P509

Presented at the 2023 ESGCT Annual Congress
Brussels, Belgium
October 24-27, 2023

Correlative findings following DSG3-CAART infusion with and without preconditioning in patients with Pemphigus Vulgaris (DesCAARTes™ study)

Cabaletta Bio®

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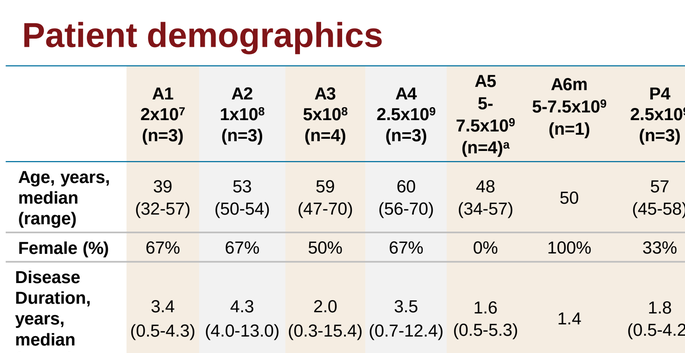
Background

Mucosal-dominant pemphigus vulgaris (mPV) is a painful blistering mucosal disease mediated by anti-desmoglein 3 autoantibodies (anti-DSG3 Ab). The current standard of care for mPV includes broadly immunosuppressive therapies (corticosteroids, MMF, & rituximab) that are not curative, require chronic administration & have risks of serious or life-threatening infection. Ideally, therapy would selectively eliminate pathogenic memory B cells that are DSG3 specific while sparing non-autoreactive immune cells. As chimeric antigen receptor engineered T cells (CAR-T) have demonstrated long lasting remission of B cell-mediated cancers, we developed engineered chimeric autoantibody receptor T (CAART) cells to assess if remission of B cell mediated autoimmune disease is possible. Currently, gene-modified autologous DSG3 specific CAART cells (DSG3-CAART) are being evaluated in patients with mPV in an open label dose escalation Phase I study (NCT 04422912). Here, we report on the phenotypic & functional characteristics of the DSG3-CAART cell infusion product and provide key correlative and clinical data from mPV patients treated with DSG3-CAART.

Methods

Flow cytometric analyses were performed on the infusion product & on post-infusion PBMC samples to assess transduction efficiency & memory phenotype. DSG3-CAART cell cytotoxicity assays were performed *in vitro* using the IncuCyte® platform. Engineered T-cell persistence was assessed by qPCR for the vector in post-infusion PBMC samples. Serum cytokines were measured via a multiplexed MSD immunoassay. Finally, anti-DSG3 Ab levels were evaluated on pre- and post- infusion serum samples via ELISA (MBL International). Pemphigus Disease Area Index (PDAI) scores were determined by investigator (physician) assessment.

DSG3-CAART Design



Overview of Dose Escalation

Cohort	Total DSG3-CAART Cell Dose	Fold Increase in Dose	Subjects per Cohort
A1	$2x10^7$	1x	3
A2	$1x10^8$	5x	3
A3	$5x10^8$	25x	3 [+ A1-1 re-treated]
A4	$2.5x10^9$	125x	3
A5	5-7.5x10^9	250 to 375x	4[a]
A6m	1-1.5x10^{10}	500 to 750x	1
P4	$2.5x10^9$ + cyclophosphamide & IVIg	125x	2 [+ A5-1 re-treated]

[a] A 4th subject was dosed in Cohort A5 to generate additional data

Patient demographics

	A1 $2x10^7$ (n=3)	A2 $1x10^8$ (n=3)	A3 $5x10^8$ (n=4)	A4 $2.5x10^9$ (n=3)	A5 5-7.5x10^9 (n=4)[a]	A6m 5-7.5x10^9 (n=1)	P4 $2.5x10^9$ (n=3)	Overall (n=21)
Age, years, median (range)	39 (32-57)	53 (50-54)	59 (47-70)	60 (56-70)	48 (34-57)	50	57 (45-58)	54 (32-70)
Female (%)	67%	67%	50%	67%	0%	100%	33%	48%
Disease Duration, years, median (range)	3.4 (0.5-4.3)	4.3 (4.0-13.0)	2.0 (0.3-15.4)	3.5 (0.7-12.4)	1.6 (0.5-5.3)	1.4	1.8 (0.5-4.2)	3.4 (0.1-15.4)
Anti-DSG3 Ab Level, U/mL, median (range)	92 (51-104)	147 (86-168)	123 (63-169)	147 (114-162)	144 (124-169)	74	145 (131-195)	143 (51-195)
Pemphigus Disease Area Index, median (range)	17 (5-20)	6 (6-14)	15 (2-20)	3 (1-4)	5 (4-18)	3	4 (3-50)	6 (1-50)
Prior use of corticosteroids (%)	3 (100%)	3 (100%)	3 (75%)	3 (100%)	3 (75%)	1	3 (100%)	19 (90%)
Prior use of mycophenolate (%)	1 (33%)	3 (100%)	2 (50%)	1 (33%)	3 (75%)	1	2 (67%)	13 (62%)
Prior use of rituximab (%)	3 (100%)	3 (100%)	1 (25%)	2 (67%)	1 (25%)	1	1 (33%)	12 (57%)

