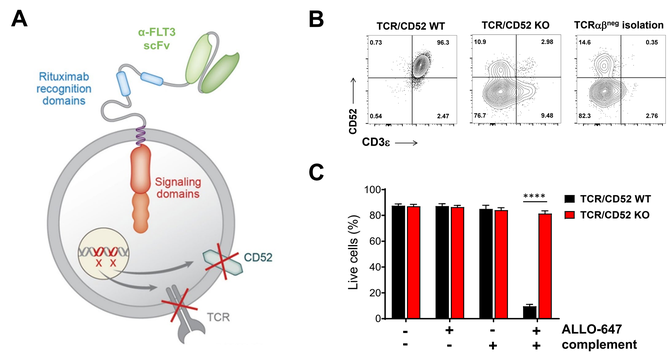
Preclinical evaluation of ALLO-819, an allogeneic CAR T cell therapy targeting FLT3 for the treatment of acute myeloid leukemia

Cesar Sommer[1], Hsin-Yuan Cheng[1], Yik Andy Yeung[2], Duy Nguyen[1], Janette Sutton[1], Zea Melton[1], Julien Valton[3], Kris Poulsen[1], Julianne Smith[3], Ivana Djuretic[2], Thomas Van Blarcom[1], Javier Chaparro-Riggers[2], Barbra Sasu[1]

[1]Allogene Therapeutics, Inc., 210 E. Grand Avenue, South San Francisco, CA 94080, USA; [2]Pfizer Inc., 230 E. Grand Avenue, South San Francisco, CA 94080, USA; [3]Cellectis Inc., 430 East 29th Street, New York, NY 10016, USA

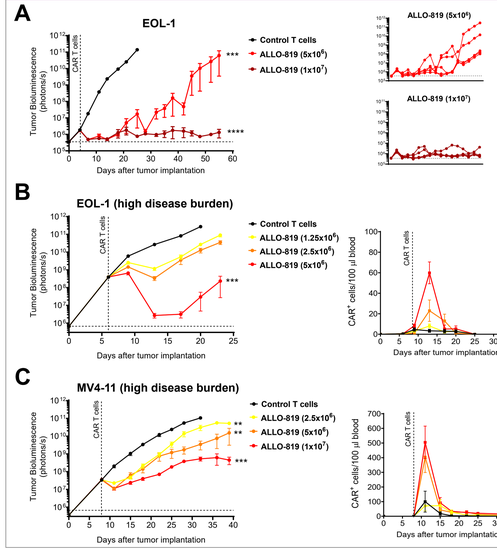
ABSTRACT: Autologous chimeric antigen receptor (CAR) T cells have achieved unprecedented clinical responses in patients with B-cell leukemias, lymphomas and multiple myeloma, raising interest in using CAR T cell therapies in AML. These therapies are produced using a patient's own T cells, an approach 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 combined with limitations associated with the autologous approach and treatment-induced lymphopenia, many patients with AML may not receive treatment. Allogeneic CAR T (AlloCAR T™) cell therapies, which utilize cells from healthy donors, may provide greater convenience with readily available off-the-shelf CAR T cells on-demand, reliable product consistency, and accessibility at greater scale for more patients. To create an allogeneic product, the *TRAC* and *CD52* genes are inactivated in CAR T cells using Transcription Activator-Like Effector Nuclease (TALEN®) technology. These genetic modifications are intended to minimize the risk of graft-versus-host disease and to confer resistance to ALLO-647, 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 variable fragments (scFvs) and the identification of a lead FLT3 CAR with optimal activity against AML cells and featuring an off-switch activated by rituximab. Here we characterize ALLO-819, an allogeneic FLT3 CAR T cell product, for its antitumor efficacy and expansion in orthotopic models of human AML, cytotoxicity in the presence of soluble FLT3 (sFLT3), performance compared with previously described anti-FLT3 CARs and potential for off-target binding of the scFv to normal human tissues. To produce ALLO-819, T cells derived from healthy donors were activated and transduced with a lentiviral construct for expression of the lead anti-FLT3 CAR followed by efficient knockout of *TRAC* and *CD52*. ALLO-819 manufactured from multiple donors was insensitive to ALLO-647 (100 µg/mL) in *in vitro* assays, suggesting that it would avoid elimination by the lymphodepletion regimen. In orthotopic models of AML (MV4-11 and EOL-1), ALLO-819 exhibited dose-dependent expansion and cytotoxic activity, with peak CAR T cell levels corresponding to maximal antitumor efficacy. Intriguingly, ALLO-819 showed earlier and more robust peak expansion in mice engrafted with MV4-11 target cells, which express lower levels of the antigen relative to EOL-1 cells (n=2 donors). To further assess the potency of ALLO-819, multiple anti-FLT3 scFvs that had been described in previous reports were cloned into lentiviral constructs that were used to generate CAR T cells following the standard protocol. In these comparative studies, the ALLO-819 CAR displayed high transduction efficiency and superior performance across different donors. Furthermore, the effector function of ALLO-819 was equivalent to that observed in FLT3 CAR T cells with normal expression of TCR and CD52, indicating no effects of TALEN® treatment on CAR T cell activity. Plasma levels of sFLT3 are frequently increased in patients with AML and correlate with tumor burden, raising the possibility that sFLT3 may act as a decoy for FLT3 CAR T cells. To rule out an inhibitory effect of sFLT3 on ALLO-819, effector and target cells were cultured overnight in the presence of increasing concentrations of recombinant sFLT3. We found that ALLO-819 retained its killing properties even in the presence of supraphysiological concentrations of sFLT3 (1 µg/mL). To investigate the potential for off-target binding of the ALLO-819 CAR to human tissues, tissue cross-reactivity studies were conducted 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 indicative of the high specificity of the lead scFv, no appreciable membrane staining 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.

