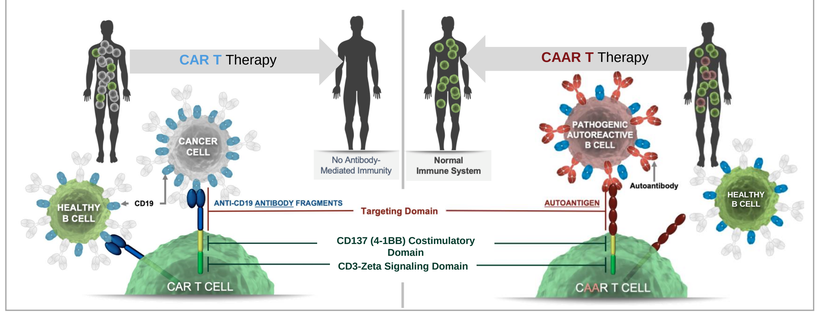
A Phase 1 Trial Of DSG3-CAART Cells In Mucosal-Dominant Pemphigus Vulgaris (mPV) Patients:
Early Cohort Clinical and Translational Data

David J. Chang[1], Samik Basu[1], Robert Micheletti[2] , Emanual Maverakis[3] , M. Peter Marinkovich[4], David Porter[2], Mehrdad Abedi[3], Wen-Kai Weng[4], Kimberly Hoffman[1], Jenell Volkov[1], Daniel Nunez[1], Michael Milone[2], Gwendolyn K. Binder[1], Aimee S. Payne[2]

[1]Cabaletta Bio, Philadelphia PA; [2]University of Pennsylvania, Philadelphia PA; [3]UC Davis, Sacramento CA; [4]Stanford University, Stanford, CA

Background

Mucosal-dominant pemphigus vulgaris (mPV) is an autoimmune blistering disease mediated by anti-desmoglein 3 (DSG3) autoantibodies. Standard of care including rituximab, steroids and other immunosuppressive agents is not curative, requires chronic administration and is associated with serious infections due to persistent immunosuppression. The ideal therapy would selectively eliminate pathogenic anti-DSG3-expressing B cells while sparing healthy B cells. Based on the long-lasting remission of B cell cancers with chimeric antigen receptor T (CART) cells, we developed chimeric autoantibody receptor T (CAART) cells to target B cell-mediated autoimmune diseases, using the same construct but switching the targeting domain to the autoantigen[1,2]. For mPV patients, autologous T cells have been genetically modified to express the DSG3 autoantigen (DSG3-CAART) and target only the anti-DSG3 B cells.



Methods

The objective of this ongoing Phase 1 open-label trial (NCT04422912) is to determine the maximum tolerated dose of DSG3-CAART in adult subjects with active, anti-DSG3 antibody-positive, biopsy confirmed mPV inadequately managed by ≥1 standard therapy. The primary endpoint is incidence of adverse events (AEs) related to DSG3-CAART within 3 months of infusion, including dose-limiting toxicities (DLTs). Secondary endpoints include DSG3-CAART persistence (qPCR), anti-DSG3 antibody levels (ELISA) and disease activity (Pemphigus Disease Area Index (PDAI) Mucous Membrane score). After discontinuing or tapering immunosuppressives, subjects in cohorts 1-4 received $2x10^7$, $1x10^8$, $5x10^8$ or $2.5x10^9$ DSG3-CAAR-transduced cells as fractionated infusions and without lymphodepletion. Retreatment at a higher dose was permitted for subjects who did not achieve remission within 6 months of therapy or flared after remission.

Engineered T cell persistence was assessed by qPCR for the vector in post-infusion PBMC samples. Anti-DSG3 antibody levels were evaluated on pre- and post- infusion serum samples by ELISA (MBL International). Flow cytometric analyses were performed on the infusion product and on post-infusion peripheral blood mononuclear cell (PBMC) samples to assess transduction efficiency and memory phenotype. Serum cytokines were measured via a multiplexed Luminex or MSD immunoassay. DSG3-CAART cell cytotoxicity assays were performed in vitro using the IncuCyte® platform.

