P370

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Characterization of DSG3-CAART cells prior to & following adoptive transfer in mucosal Pemphigus Vulgaris

Cabaletta Bio®

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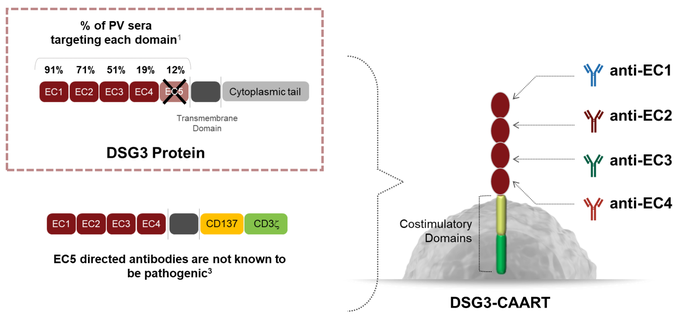
Background

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 cells (CAART) 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 [+1 A1-1 re-treated at the A3 dose]
A4	$2.5x10^9$	125x	3
A5	$5\text{-}7.5x10^9$	250 to 375x	4[a]
P4[b]	$2.5x10^9$ + cyclophosphamide & IVIg	125x	3
A6m[b]	$1\text{-}1.5x10^{10}$	500 to 750x	3

[a] A 4th subject was dosed in Cohort A5 to generate additional data
[b] Future cohorts P4 and A6m will be enrolled concurrently with prioritization of enrollment in cohort P4

Patient demographics

	Cohort A1 $2x10^7$ (n=3)	Cohort A2 $1x10^8$ (n=3)	Cohort A3 $5x10^8$ (n=3)	Cohort A4 $2.5x10^9$ (n=3)	Cohort A5 $5\text{-}7.5x10^9$ (n=4)[a]	Overall (n=16)
Age, years, median (range)	39 (32-57)	53 (50-54)	60 (47-70)	60 (56-70)	48 (34-57)	54 (32-70)
Female (%)	67%	67%	67%	67%	0%	50%
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)	1.6 (0.2-5.3)	3.4 (0.1-15.0)
Anti-DSG3 Ab Level, U/mL, median (range)	92 (51-104)	147 (86-168)	147 (63-169)	147 (114-162)	144 (124-169)	144 (51-169)
Pemphigus Disease Area Index, median (range)	17 (5-20)	6 (6-14)	12 (2-18)	3 (1-4)	5 (4-18)	6 (1-20)
Prior use of corticosteroids (%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	15 (94%)
Prior use of mycophenolate (%)	2 (67%)	3 (100%)	1 (33%)	2 (67%)	2 (50%)	10 (63%)
Prior use of rituximab (%)	3 (100%)	3 (100%)	0 (0%)	2 (67%)	1 (33%)	9 (56%)

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

Results

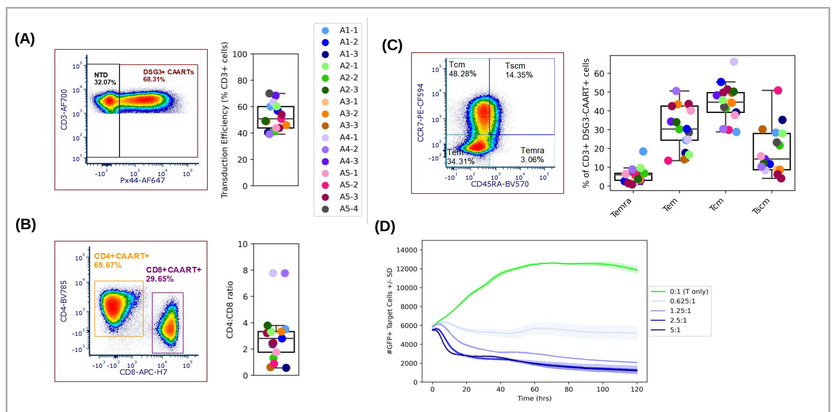


Figure 1. Infusion Product Characterization. (A) Transduction efficiency of the manufactured product (MP) measured by flow cytometry and defined as the percentage of subjects' T cells in the MP that are DSG3-CAAR$^+$. (B) Flow cytometry of DSG3-CAAR$^+$ T cells expressing CD4 and CD8 from the MP. Data 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.

