Enhancing CD117 directed bispecific T-cell engagers and activators by CD33 target-selective co-stimulation

Experimental Hematology/Oncology

Mara Hofstetter[1], Laura Volta[1], Monique Maurer[1], Abdullah Elsayed[2], Dario Neri[2], Markus G. Manz[1]

Department of Medical Oncology and Hematology – USZ/UZH[1], Philochem AG[2]

Introduction

Acute Myeloid Leukemia (AML) is an aggressive hematologic malignancy, originating in the hematopoietic stem and progenitor cell (HSPC) compartment. While AML can usually be initially controlled by current therapies most patients subsequently die due to relapse or to complications from therapy.

T-cell engaging bispecific antibodies (TCEs) have proven to be highly efficient in several lymphoid lineage hematologic malignancies (Acute Lymphoblastic Leukemia, B-cell Lymphoma, Multiple Myeloma)

However, there are two major challenges when using TCEs:

1) Specificity: Overlapping expression of AML tumor-associated antigens on healthy HSPCs
2) Efficacy: TCEs engage TCR-signaling (signal 1), but necessary co-stimulation (e.g. CD28 or 41BB, i.e. signal 2) for optimal T-cell activity, is missing[2].

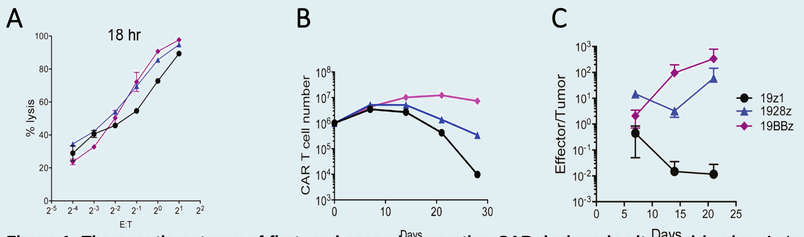

Figure 1: Therapeutic potency of first- and second-generation CAR designs *in vitro* and *in vivo*. A. *In vitro* 18h cytotoxic activity with NALM6 as target cells using bioluminescence. 19z1 = first gen. CD19 CART, 1928z = second gen. CART with CD28 signaling domain and 19BBz = second gen. CART with 41BB signaling domain B. *In vitro* cumulative cell counts of indicated CAR T cells upon weekly CD19 stimulation. C. *In vivo* effector/tumor (CART/NALM6) ratios. Sadelain, M., et al., *Cancer Cell*, 2015.

Aim

Overall aim: Investigate the role of bispecific T-cell co-stimulation antibodies in enhancing T-cell efficacy and selectivity against acute myeloid leukemia

Specific aim 1:
Generate a bispecific T-cell engager enhancer (TEE)

Specific aim 2:
Assess the functionality and safety of the bispecific TEE *in vitro*

Specific aim 3:
Assess the functionality and safety of the bispecific TEE *in vivo*

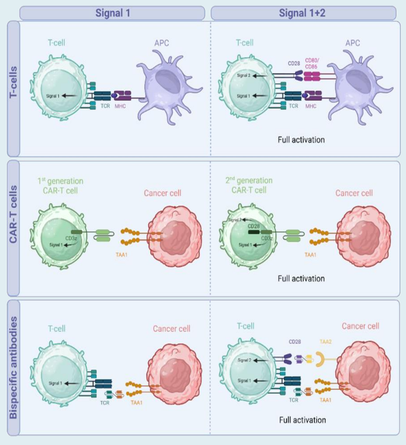

Figure 2: Schematic representation of the concept of combining CD117xCD3 TCE with agonistic TAA2xCD28

Generation of bispecific T-cell engagers

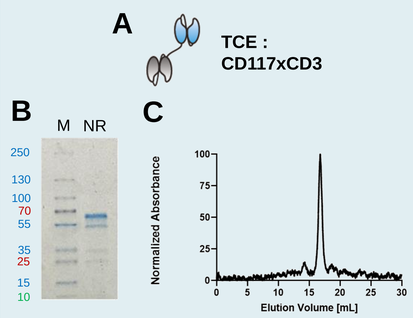

Figure 3: Characterization of CD117xCD3 TCE. A. Structure of CD117xCD3. B. SDS. C: FPLC.

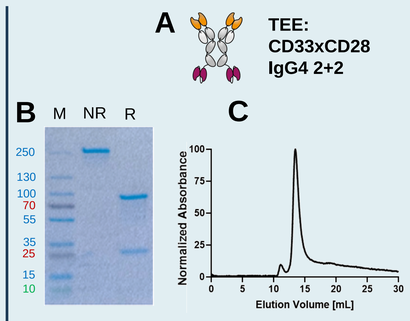

Figure 4: Characterization of CD33xCD28 IgG4 2+2 TEE. A. Structure of CD33xCD28 IgG4 2+2 TEE. B. SDS. C. FPLC