Table 1. Overview of Dose Escalation

Cohort	Total Transduced Cell Dose	Fold increase in dose	Patients per cohort
A1	2×10^7	1x	3
A2	1×10^8	5x	3
A3	5×10^8	25x	3 [+1*]
A4	2.5×10^9	125x	3
A5	$5 - 7.5 \times 10^9$	250 to 375x	3 (+3) [ongoing]
A5e**	$5 - 7.5 \times 10^9$	250 to 375x	3 (+3) [planned]
A6m***	$1 - 1.5 \times 10^{10}$	500 to 750x	3 (+3) [planned]

*Includes one A1 subject re-treated at the A3 dose
**A5e represents an enhanced manufacturing process
***A6m represents multiple infusions ≥1 week apart

Table 2. Subject Baseline Characteristics

	Cohort A1 2×10^7 (n=3)	Cohort A2 1×10^8 (n=3)	Cohort A3 5×10^8 (n=3)	Cohort A4 2.5×10^9 (n=3)	Overall (n=12)
Age, years, median (range)	39 (32-57)	53 (50-54)	60 (47-70)	60 (56-70)	55 (32-70)
Female (%)	67%	67%	67%	67%	67%
Disease duration, years, median (range)	3.4 (0.5-4.3)	4.3 (3.9-13.0)	0.7 (0.3-15.0)	3.5 (0.1-12.4)	3.7 (0.1-15.0)
Anti-DSG3 antibody level, U/mL, median (range)	92 (51-104)	147 (86-168)	147 (63-169)	147 (114-162)	130 (51-169)
Pemphigus Disease Area Index, median (range)	17 (5-20)	6 (6-14)	12 (2-18)	3 (1-4)	6 (1-20)
Prior use of corticosteroids (%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	12 (100%)
Prior use of mycophenolate (%)	2 (67%)	3 (100%)	1 (33%)	2 (67%)	8 (67%)
Prior use of rituximab (%)	3 (100%)	3 (100%)	0 (0%)	2 (67%)	8 (67%)

Table 3. Safety within 3 Months Post-Infusion

	Cohort A1 2×10^7 (n=3)	Cohort A2 1×10^8 (n=3)	Cohort A3 5×10^8 (n=3)	Cohort A4 2.5×10^9 (n=3)	Overall (n=12)
# Subjects with ≥1 AEs (%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	12 (100%)
# Subjects with ≥1 Related AEs* (%)	0 (0%)	1 (33%)	1 (33%)	1 (33%)	3 (25%)
# Subjects with ≥1 SAEs (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
# Subjects with ≥1 Related SAEs (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
# Subjects with Cytokine Release Syndrome (CRS) (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
# Subjects with Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
# Subjects with Dose-Limiting Toxicity (DLT) (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

The DSMB did not consider the overall safety profile in these cohorts to be clinically relevant to change the study design or pause the study. *Excludes dysgeusia associated with DMSO in the infusion; A2-1: mild fatigue; A3-2: mild nausea and moderate insomnia; A4-3: mild fever, palpitations, noncardiac chest pain, headache, elevated CRP, elevated LDH, moderate sinus tachycardia.

Table 4. Disease Activity

Cohort Dose	Subject	Prior RTX or IVIg*	Meds stopped or tapered prior to infusion	Screen	Pre-Inf	Mo. 1	Mo. 2	Mo. 3	Mo. 4	Mo. 5	Mo. 6
PDAI Mucous Membrane Score (0-1 = Clear or almost clear)											
2×10^7	A1-1	RTX 10m	PRD	20	10	13	33	PRD 70	IVIg 27	26	MMF 30
	A1-2	RTX 6.5m IVIg 3m		5	2	1	1	1	0	1	0
	A1-3	RTX 9m	MMF	17	4	3	1	2	6	2	13
1×10^8	A2-1	IVIg 4m		6	5	2	1	2	3 PRD	2	5
	A2-2			14	3	3	0	1	4 PRD	4	11
	A2-3	IVIg 4m		6	1	3 PRD	4	7	4 RTX	1	5
5×10^8	A3-1			2	2	0	0 PRD	0	0	0 PRD	24
	A3-2		PRD, MMF	12	10	10	22	20	20	10	21
	A3-3			18	14	8	14	17	16 PRD	6	7
# of Subjects Achieving Clear or Almost Clear				0	1	2	5	3	2	3	1

RTX=rituximab; IVIg=intravenous immunoglobulin, MMF=mycophenolate, PRD=prednisone. Systemic PV therapy changes were more permissive after month 3; new PV therapy or PRD dose increases shown in red and PRD taper starts shown in green at the time the therapy change occurred.
*RTX or IVIg within 12 months prior to infusion. RTX permitted within 12 months prior to screening if disease worsening; IVIg permitted >2 weeks prior to screening.

