Investigation of ALLO-316: A Fratricide-Resistant Allogeneic CAR T Targeting CD70 As a Potential Therapy for the Treatment of AML

Surabhi Srinivasan, Nguyen Tan, Hsin-Yuan Cheng, Yi Zhang, Silvia Tacheva-Grigorova, Tom Van Blarcom, Cesar Sommer, Duy Nguyen , Barbra Sasu, and Siler Panowski

Disclosures

- Full-time employee of Allogene Therapeutics
- Equity interest in Allogene Therapeutics

ALLO-316 (CD70) utilizes TALEN® gene-editing technology pioneered and owned by Cellectis. Allogene has an exclusive license to the Cellectis technology for allogeneic products directed at this target and holds all global development and commercial rights for this investigational candidate.

Disclaimers

This presentation is not intended for product promotion. All information is related to investigational therapies not available for commercial use. The safety and efficacy of the therapies have not been established for FDA approval.

Forward-Looking Statements

To the extent statements contained in this Presentation are not descriptions of historical facts regarding Allogene Therapeutics, Inc. ("Allogene," "we," "us," or "our"), they are forward-looking statements reflecting management's current beliefs and expectations. Forward-looking statements are subject to known and unknown risks, uncertainties, and other factors that may cause our or our industry's actual results, levels or activity, performance, or achievements to be materially different from those anticipated by such statements. You can identify forward-looking statements by words such as "anticipate," "believe," "could," "estimate," "expect," "intend," "may," "plan," "potential," "predict," "project," "should," "will," "would" or the negative of those terms, and similar expressions that convey uncertainty of future events or outcomes. Forward-looking statements contained in this Presentation include, but are not limited to, statements regarding: the ability to progress the clinical development of allogeneic CAR T (AlloCAR T™) therapies and the potential benefits of AlloCAR T™ therapy, including ALLO-316. Various factors may cause differences between Allogene's expectations and actual results as discussed in greater detail in Allogene's filings with the Securities and Exchange Commission (SEC), including without limitation in its Form 10-Q for the quarter ended September 30, 2020.

Except as required by law, we undertake no obligation to publicly update any forward-looking statements, whether as a result of new information, future events or otherwise. This Presentation shall not constitute an offer to sell or the solicitation of an offer to buy securities, nor shall there be any sale of securities in any state or jurisdiction in which such offer, solicitation or sale would be unlawful prior to registration or qualification under the securities laws of any such state or jurisdiction.

Abstract

CD70, a member of the TNF superfamily, is a type II transmembrane glycoprotein that interacts with its receptor (CD27) to promote survival of primed T cells and leads to formation of effector and memory T cells. Expression of CD70 in normal tissues is restricted to activated T and B lymphocytes and mature dendritic cells. CD70 is also widely expressed in various malignancies, including renal cell carcinoma (RCC) and acute myeloid leukemia (AML). The restricted expression pattern of CD70 in normal tissues makes it an attractive target for cancer therapeutics.

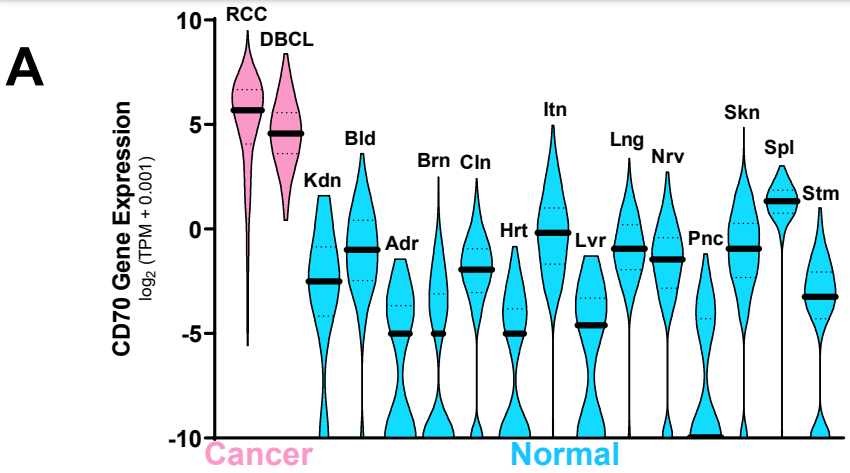
Adoptive transfer of T cells expressing chimeric antigen receptors (CARs) is an exciting new therapeutic modality showing great promise in hematologic malignancies. Approval of two CD19-targeting autologous CAR Ts, Kymriah® and Yescarta®, has been followed with promising results from BCMA autologous CAR T clinical trials, showing that activity can extend to other targets. We have previously described the functional screening of a library of anti-CD70 scFv-based CARs and the identification of lead CD70 allogeneic CAR T cells (AlloCAR T™) with robust activity against RCC cell lines both in vitro and in vivo. Here, we evaluate the anti-tumor activity and safety of a lead CD70 AlloCAR T™ (ALLO-316) for the treatment of AML.

CD70 expression was evaluated and detected on three AML cell lines and in six primary AML patient samples, with 5/6 patient samples showing expression on 24%-99% of cells. CD70 expression will be profiled in a broader subset of AML patients and preliminary data will be presented. Despite the expression of CD70 on activated T cells it was possible to generate CD70 AlloCAR T cells. No CD70 expression was observed on CAR T cells after generation, suggesting either cells are succumbing to fratricide or are being “masked” by the CAR. CD70 was also not detected on Jurkat cells expressing CARs and this data, in combination with results showing CAR expression is protective when overexpressed in RCC cells support the phenomenon of “masking”. Cellectis’ TALEN® gene-editing technology was used to inactivate the TRAC and CD52 loci with the intent 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. ALLO-316 cells were highly effective at lysing CD70-expressing target cells and eliminated greater than 99% of cells at the high effector to target (E:T) ratio and were unable to lyse AML cells in which CD70 was knocked out. Moreover, ALLO-316 cells were able to kill primary AML blasts with CD70 expression ex vivo. An orthotopic in vivo model utilizing the AML cell line MV4-11 was developed and anti-tumor activity was observed.

In addition to evaluating efficacy against AML cell lines and tumors we also explored potential toxicity liabilities related to ALLO-316 treatment. Previous studies have reported that certain AML tumor antigens can also be expressed on normal hematopoietic progenitors and such expression could potentially lead to toxicity with targeted therapeutics. No detectable CD70 was observed by flow cytometry on purified CD34 cells from 14 healthy donors. Taken together, our results support clinical development of CD70 AlloCAR T™ therapy for the treatment of AML.