[a] A 4th subject was dosed in Cohort A5 to generate additional data

Results

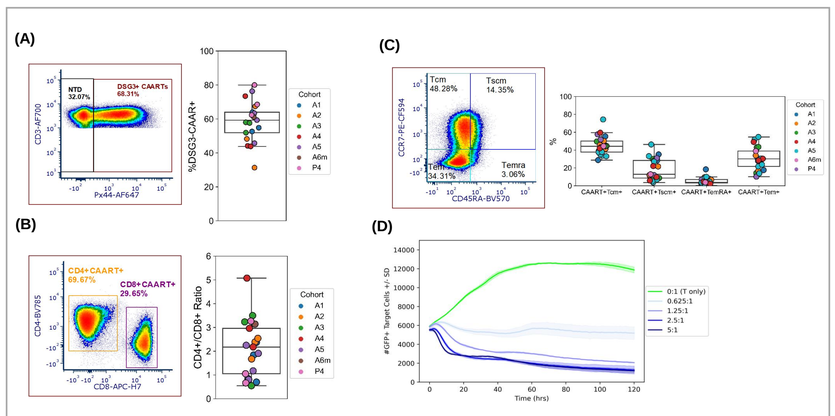


Figure 1. Infusion Product Characterization. (A) Transduction efficiency of the manufactured product (MP) measured by flow cytometry and defined as the percentage of subjects' T cells in the MP that are DSG3-CAAR+. (B) Flow cytometry of DSG3-CAAR+ T cells expressing CD4 and CD8 from the MP. Data represented as the ratio of the percentage expressing CD4+ to CD8+. (C) Flow cytometry of DSG3-CAAR+ T cells expressing CCR7 and CD45RA from subjects' MP. Data represented as the percentage of DSG3-CAART+ T cells that are T_{EM} (CD45RA− CCR7−), T_{EMRA} (CD45RA+CCR7−), T_{CM} (CD45RA− CCR7+), and T_{SCM} (CD45RA+CCR7+). (D) Representative antigen-specific lysis of GFP+ anti-DSG3 surface immunoglobulin-expressing NALM6 target cells by DSG3-CAAR+ effector cells from patients' MP. Cell lysis curves show the number of GFP+ target cells present (±SD) at effector to target ratios ranging from 0:1 to 5:1 over 120 hours.

