

4th Cuneo City ImmunoTherapy Conference (CCITC)

Immunotherapy in Hematological Malignancies 2024

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Tumor-host interactions as determinants of immunotherapy responses in CLL

Martina Seiffert

German Cancer Research Center, Heidelberg

Organized by Prof. Massimo Massaia, SC Ematologia AO S.Croce e Carle, Cuneo - Italy and
Centro Interdipartimentale di Biotecnologie Molecolari "Guido Tarone" (MBC), Torino - Italy

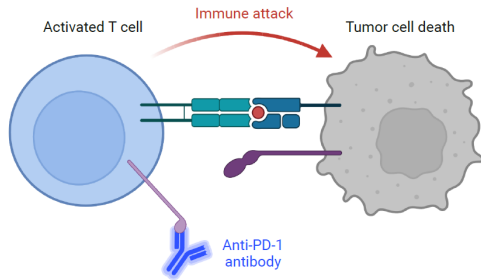
Immunotherapy in Hematological Malignancies 2024

Disclosures of Martina Seiffert

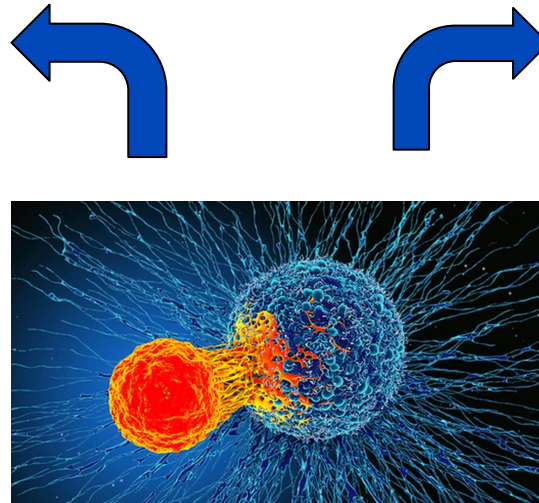
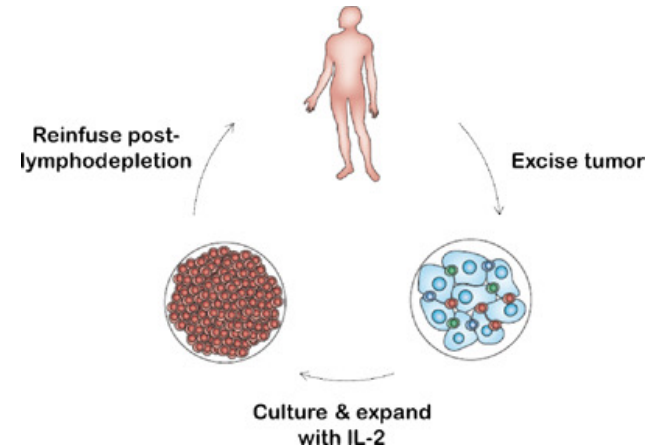
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
AbbVie						x	

T cells attacking cancer cells are in the center of novel immunotherapies

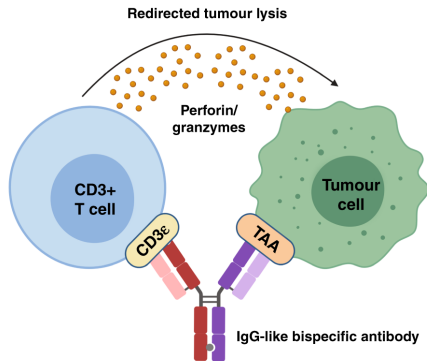
Immune checkpoint blockade



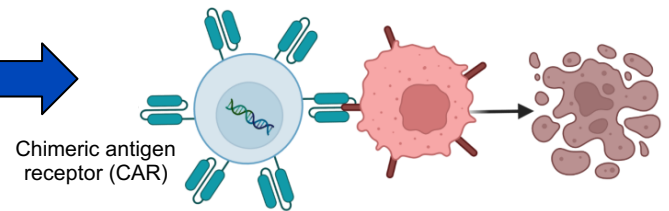
Adoptive T-cell therapy



T-cell engaging bispecific antibodies

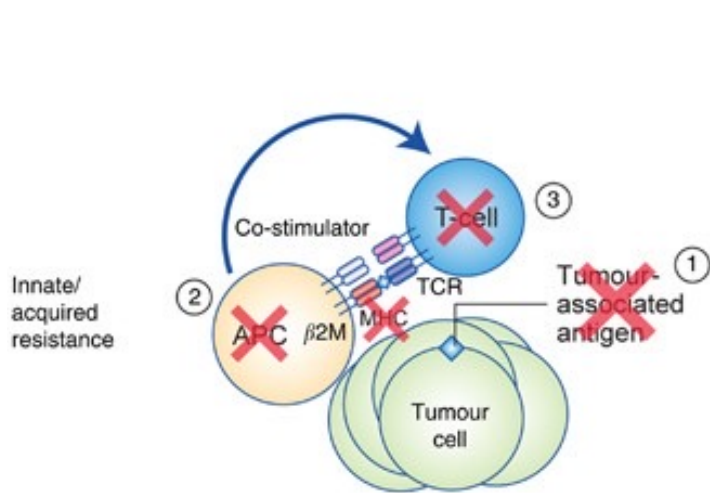


CAR T-cell therapy

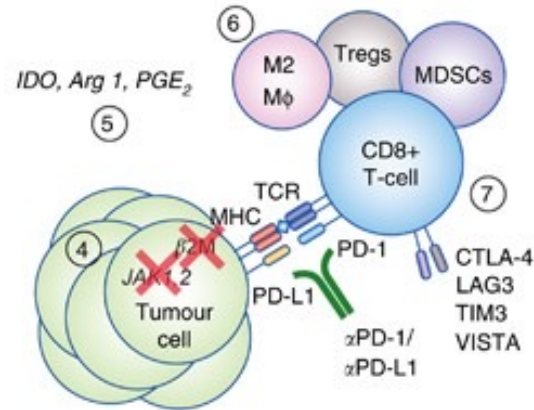


Why are patients (with CLL) not responding to immunotherapy?