CD70 is Highly Expressed in Renal Cell Carcinoma and Hematological Malignancies

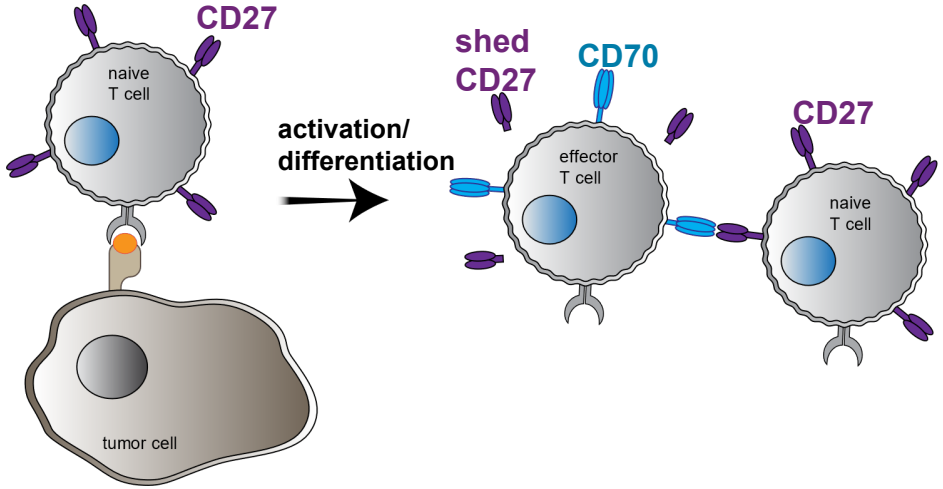
CD70 is expressed in renal cell carcinoma (RCC) and hematological malignancies with limited normal tissue expression



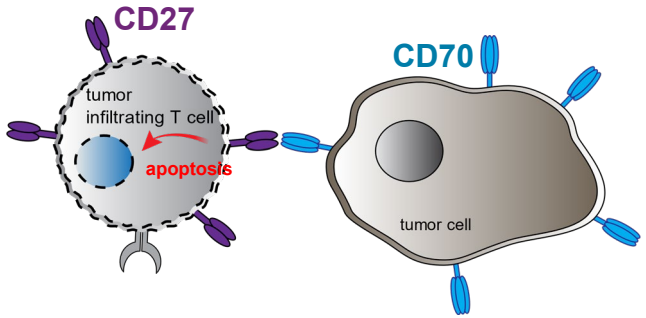
- Normal CD70 expression is limited to activated lymphocytes and APCs[1,2]
- CD70 expression in solid tumors and hematological cancers[3]:
  - RCC tumor samples (72-80%)
  - AML (96%), DBCL (71%), MM (63%), CLL (50%)

(A) RNA expression data from TCGA and GTeX reveal that CD70 is highly expressed in renal cell carcinoma (RCC) and lymphoma, but lower/absent in normal tissues. **(B)** Activation of naïve T cells and differentiation into effector cells results in shedding of CD27 and upregulated CD70 expression. CD70 on effector cells can engage CD27 on neighboring naïve cells and provide co-stimulation to support survival, persistence, and memory formation, resulting in enhanced anti-tumor immune responses. **(C)** However, CD70 expression on solid tumors such as RCC and GBM is known to induce apoptosis of T cells via direct and prolonged binding to CD27 in the absence of additional co-stimulatory signaling[4-6].

