UTR optimization enhances antigen presentation and CD8⁺ T-cell immunity in mRNA cancer vaccines

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Background: mRNA vaccines are a promising cancer immunotherapy platform due to their rapid manufacturability and ability to generate potent immune responses, yet many current approaches elicit insufficient CD8⁺ T-cell responses, limiting their efficacy against tumors. While codon optimization and nucleoside modification can enhance mRNA performance, the contribution of untranslated regions (UTRs) to antigen presentation and T-cell immunity remains poorly understood. Methods: We generated mRNA constructs encoding eGFP, ovalbumin (OVA), or the melanoma antigen TRP2 with alternative UTRs and formulated them into lipid nanoparticles (LNPs) based on approved BioNTech/Pfizer formulations. Translation and MHC I antigen presentation were assessed in dendritic cells by flow cytometry, and bulk RNA sequencing characterized UTR-dependent transcriptional programs. In vivo studies in C57BL/6 mice evaluated antigen-specific CD8⁺ T-cell priming, memory formation, and antitumor efficacy in prophylactic and therapeutic B16F10 melanoma models, alone or combined with immune checkpoint blockade (ICB). Results: Among the constructs tested, UTR6 emerged as the lead candidate, significantly enhancing antigen expression and MHC I presentation relative to UTRs used in clinically approved or clinicalstage mRNA vaccine platforms, including those from BioNTech and Moderna. RNA sequencing revealed that UTR identity differentially shaped transcriptomic responses, modulating pathways involved in interferon signaling, antigen processing, and inflammation, with specific UTRs attenuating inflammatory programs associated with mRNA reactogenicity. Unmodified UTR6- containing mRNA-LNPs elicited stronger and more durable antigen-specific CD8⁺ T-cell responses, marked by increased IFN-γ and TNF-α production and greater polyfunctionality. In both prophylactic and therapeutic B16F10 models, UTR6-based constructs delayed tumor progression and improved survival, with superior efficacy maintained in aged mice with prior vaccination against unrelated antigens. Further therapeutic benefit was observed in combination with checkpoint blockade. Conclusions: These findings establish UTR engineering as a powerful strategy for optimizing nextgeneration mRNA cancer vaccines, improving antigen presentation and sustaining functional CD8⁺ T-cell immunity to strengthen therapeutic efficacy.
Keywords: mRNA vaccines; Cancer vaccines, UTR engineering; CD8⁺ T cells; cancer immunotherapy.