Antibody affinity modulation and optimization of activation signals for the development of a new generation of bispecific CAR-T cells

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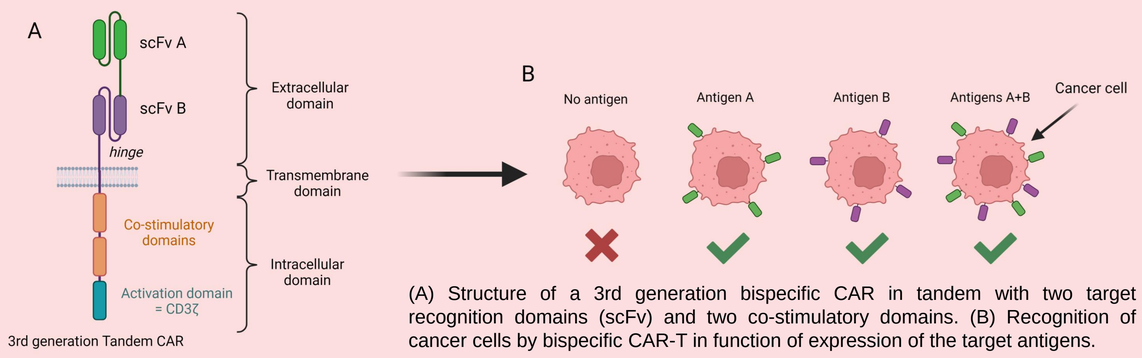
Introduction

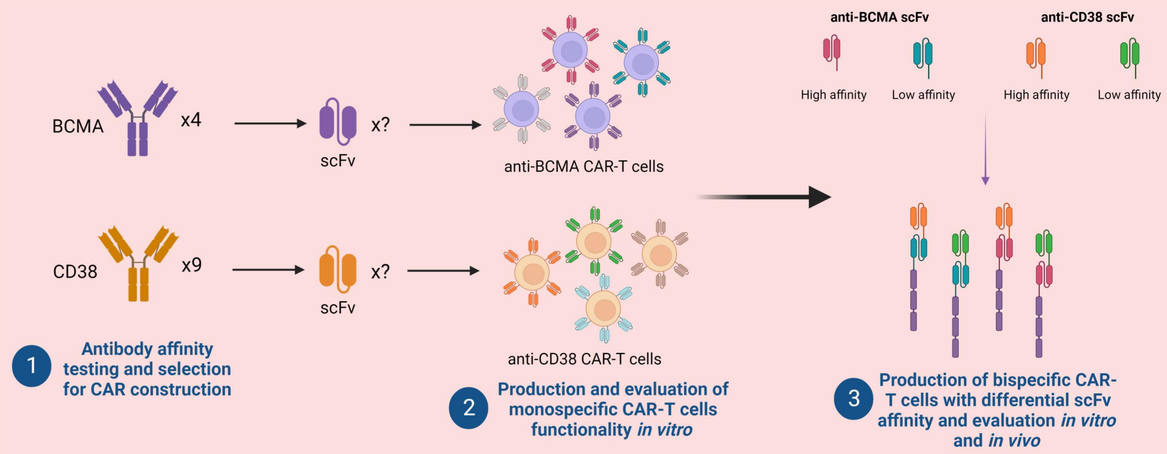
CAR-T cell strategy has proven to be very effective in the treatment of malignant hemopathies. However, relapses occur in treated patients. These can be due to either a lack of CAR-T efficacy and persistence, or the escape of cancer cells from CAR-T recognition through loss of expression of the target protein. Bi-specific CAR-T strategy, using the recognition of two targets simultaneously, has shown to be effective to overcome this resistance mechanism *(Zah et al., Cancer Immunol Res., 2016, Dai et al., J Hematol Oncol. 2020)*. In addition, it has been shown that scFv affinity can influence antigen loss as well as CAR-T cell activation, persistence and toxicity towards healthy cells *(Olson et al., Leukemia, 2022, Ghorashian et al., Nat Med, 2019, Drent et al., Mol. Ther., 2017)*.



(A) Structure of a 3rd generation bispecific CAR in tandem with two target recognition domains (scFv) and two co-stimulatory domains. (B) Recognition of cancer cells by bispecific CAR-T in function of expression of the target antigens.