CD70 is a T cell co-stimulatory ligand and induced upon T cell activation



CD70 Expression on Tumors Can Mediate Immune Suppression

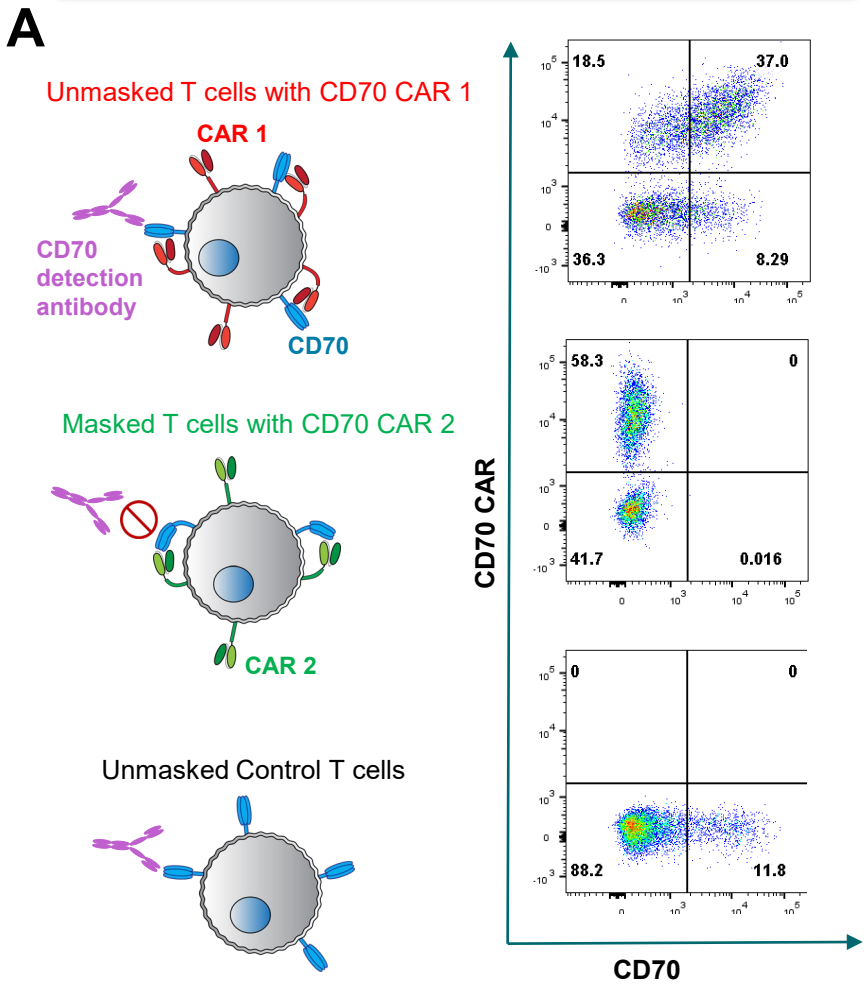


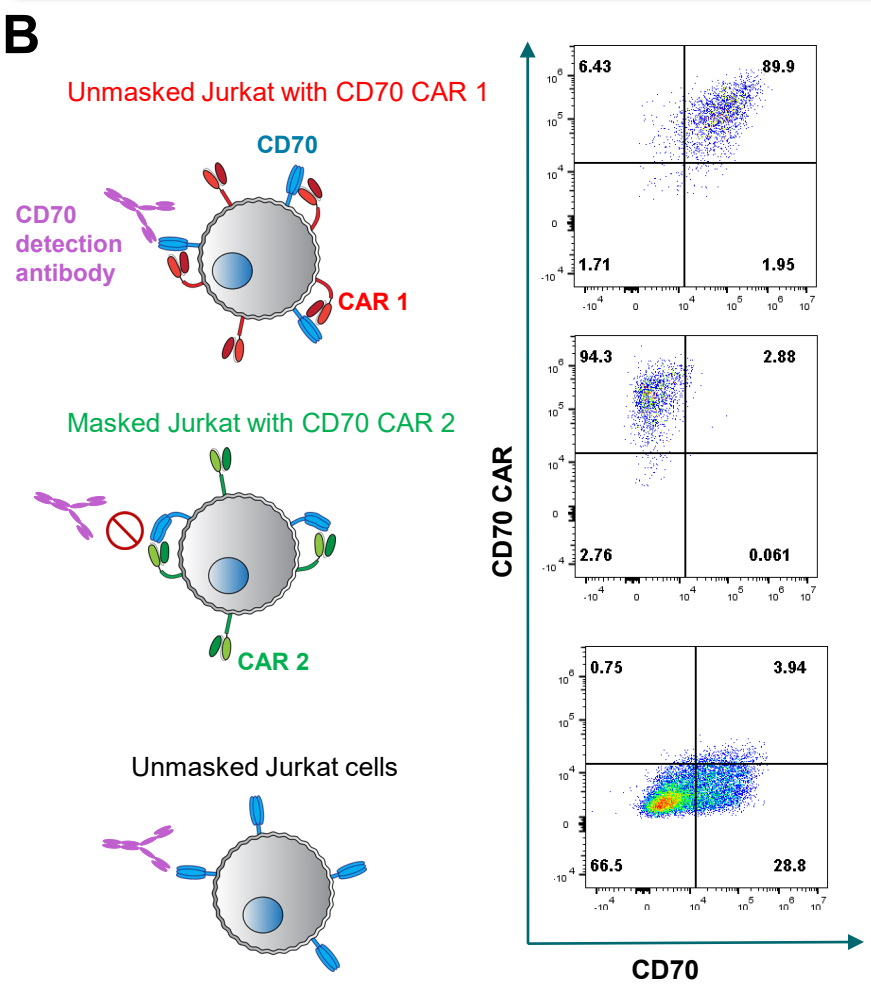
[1]Nolte et al., Immunology Review. 2009
[2]Keller et al., Proceedings Natl Acad Sciences. 2007
[3]Grewal, Expert Opin Ther Targets. 2008
[4]Diegmann et al., Neoplasia. 2006
[5]Jin et al., Neuro-Onc. 2018
[6]Yang et al., Blood. 2007

CD70 CARs Can Mask CD70 on the Cell Surface

CD70 is not detected on T cells transduced with some but not other CD70 CARs

Lack of CD70 detection cannot be attributed wholly to fratricide since the same phenomenon is observed in Jurkat cells



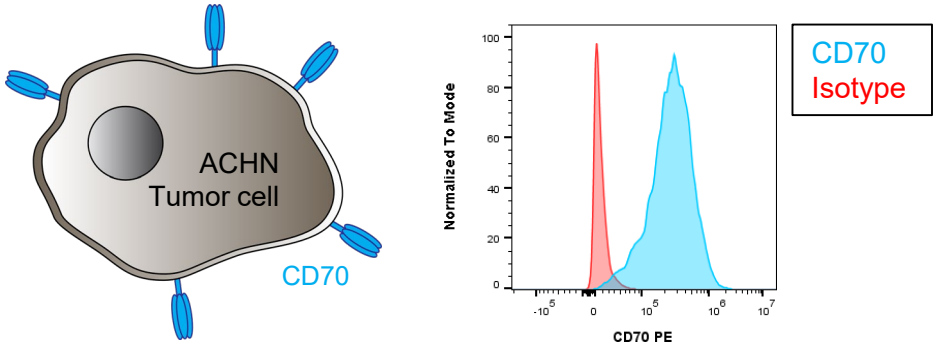


(A) CD70 expression was detected on non-transduced T cells and a subset of CD70 CAR T cells (CAR 1 shown as an example), whereas CD70 was not observed on T cells when transduced with a different of subset of CD70 CARs (CAR 2 shown as an example). **(B)** Since lack of detection could be due to either masking or killing, experiment was repeated in Jurkat cells, which are not capable of effector function. Similar findings were observed.

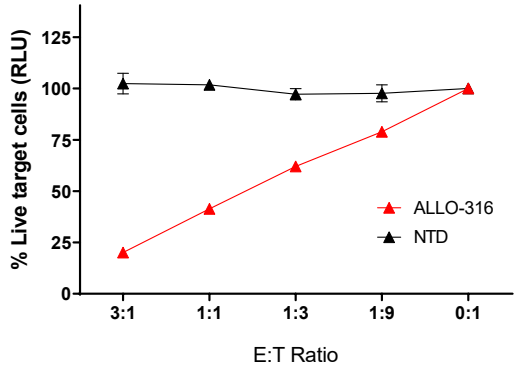
ALLO-316 (Lead CD70 CAR) Can Mask CD70 in *Cis* on the Cell Surface and Prevent Both Detection and Killing

Unmasked ACHN are killed by ALLO-316 CAR T cells

A

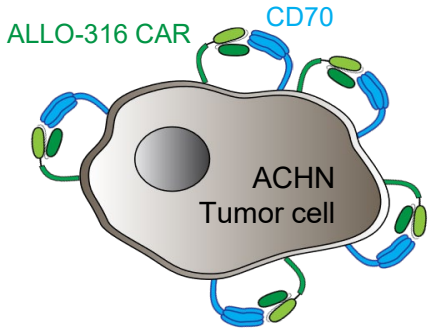


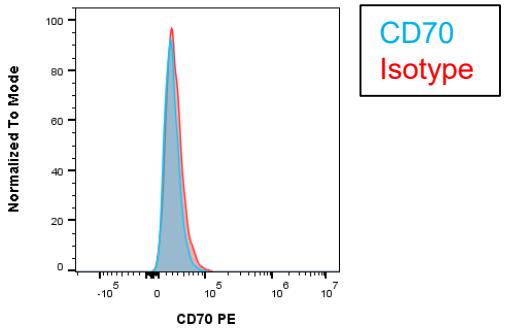
CD70
Isotype



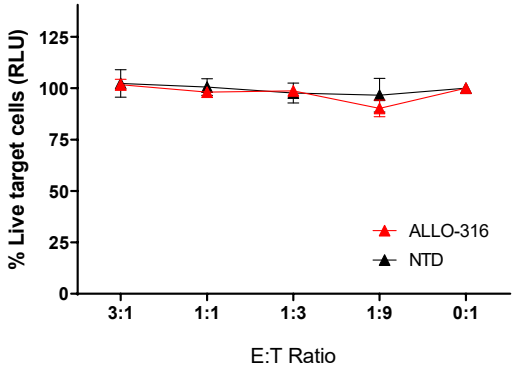
Masked ACHN cells expressing ALLO-316 CAR are protected from killing by ALLO-316 CAR T cells