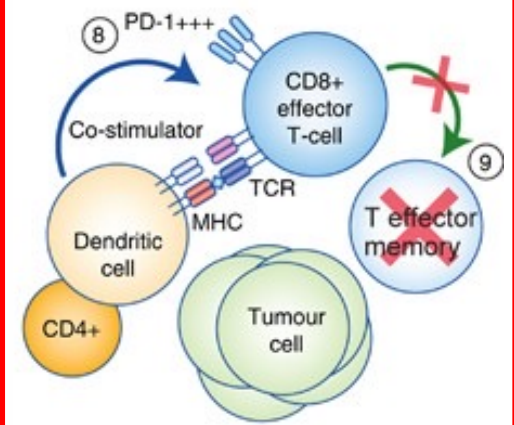
No proper stimulation of immune cells



Immune suppressive microenvironment



Dysfunctional effector T cells

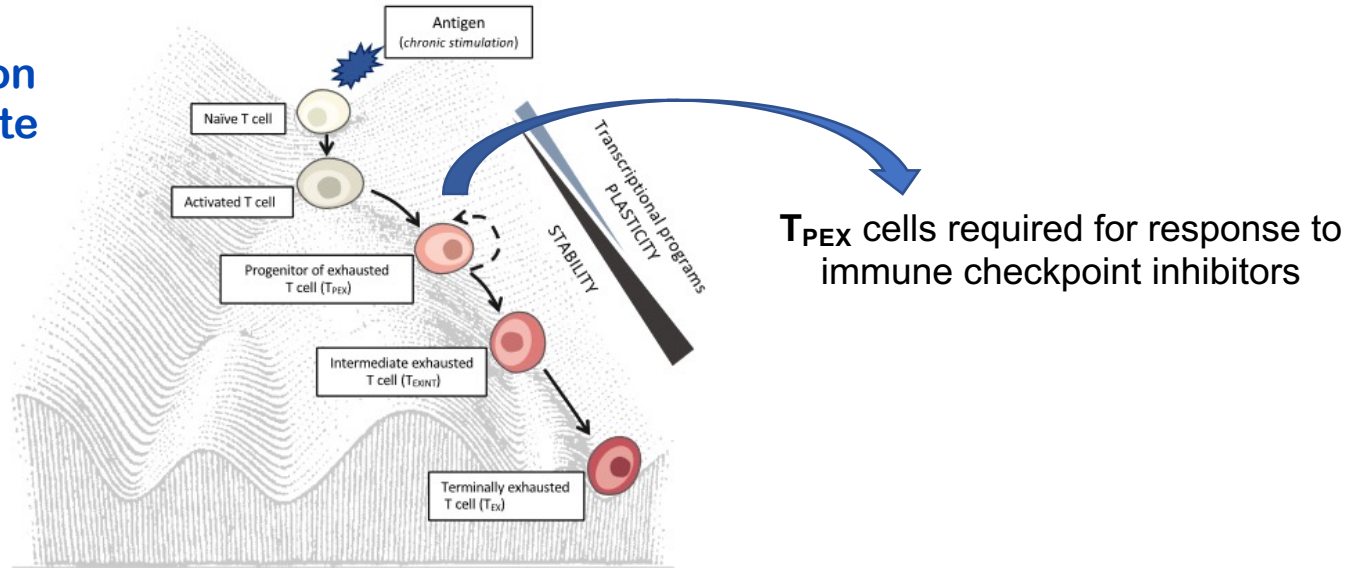


Jenkins et al. Br J Cancer, 118, 9–16 (2018)

CD8 T_{PEX} are important to maintain anti-tumor immunity

T-exhaustion =
progressive acquisition
of a dysfunctional state

Model of progressive exhaustion



Modified from Figure 2 of *Epigenetics and CD8+ T cell memory*
Montacchiesi G and Pace L. *Immunological Reviews*. 2022; 305: 77-89.

