DRUŠTVO ZA CELIČNO IN TKIVNO INŽENIRSTVO SLOVENIJE
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‘Next-generation Chimeric Antigen Receptor (CAR) T Cells’
‘Naslednja generacija celic CAR-T’

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Abstract: T cell-intrinsic dysfunctions and immunosuppressive tumor microenvironment (TME) influence clinical efficacy of CAR T cells. We recently discovered that inducible expression of transcription factors or immunostimulatory molecules improved functional qualities and augmented anti-tumor activity of CAR T cells in preclinical *in vivo* models. We have developed genetic approach that combines autonomous antigen-triggered production of an accessory molecule, along with constitutive CAR expression in a single lentiviral vector - **Uni-Vect**. By knocking out the endogenous TCR we render CAR signaling an exclusive activator of the system. To modulate CAR T cell-intrinsic features we implemented Uni-Vect for transient, activation-inducible transcription factor expression (iTF-CAR T). In a second model, we introduced inducible expression of IL-12 (iIL-12-CAR T) to overcome immunosuppressive TME. **iTF-CAR T** cells demonstrated enhanced antigen-dependent proliferation and a less differentiated phenotype following repeated stimulations with cancer cells *in vitro*. CyTOF analysis of iTF-CAR T cells showed that antigen-inducible expression of a single TF favorably affected T cell markers of efficacy. Finally, we tested activity *in vivo* in tumor xenografts models where iTF-CAR T cells demonstrated significantly increased expansion in the blood compared to control CAR T cells. Importantly, T cells expansion was transient and ultimately contracted to the normal levels after tumor was cleared. iTF-CAR T approach addresses challenges related to intrinsic CAR T cell dysfunctions, however, it may not directly counteract immunosuppressive TME. Therefore, we have developed an **iIL-12-CAR T** system and demonstrated that only iIL-12-secreting, and not conventional CAR T cells, were capable of eradicating solid tumors *in vivo*. Together, we demonstrated that inducible TF expression equips CAR T cells with improved therapeutically relevant T cell states, which translates into improved *in vivo* T cell expansion, and that iIL-12 expression remarkably enhanced anti-tumor responses in established solid tumors *in vivo*. With these contributions, we have established a foundation for more effective next-generation cellular immunotherapies.

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