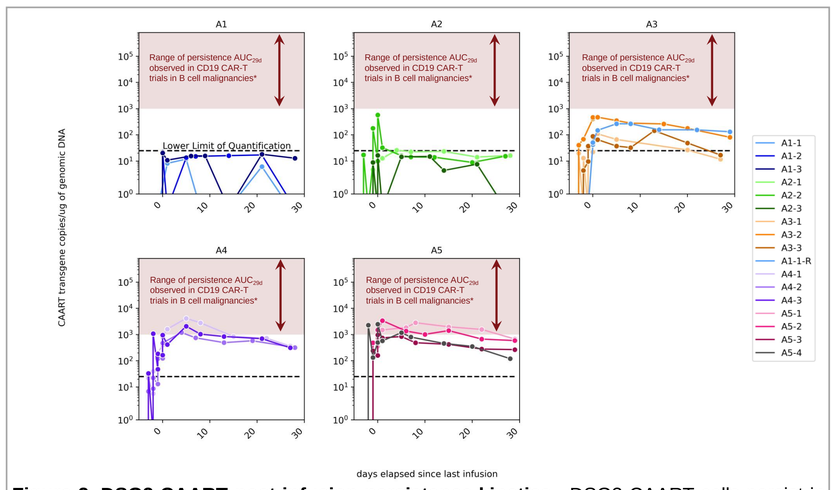


Figure 2. DSG3-CAART post-infusion persistence kinetics. DSG3-CAART cells persist in subjects following infusion without lymphodepletion. Post-infusion DSG3-CAART cell persistence was measured by qPCR as copies of CAART transgene/µg of genomic DNA, extracted from peripheral blood mononuclear cells in 16 subjects from the first 5 dosing cohorts of CAB-101. X-axis corresponds to days elapsed since last infusion. Upper left panel, 3 subjects enrolled in cohort A1. Upper middle panel, 3 subjects enrolled in cohort A2. Upper right panel, 3 subjects enrolled in cohort A3. Patient A1-1 from cohort A1 was re-treated with 5 x 10^8 DSG3-CAAR T cells and is included with the cohort A3 patients. Lower left panel, 3 subjects enrolled in cohort A4. Lower right panel, 4 subjects enrolled in cohort A5.

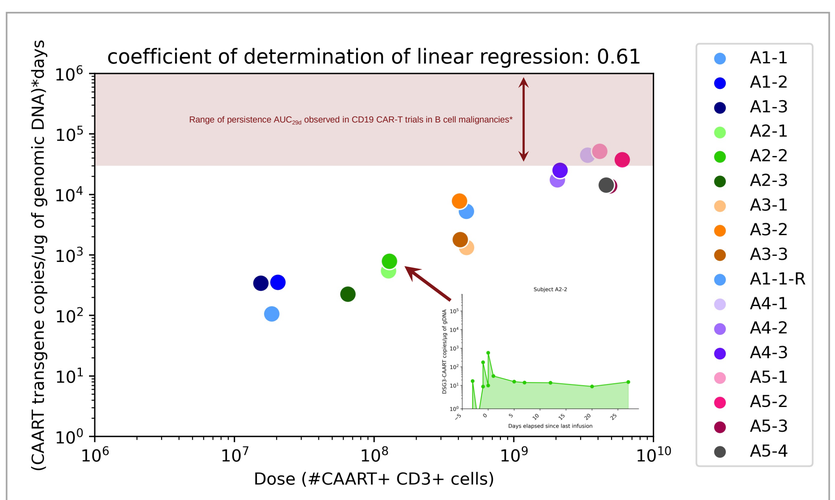


Figure 3. Post-infusion persistence is dose dependent up to the A4 dose. Persistence increases in a dose dependent manner following DSG3-CAART infusion and levels off at doses > 2.5 x 10^9 DSG3-CAART cells. Scatterplot depicting subjects' post-infusion persistence area under the curve for the first 29 days (AUC_{29d}) vs. dose administered of DSG3-CAART cells across 16 subjects from the first five dosing cohorts of CAB-101. Inset, AUC_{29d} for subject A2-2. The coefficient of determination of a linear regression using dose as the independent variable is 0.61.