Deciphering the T-cell landscape in CLL blood and tissue samples



Laura Llaó Cid



John KL Wong



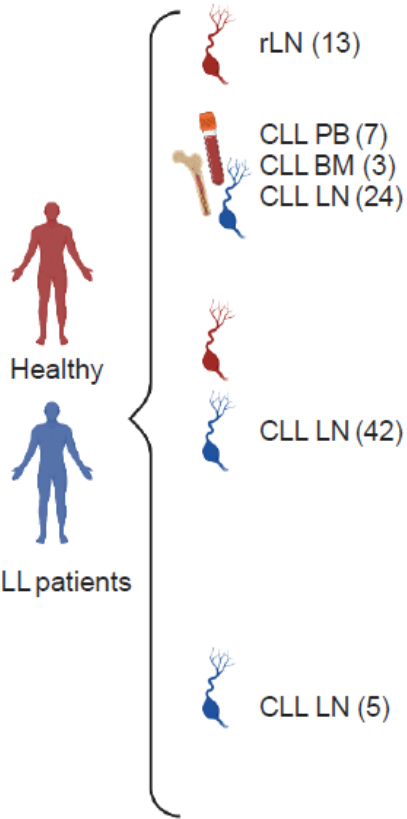
Etienne Moussay
Jérôme Paggetti



Elias Campo
Dolors Colomer

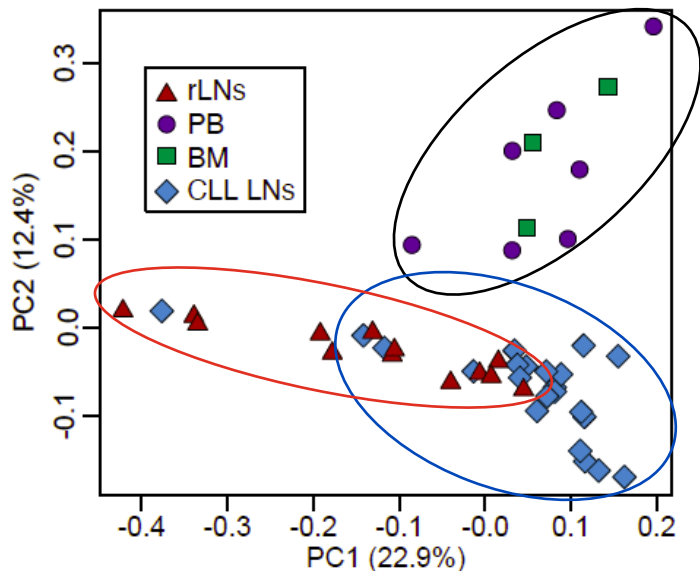


Sascha Dietrich

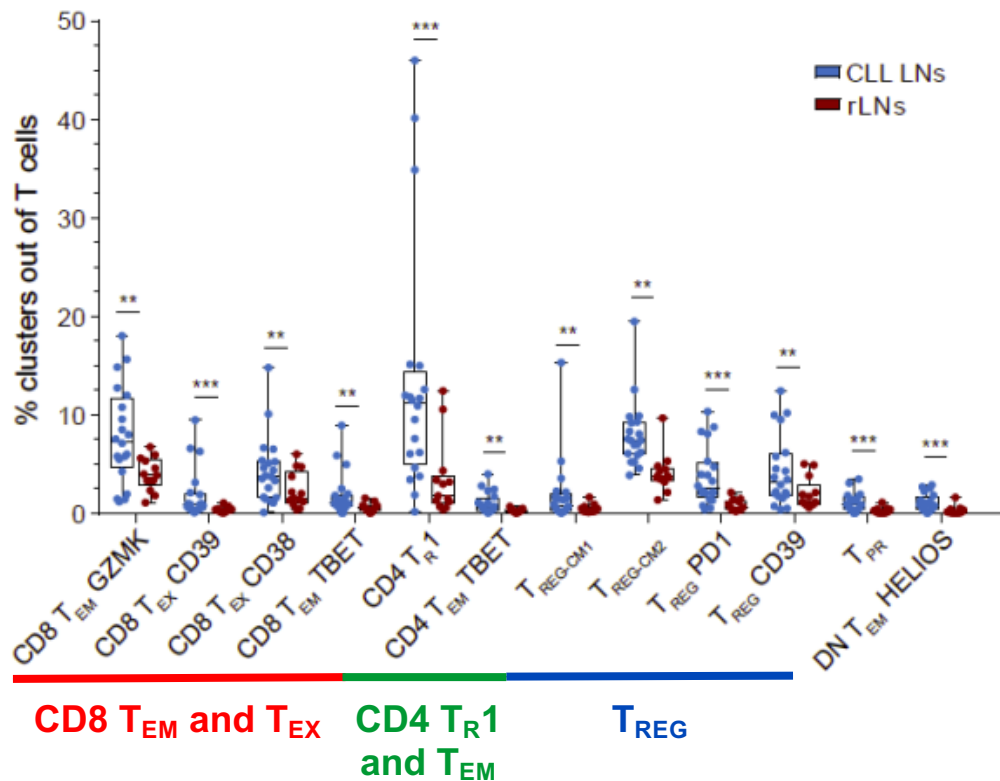


CLL LN are distinct and enriched in T_{REG}, T_{EM} and exhausted T cells

Principal component analysis of mass cytometry data

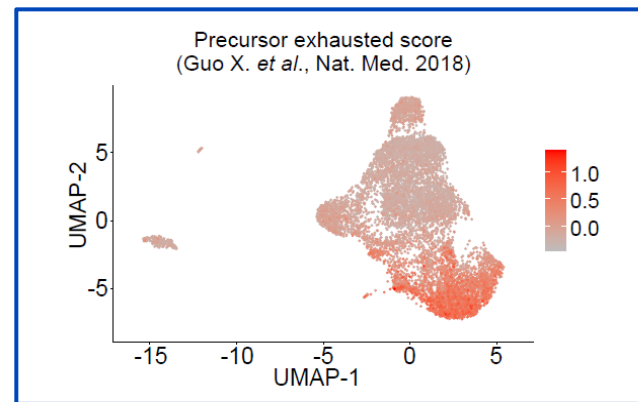
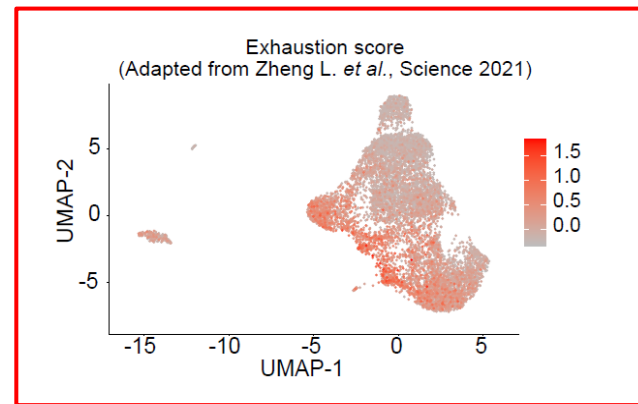
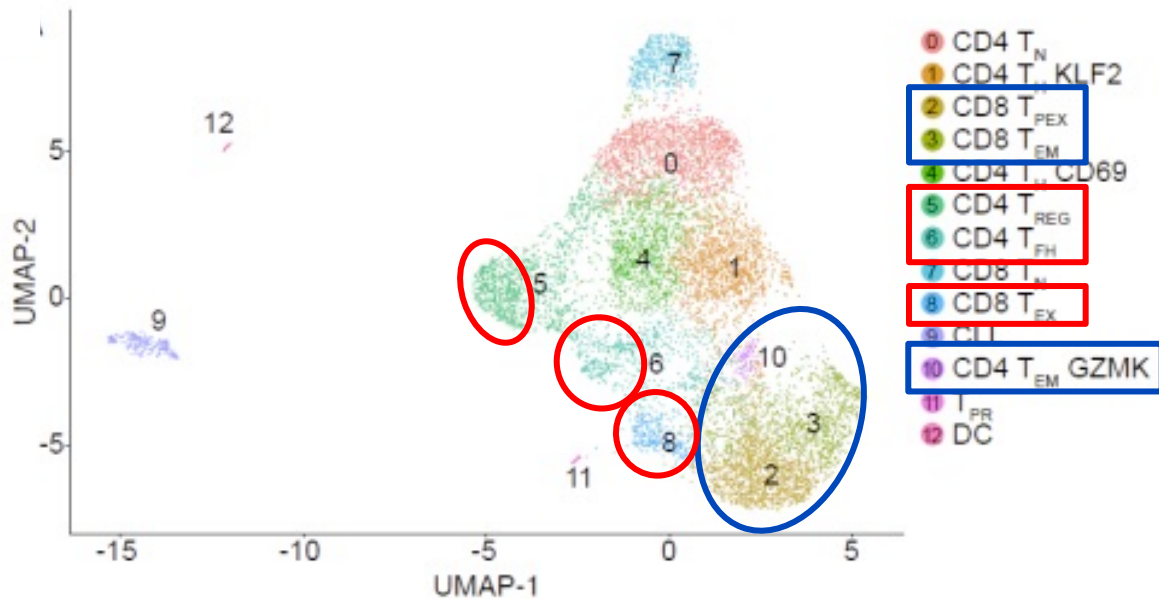


Cluster frequencies