B



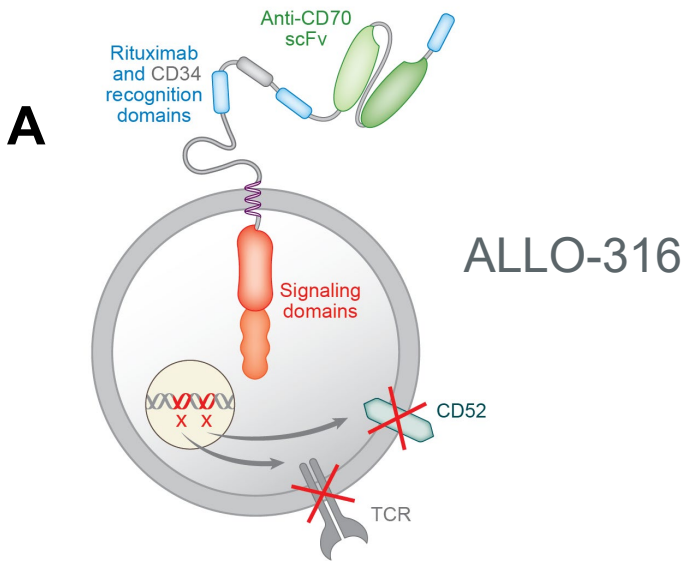


CD70
Isotype

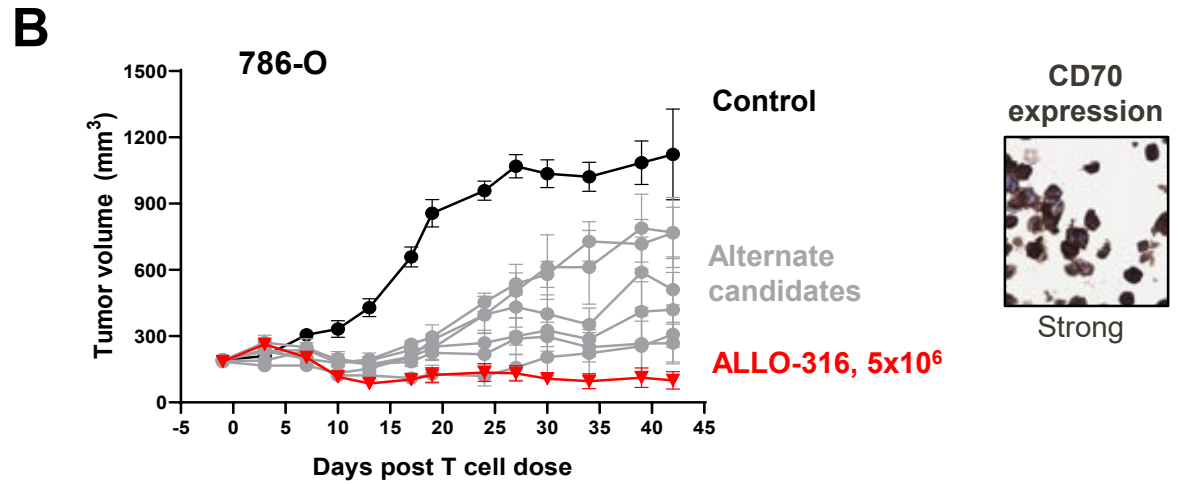


(A) CD70 expression was detected on unmasked RCC parental ACHN cells. These unmasked ACHN cells were killed in a dose-dependent manner by ALLO-316 CAR T cells. **(B)** When the tumor cells were transduced with ALLO-316 CAR, detection of CD70 protein on the cell surface was not observed, suggesting ALLO-316 CAR is binding to CD70 in *cis* and masking detection. Masked ACHN cells were protected from lysis by ALLO-316 CAR T cells.

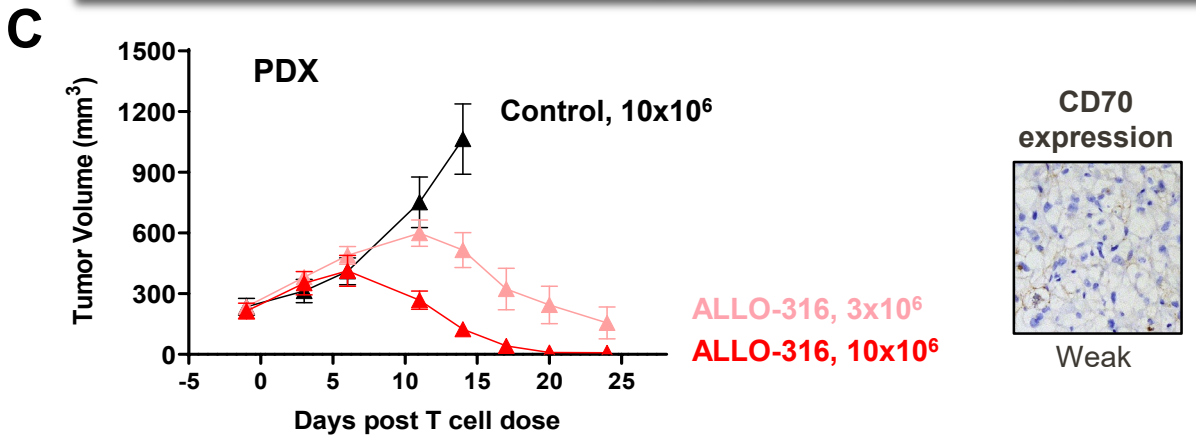
ALLO-316 Can Mediate Efficient Killing of both CD70 High and Low Cells In Vivo



ALLO-316 cells display superior antitumor activity in a CD70-high RCC xenograft model



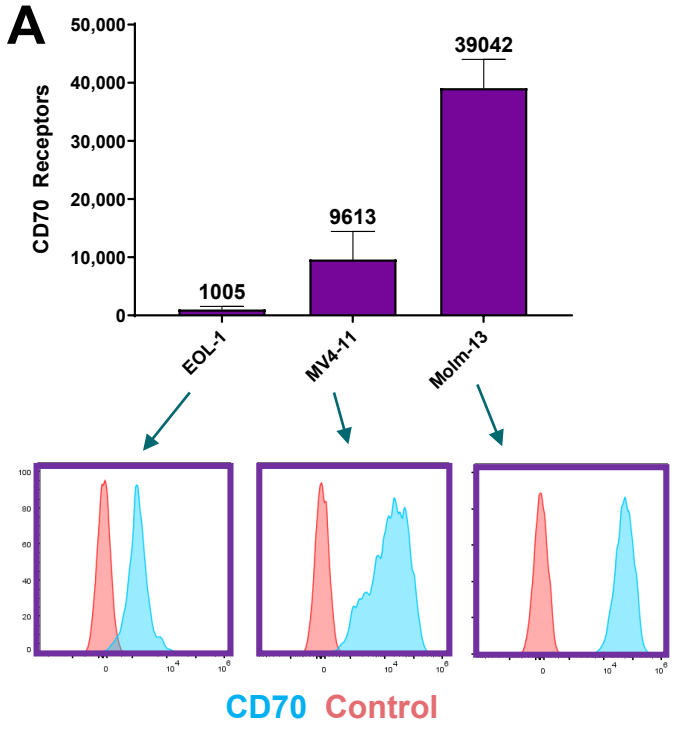
ALLO-316 cells eliminate tumors in a highly relevant RCC PDX xenograft model



