ELSEVIER

Blood 142 (2023) 2073

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Deploying an RNA-Based Gene Writer System and Lipid Nanoparticle (LNP) Delivery to Generate Functional Chimeric Antigen Receptor (CAR) T Cells with in Vitro and In Vivo Anti-Tumor Activity

Michael S Magee[1], *Alberto De Iaco, PhD*[1], *Jason Rodriguez, PhD*[1], *James B. Rottman*[1], *Donghui Liu, PhD*[1], *Giulia Schiroli*[1], *Pooja Sabhachandani, PhD*[1], *Yu Cao*[1], *Rebecca Levy, MS*[1], *Mohit Gupta*[1], *Michael T. Monte*[1], *Jane Wang, PhD*[1], *Bill Querbes, PhD*[1], *Cecilia Cotta-Ramusino, PhD*[1], *Madhusudan V Peshwa, PhD*[1], *Michael C Holmes, PhD*[1]

[1] Tessera Therapeutics, Somerville, MA

The FDA approval of autologous Chimeric Antigen Receptor (CAR) T-cell therapies have added a powerful tool in the armamentarium to treat relapsed and refractory leukemias and lymphomas. However, significant challenges limit these therapies from accommodating current patient demand including supply chain limitations and wait times associated with availability of GMP quality viral vectors and long needle-to-needle time from initiation of treatment to receiving the final drug product. As of yet other competitive therapies including bi-specific antibodies and allogeneic CAR-T have not achieved the same level of therapeutic efficacy as conventional autologous T cell therapies highlighting the critical unmet need for same day treatment options for autologous CAR-T products.

RNA Gene Writers leverage target-primed reverse transcription (TPRT) biochemistry evolved from non-LTR retrotransposon mobile genetic elements to modify the genetic information in cells using RNA templates and without the need to introduce DNA breaks. Moreover, RNA Gene Writers can be engineered to catalyze a variety of editing reactions, such as the introduction of gene-length DNA sequences, substitutions, insertions, and deletions. These edits can be achieved with all-RNA delivery in primary cells and *in vivo*, eliminating the necessity for viral vectors and DNA template-based gene editing.

LNP-RNA delivery of RNA Gene Writers resulted in the integration and expression of transgenes in >60% of primary human T cells *in vitro*. Furthermore, our LNPs deliver RNA to T cells *in vivo*, with 80% reporter expression in a humanized mouse model and 45% in non-human primates (NHP). We have demonstrated LNP delivery of RNA Writers into primary human T cells with an RNA template encoding a CAR cassette can achieve >20% CAR+ T cells without detriments to cell viability or proliferative capacity and have demonstrated ability to mediate tumor cell killing *in vitro*. Moreover, RNA Gene Writer derived CAR-T cells can be introduced into mouse xenograft models to clear antigen specific tumors *in vivo*. CAR mRNA can also be packaged into proprietary LNP formulations and delivered to primary human T cells in a mixture of lymphocytes commonly found in patient leukapheresis to generate CAR-T cells *in vitro*. This opens the possibility of using RNA Gene Writers to develop a same-day CAR-T treatment.

In addition, the modularity of our RNA Gene Writing technology allows multiplex editing to co-introduce multiple genetic changes including generation of universal and more potent CAR-T cells through the knock-out of B2M and TRAC at comparable levels (>80% double knockout) to Cas9 nucleases. Further, we show that we can achieve both edits simultaneously in 80% of T cells that have achieved CAR transgene insertion via another RNA Gene Writer enzyme. Importantly, we have demonstrated successful B2M and TRAC double knock-out without inducing translocations (undetectable vs 8% translocations with Cas9).

RNA Gene Writing technology is uniquely positioned for same-day delivery of all-RNA components toenable a wide range of editing applications, from gene knock-out to gene integration. The ability to package the RNA Gene Writer system into LNPs unlocks the potential for the genetic engineering of autologous T cell therapies to enable same-day delivery a current limitation of conventional lentiviral-based CAR-T therapies.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-184755