128 DEVELOPMENT OF AN M1-POLARIZED, NON-VIRAL CHIMERIC ANTIGEN RECEPTOR MACROPHAGE (CAR-M) PLATFORM FOR CANCER IMMUNOTHERAPY

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Background We have previously developed CAR-M as a novel cell therapy approach for the treatment of solid tumors.[1] CAR-M have the potential to overcome key challenges that cell therapies face in the solid tumor setting – tumor infiltration, immunosuppression, lymphocyte exclusion – and can induce epitope spreading to overcome target antigen heterogeneity. While macrophages transduced with the adenoviral vector Ad5f35 (Ad CAR-M) traffic to tumors, provide robust anti-tumor activity, and recruit and activate T cells, we sought to identify a robust non-viral method of macrophage engineering in order to reduce the cost of goods, manufacturing complexity, and potential immunogenicity associated with viral vectors.

Methods As innate immune cells, macrophages detect exogenous nucleic acids and respond with inflammatory and apoptotic programs. Thus, we sought to identify a means of mRNA delivery that avoids recognition by innate immune sensors. We screened a broad panel of mRNA encoding an anti-HER2 CAR comprising multiplexed 5'Cap and base modifications using an optimized and scalable electroporation approach and evaluated the impact of interferon-β priming on CAR-M phenotype and function.

Results We identified the optimal multiplexed mRNA modifications that led to maximal macrophage viability, transfection efficiency, intensity of CAR expression, and duration of expression. Non-viral HER2 CAR-M phagocytosed and killed human HER2+ tumor cells. Unlike Ad CAR-M, mRNA CAR-M were not skewed toward an M1 state by mRNA electroporation. Priming non-viral CAR-M with IFN-β induced a durable M1 phenotype, as shown by stable upregulation of numerous M1 markers and pathways. IFN-β priming significantly enhanced the anti-tumor activity of CAR but not control macrophages. IFN-β primed mRNA CAR-M were resistant to M2 conversion, maintaining an M1 phenotype despite challenge with various immunosuppressive factors, and converted bystander M2 macrophages toward M1. Interestingly, priming mRNA CAR-M with IFN-β significantly enhanced the persistence of CAR expression, overcoming the known issue of rapid mRNA turnover. RNA-seq analysis revealed that IFN-β priming affected pathways involved in increasing translation and decreasing RNA degradation in human macrophages.

Conclusions We have established a novel, optimized non-viral CAR-M platform based on chemically modified mRNA and IFN-β priming. IFN-β priming induced a durable M1 phenotype, improved CAR expression, improved CAR persistence, led to enhanced anti-tumor function, and rendered resistance to immunosuppressive factors. This novel platform is amenable to scale-up, GMP manufacturing, and represents an advance in the development of CAR-M.

REFERENCE

1. Klichinsky M, Ruella M, Shestova O, *et al*. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 2020;**38**(8):947–953.

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129 A NOVEL CAR CONDUCTING ANTIGEN-SPECIFIC JAK-STAT SIGNALS DEMONSTRATES SUPERIOR ANTITUMOR EFFECTS WITH MINIMAL UNDESIRED NON-SPECIFIC ACTIVATION

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Background Despite recent impressive successes in chimeric antigen receptor (CAR)-T cell therapy, there are still considerable clinical challenges. To improve T cell persistence and antitumor effect, which are critical for clinical responses, various efforts have been made to optimize the CAR design such as the inclusion of a costimulatory domain(s). It is known that non-specific activation of CAR-T cells is greatly influenced by the CAR design, and excessive T cell activation leads exhaustion of T cells and depletion of naïve/memory subsets important for durable clinical responses. Thus, the CAR construct needs to be optimized so that transduced T cells persist and induce potent antigen-specific response with reduced non-specific activation. For optimal T cell activation and proliferation, three signals including TCR (signal 1), co-stimulatory (signal 2), and cytokine (signal 3) signals, are essential. The conventional second and third generation CARs containing CD3ζ and a co-stimulatory domain such as a signal domain of CD28 and 4-1BB can conduct signal 1 and 2, but not signal 3. Recently, we have developed a new generation JAK-STAT CAR composed of a truncated cytoplasmic domain of the IL-2 receptor β chain and STAT3/5 binding motifs, CD28 co-stimulatory domain, and CD3ζ domain. The novel anti-CD19 JAK-STAT CAR-T cells showed antigen-specific activation of the JAK-STAT signaling pathway, enhanced proliferation, and limited terminal differentiation in vitro compared to second generation 28ζ CAR or 4-1BBζ CAR-transduced T cells. Furthermore, the anti-CD19 JAK-STAT CAR-T cells demonstrated superior in vivo persistence and antitumor effect in mouse models.[1] In addition, we previously showed that a hinge region and the composition of a single chain variable fragment (scFv) such as the order of VH and VL regions critically influence not only antigen-dependent activation but also undesired antigen-independent activation known as tonic signaling.[2]

Methods In this study, we have optimized the scFv design in 28ζ CAR and JAK-STAT CAR constructs to show superior antigen-specific activation and reduced tonic signaling for several targets (CD19, CD20, Mesothelin, and GD2). And we have evaluated the feature of JAK-STAT CAR-T cells compared to 28ζ CAR-T cells.

Results JAK-STAT CAR-T cells showed superior antigen-specific proliferation with less differentiated status, whereas 28ζ CAR-T cells showed antigen-independent proliferation and displayed higher exhaustion marker expression after repetitive stimulations.

Conclusions These results suggest that our JAK-STAT-CARs with enhanced antigen-specific response with minimized tonic signaling targeting various antigens has the potential to demonstrate improved clinical efficacy.

REFERENCES

1. Kagoya Y, *et al*. A novel chimeric antigen receptor containing a JAK–STAT signaling domain mediates superior antitumor effects. *Nat Med* 2018;**24**:p352–359.
2. Okamoto S, *et al*. Detail analysis of non-specific activation of CD19 CAR-T cells caused by CAR design. ASGCT (2015)

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0129

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