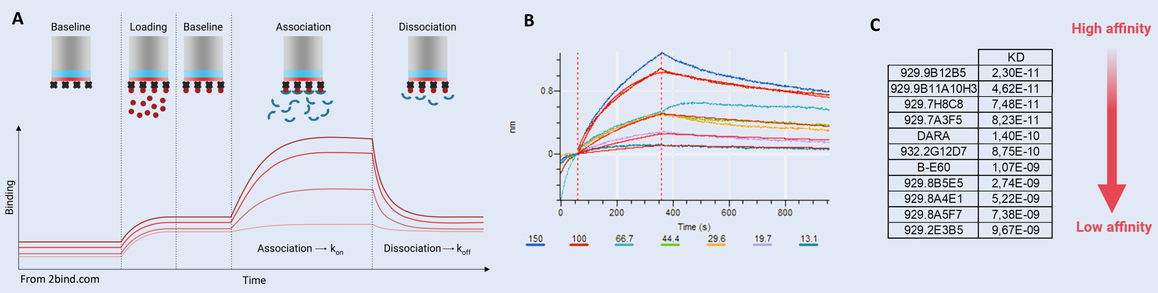
Objective

Our goal is to combine bi-specific CAR-T strategy and scFv affinity tuning by developing a **new generation of bispecific CAR-T targeting BCMA and CD38 in the context of multiple myeloma**. Therefore, we hope to limit cancer cell escape and boost CAR-T cell efficiency by finding the optimal combination of scFv affinity for each target. We are also planning to optimize activation signals by testing different hinge and co-stimulatory domains combinations.



Preliminary results

1. Antibody affinity testing and scFv selection

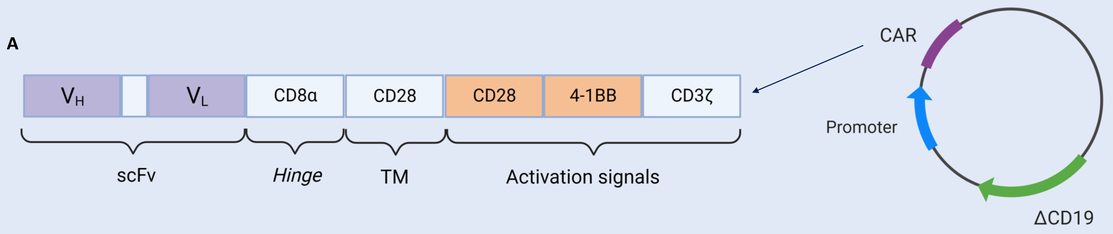


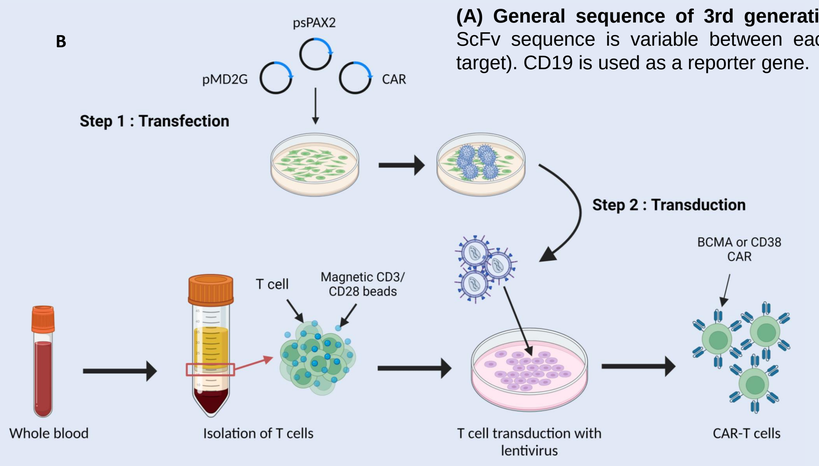
	KD
929.9B12B5	2,30E-11
929.9B11A10H3	4,62E-11
929.7H8C8	7,48E-11
929.7A3F5	8,23E-11
DARA	1,40E-10
932.2G12D7	8,75E-10
B-E60	1,07E-09
929.8B5E5	2,74E-09
929.8A4E1	5,22E-09
929.8A5F7	7,38E-09
929.2E3B5	9,67E-09

