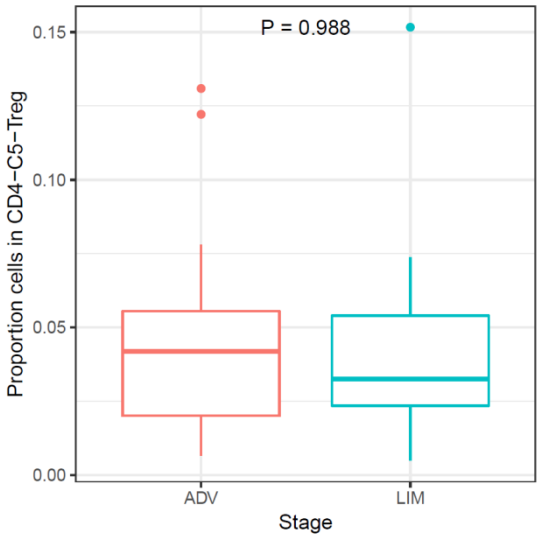
Cell proportion and Stage

QuestionIn cHL, does the proportion of different cell types correlated with the stage of the disease?









Maybe, Yes for some population, but not been investigated the detail. (Chemokine/cytokine expression that is associated with tumor growth/migration)

IHC/IMC might be better tool to Investigate.

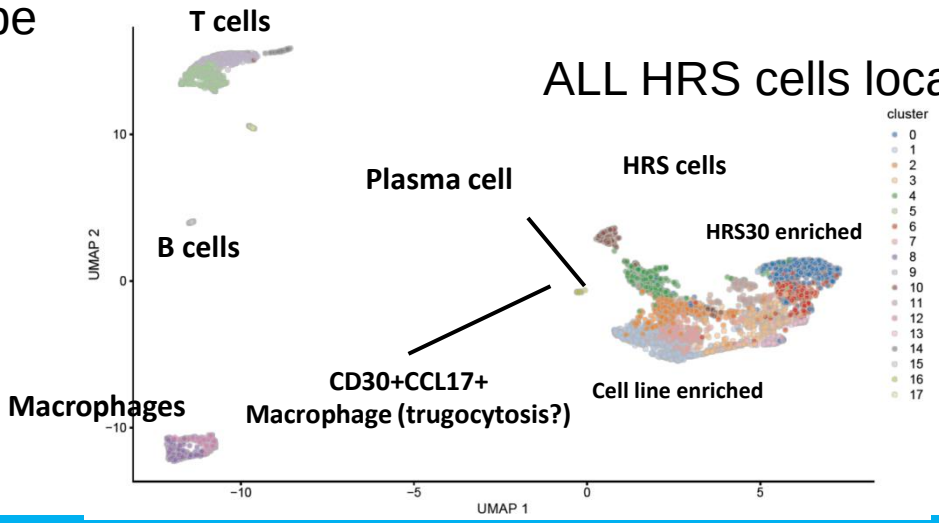
Cancer Discovery cohort

Normal B cells/Tumor B cells

QuestionIn single-cell FL samples we see patient specific malignant B-cells and normal B-cells, which are present in all samples/patients. Why is the malignant B-cell sample specific but not the normal B-cells?

I think the results could be changed according to several factors such as

1. Normalizatioh/batch correction methods
2. Cancer Type



Normal B cells generally share the phenotype among samples (e.g. Naïve/Memory/GCB/Plasma)

Tumor B cells showed various expression pattern according to
- cell of origin
- mutational status

You can explore using FL/HL data

Question: Why are CD4 cells considered "more important" than CD8 cells in Hodgkin Lymphoma?

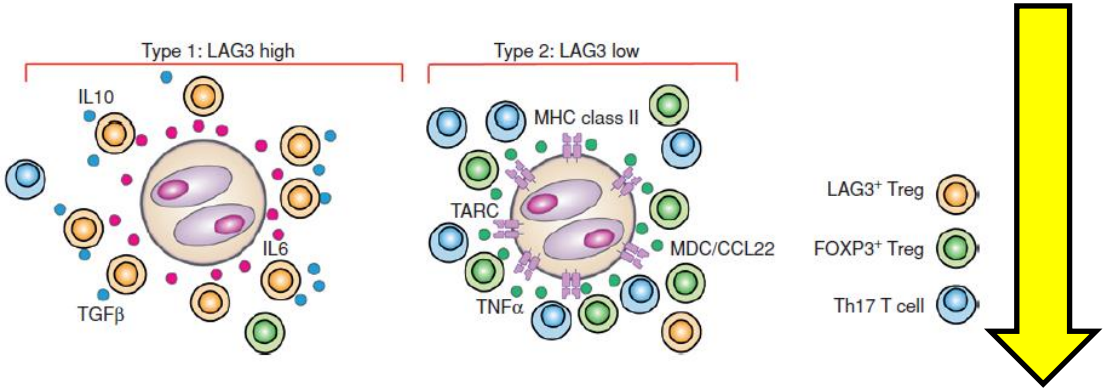
1. With few exceptions, HRS cells retain only B-cell features associated with antigen-presenting functions and interaction with TH cells, such as expression of major histocompatibility (MHC) class II (MHC-II), CD40 and CD80.

