VariantFind™ for CAR-T Immunotherapy Applications

BACKGROUND:

A chimeric antigen receptor (CAR) is a fusion protein consisting of an extracellular antigen-recognition domain and an intracellular T-cell activation/signalling domain. This bi-functional protein couples the antigen recognition properties of antibodies with the cytotoxic activity of T-cells. Synthetic CARs can be introduced into patient derived T-cells, resulting in a CAR-T cell, which effectively reprograms the T-cell to target malignancies. A well-characterized example of CAR-T immunotherapy is the adoption of anti-CD19 CAR-T cells to treat B-cell lymphoma in patients. Three key features of CAR-Ts are their anti-tumour efficacy (i.e. tumour elimination), their sustained proliferation and expansion, and their ability to persist in the patient. There are currently three generations of CARs, which differ in their intracellular T-cell activation/signalling domain. First generation CARs employ the CD3ζ chain of the T-cell receptor complex, while second and third generation CARs employ additional one or two co-stimulatory domains, respectively. Co-stimulatory domains such as CD28 and 4-1BB serve to improve CAR-T anti-tumour efficacy, proliferation/expansion, and persistence.

VariantFind™ is a DNA variant library production platform that enables transformative discovery. By providing our partners with DNA variant libraries, VariantFind™ enables rapid high-throughput engineering and optimization of CAR molecules. Our pipeline can assist in the following applications:

APPLICATION 1: Targeting the extracellular antigen-recognition domain.

Most extracellular domains of CARs are engineered to recognize and bind tumour cell markers (e.g. anti-CD19). These extracellular domains are derived from the single chain variable fragment of any known monoclonal antibody. To improve specificity for the targeted antigen, VariantFind™ offers saturation, combinatorial and scanning variant libraries of the antigen-binding domain. VariantFind™ libraries are used to screen (e.g. by phage display) for antigen-recognition domains with improved affinity, specificity, and recognition for the antigen. Enhanced binding ultimately improves the ability of CAR-T cells to target malignant cells and ignore healthy cells.

APPLICATION 2: Targeting the intracellular T-cell activation and signalling domains.

The intracellular moiety of CARs is critical for successful T-cell activation, proliferation, and persistence. The addition of co-stimulatory domains in second and third generation CARs illustrates the importance of engineered co-stimulation in the success of CAR-T cell therapy. Using proprietary technology to build saturation, combinatorial and scanning variant libraries of co-stimulatory domains, VariantFind™ offers a systematic approach to engineering the intracellular moiety of CARs for enhanced tumour elimination, CAR-T cell proliferation and persistence.

APPLICATION 3: Targeting promoters of CARs.

A frequently used approach for integration of DNA encoding CARs into T-cell genomes is viral transduction. A critical control element in the expression of CAR proteins is the promoter DNA element upstream of the CAR. In addition to building libraries on coding sequence, VariantFind™ technology is also applicable to non-coding DNA sequence. Using VariantFind™ to generate a library of promoter DNA variants, expression of CARs may be enhanced to improve antigen binding and tumour elimination.