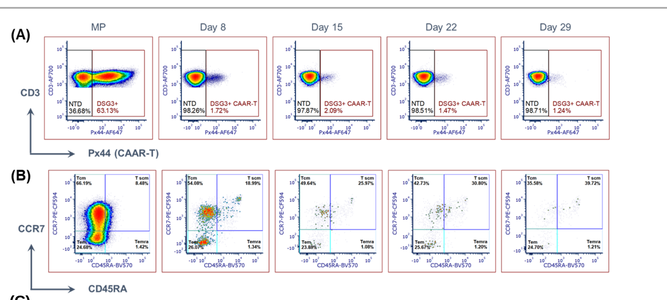


Figure 4. Phenotype of DSG3-CAART cells following infusion. Cohort A4 and A5 DSG3-CAART+ cells compromise 0 to 5% of all peripheral blood T cells following infusion and are mostly T_{SCM} or T_{CM} following infusion. (A) Enumeration of subject A4-1's DSG3-CAART+ cells from the manufactured product (MP) or PBMCs from selected timepoints following infusion. (B) 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. (C) Line graphs from all A4 subjects depicting percentage of T cells that are DSG3-CAART+ (left panel); the percentage of DSG3-CAART+ cells that are T_{CM} (middle panel); and the percentage of DSG3-CAART+ cells that T_{SCM} (right panel) following infusion. Note: T_{EM} and T_{EMRA} DSG3-CAART+ cells were less reliably detected by flow cytometry due to low frequency of events.

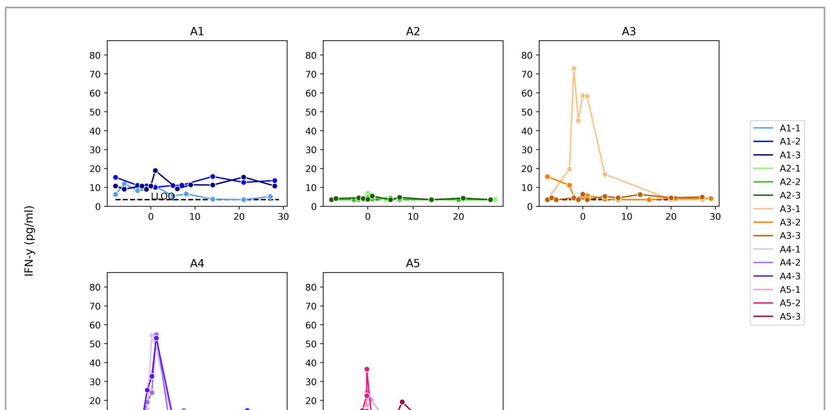


Figure 5. Post-infusion serum IFNγ levels. Serum IFNγ is transiently elevated following infusion in subjects at higher dose cohorts (A4 and A5). Screening and post-infusion serum samples were analyzed for cytokines via MSD multiplex immunoassay. X-axis corresponds to days elapsed since last infusion. Dashed line depicts lower limit of quantification (LLOQ) of assay. *Subject A3-1 was diagnosed with SARS-CoV2 infection shortly after DSG3-CAART cell infusion via PCR assay.