(A) Principle of antibody affinity assay by bio-layer interferometry (BLI) with the Sartorius Octet. The curve is an example of data generated with the Octet showing interaction kinetics between the antibody and its target. Measurement of association (Ka) and dissociation (Kd) rates allows kD calculation. (B) Kinetics of interaction between anti-CD38 antibody 929.2E3B5 and recombinant human CD38 protein. (C) kD values for the 9 anti-CD38 antibody candidates as measured with Octet, from highest to lowest affinity.

→ 4 anti-CD38 antibodies with different affinities were chosen to generate scFvs for CD38 CAR-T cells candidates : 8B5E5, 2G12D7, 7A3F5, 9B11A10H3
→ The anti-BCMA antibodies are currently being tested for affinity using the same protocol

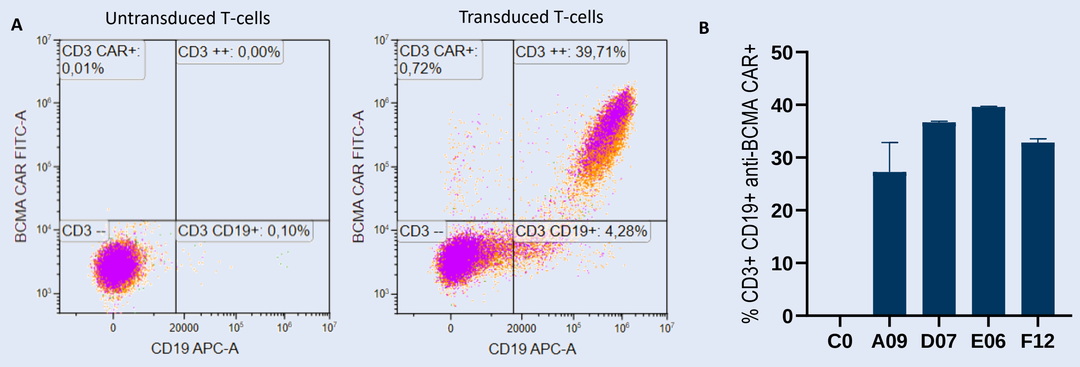
2. CAR-T cell production





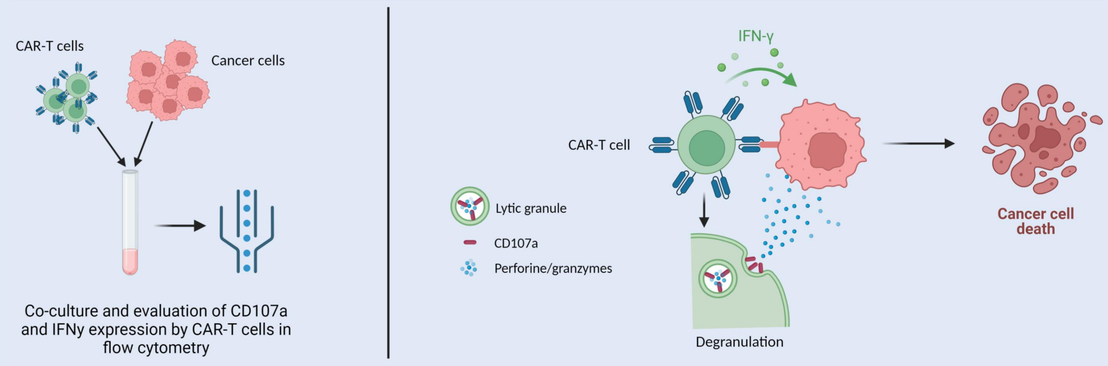
(A) General sequence of 3rd generation CARs used in this project. ScFv sequence is variable between each CAR-T candidate (4 for each target). CD19 is used as a reporter gene.

