CD20-targeting bispecific antibodies improve response to CD19-CAR T-cells in B-cell malignancies

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INTRODUCTION

Chimeric Antigen Receptor (CAR) T-cells targeting the B-cell lineage marker CD19 have emerged as a promising treatment option for patients with relapsed of refractory B-cell malignancies. Nevertheless, major challenges such as incomplete responses after therapy and loss of target antigen expression remain, so that patients suffer from disease progression or relapse. Lack of CAR persistence, linked to exhaustion in the immunosuppressive tumor-microenvironment, has been associated with inferior clinical response. Novel strageties and combination therapies are needed for therapy-resistant cases.

Co-targeting of multiple tumor-associated antigens may reduce the risk of antigen escape. We exploit bispecific antibodies (BsAb) binding CD20 and CD3 to provide dual targeting, while engaging endogenous infiltrating T-cells in addition.

Human: Third-generation CD19 CAR
Mouse: CD19 CAR with tEGFR
Paszkiewicz *et al.* (2016)

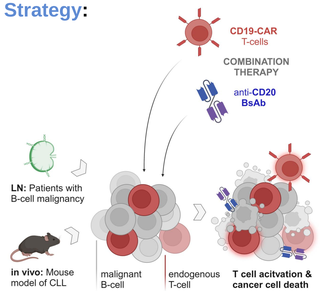
Human: CD20 BsAb (Genentech)
Mouse: CD20 BsAb (bAb0185, absolute antibody) knob-into-hole bispecific
Ramos *et al.* (2018)

CAR – Chimeric Antigen Receptor
NT – Non-Transduced
BsAb – Bispecific Antibody
Eμ-TCL1 – Transgenic CLL mouse model

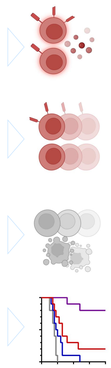
HYPOTHESIS

CD20-targeting BsAb support anti-tumor efficacy of CD19-CAR T-cell therapy via targeting of an **additional antigen** & **T-cell stimulation.**

Strategy:

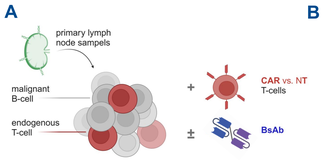


Readout:



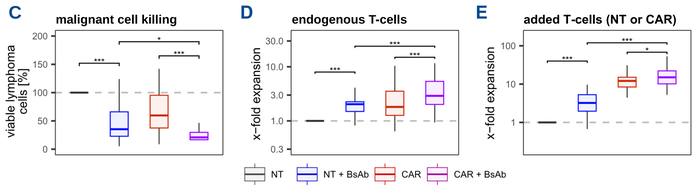
RESULTS

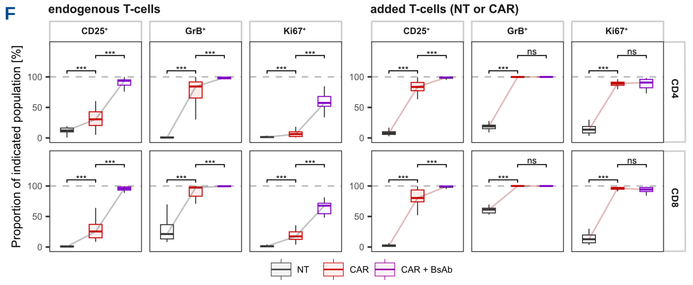
Patient samples *in vitro*:
CD20-BsAb **increase killing** by and **expansion** of CD19-CAR T-cells



B

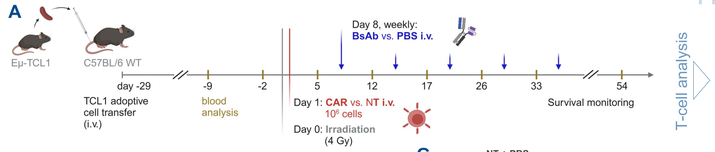
Entity	# Samples (Initial diagnosis / Relapse)	B-cells (median)	T cells (median)	CD8/CD4 (median)
CLL	4 (3 / 1)	77-95% (90)	4-22% (9)	0.15 - 0.43 (0.31)
DLBCL	5 (1 / 4)	75-93% (88)	4-22% (11)	0.11 - 5.38 (0.70)
FL	10 (6 / 4)	22-82% (65)	16-67% (30)	0.06 - 0.44 (0.28)
MCL	5 (4 / 1)	50-96% (86)	4-49% (13)	0.10 - 0.99 (0.57)