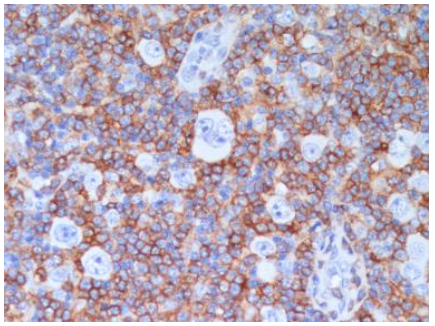
2. CD4+ T cells is the largest population of infiltrating non-tumour cells, which often form T cell rosette.

*MHC-II is generally negative for Solid Cancer cells

3. HRS cells recruit/educate CD4 T cells.

4. Interestingly, the expression of MHC class II (MHC-II), but not MHC class I (MHC-I), on HRS cells was predictive of complete remission (CR) after nivolumab treatment.





CD4 IHC

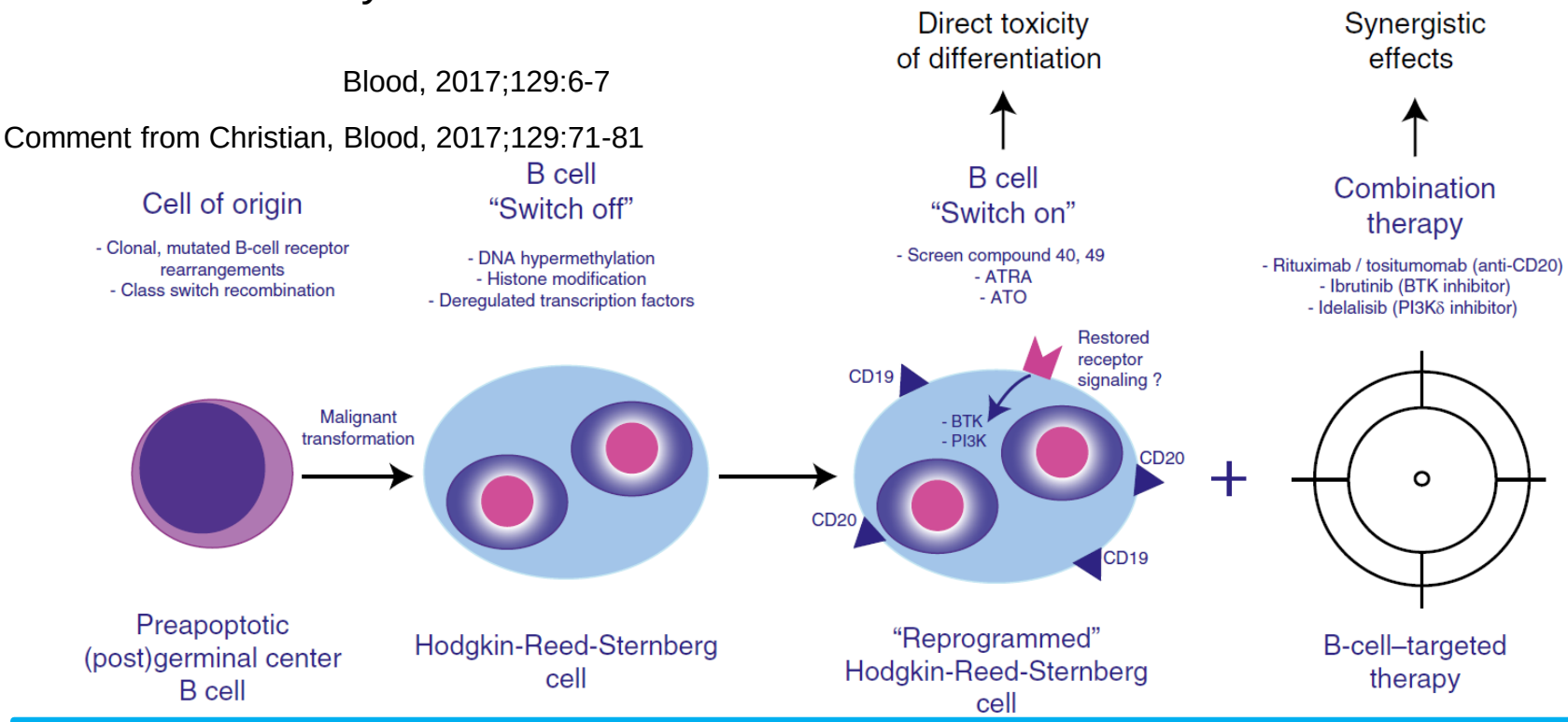
This suggests that communication with CD4 T cells is important

