An Intrinsic Safeguard Chimeric Antigen Receptor Architecture for T-Cell Immunotherapy

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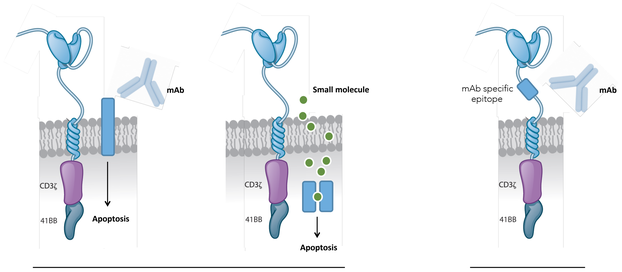
Abstract

Adoptive CAR T-cell therapies have been reported to be highly efficient at killing tumor cells and are on the verge of revolutionizing the field of cancer treatment. However, there are safety concerns with CAR T-cell treatments which would require intervention, including on-target off-tumor effects, cytokine release and tumor lysis syndromes. Thus, avoiding the aforementioned acute and long term adverse events appears to be mandatory to improve the safety of adoptive cell immunotherapies. Here we developed a novel CAR architecture containing an integrated safeguard component. We demonstrated that this CAR architecture was efficiently and rapidly depleted by the FDA-approved Rituximab (RTX) *in vitro*, shows cytolytic activity toward tumor cells and allows for universal enrichment and detection of CAR T-cells from complex cellular populations.

An intrinsic Safeguard for safer adoptive CAR T-cell immunotherapies



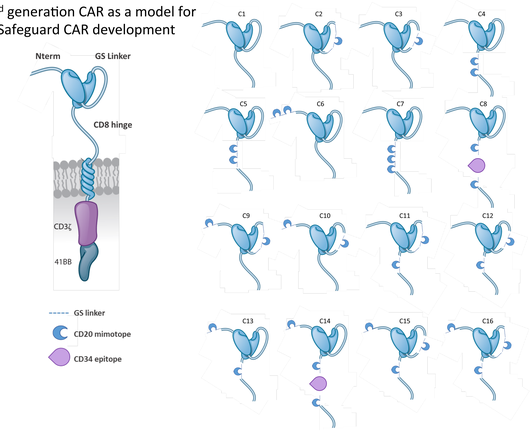
Conventional CAR/switch OFF multi-component systems

CAR/switch OFF Mono-component system

Schema of CAR/switch-OFF systems

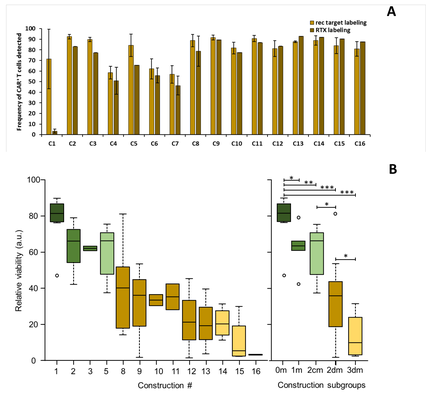
Assembly of Safeguard CAR architectures

A 2nd generation CAR as a model for Safeguard CAR development



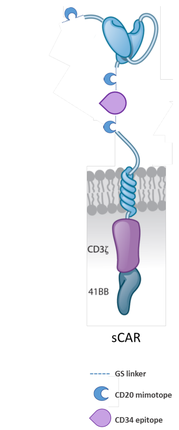
Schema of the different safeguard CAR architectures assembled. 1, 2 or 3 consecutive or distant CD20 mimotopes and an additional CD34 epitope (*Philip. B et al, Blood 2014*) were engrafted at different positions of the extracellular domain of a CAR model. A total of 16 constructions were assembled.

Identification of an optimal Safeguard CAR architecture



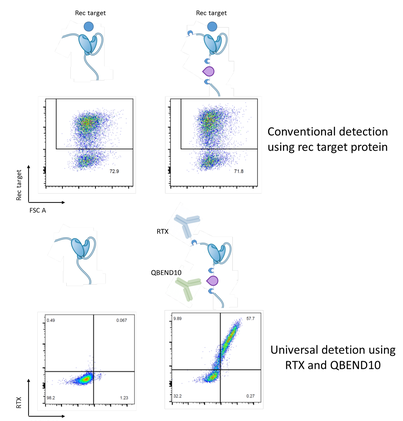
Expression and depletion screening of safeguard CAR architectures. Primary T-cell transiently expressing CAR construction were analyzed by flow cytometry after being labeled by the recombinant protein target or Rituximab **(A)**. CAR T-cells treated with 100 µg/mL RTX and complement for 150 min were analyzed by flow cytometry to determine their viability relative to an untreated control. Relative viability is displayed for each individual CAR construction, or for subgroups of constructions containing 1 mimotope (1m), 2 consecutive mimotopes (2cm) and 2 or 3 distant mimotopes (2dm and 3dm respectively) **(B)**.