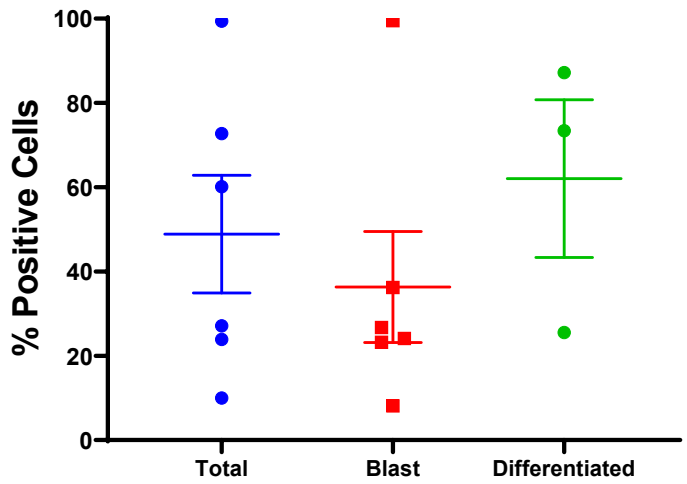
(A) ALLO-316 is comprised of a fully human scFv specific to CD70, a rituximab-based off-switch, and 4-1BB and CD3ζ signaling domains. TALEN® gene editing technology is used to specifically inactivate the T cell receptor (TRAC) and CD52. **(B)** ALLO-316 demonstrated superior antitumor activity as compared to other clones against the 786-0 model, which has high CD70 expression (illustrated by IHC staining on the right). NSG mice (n=6-8) subcutaneously implanted with renal cell carcinoma (RCC) 786-0 cells were dosed intravenously (IV) with different CD70 CAR clones ($5x10^6$ CAR T cells) when the tumor reached approximately 200 mm^3 in size. **(C)** ALLO-316 CAR T cells eliminate RCC PDX tumor cells in a dose-dependent manner. The activity of ALLO-316 cells was evaluated using a subcutaneous RCC PDX xenograft model with weaker CD70 expression compared to 786-0 cells (illustrated by IHC staining on the right). NSG mice (n=8) subcutaneously implanted with RCC PDX fragments were dosed IV with ALLO-316 cells at two different doses (3 and $10x10^6$ CAR T cells) when tumor reached approximately 225 mm^3 in size.

CD70 is Expressed on AML Cell Lines and Primary AML Patient Samples

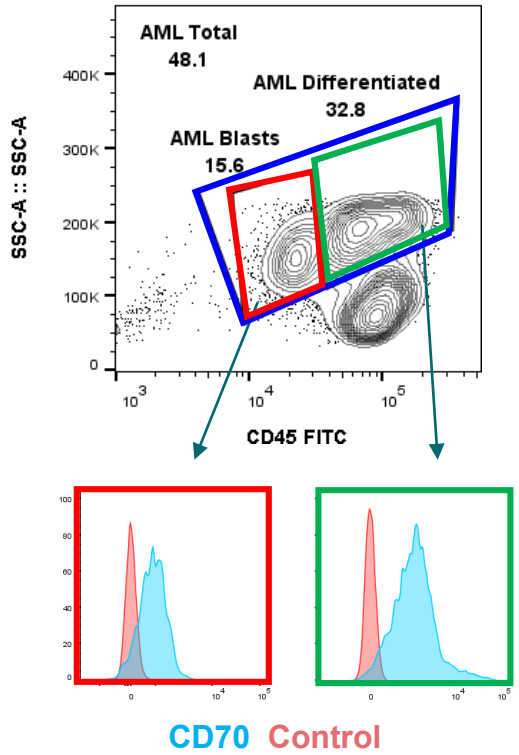
CD70 receptor density on AML cell lines



CD70 expression on multiple primary AML patient samples



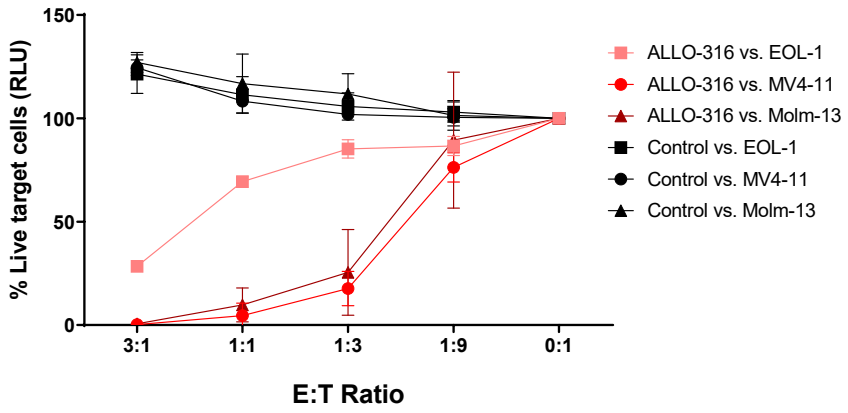
CD70 expression on Primary AML



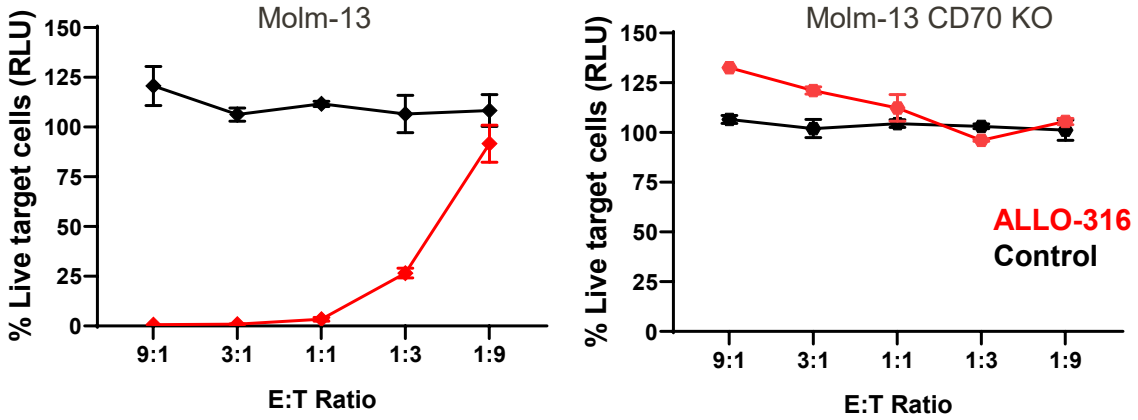
(A) CD70 expression was evaluated on three different AML cell lines by flow cytometry. CD70 expression was also determined using either peripheral blood or apheresis samples from six different AML patients. **(B)** The AML samples were gated on total AML cells (blue), AML blasts (red), or differentiated AML cells (green; only 3 patient samples had differentiated cells). Gating for CD70 expression (light blue) as compared to control (fluorescence minus one; pink) is shown as an example **(C)**.