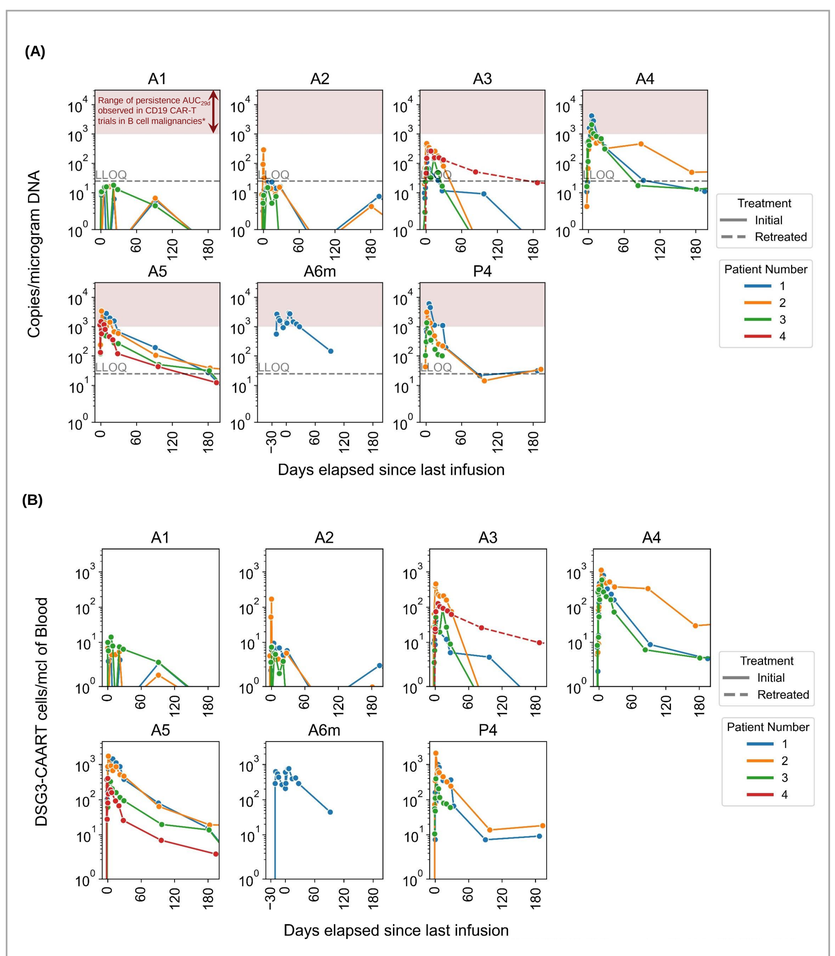


Figure 2. DSG3-CAART post-infusion persistence. Post-infusion DSG3-CAART cell persistence was measured by qPCR on genomic DNA extracted from peripheral whole blood samples. (A) Persistence plotted as copies of CAART transgene/µg of DNA. (B) Persistence plotted as DSG3-CAART cells/µL of blood, which is calculated using leukocyte counts at each visit and vector copy number of the drug product, in addition to copies of CAART transgene/µg of DNA. X-axis corresponds to days elapsed since last infusion.

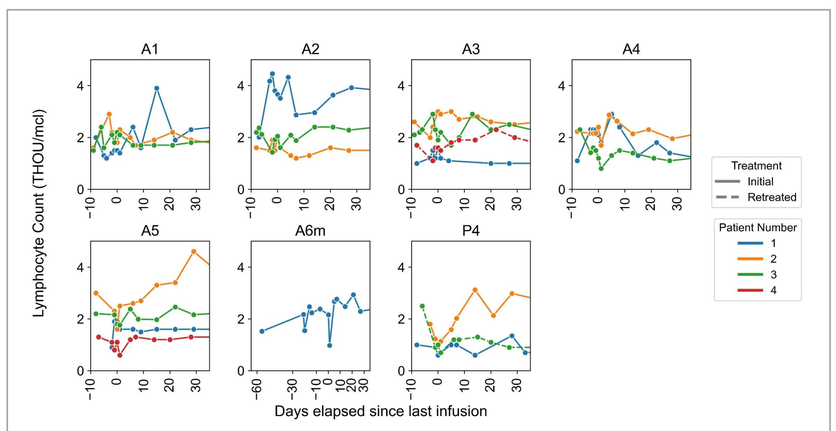


Figure 3. Lymphocyte counts. Complete white blood cell counts were clinically assessed before and after DSG3-CAART infusion. X-axis refer to timepoints pre- & post- infusion.

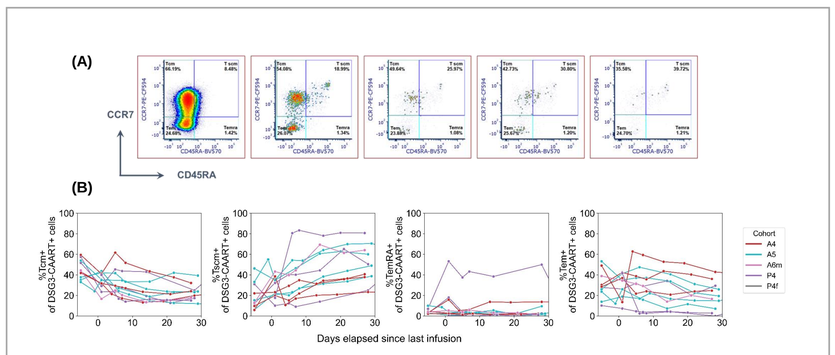


Figure 4. Phenotype of DSG3-CAART cells following infusion. (A) Flow cytometry of DSG3-CAART+ T cells from subject A4-1 expressing CCR7 and CD45RA from the MP or PBMCs from selected timepoints following infusion. (B) Line graphs from subjects with significant percentage of T cells that are DSG3-CAART+; the percentage of DSG3-CAART+ cells that are T_{CM}, T_{SCM},T_{EMRA} and T_{EM}. following infusion.