ALLO-819 is designed to minimize the risk of GvHD and to exhibit resistance to the lymphodepleting α-CD52 antibody ALLO-647



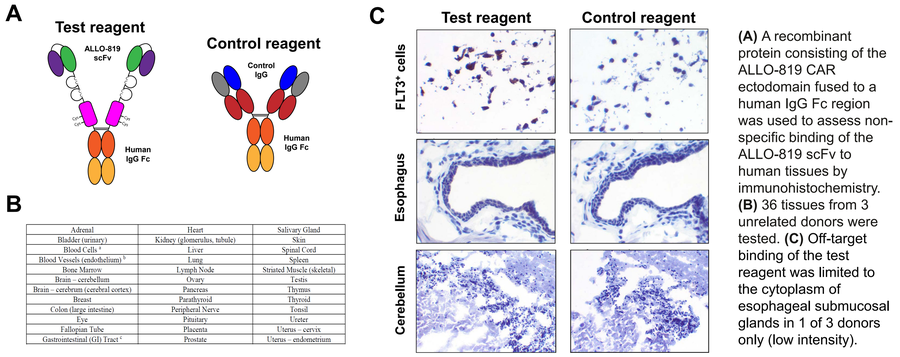
(A) ALLO-819 is manufactured using healthy donor T cells that are engineered to express a CAR directed against FLT3, a receptor tyrosine kinase with high expression in AML stem cells (PMID:15797998). CAR-expressing cells are further modified using TALEN® gene-editing technology to disrupt TCRα and CD52 expression, potentially minimizing the risk of GvHD and increasing CAR T cell persistence in patients undergoing α-CD52 antibody-based conditioning regimens. An off-switch activated by rituximab is included between the scFv and the linker regions of the CAR to enable CAR T cell depletion via CDC and ADCC. **(B)** Representative FACS plots show expression of CD3ε (as a surrogate for the TCRαβ complex) and CD52 in non gene-edited (TCR/CD52 WT) and gene-edited (TCR/CD52 KO) CAR T cells before and after TCRαβ$^+$ cell depletion. **(C)** ALLO-819 was resistant to the α-CD52 antibody ALLO-647, whereas non-gene-edited CAR T cells were depleted. CAR T cells were incubated for 3 hours with complement and ALLO-647 (100 µg/ml) and residual live cells were measured by flow cytometry. Results are expressed as mean ± SEM. N=4 donors (****p<0.0001, t-test).

