CRS ASSESSMENT POST FAST-TRACK – CAR T CELLS
APPLICATION IN NSG-SGM3 MOUSE MODEL

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Background CRS (Cytokine Release Syndrome) is considered as a recurrent side effect of CAR (Chimeric Antigen Receptor) T cell therapy. An experimental animal model for better investigating different aspects of CRS is still in demand. Besides CAR T cells’ astonishing achievements, numerous efforts are determined on manufacturing hindrance, notably shortening production procedures. Here, we introduce a novel CRS mouse model employing the NSG-SGM3 strain to address adverse effects of FT- CAR T cells (Fast-Track CAR T).

Methods CD19-specific FT- CAR T cells were produced from 48 hours activated human PBMC plus 24h incubation with lentiviral vectors (LV). Cytotoxic activity of FT- CAR T cells was determined against Nalm-6 cells +/- same donor-derived monocyte supplementation allowed in vitro CRS assessment. Next, to launch a CRS mouse model, FT-CAR T cells were administered into NSG-SGM3 mice engrafted with Nalm-6 cells. Mice received 1x10^7 FT-CAR or activated T cells intravenously. Health condition assessment and body index calculation were regularly monitored using weight, and body temperature measurements. Human cytokines in plasma were determined by the LEGENDplex kit.

Results Flow cytometric analysis revealed that the majority of FT-CAR T cells were positive for the VSV-glycoprotein. Upon reactivation and further cultivation, 65% of all T cells converted into CAR-positive T cells by day 6. The FT-CAR T cells indicated significant cytotoxic activity against tumor cells compared to T cell control, without any contraction in presence of monocytes. The co-culture supernatant displayed significantly elevated amounts of pro-inflammatory cytokines, including IL-6, INF-γ, TNF-α, and IL-10. Next, we appointed the NSG-SGM3 mice to refine FT-CAR T cell competence in CRS induction. While all control mice were in good condition, detrimental side effects came up rapidly within 24h for all FT-CAR T-treated mice. Tremendous temperature change over 2°C and more than 10% weight loss led to termination of this group. Highly elevated cytokine levels were observed, notably enhanced for IFN-γ, TNF-α, IL-2, IL-10 (all P<0.0001), and IL-6 (P<0.0121).

Conclusions Our study introduces an appropriate CRS mouse model to substantiate the acute side effects of FT-CAR T cells. The key feature of this model is innate myeloid cells releasing cytokines upon interaction with CAR T cells. Our results suggest that FT-CAR T cells carry residual LV components and can induce at least as severe CRS as conventional CAR T cells.