B cell Switch off on HRS cells

Question: What is the most likely evolutionary path that caused HRS cells to lose their B-cell identity?

Blood, 2017;129:6-7

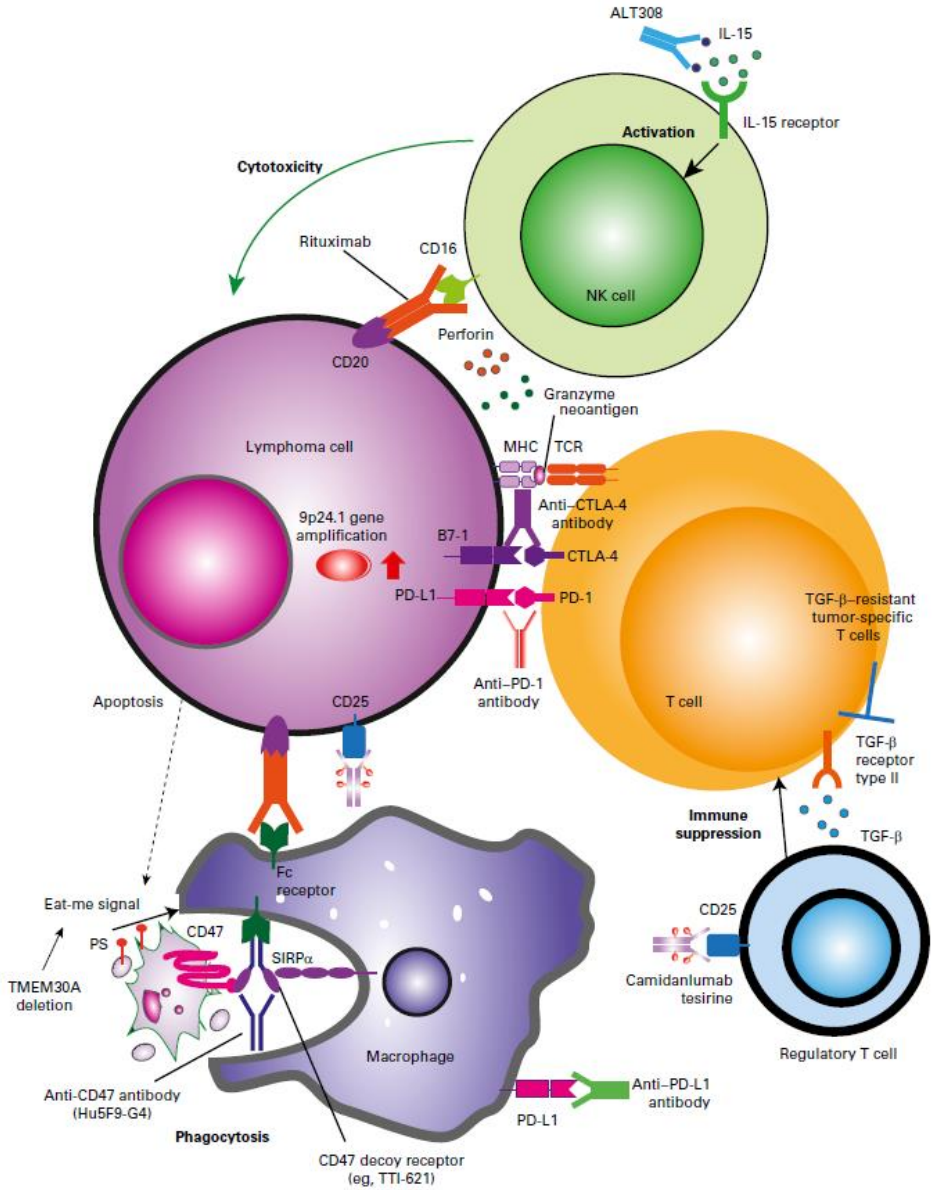
Comment from Christian, Blood, 2017;129:71-81



Among the most important and widely discussed mechanisms of HRS cell reprogramming are

1. Promoter hypermethylation
2. Aberrant histone modifications
3. Altered expression of key B-cell differentiation factors that in conjunction might lead to lineage-inappropriate gene expression profiles.