Single-cell RNA-seq identifies T_{PEX} und T_{EX} cells in CLL LN

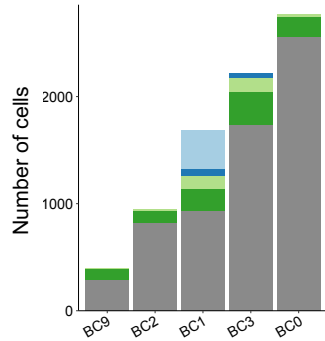
UMAP of 5 CLL LN



Llaó Cid & Wong *et al.* *Nat Comms*, in review

CD8 T_{PEX} are clonally expanded in CLL

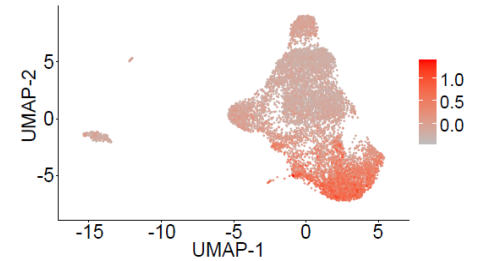
All T cells



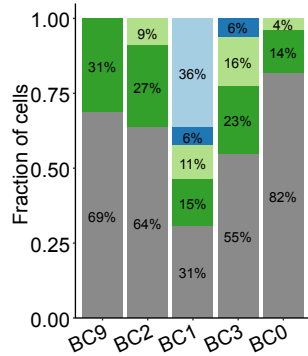
Clone type

- Hyperexpanded ($100 < X \leq 500$)
- Large ($20 < X \leq 100$)
- Medium ($5 < X \leq 20$)
- Small ($1 < X \leq 5$)
- Single ($0 < X \leq 1$)

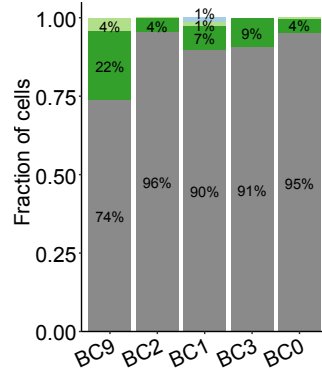
Precursor exhausted score
(Guo X. *et al.*, Nat. Med. 2018)



