Cyclosporine A suppresses IFN-γ release and reduces exhaustion while preserving CAR-T potency in a CRS model

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Background: Cytokine release syndrome (CRS) is a frequent, potentially life-threatening toxicity of CAR-T therapy. Broad immunosuppression used to control severe CRS can blunt CAR-T expansion and anti-tumor activity, potentially worsening outcomes. Strategies that dampen cytokine output without impairing tumor killing are therefore needed. Calcineurin inhibition limits NFAT/TOX-driven terminal T-cell exhaustion in preclinical transplant models,[1] while sparing NFAT-independent cytolytic function.[2] We asked whether cyclosporine A (CsA) can uncouple IFN-γ release from cytotoxic function in anti-CEACAM6 CAR-T cells.

Methods: Second-generation anti-CEACAM6 CAR-T cells as bulk, or $CD4^+$- or $CD8^+$-selected CAR^+ populations (immunomagnetic selection) were co-cultured with $CEACAM6^+$ BxPC3 pancreatic carcinoma cells, with or without CsA (1250 ng/mL). After 48 h, IFN-γ, cytotoxicity, and phenotype were assessed; cells were then washed, transferred to fresh BxPC3, and the cycle repeated. At each time point IFN-γ was measured by chemiluminescent immunoassay (CLIA), cytotoxicity by live-cell imaging (target-confluence loss) and LDH release, and exhaustion markers (PD-1, LAG-3, TIM-3) by flow cytometry.

Results: CsA reduced bulk IFN-γ ~30-fold versus untreated cells without compromising cytotoxicity. The effect was largely confined to $CD4^+$ CAR-T cells, the dominant IFN-γ source (78,334 vs 2,244 RLU; ~35-fold), whereas $CD8^+$ cells produced lower baseline IFN-γ (25,962 RLU) that CsA reduced only ~8-fold (3,458 RLU). The proportion of CAR^+ lymphocytes co-expressing PD-1, LAG-3, and TIM-3 fell from ~20% to ~6% with CsA. CsA-exposed CAR-T transferred to fresh BxPC3 remained potent and eliminated all targets.

Conclusion: CsA selectively suppressed CD4-derived IFN-γ potentially reducing CRS risk while preserving cytotoxicity in both $CD4^+$ and $CD8^+$ CAR-T cells, and reduced exhaustion-marker expression. Calcineurin inhibition is a candidate strategy to mitigate CRS without compromising CAR-T anti-tumor potency.

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