Designing the next generation 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) comprise an extracellular binding domain, frequently a single chain Fv (scFv), and an intracellular signaling domain. Whereas mainly described so far to generate tumor-specific T effector (Teff) cells, this approach is also promising to develop antigen-specific regulatory T (Treg) cell-based therapies. scFv can be generated from virtually any murine or human antibody. However, scFv stability and/or folding properties affect its surface expression level, CAR Teff and Treg function as well as antigen-independent tonic signaling. 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. Using computational modeling, we established an iterative process for reverting hypermutations to germline consensus and introducing stability-improving amino acids into weak germline sequences. We will present the optimization of mCD19 hybridoma 1D5 derived scFv, which leads to improved surface expression and enhanced functionality in the CAR construct. 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.

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