Safety of CD33xCD28 TEE

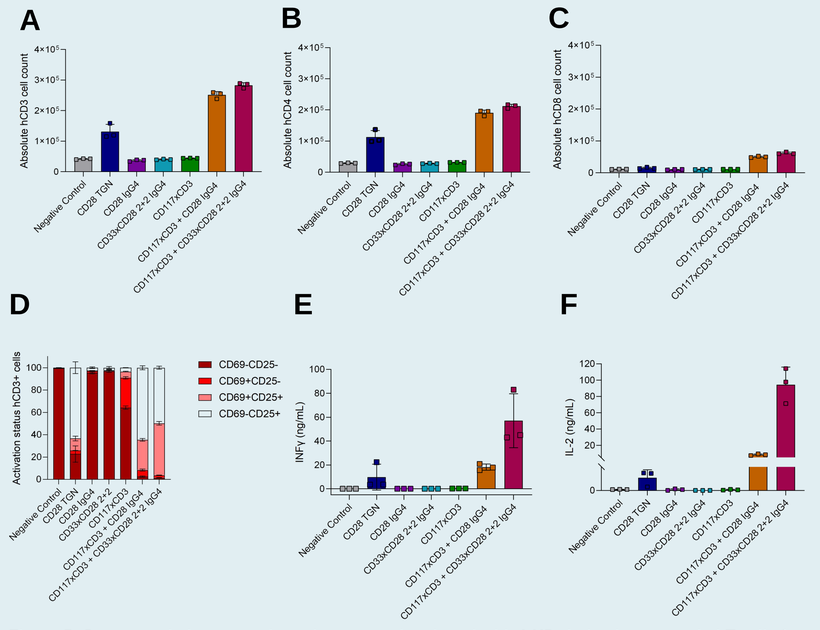

Figure 5: Superagonistic assay where drugs were incubated with 1 HD-derived unexpanded T-cells for 96h. A. Absolute hCD3+ cell count. B. Absolute hCD4+ cell count. C. Absolute hCD8+ cell count. D. Activation status of hCD3+ cells. E. INFγ cytokine release. F. IL-2 cytokine release.

Efficacy of CD33xCD28 TEE *in vitro*

1) CD33xCD28 TEE enhances CD117xCD3 T-cell mediated lysis on cell lines expressing various levels of TAA1

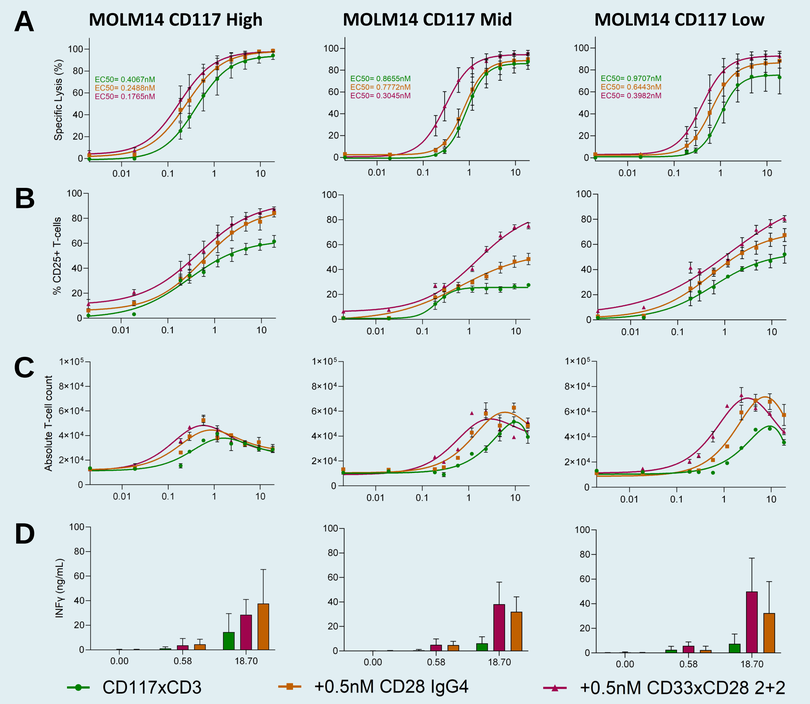

Figure 6: Killing assay with a 1:1 ratio of 3 HD-derived unexpanded T-cells and MOLM14 CD117 high, intermediate and low expressing cells at 96h. A. Specific lysis. B. T-cell activation status. C. T-cell proliferation. D. INFγ release.

2) CD33xCD28 TEE enhances CD117xCD3 T-cell mediated lysis of primary AML sample

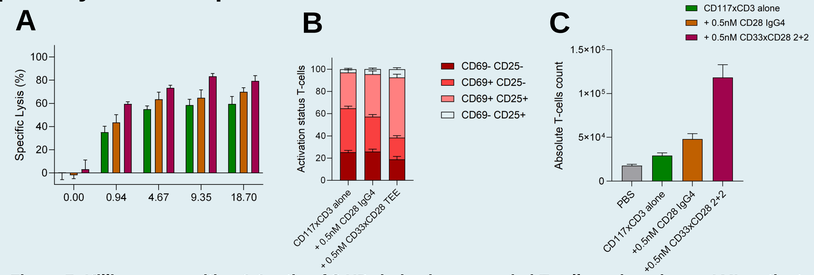

Figure 7: Killing assay with a 1:1 ratio of 2 HD-derived unexpanded T-cells and a primary AML patient sample. A. Specific lysis at 48h. B. T-cell activation status at a concentration of C. T-cell proliferation

Conclusions

- Successful production of CD117xCD3 TCE and CD33xCD28 TEE
- Safety: CD33xCD28 TEE does not have superagonistic properties
- Efficacy: the addition of CD33xCD28 TEE to CD117xCD3 enhances T-cell mediated killing by delivery of co-stimulation via a second TAA, thereby likely also enhancing specificity

Contact Information

Mara Hofstetter: PhD Candidate,
E-mail: mara.hofstetter@usz.ch

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