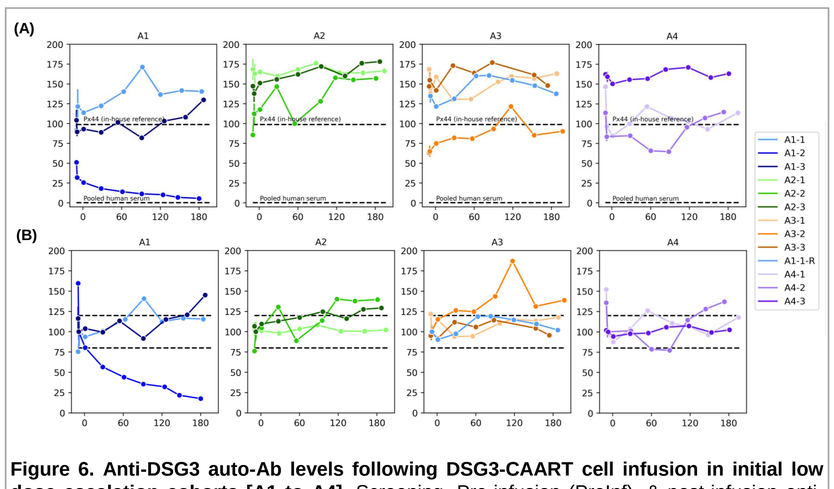


Figure 6. Anti-DSG3 auto-Ab levels following DSG3-CAART cell infusion in initial low dose escalation cohorts [A1 to A4]. Screening, Pre-infusion (PreInf), & post-infusion anti-DSG3 auto-Ab levels were determined by ELISA as U/mL from serum isolated from 12 subjects of the first 4 dosing cohorts of CAB-101. X-axis refers to timepoints pre- & post-infusion. (A) Line graphs depicting absolute values of anti-DSG3 auto-Ab levels over time. Dashed line depicts antibody control for assay. (B) Line graphs depicting relative anti-DSG3 auto-Ab levels over time normalized to the PreInf timepoint. Dashed lines represent changes from the PreInf timepoint > ± 20% which are considered significant in this assay. *Subject A1-2 was treated with rituximab within 12 months of infusion (rituximab excluded within 12 months of screening unless disease worsening).

Cohort (Dose)	Subject	Prior RTX or IVIg*	Meds stopped or tapered prior to inf.	Screen	Pre-Inf	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
A1	A1-1	RTX 10m	PRD	20	10	13	33 PRD	70 IVIg	27	26 MMF	30
A1	A1-2	RTX 6.5m IVIg 3m		5	2	1	1	1	0	1	0
A1	A1-3	RTX 9m	MMF	17	4	3	1	2	6	2	13
A2	A2-1	IVIg 4m		6	5	2	1	2	3 PRD	2	5
A2	A2-2			14	3	3	0	1	4 PRD	4	11
A2	A2-3	IVIg 4m		6	1	3 PRD	4	7	4 RTX	1	5
A3	A3-1			2	2	0	0 PRD	0	0	0 PRD	24
A3	A3-2		PRD, MMF	12	10	10	22	20	20	10	21
A3	A3-3			18	14	8	14	17	16 PRD	6	7
A4	A4-1		PRD, MMF	3	5	3	6 IVIg	4	2	12	7
A4	A4-2			1	1 PRD	1	1	0	0 PRD	8	0
A4	A4-3			4	5	4	5	4 PRD	5	4	8
# Subjects with PDAI=0 or 1 (Clear/Almost Clear)				1	2	3	6	4	3	3	2

Table 1. Disease Activity (PDAI Mucosal Score) following DSG3-CAART infusion. Pemphigus Disease Area Index (PDAI) Mucosal score was clinically assessed for each subject at the multiple timepoints: screening, pre-infusion, and post-infusion. RTX=rituximab; IVIg=intravenous immunoglobulin; MMF=mycophenolate; PRD=prednisone. *RTX or IVIg within12 months prior to infusion. RTX permitted within 12 months prior to screening if disease worsening; IVIg permitted >2 weeks prior to screening. 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.

Conclusions

- A 100% manufacturing success rate has been achieved to date across the 16 subjects treated in cohorts A1 to A5 in CAB-101
  - The infusion product has a median CD4:CD8 ratio of 2.8 (range 0.57-7.77) & median transduction percentage of 50.75% (range 39.2% - 70.0%)
  - The infusion product is largely composed of memory cells (T_{CM}, T_{SCM}, & T_{EMRA}) and has strong cytolytic capacity *in vitro*
- DSG3-CAART cells persist in subjects with known anti-DSG3 auto-immunity up to and including 29 days in the absence of lymphodepletion – no immune mediated rejection observed to date
  - There is a dose dependent increase in persistence and persistence AUC_{29d} across 16 subjects (in the absence of lymphodepletion) 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 and A5), persistence approached that which is observed in hematologic CAR-T trials (>1000 copies / ug DNA)
  - Elevations in serum cytokines are observed following DSG3-CAART infusion
- To date, in cohorts A1 to A4, there is no clear pattern of changes in anti-DSG3 auto-antibody levels or clinical disease activity scores
  - Patient A4-2 had a decrease in anti-DSG3 Ab titers at month 2 & 3**
- Initial results warrant further exploration of DSG3-CAART either through combination regimens or multi-dosing strategies.

*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).
** Data presented in detail at 31st Annual EADV conference

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