Therapeutic targets



Question: According to you, what are the most promising therapeutic targets in the ME of cHL or other types of HL? Since these targets are (presumably) not tumor-specific, are side effects a major concern?

CD25: Targeting HRS cells and Treg

Aoki et al, J Clin Oncol, 2021

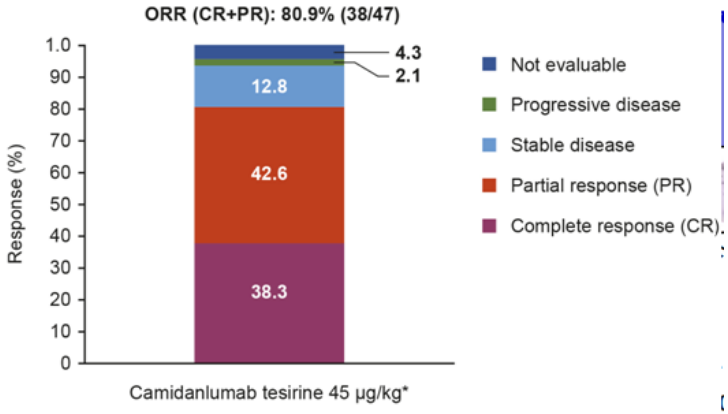
474 Preliminary Results of a Phase 2 Study of Camidanlumab Tesirine (Cami), a Novel Pyrrolobenzodiazepine-Based Antibody-Drug Conjugate, in Patients with Relapsed or Refractory Hodgkin Lymphoma

Table 1. Baseline demographics and clinical characteristics of study patients

Characteristic	Total (N=47)
Sex, n (%)	
Female	14 (29.8)
Male	33 (70.2)
Race, n (%)	
White	41 (87.2)
Black or African American	3 (6.4)
Native Hawaiian or Pacific Islander	1 (2.1)
Other (not specified)	2 (4.3)
Age, years, median (min, max)	36 (23, 74)
Histology	
Nodular sclerosis cHL	34 (72.3)
Mixed cellularity cHL	2 (4.3)
Lymphocyte-rich cHL	1 (2.1)
Other cHL – Subtype not specified/unknown	10 (21.3)
Eastern Cooperative Oncology Group status, n (%)	
0	26 (55.3)
1	18 (38.3)
2	3 (6.4)
Number of prior systemic therapies,* median (min, max)	7 (3, 20)
Disease status after first-line therapy, n (%)†	
Relapsed	31 (66.0)
Refractory	10 (21.3)
Other	6 (12.8)
Refractory to last systemic therapy, n (%)	24 (51.1)
Prior stem cell transplantation, n (%)	
Autologous	28 (59.6)
Allogeneic	2 (4.3)
Both	3 (6.4)
Other – Tandem autologous stem cell transplantation	1 (2.1)

*Includes prior stem cell transplantation; †Relapsed: complete or partial response, followed by relapse; Refractory: stable disease or progressive disease; Other: missing or not evaluable.
cHL, classical Hodgkin lymphoma.

Figure 1. Responses to camidanlumab tesirine in patients with classical Hodgkin lymphoma (central review)



*45 µg/kg for 2 cycles, then 30 µg/kg for subsequent cycles.
ORR, overall response rate.