Figure 1. DSG3-CAART Persistence

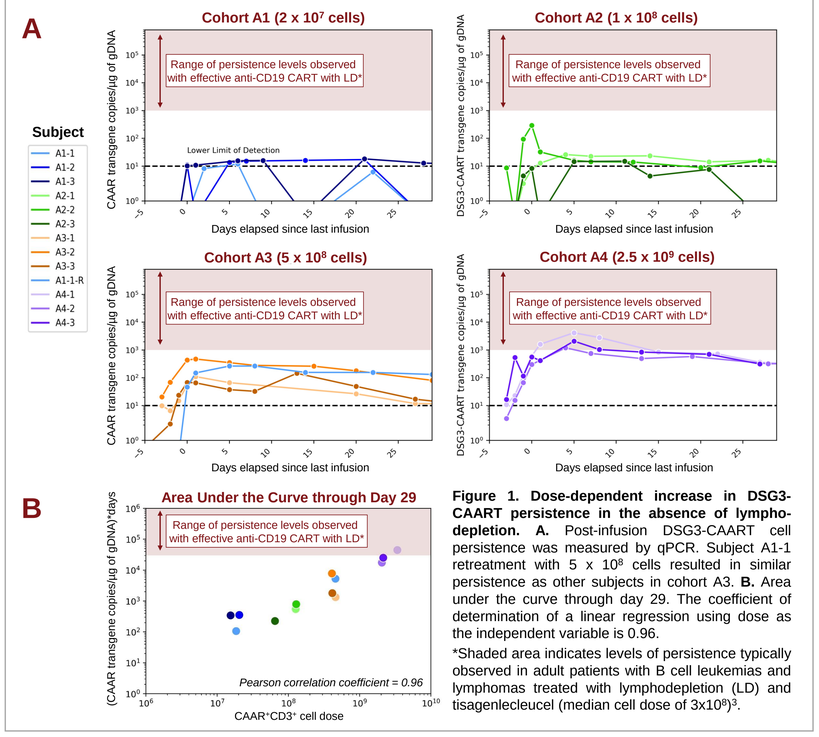


Figure 1. Dose-dependent increase in DSG3-CAART persistence in the absence of lympho-depletion. A. Post-infusion DSG3-CAART cell persistence was measured by qPCR. Subject A1-1 retreatment with 5 x 10^8 cells resulted in similar persistence as other subjects in cohort A3. **B.** Area under the curve through day 29. The coefficient of determination of a linear regression using dose as the independent variable is 0.96.
*Shaded area indicates levels of persistence typically observed in adult patients with B cell leukemias and lymphomas treated with lymphodepletion (LD) and tisagenlecleucel (median cell dose of $3x10^8$)[3].

Figure 2. Anti-DSG3 Antibody Levels

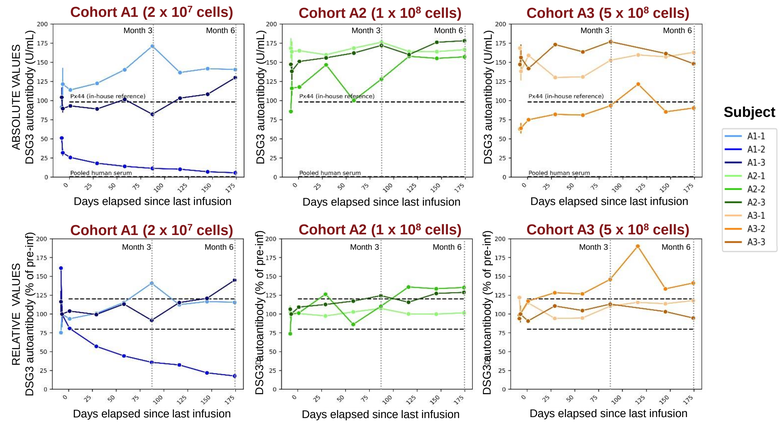


Figure 2. Absolute and relative anti-DSG3 antibody levels over time. (A) Absolute ELISA index value (U/mL). Dashed lines indicate Px44 monoclonal anti-DSG3 antibody positive control and pooled normal human serum antibody negative control. (B) Relative ELISA index value (% of pre-infusion timepoint). Dashed lines indicate ± 20%, which is considered the margin for relevant change. At month 3, subject ELISA values decreased (n=1), remained stable (n=6), or increased (n=2). At month 6, subject ELISA values decreased (n=1), remained stable (n=4), or increased (n=4). Systemic PV therapy changes were more permissive after Month 3. Concomitant systemic PV medications are shown in Table 4.