ALLO-316 Displayed Cytotoxicity Against AML Cell Lines and Primary Patient Samples

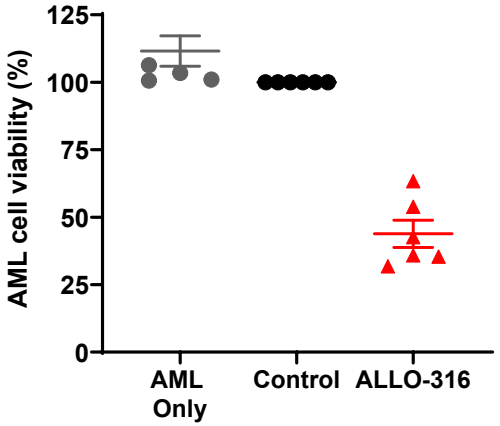
A ALLO-316 was cytotoxic against AML cell lines



B ALLO-316 specifically killed CD70 positive but not CD70 KO targets

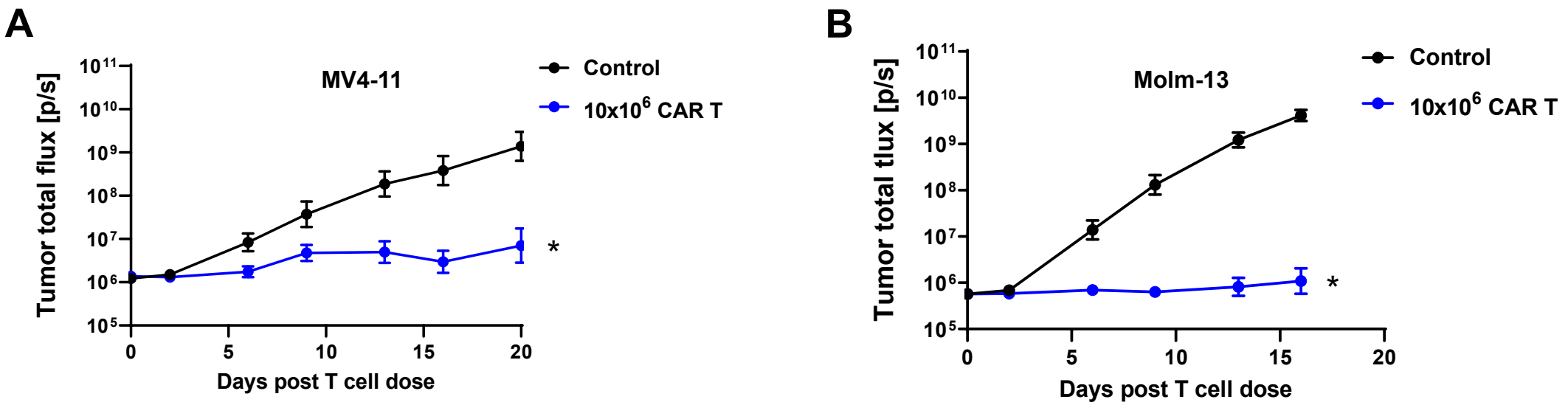


C ALLO-316 eliminated primary AML blasts



(A) The activity of ALLO-316 cells was evaluated in vitro against AML cell lines. ALLO-316 cells eliminated all three AML cell lines in a dose-dependent manner. **(B)** CD70 knockout was performed using TALEN® gene editing technology on Molm-13 cells with >99% efficiency, and the activity of ALLO-316 cells was further tested. The data demonstrated ALLO-316 cells specifically kill CD70 positive but not CD70 KO targets. **(C)** ALLO-316 (red) was active against primary AML blasts as compared to control T cells (black) or AML cells alone (gray). Effector:target (E:T) ratio was 1:1 and cells were co-cultured for 48 hours.

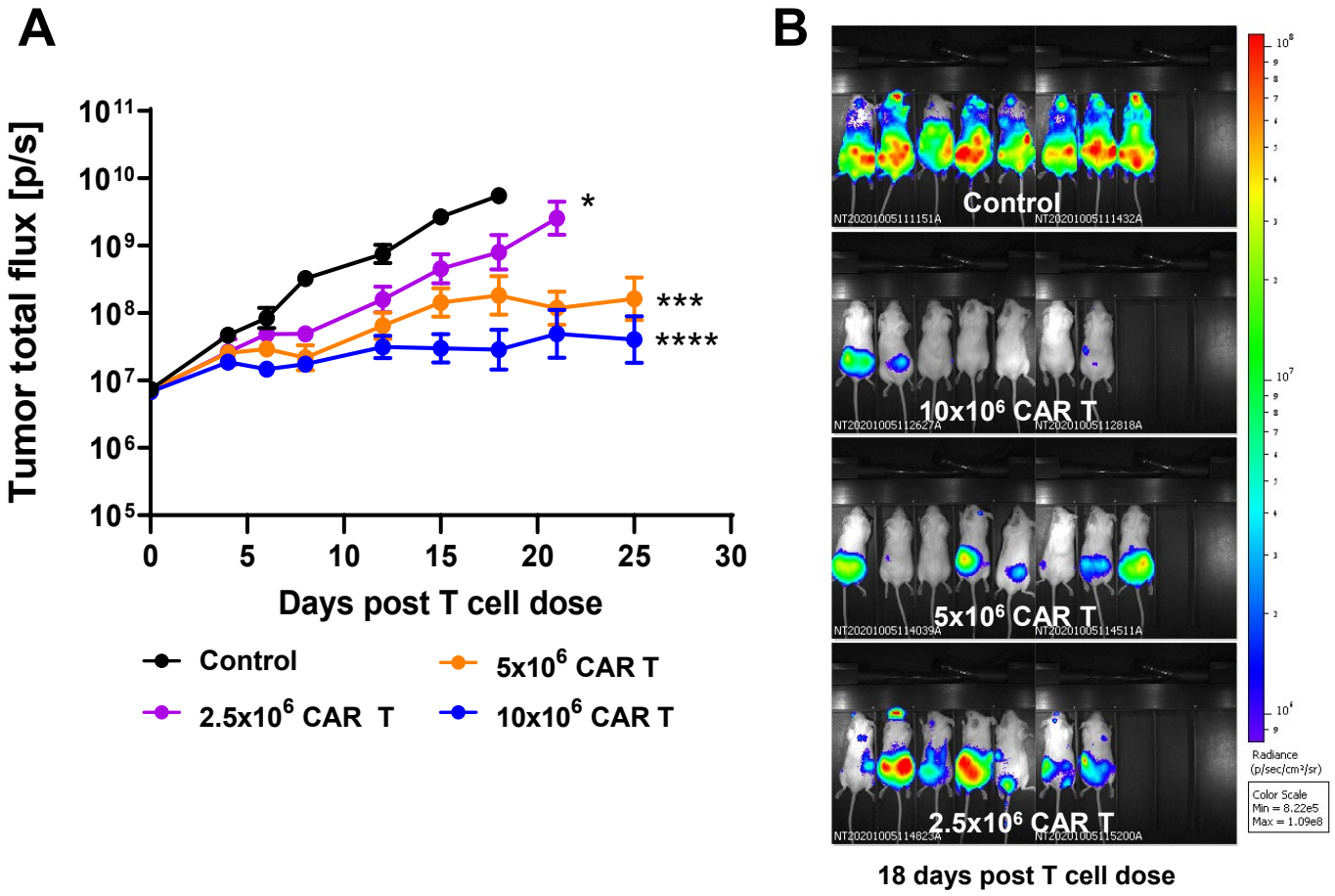
ALLO-316 Displayed Robust Antitumor Activity Against Two Orthotopic AML Tumor Models