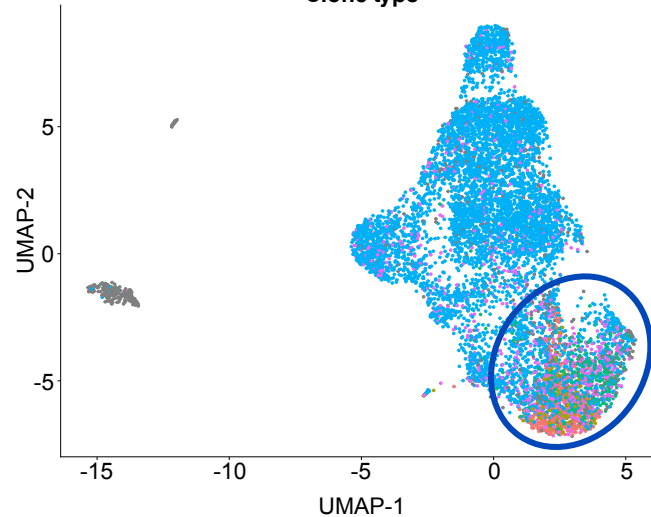
CD8⁺ T cells



CD4⁺ T cells



Clone type



- Hyperexpanded ($100 < X \leq 500$)
- Large ($20 < X \leq 100$)
- Medium ($5 < X \leq 20$)
- Small ($1 < X \leq 5$)
- Single ($0 < X \leq 1$)
- NA



Are these clonally expanded T_{PEX} cells CLL-reactive?

Llaó Cid & Wong *et al.* Nat Comms, in review

Conclusions – Take-home messages – Part 1

- **CD8 T-cells are clonally expanded in CLL patients**
- **CD8 T-cells show signs of exhaustion**
 - **both T_{PEX} und T_{EX} cells are enriched in CLL LN**
 - **T_{PEX} cells are clonally expanded and likely CLL-reactive**

CAR T cells: The success story of Emily Whitehead



First pediatric ALL patient treated with CD19-CAR T-cell therapy



How can we improve CAR T-cell therapy for patients with CLL?

- **Combination of CAR T-cell therapy: A valid strategy in CLL**

- CAR T after ibrutinib → enhances CAR T-cell function (Fraietta et al., Blood 2016)
- CAR T after failure of ibrutinib → durable remissions (Turtle et al., JCO 2017)
- CAR T with concurrent ibrutinib → high rates of MRD-negative response and lower CRS (Gauthier et al., Blood 2020)
- CAR T in patients without CR on ibrutinib → improved CR (Gill et al. Blood Adv. 2022)
- **TRANSCEND CLL 004:** Complete response / remission in 18 % (Siddiqi et al. Lancet 2023)
- March 2024: **FDA approves first CAR T-cell therapy for treatment of refractory CLL**

- **CAR T-cells and bispecific antibodies**

- Circumvent antigen escape (Choi et al., Nat Biotech 2019)

Steering CARs in the right direction

(A. Dart, Nature Rev Cancer 2019)



Credit: Carl Conway/Springer Nature Limited

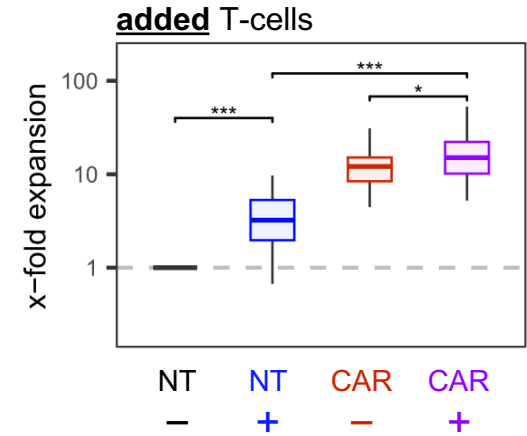
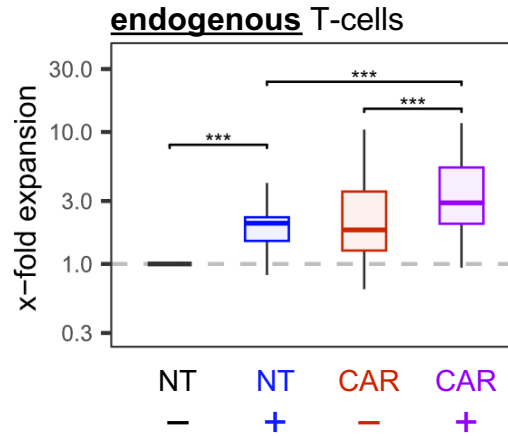
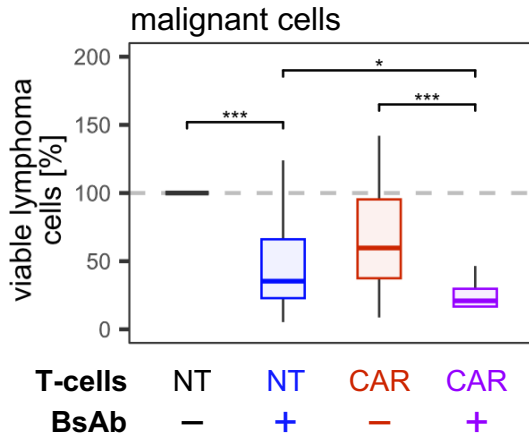
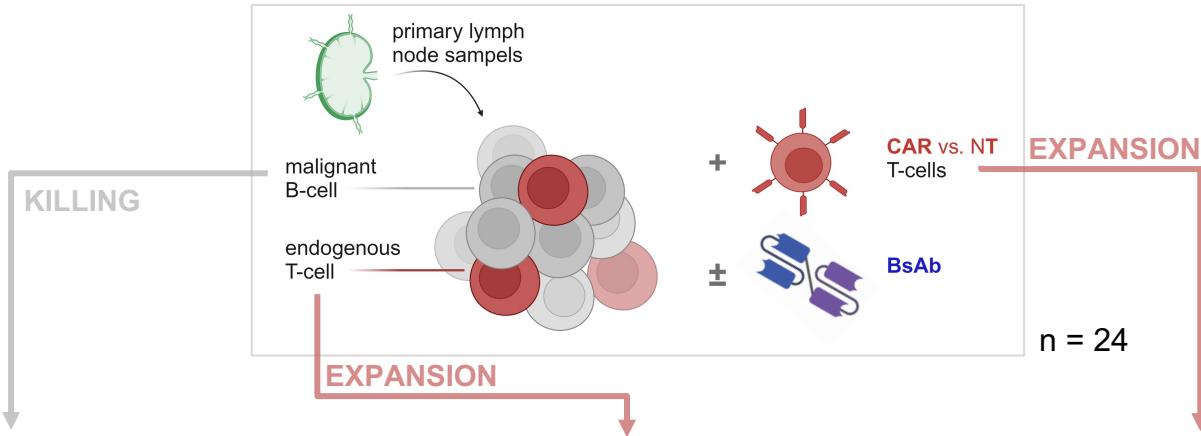
CD20-BsAb increase killing by & expansion of CD19-CAR *in vitro*