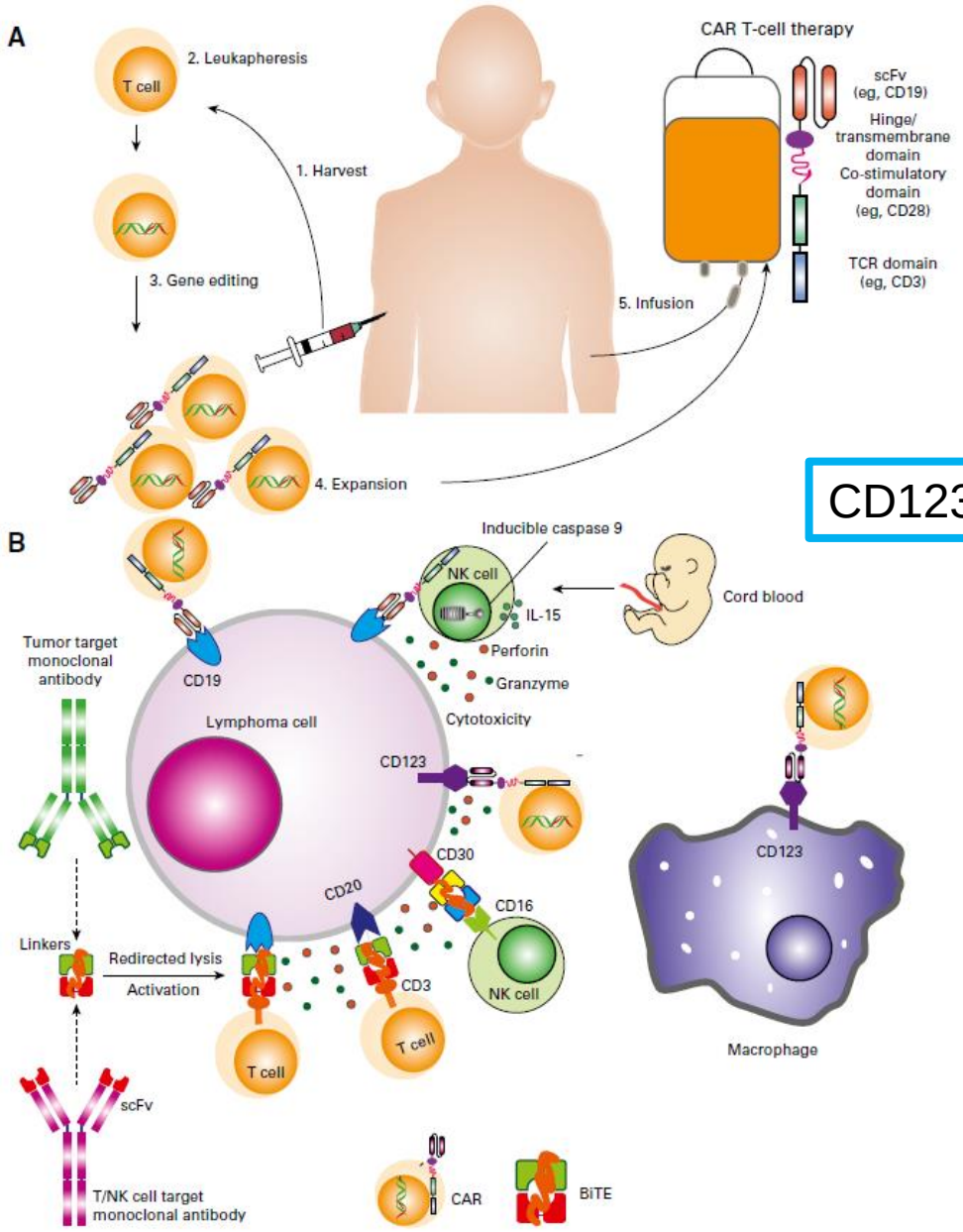
Cami has encouraging anti-tumor activity in heavily pretreated pts with R/R cHL.

Due to targeting CD25 T-reg?

There were 3 (6.4%) pts with GBS/polyradiculopathy (Preferred Terms: grade 4 subacute inflammatory demyelinating polyneuropathy, grade 2 radiculopathy, and grade 2 peripheral motor and sensory neuropathy updated to GBS after data cut-off date).

