

Designing the next generation of chimeric Antigen receptors for Regulatory T cell therapy through in silico modeling-guided single chain Fv engineering.

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Chimeric antigen receptors (CAR) are synthetic receptors comprising an extracellular binding domain, frequently a single chain Fv (scFv) and an intracellular signaling domain. Whereas mainly described for the generation of tumor-specific T effector (Teff) cells, this approach represents a promising novel approach for generating antigen-specific regulatory T (Treg) cell-based therapies. scFv can be generated from virtually all antibodies from murine and human. However, scFv stability and/or folding properties affect its surface expression level, CAR Teff and Treg function as well as tonic signaling triggered by antigen-independent clustering of scFv molecules on cell surface. The differences in stability and biophysical properties are determined by their germline sequence and influenced by somatic hypermutations in the framework regions. Therefore, appropriate scFv engineering could be a powerful solution to improve CAR function. We established an iterative process for reverting hypermutations to germline consensus and introducing stability improving amino acids into weak germline sequences using computational modeling to work out the influence of biophysical scFv properties on CAR function. We will present the optimization of mCD19 hybridoma 1D5 derived scFv, which leads to improved surface expression and enhanced functionality in Jurkat cells as well as T eff and T reg cells. It is worth noting that the tonic signaling is reduced compared to the original scFv derived CAR. The potential mechanisms underlying the improved folding stability will be discussed.