Berit Brinkmann



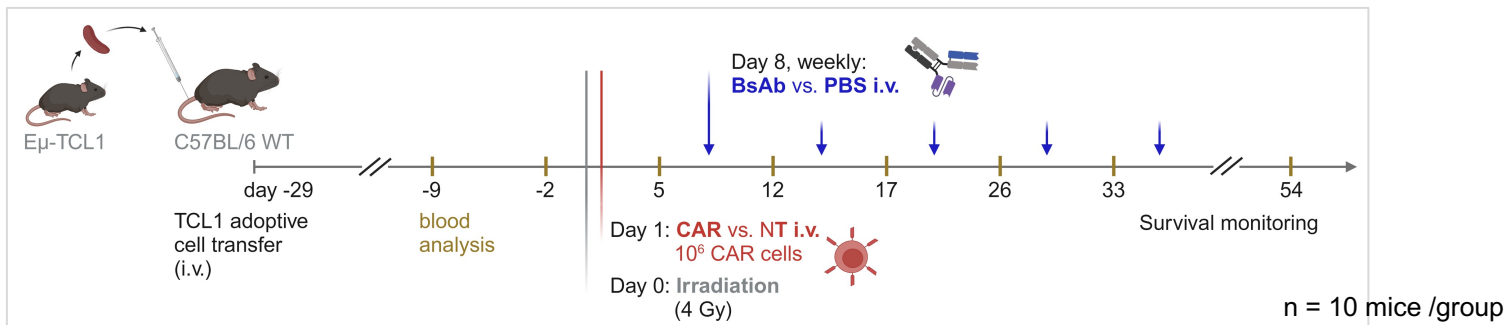
Sascha Dietrich



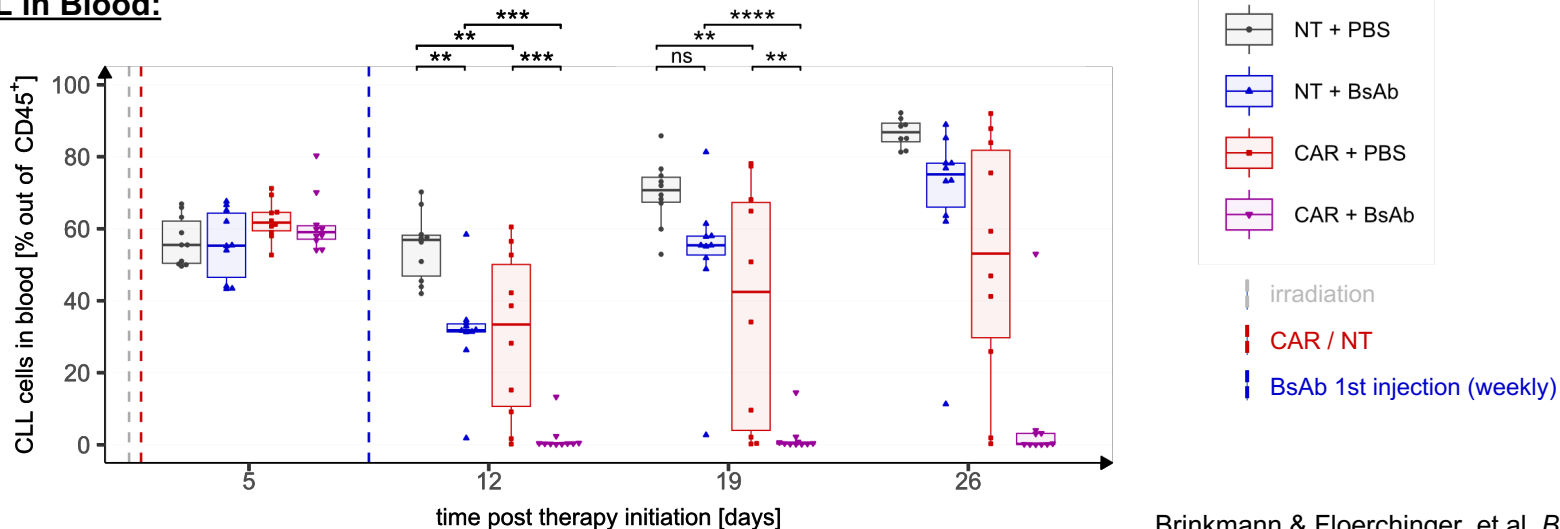
CD20-BsAb enhance anti-tumor efficacy of CD19-CAR *in vivo*



