The RevCAR T cell platform: a switchable and combinatorial therapeutic strategy for glioblastoma

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The novel RevTMs can bind bispecifically to both the RevCAR T cells and the GBM cells. Importantly, the RevCAR T cells can be activated to efficiently kill GBM both in vitro and in vivo. In conclusion, the switchable RevCAR platform is a novel therapeutic approach that provides improved safety and allows combinatorial targeting of GBM.

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Conclusion

The novel RevTMs can bind bispecifically to both the RevCAR T cells and the GBM cells. Importantly, the RevCAR T cells can be activated to efficiently kill GBM cells via the RevTMs. Moreover, we were able to prove that the Dual-RevCAR T cells can be activated only upon recognition of both EGFR and GD2, thereby allowing a highly specific and selective killing of GBM.

Figure 2. Binding of RevTMs on glioblastoma cells and RevT cells. (A) Binding of the RevTMs to RevCAR-E5B9 T cells (EGFPlow) was confirmed by staining with anti-GD2 or anti-EGFR mAb and Pacific Blue-goat anti-mouse secondary Ab. Moreover, the binding of GD2- and EGFR-specific RevTMs to U251 Luc cells was detected with APC-conjugated anti-His Ab. In histograms, light lines indicate negative or isotype control and dark lines indicate the stained cells.

Figure 3. In vitro functionality of redirected mono-RevCAR T cells. (A) U251 Luc (GD2+ EGFR+), U343 Luc (GD2+ EGFR+) or HEK 293T Luc (GD2- EGFR-) were co-cultured with RevCAR-E5B9 T cells at E:T ratio of 1:4 in the absence or the presence of 25 nM of each RevTM. Target cell lysis was then determined after 18-20 h of incubation using luminescence-based assay. (B) Cytokines were analyzed in supernatants collected from the co-culturing experiment using ELISA. (C) RevTMs were titrated in co-culture of RevCAR-E5B9 T cells and U251 Luc cells in order to determine the half-maximal effective concentration (EC50). Results are shown as mean ± SD for three independent T-cell donors (*** p < 0.0021, **** p < 0.0001; comparison to sample W/O TM; One-way ANOVA with Dunnett’s multiple comparison test).

Figure 4. In vitro and in vivo functionality of Dual-RevCAR T cells. (A) Dual-RevCAR T cells were co-cultured with either U251 Luc (GD2+ EGFR+), U343 Luc (GD2+ EGFR+) or HEK 293T Luc (GD2- EGFR-) at E:T ratio of 1:2 in the presence of RevTMs combinations or the single RevTMs as indicated in the figure (25 nM each). After 18-20 h, cytotoxicity was determined using luminescence-based assay. (B) Supernatants were collected and cytokines were analyzed using ELISA. Results are shown as mean ± SD for three independent T-cell donors (** p < 0.0021, **** p < 0.0001; comparison to sample W/O TM; One-way ANOVA with Dunnett’s multiple comparison test). (C) Five groups of NXG mice were co-injected with mixtures containing either: U251 Luc alone (Gr.1), U251 Luc + RevTM GD2-IgG4-5B9 (Gr.3) or RevTM EGFR-7B6 (Gr.4) or a combination of both RevTMs (Gr.5).

Figure 1. Schematic representation of the RevCAR system. (A) RevCAR T cells are second generation CARs which contain an epitope in their intracellular domain instead of the conventional variable chains of an antibody (Ab). RevCAR T cells can only be activated via bispecific target modules (RevTM) which recognize the RevCAR T cells on one side and glioblastoma (GBM) cells on the other side. Once these RevTMs are eliminated, RevCARs are switched off. (B) In addition, we have developed dual-targeting RevCARs which allow the control of T cells according to the AND gate logic of Boolean algebra. Dual-RevCARs have their intracellular signaling (SD) and costimulatory domain (CSD) separated into two receptors. Only when both receptors are activated, the T cells can be fully induced to kill the tumor cells.