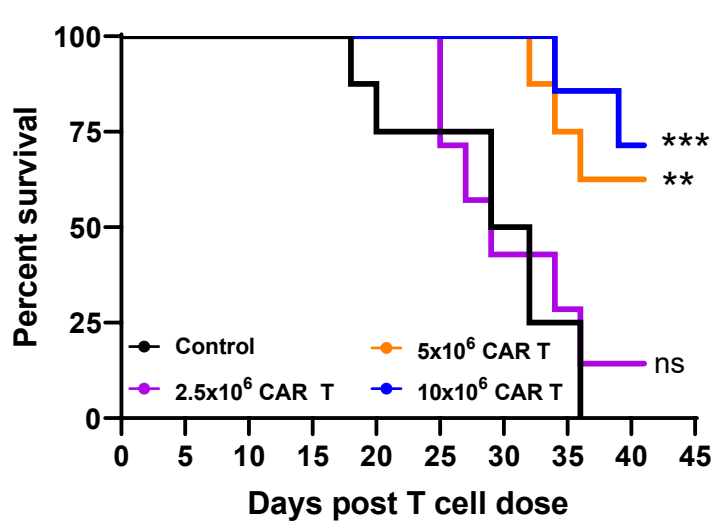


(A-B) The activity of ALLO-316 cells was investigated in two different orthotopic AML tumor models. NSG mice (n=5-6) intravenously (IV) infused either with luciferase-labeled MV4-11 ($1x10^6$) or Molm-13 ($2.5x10^4$) cells received $10x10^6$ ALLO-316 cells 4 days later. Tumor burden was monitored by bioluminescence, and the results demonstrated significant anti-tumor activity in both models. Statistics represent paired t test with two-tailed (* $p<0.05$)

ALLO-316 Demonstrated Dose-Dependent Antitumor Activity in an MV4-11 Orthotopic AML Model with Well Established Tumor (Higher Tumor Burden)

ALLO-316 CAR T cells demonstrate dose-dependent antitumor activity in MV4-11 orthotopic model



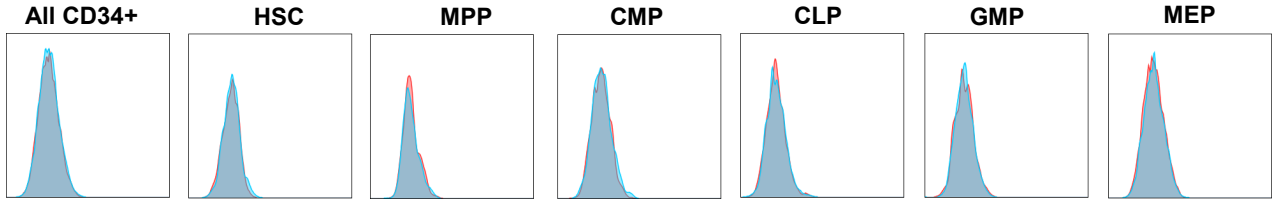


The activity of ALLO-316 CAR T cell was investigated in NSG mice (n=7-8) bearing MV4-11 tumors. Mice were inoculated with luciferase-labeled MV4-11 ($1x10^6$) cells IV, followed 8 days later with ALLO-316 cells at the indicated doses. Tumor burden was monitored by bioluminescence, and the results showed significant anti-tumor activity in a dose-dependent manner **(A-B)**. The benefit from receiving ALLO-316 cells was further demonstrated by increased survival **(C)** at the two highest doses. Statistics represent RMANOVA with Dunnett's post-hoc test **(A)** or log-rank test **(C)**, all groups compared to Control (* $p<0.05$, ** $p<0.005$, *** $p<0.001$, **** $p<0.0001$); not significant (ns)

CD70 Expression was Not Detected on CD34+ HSCs or Progenitors but Depletion of MEPs was Observed Upon Culture with CD70 CAR T Cells

A

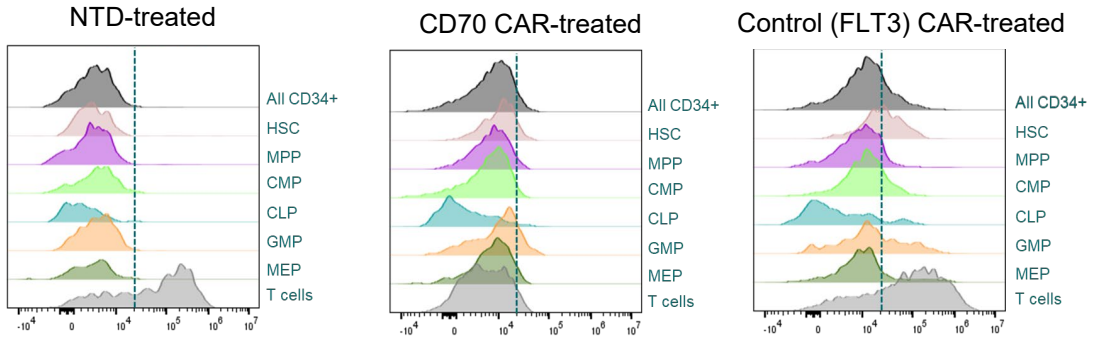
CD70 was not expressed on any bone marrow hematopoietic lineage at baseline



CD70 Isotype 14 donors tested, one example is shown

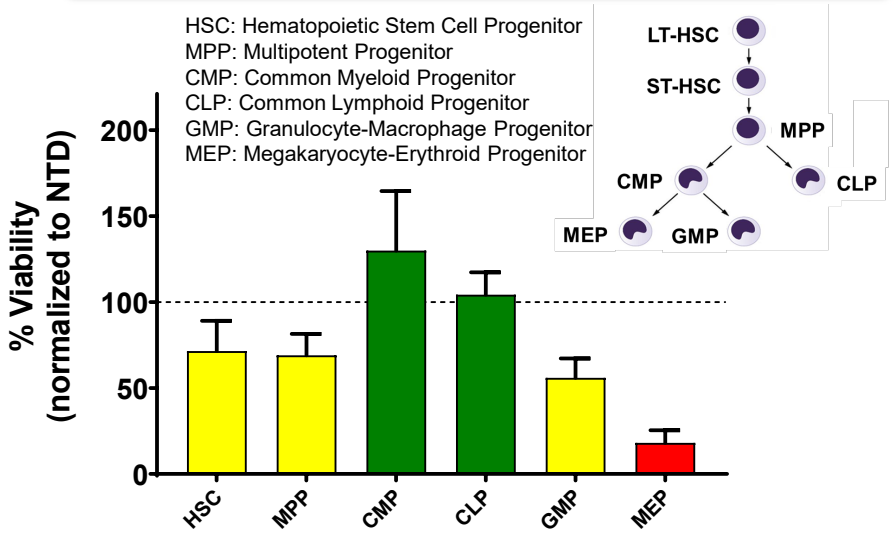
B

CD70 was upregulated upon CAR treatment



C

ALLO-316 depleted some MEP in vitro
(Average of 4 Donors)