ASH 2020 Abstract # 474

Therapeutic targets



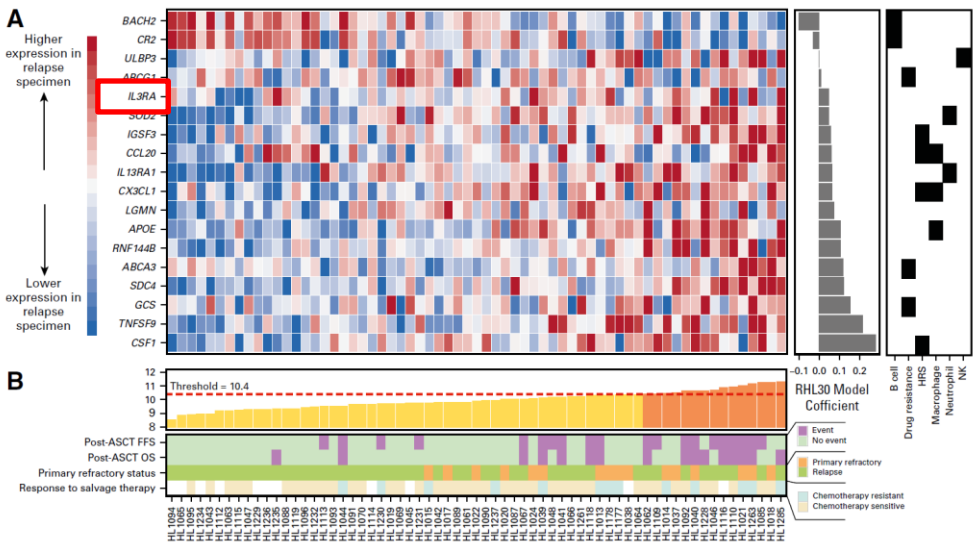
Genetically engineered T and NK cells modified with chimeric antigen receptors (CARs) represent a breakthrough in lymphoma therapy. Importantly, antigen recognition by CAR cells relies on the binding of the scFv to intact surface antigens such as CD19. The mechanism of action is **not restricted to effective MHC dependent antigen presentation**, CAR T-cell therapy may be effective even in lymphomas that lack surface MHC molecules.

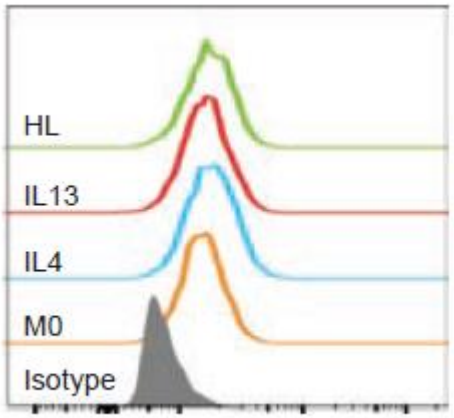
CD123: Targeting HRS cells and TAM/pDC

The fundamental concept of bispecific T-cell engagers (BiTE) is based on the enhancement of antitumor immune response using antibodies that have high affinity to multiple antigens on the surface of both tumor cells and immune cells, such as CD3 on T cells and CD16 on NK cells. This approach allows all T or NK cells to transiently link cancer cells in **an antigen presentation–independent manner** and to elicit cytotoxic activity in response to BiTE stimulation **without additional co-stimulatory signals.**

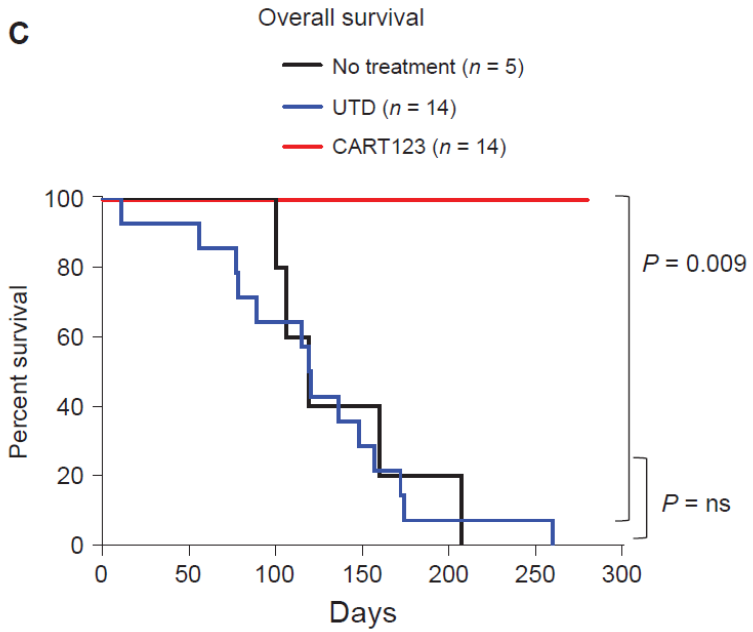
Aoki et al, J Clin Oncol, 2021

Higher expression of Macrophage signatures are associated with poor prognosis for post-ASCT FFS in relapse cHL





CD123+Mac can be induced by HL cell line supernatant



Anti-CD123 chimeric antigen receptor T cells target both the malignant cells and TAMs in Hodgkin lymphoma, thereby eliminating an important immunosuppressive component of the tumor microenvironment

Chan et al. J Clin Oncol, 2018;
Cancer Discov, 2017; 7(10); 1154–67

Thank you very much for your attention!