ALLO-819 shows dose-dependent expansion and antitumor activity in orthotopic models of AML



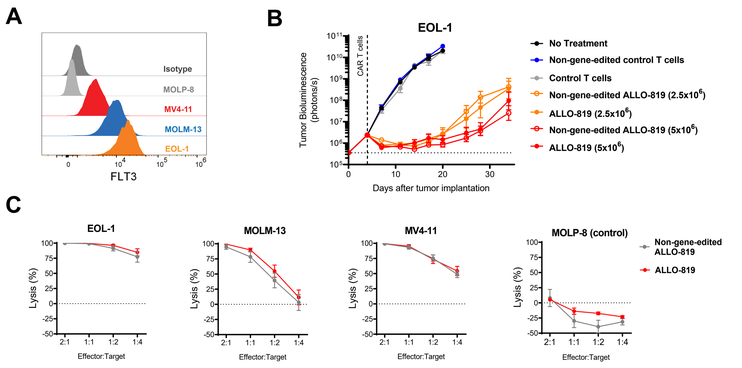
(A) NSG mice were inoculated with luciferase-expressing EOL-1 cells and tumor burden was monitored by bioluminescence (BLI). ALLO-819 showed dose-dependent antitumor activity and induced sustained responses in mice treated at the highest dose. **(B)** A similar study as shown in (A) was conducted by treating mice with higher tumor burden, as determined by BLI, with sub-optimal doses of ALLO-819. Peak CAR T cell expansion, measured by flow cytometry analysis of peripheral blood, correlated with maximal antitumor activity. **(C)** ALLO-819 exhibited dose-dependent antitumor efficacy and CAR T cell expansion in the MV4-11 orthotopic model. Results in **(B)** and **(C)** are representative of two experiments with different donors. Values are expressed as mean ± SEM (**p<0.01, ***p<0.001, ****p<0.0001, one-way ANOVA with Dunnett's post-hoc test for multiple comparisons versus control T cells).

ALLO-819 scFv shows limited off-target binding to human tissues in tissue cross-reactivity studies



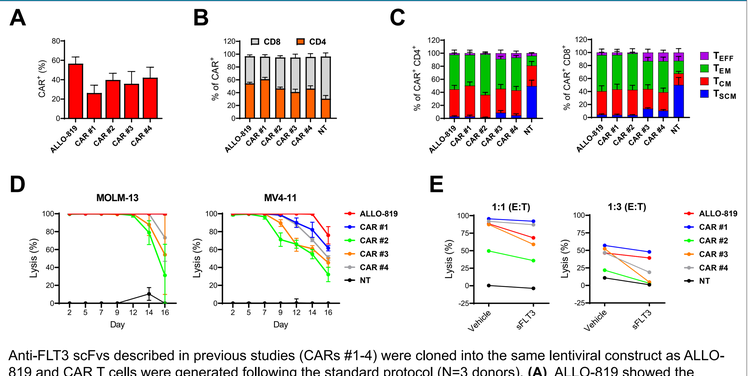
(A) A recombinant protein consisting of the ALLO-819 CAR ectodomain fused to a human IgG Fc region was used to assess non-specific binding of the ALLO-819 scFv to human tissues by immunohistochemistry. **(B)** 36 tissues from 3 unrelated donors were tested. **(C)** Off-target binding of the test reagent was limited to the cytoplasm of esophageal submucosal glands in 1 of 3 donors only (low intensity).

ALLO-819 demonstrates equivalent effector function as its non-gene-edited counterpart



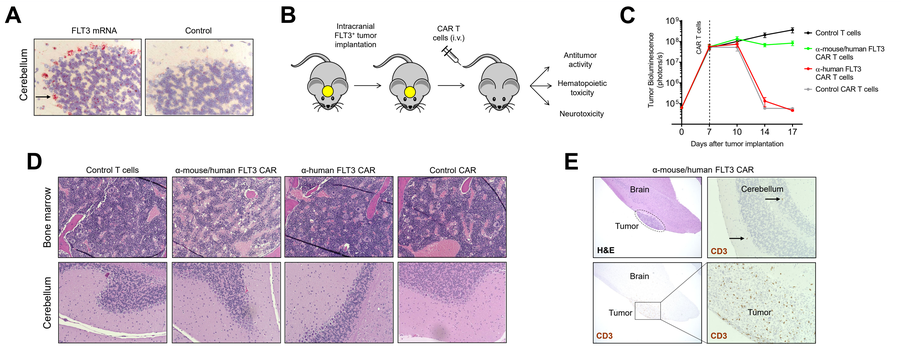
(A) FLT3 expression in AML cell lines (EOL-1, MOLM-13, MV4-11) and a FLT3-negative control cell line (MOLP-8) was determined by flow cytometry. **(B)** Gene-editing did not alter FLT3 CAR T cell efficacy *in vivo*. NSG mice engrafted with luciferase-expressing EOL-1 cells were treated with ALLO-819 or non-gene-edited ALLO-819 at the indicated dose and tumor burden was monitored by bioluminescence. Values are expressed as mean ± SEM (N=10 animals/group). **(C)** Gene-editing did not alter FLT3 CAR T cell cytotoxicity *in vitro*. The cytotoxicity assays were set up with FLT3 CAR T cells that had been exposed to EOL-1 target cells for 7 days. CAR T cells were cultured with luciferase-expressing target cells at the indicated ratios for 24 hours and residual luciferase activity was measured by luminescence.