Alessia Floerchinger



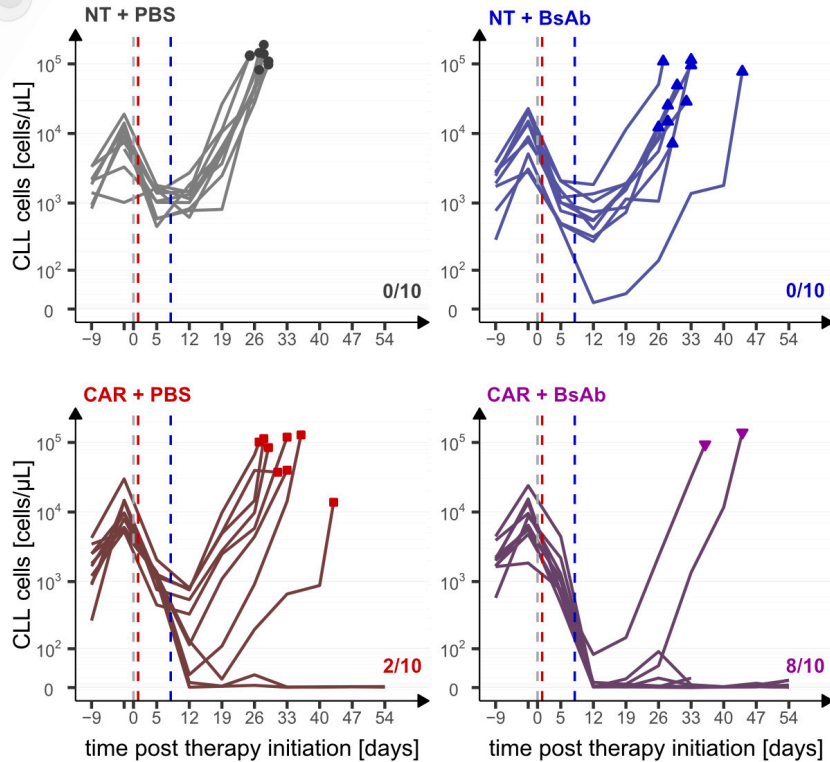
CLL in Blood:



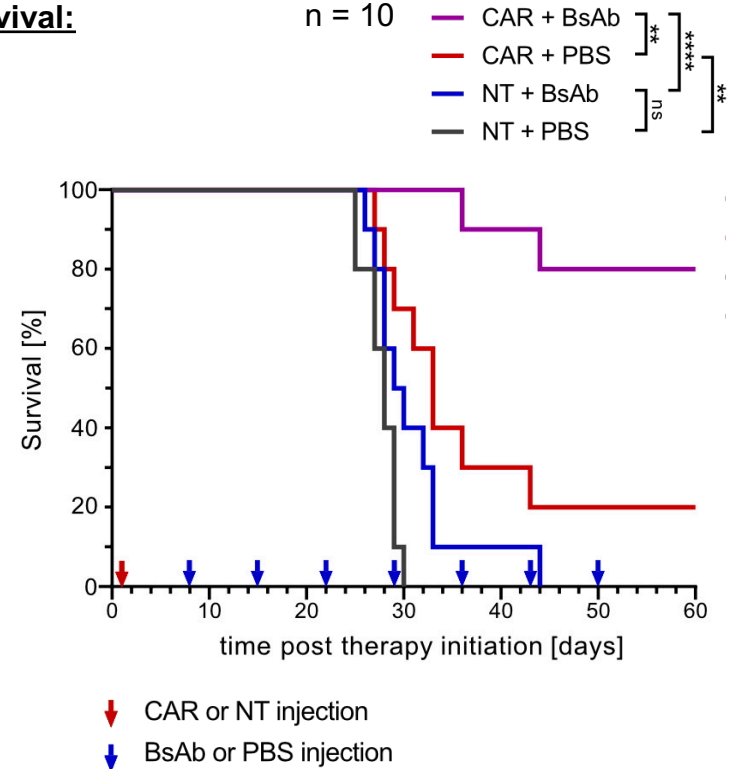
Brinkmann & Floerchinger, et al. *Blood* 2024

CD20-BsAb combined with CD19-CAR prolong survival *in vivo*

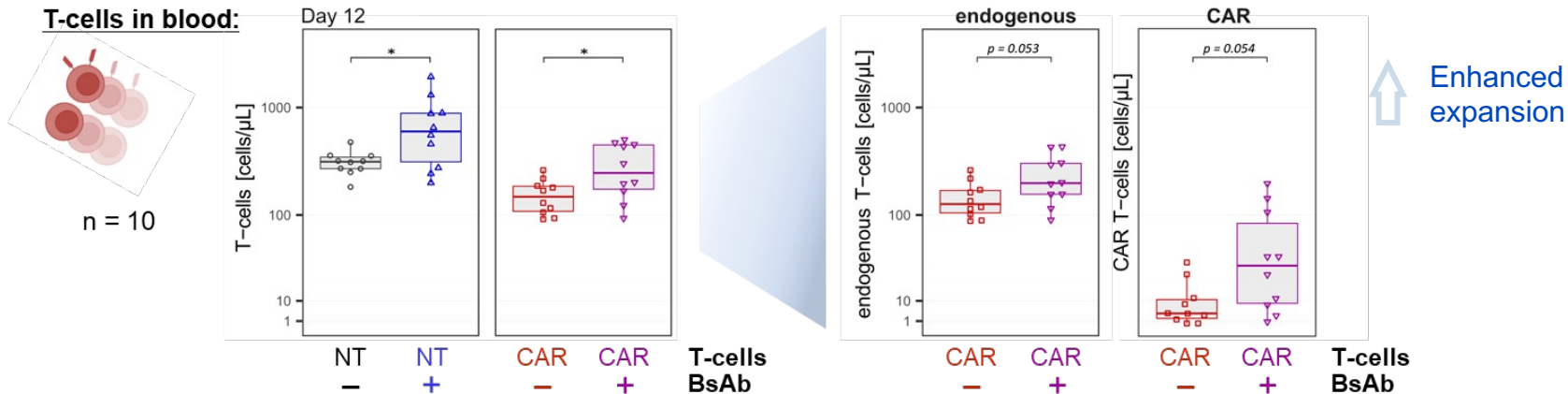
CLL in Blood:



Survival:



CD20-BsAb enhance endogenous T-cell and CD19-CAR expansion and activity *in vivo*



Endogenous T-cells

Enhanced activation and proliferation



PD-1 expression [%]

Ki67^{high} [%]

CAR - CAR +

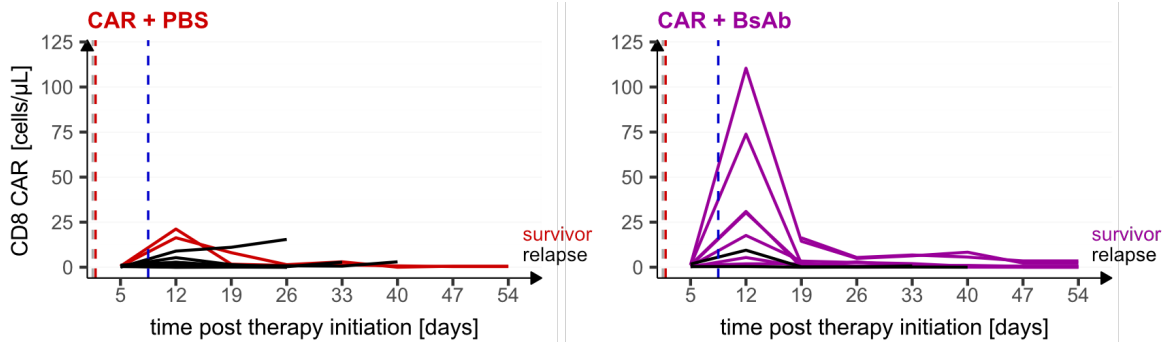
T-cells BsAb

$***$

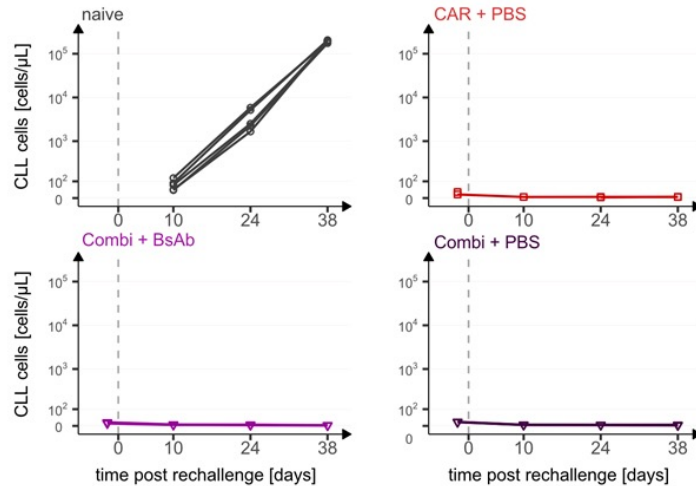
$***$

Detailed description: This figure shows two box plots. The first plot shows PD-1 expression [%] for CAR - and + groups. The + group shows a significant increase in PD-1 expression compared to the - group (p < 0.001, indicated by ***). The second plot shows Ki67^{high} [%] for CAR - and + groups. The + group shows a significant increase in Ki67^{high} expression compared to the - group (p < 0.001, indicated by ***). A blue arrow on the left points upwards, labeled 'Enhanced activation and proliferation'.

BsAb/CD19-CAR combination leads to cure and immunity against CLL in mice

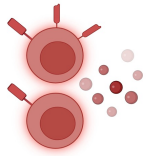


➔ Cure of mice is linked to strong initial expansion of CAR-T cells



➔ Rechallenge of cured mice with TCL1 leukemia prevents CLL development

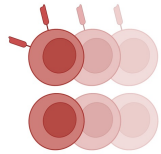
Addition of CD20-BsAb to CD19-CAR T-cell therapy



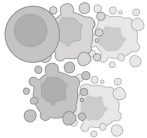
Activity (CD25) of endogenous T-cells and CAR *in vitro*



Cytotoxicity (GrB) of endogenous T-cells *in vitro*



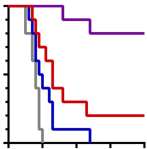
Proliferation upon BsAb therapy (NT and CAR)
in vitro and *in vivo*



Killing of malignant cells *in vitro*



Reduced CLL load *in vivo*



Prolonged survival *in vivo*

8/10 mice with no detectable CLL and immunity against CLL upon rechallenge

Conclusions – Take-home messages – Part 2

- CD8 T-cells are clonally expanded in CLL patients
- CD8 T-cells show signs of exhaustion
 - both T_{PEX} und T_{EX} cells are enriched in CLL LN
 - T_{PEX} cells are clonally expanded and likely CLL-reactive
- **CAR T-cell therapy works only in a subset of CLL patients**
 - **benefits from combinations**
 - **combining CAR T-cells and bispecific antibodies is highly effective**
 - **better expansion and activation of both CAR and endogenous T cells**
→ cures mice with CLL

THANKS



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