SafeCAR



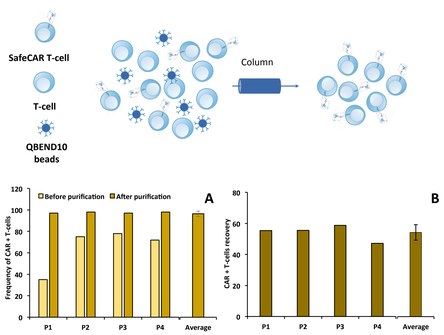
Scheme of the safeguard CAR (SafeCAR) architecture selected

Dual detection of SafeCAR



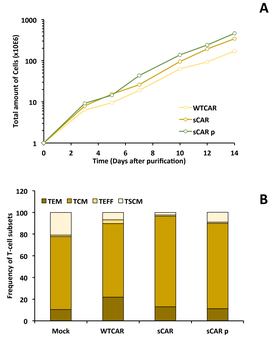
Detection of WTCAR and SafeCAR by flow cytometry using recombinant target protein or RTX/QBEND10 mAbs

Purification of SafeCAR T-cells



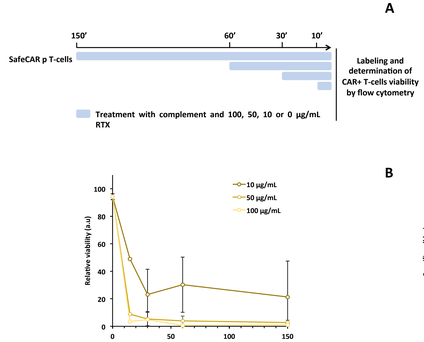
Engineered SafeCAR T-cells are efficiently purified using CD34 isolation kit. Primary T-cells transduced by lentiviral particles encoding SafeCAR (MOI 2, 3 days post CD3/CD28 activation) were grown for 4 days before being purified using CD34-specific beads. The presence of the CD34 epitope in the SafeCAR architecture enables purification to >96% homogeneity **(A)** with an average rate of recovery of ~55 % **(B)**. Results from four independent purification (P1-P4) are shown.

Proliferation of SafeCAR T-cells



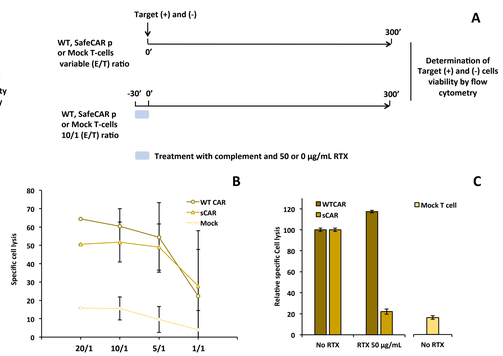
Engineered SafeCAR T-cells retain their proliferative potential. Purified (p) and non purified primary T-cells transduced by WTCAR or SafeCAR were grown for 14 days. The evolution of cell number is displayed as a function of time **(A)**. After 14 days of culture, cells were recovered and analyzed by flow cytometry to determine their composition in TSCM, TCM, TEM and TEFF subsets (respectively, T stem cell memory, T central memory, T effector memory and T effector) using CD62L and CD45RO surface markers **(B)**

Depletion of SafeCAR T-cells



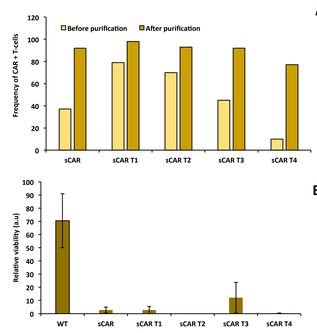
Engineered SafeCAR T-cells are efficiently and rapidly depleted by RTX. Purified SafeCAR (p) T-cells were incubated with increasing concentration of RTX (0-100 µg/mL) and complement for 150, 60, 30 or 10 min before being analyzed by flow cytometry to determine their relative viability with respect to untreated control **(A)**. Kinetic of purified SafeCAR T-cells depletion is illustrated as a function of RTX concentration **(B)**.

Cytolytic activity of SafeCAR T-cells



Engineered SafeCAR T-cells display cytolytic activity toward tumor cells and are readily inhibited by RTX treatment. Primary T-cells expressing similar level of WTCAR or SafeCAR were incubated for 5H in the presence of a stoichiometric mixture of target+ and target- labelled cells lines, at variable effector/target (E/T) ratios **(A, upper panel)**. Flow cytometric analysis of target viabilities allowed the determination the specific cell lysis that is illustrated as a function of E/T ratio **(B)**. WTCAR or SafeCAR T-cells pretreated with 50 µg/mL RTX and complement for 30 min were used to performed the activity test described above at an apparent E/T ratio of 10/1 **(A, lower panel)**. Specific cell lysis obtained after 5H incubation and relative to the one obtained without RTX pretreatment is illustrated **(C)**.

Transportability of SafeCAR architecture



SafeCAR architecture is transportable. SafeCAR architecture was implemented in four different scFvs targeting surface antigens implicated in cancer development (SafeCAR T1-T4). Primary T-cells transduced with SafeCAR were able to be purified **(A)** and were efficiently depleted by a 150' incubation in the presence of complement and 100 µg/mL RTX **(B)**.

Conclusions

Here we report the development of a novel CAR architecture (SafeCAR) comprising an integrated safeguard component which enables fast and efficient depletion of CAR T cells in the presence of the FDA-approved monoclonal antibody Rituximab. SafeCAR T cells are purifiable and detectable with commercially available antibodies, retain their proliferative potential and display cytolytic activity toward tumor cells. Finally, the SafeCAR architecture is transportable to other scFvs, making it potentially compatible with multiple tumor treatments.

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