Engineered mRNA backbones for gene expression in human T cells

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Background: mRNA-based CAR-T cell engineering offers a safer, transient, and redosable alternative to viral gene delivery, but conventional α-globin (HBA1) 5' untranslated regions (UTRs) used in most mRNA constructs are not optimized for T cell biology. We hypothesized that 5' UTRs derived from genes intrinsically regulated during T cell activation and exhaustion could be repurposed to fine-tune transgene expression and function in engineered T cells. Method: A library of 13 candidate 5' UTRs derived from T cell cytokines (IL2, IFNG, TNF), effector molecules (GZMB, CD39), and checkpoint genes (PD1, TIM3, TIGIT, LAG3, others) replaced the HBA1 5' UTR in in vitrotranscribed, pseudouridine-modified mRNA encoding EGFP, luciferase, or a CD19-CAR. Constructs were electroporated into primary human T cells, tumor-infiltrating lymphocytes, and HEK293 cells, and evaluated by flow cytometry, luminescence, RNAfold-based structure/stability prediction, and functional co-culture assays against CD19-positive and CD19-knockout NALM6 targets Results: Reporter expression varied substantially by UTR identity, with IFNG UTR enhancing and TNF UTR reducing expression; these effects did not correlate with predicted RNA secondary-structure stability and were T cell-specific, as HEK293 cells did not reproduce the pattern. In CD19-CAR constructs, the TIGIT 5' UTR produced the highest antigen-specific reactivity despite CAR expression comparable to or lower than HBA1, while limiting tonic signaling and exhaustion marker upregulation (TIM-3, PD-1/TIM-3 double-positivity) compared with HBA1- and TNF-based constructs. Conclusion: 5' UTR engineering enables empirical, expression-independent fine-tuning of CAR-T reactivity and exhaustion phenotype that cannot be predicted from RNA stability alone. The TIGIT 5' UTR offers a favorable balance of high antigen reactivity and low tonic signaling, supporting UTR selection as a practical tool for optimizing safety and efficacy in nonviral, mRNA-based CAR-T platforms.