ALLO-819 scFv compares favorably to anti-FLT3 scFvs used in previous studies and is active in the presence of soluble antigen



Anti-FLT3 scFvs described in previous studies (CARs #1-4) were cloned into the same lentiviral construct as ALLO-819 and CAR T cells were generated following the standard protocol (N=3 donors). **(A)** ALLO-819 showed the highest transduction efficiency as determined by flow cytometry analysis using soluble FLT3 (sFLT3) protein. The CD4:CD8 ratio **(B)** and CAR T cell differentiation status **(C)** were comparable among CARs. Phenotypes were determined as stem cell memory (CD45RO$^-$/CD62L$^+$), central memory (CD45RO$^+$/CD62L$^+$), effector memory (CD45RO$^+$/CD62L$^-$), and effector cells (CD45RO$^-$/CD62L$^-$). Results are shown as mean +/- SEM. **(D)** ALLO-819 showed superior cytolytic activity after multiple encounters with luciferase-expressing FLT3$^+$ AML cell lines (mean +/- SEM). **(E)** The effector function of ALLO-819 is not compromised by soluble FLT3 (sFLT3). ALLO-819 and CAR T cells #1-4 were cultured with luciferase-expressing MV4-11 cells in the presence of sFLT3 (1 µg/ml) at two different effector to target ratios and residual viable cells were measured by luminescence.

FLT3 CAR T cells exhibit off-tumor activity against hematopoietic cells but no neurological damage was seen despite expression of FLT3 mRNA in cerebellum



(A) FLT3 mRNA was detected in the granular layer and Purkinje cells (arrow) in the mouse cerebellum by in-situ hybridization (ISH). **(B)** NSG mice were inoculated with LN229 glioblastoma cells overexpressing human FLT3 directly in the intracranial space and then treated with non-gene-edited T cells expressing a cross-reactive (α-mouse/human) FLT3 CAR, a non-cross-reactive (α-human) FLT3 CAR (ALLO-819 CAR), or a CAR with activity against LN229 cells (control CAR). Non-transduced T cells were used as negative control. **(C)** Treatment with α-human FLT3 CAR T cells or control CAR T cells resulted in complete tumor elimination whereas mice that received α-mouse/human FLT3 CAR T cells showed only a minimal response. Results are expressed as mean ± SEM (N=10 animals/group). **(D)** Mice treated with α-mouse/human FLT3 CAR T cells displayed reduced cellularity in the bone marrow but no noticeable damage in cerebellum or any other tissues (not shown). These mice showed increased T cell infiltration into intracranial tumors with occasional T cells found in adjacent brain and cerebellum, as determined by immunohistochemistry using an anti-human CD3 antibody **(E)**. Taken together, these findings corroborate previous observations and suggest that the on-target off-tumor activity of FLT3 CAR T cells in mice is confined to FLT3-expressing hematopoietic progenitors.

CONCLUSIONS

ALLO-819 exhibits robust antitumor 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 and the absence of apparent neurotoxicity observed in preclinical models indicate that off-tumor effects of ALLO-819 may be restricted to a subset of hematopoietic stem and progenitor cells in the bone marrow. These results support clinical development of ALLO-819 as a novel and effective off-the-shelf CAR T cell therapy for the treatment of AML.

Acknowledgements: ALLO-819 utilizes Cellectis' technologies (including TALEN® gene-editing technology pioneered and controlled by Cellectis). Allogene holds the global development and commercial rights.