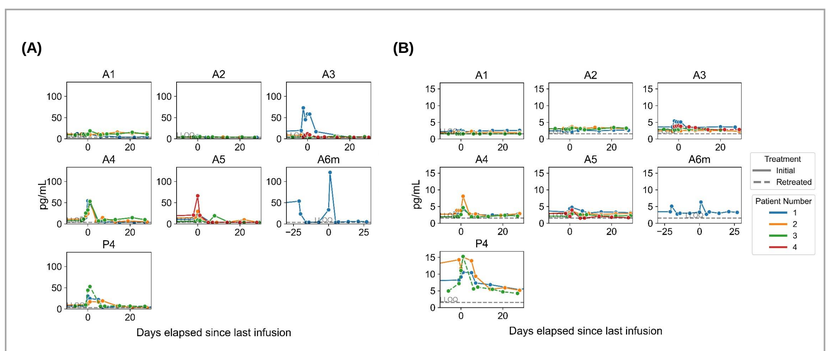


Figure 5. Post-infusion serum cytokine levels. Screening and post-infusion serum samples were analyzed for (A) IFN-γ and (B) IL-15 via MSD multiplex immunoassay. X-axis corresponds to days elapsed since last infusion. Dashed line depicts lower limit of quantification (LLOQ) of assay.

