332 CRISPR/CAS9-BASED INTEGRATION OF A LARGE AND MODULAR CASSETTE INTO A SAFE HARBOR SITE TO IMPROVE CAR T CELL THERAPY EFFICACY AND SAFETY

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Background Chimeric antigen receptor (CAR) T cell therapy has emerged as an important new tool in the treatment of cancers. However, the complexity of the enhancements used is limited by the amount of genetic information that can be integrated into the genome. Our approach utilizes Integrated Circuit T (ICT) cells, which are engineered to include a large DNA cassette that includes: receptor strategies to target multiple tumor antigens; transcriptional modifications that alter cell state; engineered cytokines and chemokines and variations in the CAR binding and signaling domains.

Our first ICT clinical program, AB-1015, is an autologous cell product for the treatment of ovarian cancer. The AB-1015 transgene cassette consists of a logic gate directed against ALPG/P and MSLN and an shRNA-miR module targeting FAS and PTPN2 that enhance potency and confer resistance to the tumor microenvironment. This transgene is delivered into primary T cells via non-viral, site-specific editing into a safe-harbor locus via CRISPR integration of transgenes by electroporation (CITE). CITE has many advantages over viral and other non-viral random integration methods, including more predictable transgene expression and function, reduced risk of unsafe insertional mutagenesis, and efficient integration of large cassettes.

Methods To identify candidate genomic loci for CITE-directed gene insertion we used epigenetic analysis, transcriptional profiling, and high-throughput gene-editing of primary T cells. Loci were further characterized using T cell functional assays. Knock-in efficiency and transgene expression stability in primary human T cells were evaluated for all loci. Lead candidate loci were tested for compatibility with complex T cell programs embodied by our integrated circuits, containing a priming receptor (PrimeR, ALPG/P) that triggers the expression of a CAR (MSLN) in response to a priming antigen. The top insertion site, GS94, was further characterized using in silico and empirical approaches.

Results GS94 was identified as an optimal locus for CITE-directed gene insertion based upon: 1) stable and high PrimeR expression; 2) high and inducible CAR expression; and 3) a superior T cell cytotoxic and cytokine secretion profile. We were unable to identify any off-target events generated by CITE at GS94, including off-target editing, knock-in and translocations, using a suite of molecular assays including iGUIDE, rhAMPseq, deep whole genome sequencing, and anchored-PCR.

Conclusions CITE editing at GS94 is specific and generates highly functional ICT cells. This novel approach to engineering tumor-specific T cells enables the generation of exceptional clinical candidates that both target new cancer types and improve efficacy.

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