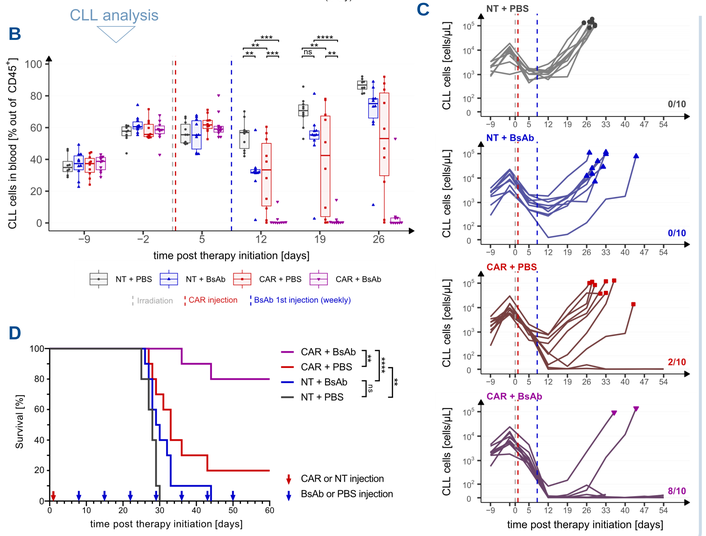


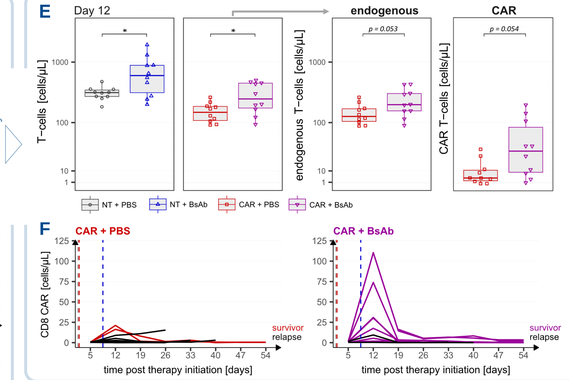


A) Schematic overview of *in vitro* study: LN-derived malignant B-cells and endogenous T-cells are cocultured with CAR or NT in combination with BsAb for 4 days. **B**) Clinical B-cell malignancy LN samples (n=24) used for *in vitro* experiments. **C-E**) Percentage of viably lymphoma cells (C), x-fold expansion of endogenous T-cells (D), and x-fold expansion of added CD19-CAR or NT T-cells (E) in co-cultures of B-NHL primary samples with CD19-CAR or NT T-cells in the presence or absence of 1 µg/mL CD20-BsAb. **F**) Percentage of CD25+, GrB+, and Ki67+ among CD4+ or CD8+ endogenous or CD19-CAR or NT T-cells in the presence or absence of 1 µg/mL CD20-BsAb.
Two-sided, paired (C-E) and unpaired (F) Wilxocon's test.
*** p ≤ 0.001, ** p ≤ 0.01, * p ≤ 0.05, ns, not significant.

CLL model *in vivo* (Eμ-TCL1):
CD20-BsAb **enhance anti-tumor efficacy** of CD19-CAR T-cells





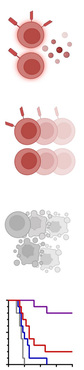


A) Schematic overview of *in vivo* study: C57BL/6 mice (n = 10 mice per group) were transplanted with 10^7 Eμ-TCL1 splenocyte-derived CLL cells i.v. and disease progression was monitored in blood. After CLL establishment, mice were conditioned with sublethal 4 Gy irradiation (Day 0). Injection with 10^6 CD19-CAR or NT T-cells was performed on Day 1 and weekly CD20-BsAb or PBS therapy initiated on Day 8. **B-C**) Frequency in blood (B) and absolute concentration (C) of $CD5^+$ $CD19^+$ CLL cells prior to and during treatment. In (C), symbols represent endpoint analysis. **D**) Survival curves for treatment with CD19-CAR plus CD20-BsAb combination, respective monotherapies or NT + PBS control.
One-way ANOVA with Tukey's HSD multiple comparison test (B) and Mantel-Cox Log-rank test (D).
***, p ≤ 0.001. **, p ≤ 0.01. *, p ≤ 0.05. ns, not significant.

E) Effect of CD20-BsAb treatment start (Day 8) on T cell expansion (Day 12): Total T-cell concentrations (left) and endogenous vs. CAR T-cells (right) are shown. **F**) Concentration of CD8+ CAR in blood over time, with relapsing mice shown in black, cured mice shown in respective colors. Unpaired t-test (E-F).

CONCLUSION

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 in complete remission

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