Figure 3. Infusion Product Characterization

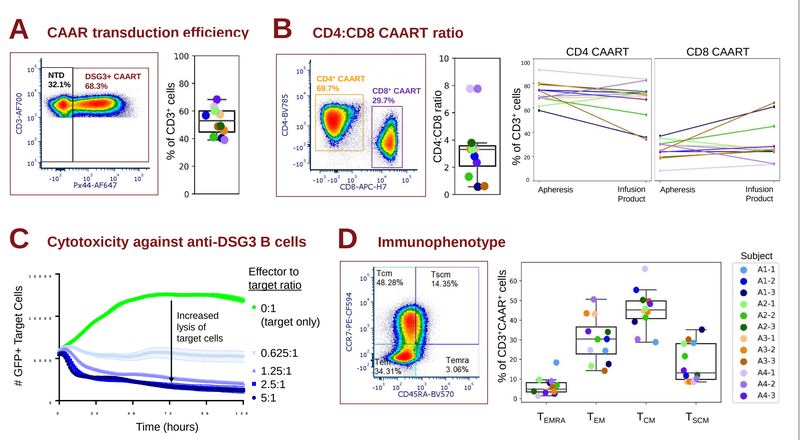


Figure 3. Infusion Product Characterization. (A) CAAR transduction efficiency (median 53%, range 39.1% - 68.3%), measured by flow cytometry and defined as the percent of $CD3^+$ cells in the infusion product that are DSG3 $CAAR^+$. (B) Flow cytometry of CD4 and CD8 $CAAR^+$ T cells in the infusion product. The ratio of CD4:CD8 $CAAR^+$ T cells in the infusion product (median 3.3, range 0.6-7.8) reflected a similar CD4 predominance in the apheresis product. (C) Representative cytotoxicity assay against GFP^+ anti-DSG3 surface immunoglobulin-expressing Nalm-6 target cells DSG3 $CAAR^+$ effector T cells in the infusion product. Curves plot the number of GFP^+ target cells present (± standard deviation) over 120 hours at effector to target cell ratios ranging from 0:1 to 5:1. (D) Flow cytometry of DSG3-$CAAR^+$ T cells expressing CCR7 and CD45RA in the infusion product. Data show the percentage of DSG3-$CAAR^+$ T cells that are T_{EM} ($CD45RA^-CCR7^-$), T_{EMRA} ($CD45RA^+CCR7^-$), T_{CM} ($CD45RA^-CCR7^+$), and T_{SCM} ($CD45RA^+CCR7^+$).

Conclusions

- Early cohort data from the first-in-human trial of DSG3-CAART, a novel investigational precision cell therapy for the autoimmune disease mPV, demonstrate that doses up to $2.5x10^9$ DSG3-CAART cells were well-tolerated with no CRS, ICANS, or related SAEs.
- A 100% manufacturing success rate has been achieved to date across the 12 subjects in cohorts A1-A4.
  - The infusion product has a median CD4:CD8 ratio of 3.3 (range 0.6-7.8) & median transduction percentage of 53% (range 39.1% - 68.3%)
  - The infusion product is largely composed of memory T cell subsets
  - All infusion products manufactured to date have strong cytolytic capacity *in vitro*
- There is a dose-dependent increase in persistence of DSG3-CAART in the absence of lymphodepletion.
  - Re-treatment of one A1 subject at the cohort A3 dose achieved similar DSG3-CAART persistence levels as other subjects in cohort A3, suggesting a lack of immune-mediated rejection of DSG3-CAART cells
  - Persisting DSG3-CAART cells are predominantly T_{CM} and T_{SCM}, which are associated with better clinical outcomes in clinical studies of anti-CD19 CART in B cell malignancies
- Transient clinical improvements are observed in some subjects; effects on anti-DSG3 antibody titer in cohorts A1 to A3 are variable
  - A3 dose (5×10^8 DSG3 $CAAR^+$ T cells) is 10-15-fold lower than the A5 dose ($5-7.5 \times 10^9$ DSG3 $CAAR^+$ T cells) and 20-30-fold lower than the highest planned cohort dose
- DSG3-CAART persistence in cohort A4 approaches the lower end of the range observed with anti-CD19 CART therapy plus lymphodepletion for B cell malignancies, providing rationale to evaluate:
  - Higher doses to increase in vivo presence of DSG3-CAART
  - Manufacturing enhancement designed to increase product potency and trafficking to tissue where the target B cells reside

References

1. Ellebrecht CT, Bhoj VG, Nace A, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science. 2016;353(6295):179-184. doi:10.1126/science.aaf6756
2. Lee J, Lundgren DK, Mao X, et al. Antigen-specific B cell depletion for precision therapy of mucosal pemphigus vulgaris. J Clin Invest. 2020;130(12):6317-6324. doi:10.1172/JCI138416
3. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. N Engl J Med. 2019;380(1):45-56. doi:10.1056/NEJMoa1804980

Acknowledgments: Clinical Cell and Vaccine Production Facility (DSG3-CAART manufacturing); Translational and Correlative Studies Laboratory (qPCR, ELISA, flow cytometry analyses); Eun Jung Choi, Silvio Manfredo-Vieira (production and validation of Px44 anti-DSG3 monoclonal antibody); DesCAARTes™ clinical trial investigators and coordinators; Cabaletta Bio for study sponsorship. **Conflict of interest:** MCM and ASP are co-founders and co-chairs of the Scientific Advisory Board for Cabaletta Bio.