Preclinical evaluation of ALLO-819, an allogeneic CAR T cell therapy targeting FLT3 for the treatment of acute myeloid leukemia

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ABSTRACT: Autologous chimeric antigen receptor (CAR) T cells have achieved unprecedented clinical responses in patients with B-cell malignancies, lymphomas and multiple myeloma, raising interest in using CAR T cell therapies in AML. These therapies are produced using a patient’s own T cells, an approach that has inherent challenges, including requiring significant time for production, complex supply chain logistics, separate GMP manufacturing for each patient, and variability in performance of patient-derived cells. Given the rapid pace of disease progression compared with limitations associated with the autologous approach and treatment-induced toxicities, many patients with AML may not receive treatment. Allogeneic CAR T (ALLO-819 T) cell therapies, which utilize cells from healthy donors, may provide greater convenience with readily available off-the-shelf CAR T cell therapy providing reliable product consistency, and accessibility at greater scale for more patients. To create an allogeneic product, the TSCC and CD52 genes are inactivated via Transfection Activation Defective Enzyme Nuclease (TALEN®) technology. These genomic modifications are intended to minimize the risk of graft-versus-host disease and to confer resistance to ALLO-819, an anti-CD52 antibody that can be used as part of the conditioning regimen to deplete host alloreactive immune cells potentially leading to increased persistence and efficacy of the infused allogeneic cells.

We have previously described the functional screening of a library of anti-FLT3 single chain fragment variable (scFvs) and the identification of a lead FLT3 CAR with optimal antigen specificity and activity. circuits were cultured ex vivo with luciferase-expressing FLT3+ AML target cells, and tumor burden was monitored by bioluminescence. Values are expressed as mean ± SEM (N=10 animals/group). (B) ALLO-819 manufactured from multiple donors was insensitive to ALLO-647 (100 µg/mL) in vitro and in vivo, indicating no effects of TALEN® treatment on CAR T cell activity. Plasma levels of FLT3 are frequently increased in patients with AML and correlate with tumor burden, raising the possibility that ALLO-819 may act as a decay of FcεR1 on macrophages. To rule out an inhibitory effect of ALLO-819 on FLT3 expression, a luciferase expression assay was performed using a recombinant protein consisting of the extracellular domain of the CAR fused to human IgG Fc. Consistent with the limited expression pattern of FLT3 and the indication of the high specificity of the lead scFv, no appreciable membrane binding was detected in any of the 36 normal tissues tested (n=3 donors). Taken together, our results support clinical development of ALLO-819 as a novel and effective CAR T cell therapy for the treatment of AML.

(1) ALLO-819 is manufactured using healthy donor T cells that are engineered to express a CAR directed against FLT3, a receptor tyrosine kinase with high expression in AML (97% & 99.7%). CAR expression was further modified using TALEN®-gene editing technology to reduce TCR and CD52 expression, potentially minimizing the risk of GvHD and increasing CAR T cell persistence in patients undergoing αβ T cell depletion. ALLO-819 was less sensitive to CD52 depletion via CDC and ADCC. (B) ALLO-819 was resistant to the αβ-50µg/mL ALLO-647 antibody ALLO-819, whereas non-gene-edited CAR T cells were sensitive to ALLO-647. (C) ALLO-819 CD52 KO is isolation ALLO-819 as a novel and effective off-the-shelf CAR T cell therapy for the treatment of AML.

CONCLUSIONS: ALLO-819 exhibits robust antigen activity in vitro and in vivo. The efficacy of ALLO-819 is not affected by treatment with TALEN®, and is comparable to or higher than that observed with previously characterized anti-FLT3 scFvs. The high specificity of the scFv’s and the absence of apparent neurological toxicity observed in preclinical models indicate that off-target effects of ALLO-819 may be limited, and that ALLO-819 is a promising therapeutic option for patients with AML.