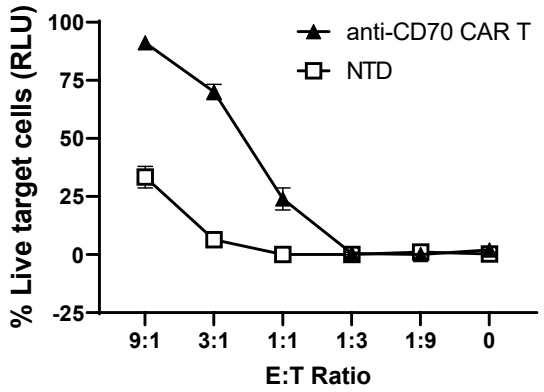
(A) CD70 expression on human CD34+ bone marrow cells from 14 healthy donors was evaluated by flow cytometry and one example of staining is shown. Hematopoietic stem and progenitor cell (HSPC) populations were gated as described in Weissman et al, 2008 cells. No CD70 expression was observed. **(B)** CD70 expression was induced on HSPCs by co-culture with control (FLT3) CAR T cells in vitro at the high E:T ratio of 1:1. No CD70 expression was seen on non-transduced (NTD) control or CD70 CAR treated HSPCs. **(C)** Cytotoxic activity of ALLO-316 cells against HSPCs from 4 healthy bone marrow donors was evaluated by co-culturing ALLO-316 with CD34+ cells at an effector-to-target ratio of 1:1 for 48 hours. ALLO-316 exhibited cytotoxicity against the MEP population in all four donors tested, with a mean depletion of 82%.

HSPC Toxicity or Depletion was not Observed in an In Vivo Mouse Toxicity Study Utilizing Cross-Reactive CAR T Cells

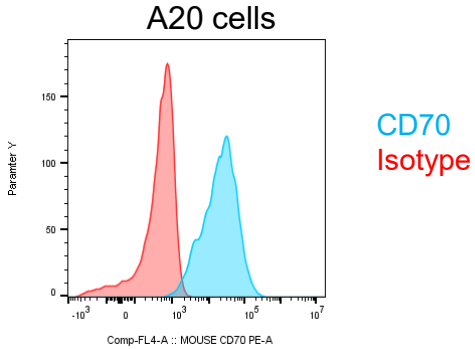
Mouse cross-reactive CAR T cells eliminated cell endogenously expressing mouse CD70

No depletion of hematopoietic stem and progenitor cells was observed upon treatment with cross-reactive anti-CD70 CAR T cells in vivo

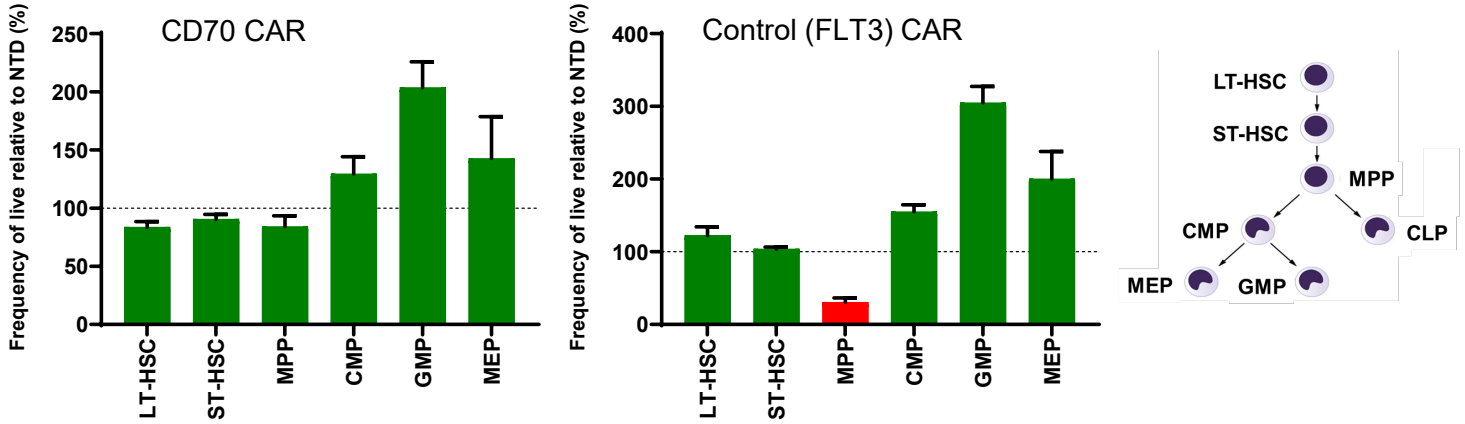
A



B



C



Human CAR T cells with a mouse cross-reactive anti-CD70 CAR utilizing mouse CD27 (mCD27) exhibited dose-dependent cytotoxicity **(A)** against mouse A20 B cell lymphoma cells with endogenous mouse CD70 expression **(B)**. **(C)** Effects of human CAR T cells targeting mouse CD70 or mouse FLT3 on HSPCs were evaluated in a non-tumor-bearing in vivo model. CAR T cells were dosed via IV at $5x10^6$ CAR+ cells per animal (n=6 for CD70, n=3 for FLT3). The cells were supplemented with IL-15 in vivo in order to enhance activity and persistence. NTD cells were also used as controls (n=5). On Day 7 relative to CAR T cell administration, CAR^+ cells were detected in the bone marrow, however, no cytotoxicity of mouse HSPCs was observed in the CD70 CAR T-treated group compared to NTD control-treated group. CD70 CAR T-treated mice showed > 80% viability across all HSPC subtypes. In contrast, FLT3 CAR T cell treatment caused considerable depletion of multipotent progenitor (MPP) population. In mice, FLT3 is known to be expressed on MPP population of bone marrow cells thus leading to depletion of MPP cells by anti-FLT3 CAR T cells in vivo (Sommer et al., 2020).

Conclusions

- CD70 is highly expressed on renal cell carcinoma and hematological malignancies, making it an attractive target for cancer therapeutics
  - CD70 expression is observed on AML cell lines and on primary patient samples
- Masking of CD70 on the cell surface by CD70 CAR appears to provide protection against fratricide, allowing for CD70 CAR T generation without the need for performing CD70 knockout
- Lead anti-CD70 AlloCAR T™ candidate, ALLO-316, demonstrated robust anti-tumor activity in vitro and in vivo in the absence of CD70 knockout
- ALLO-316 displayed an acceptable toxicity profile in preclinical studies
  - Expression of CD70 on HSPCs is absent at baseline, but may be induced to low levels by incubation with CAR T cells at high E:T ratios in vitro, resulting in partial depletion of the MEP population in vitro
  - No depletion of early progenitor cells was observed in an in vivo mouse safety study
- Results support clinical development of ALLO-316 as novel therapy for the treatment of AML