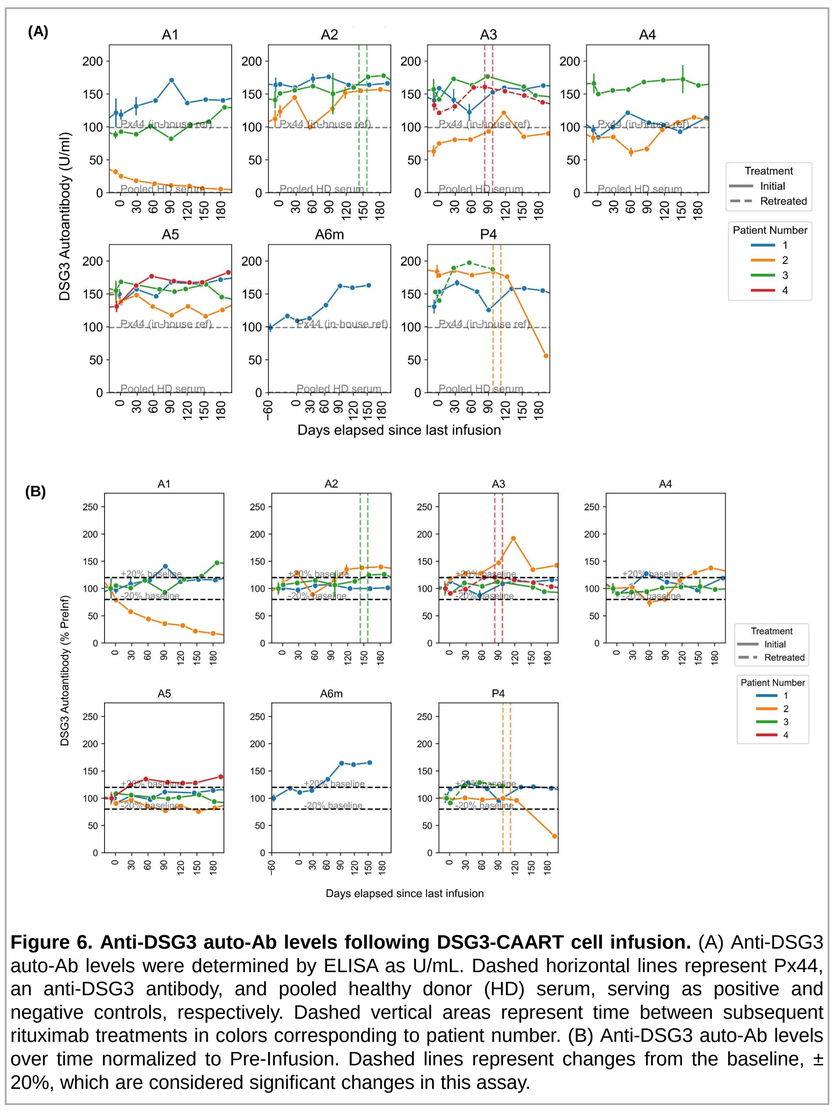


Figure 6. Anti-DSG3 auto-Ab levels following DSG3-CAART cell infusion. (A) Anti-DSG3 auto-Ab levels were determined by ELISA as U/mL. Dashed horizontal lines represent Px44, an anti-DSG3 antibody, and pooled healthy donor (HD) serum, serving as positive and negative controls, respectively. Dashed vertical areas represent time between subsequent rituximab treatments in colors corresponding to patient number. (B) Anti-DSG3 auto-Ab levels over time normalized to Pre-Infusion. Dashed lines represent changes from the baseline, ± 20%, which are considered significant changes in this assay.

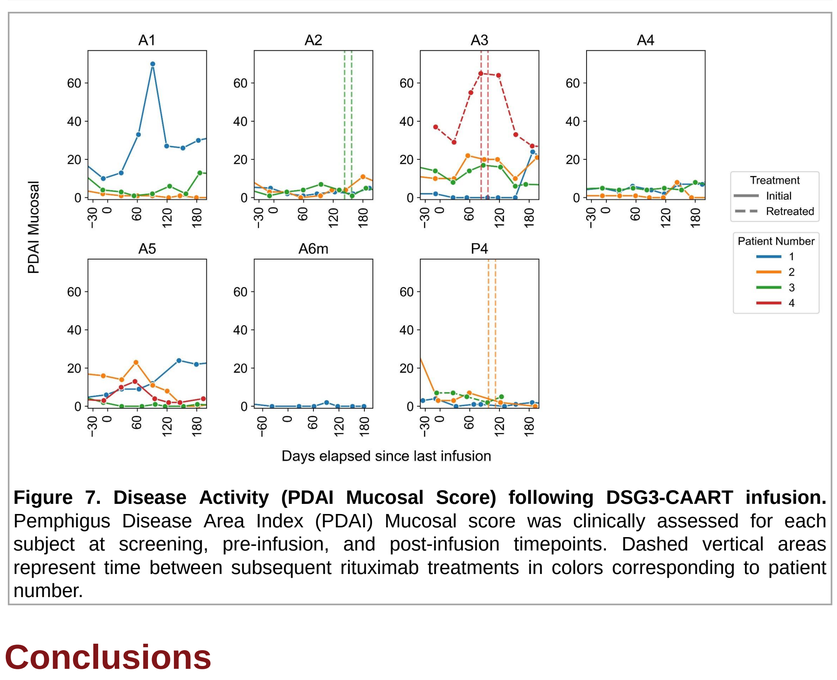


Figure 7. Disease Activity (PDAI Mucosal Score) following DSG3-CAART infusion. Pemphigus Disease Area Index (PDAI) Mucosal score was clinically assessed for each subject at screening, pre-infusion, and post-infusion timepoints. Dashed vertical areas represent time between subsequent rituximab treatments in colors corresponding to patient number.

Conclusions

- A 100% manufacturing success rate has been achieved to date across the 23 subjects treated in cohorts A1 to P4 in CAB-101
- DSG3-CAART cells persist in subjects with known anti-DSG3 auto-immunity up to and including 29 days in the presence or absence of lymphodepletion
  - There is a dose dependent increase in persistence and persistence AUC_{29d} across Cohorts A1 through A4 that levels off at doses ≥ 2.5 x 10^9 DSG3-CAART cells; persisting cells are predominately T_{SCM} and T_{CM}
  - At higher dose cohorts (A4, A5, A6m, and P4), persistence approached that which is observed in hematologic CAR-T trials (>1000 copies / ug DNA)
  - Elevations in serum IFNγ are observed following DSG3-CAART infusion
- Leukocyte numbers are temporarily reduced following cyclophosphamide and IVIg lymphodepletion, but persistence is not meaningfully impacted
  - Serum IL-15 is elevated following cyclophosphamide and IVIg lymphodepletion
- To date, in cohorts A1 to P4, there is no clear pattern of changes in anti-DSG3 auto-antibody levels or clinical disease activity scores
- Future cohorts will explore DSG3-CAART in the context of lymphodepletion with fludarabine, cyclophosphamide, and IVIg.

*The shaded area indicates levels of persistence typically observed in adult patients who have B-cell derived hematologic malignancies treated with CD19 CART cells combined with lymphodepletion (at a median dose of tisagenlecleucel of 3 x 10^8 cells).