(B) CAR-T cell production protocol. First, lentivirus are produced by transfection of 293T cell line with plasmid containing CAR sequence and helper plasmids pMD2G and psPAX2. Culture supernatant is harvested and titrated for functional titer. Then, PBMCs are harvested from healthy donors' blood and T cells are isolated and activated using CD28/CD3 coated magnetic beads. T cells are transduced with lentivirus at MOI 10 and expanded in vitro with IL-2.

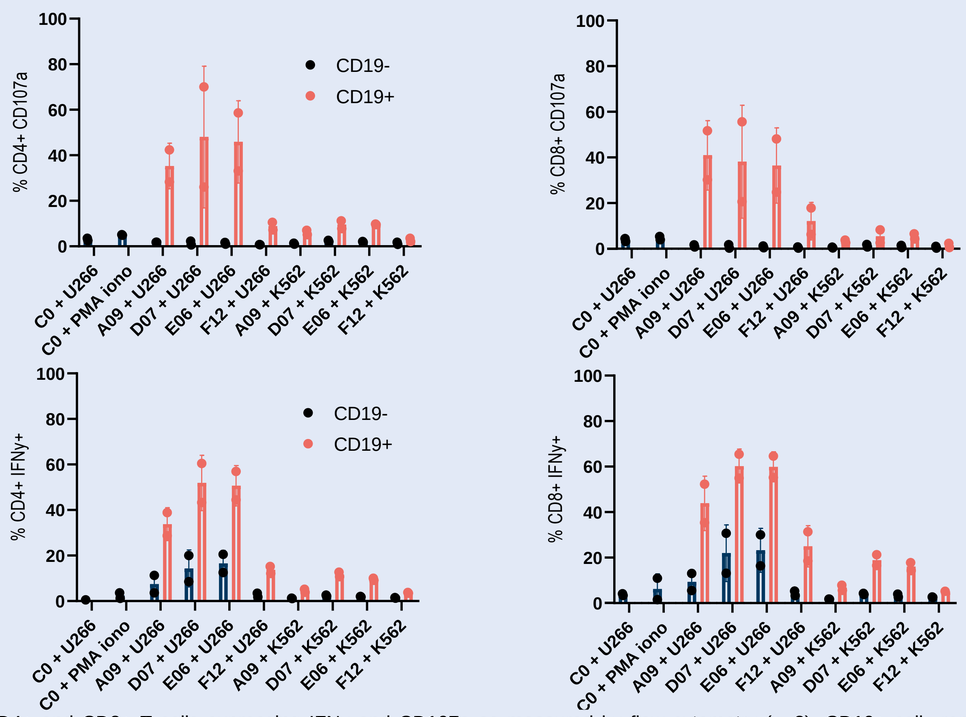


(A) Representative flow cytometry plots showing co-expression of the BCMA targeting CAR (using BCMA protein conjugated to FITC) and CD19 (reporter gene) by healthy donor T cells untransduced or transduced with A09 anti-BCMA CAR lentiviral supernatant (MOI = 10), at day 7 post-transduction. (B) Transduction efficiency determined in flow cytometry by the percentage of CD3+ cells co-expressing CD19 and anti-BCMA CAR for the four anti-BCMA CAR-T cell candidates (A09, D07, E06, F12) (n=2). C0 = untransduced control.

3. CAR-T cell functionality assessment



Flow cytometry measurement of membrane expression of CD107a (marker of degranulation) and intracellular expression of IFNγ to evaluate cytokine production after 5h (CD107a) or 18h (IFNγ) co-culture with multiple myeloma cells expressing BCMA.



% of CD4+ and CD8+ T cells expressing IFNγ and CD107a as measured by flow cytometry (n=2). CD19+ cells represent the fraction of T cells efficiently transduced = CAR-T cells. C0 = untransduced control. U266 = BCMA+ myeloma cell line. K562 = BCMA- cell line.

Perspectives

- Follow-up functionality testing of monospecific CAR-T cells : specific lysis ability, functionality against cell lines with differential expression of target antigens
- Selection of scFv for bispecific CAR-T cell production based on affinity and functionality
- Transcriptomic analysis of bispecific CAR-T to evaluate the impact of scFV affinity on activated signaling pathways
- Functional analysis of bispecific CAR-T *in vitro* followed by *in vivo* experiments to select optimal scFV combination and backbone