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Characterization of DSG3-CAART Cells Prior to & Following Adoptive Transfer in Mucosal Pemphigus Vulgaris

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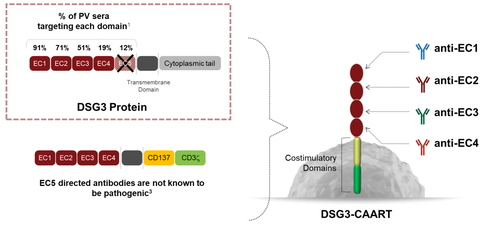
Background

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 along with phenotypic studies of T cells & sera from mPV patients treated with DSG3-CAART cells.

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).

DSG3-CAART Design



Overview of dose escalation

Cohort	Total Dose	Fold increase in dose	Patients per cohort
A1	2 x 10^7 DSG3-CAART	1x	3
A2	1 x 10^8 DSG3-CAART	5x	3
A3	5 x 10^8 DSG3-CAART	25x	3 [+1 A1-1 re-treated at the A3 dose]
A4	2.5 x 10^9 DSG3-CAART	125x	3
A5	5 - 7.5 x 10^9 DSG3-CAART	250 to 375x	3 (+3) [ongoing]
A5e*	5 - 7.5 x 10^9 DSG3-CAART	250 to 375x	3 (+3) [planned]
A6m**	1 – 1.5 x 10^{10} DSG3-CAART	500 to 750x	3 (+3) [planned]

*A5e represents an enhanced manufacturing process **A6m represents multiple infusions ≥ 1 week apart

Results

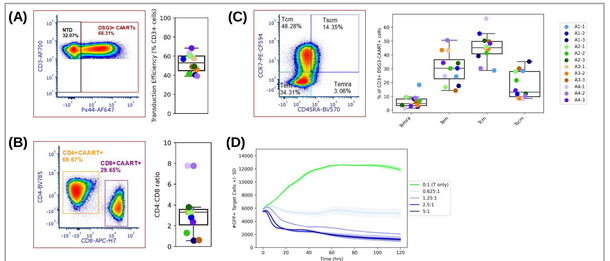


Figure 1. Infusion Product Characterization. (A) Transduction efficiency of the manufactured product (MP) measured by flow cytometry and defined as the percentage of subjects' T cells in the MP that are DSG3-CAAR+. (B) Flow cytometry of DSG3-CAAR+ T cells expressing CD4 and CD8 from the MP. Data represented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

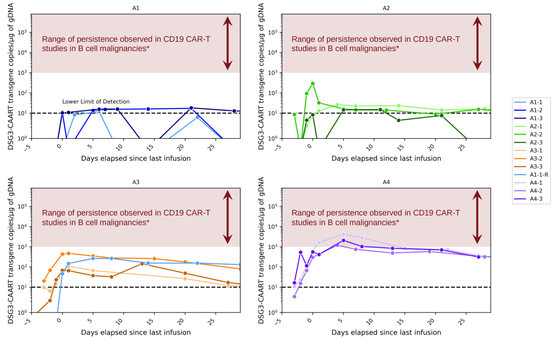


Figure 2. DSG3-CAART post-infusion persistence kinetics. DSG3-CAART cells persist in subjects following infusion in the absence of lymphodepletion. Post-infusion DSG3-CAART cell persistence was measured by qPCR as copies of CAART transgene per microgram of genomic DNA, extracted from peripheral blood mononuclear cells (PBMCs), in 12 subjects from the first four dosing cohorts of CAB-101. X-axis corresponds to days elapsed since last infusion. Upper left panel, three subjects enrolled in cohort A1 (target dose was 2x10^7 DSG3-CAAR T cells). Upper right panel, three subjects enrolled in cohort A2 (target dose was 1x10^8 DSG3-CAART cells). Lower left panel, three subjects enrolled in cohort A3 (target dose was 5x10^8 DSG3-CAART cells). 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 right panel, three subjects enrolled in cohort A4 (target dose was 2.5 x 10^9 DSG3-CAART cells). *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).

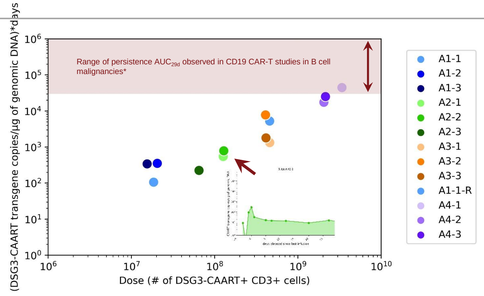


Figure 3. Post-infusion persistence is dose dependent. Persistence increases in a dose dependent manner following DSG3-CAART infusion. Scatterplot depicting subjects' post-infusion persistence area under the curve for the first 29 days (AUC_{29d}) vs. dose administered of DSG3-CAART cells across 12 subjects from the first four 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 0.96. *The shaded area indicates levels of AUC_{29d} 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).

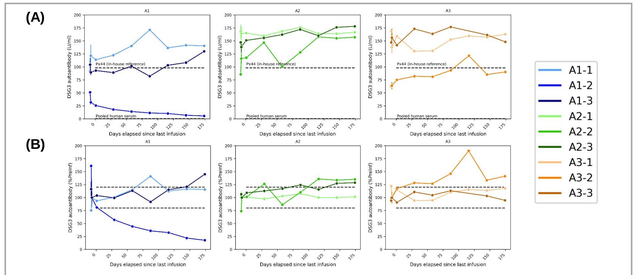


Figure 5. Anti-DSG3 auto-Ab levels following DSG3-CAART cell infusion in initial low dose escalation cohorts [A1 to A3]. Screening, Pre-infusion (PreInf), & post-infusion anti-DSG3 auto-Ab levels were determined by ELISA as U/mL from serum isolated from 9 subjects of the first three dosing cohorts of 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).

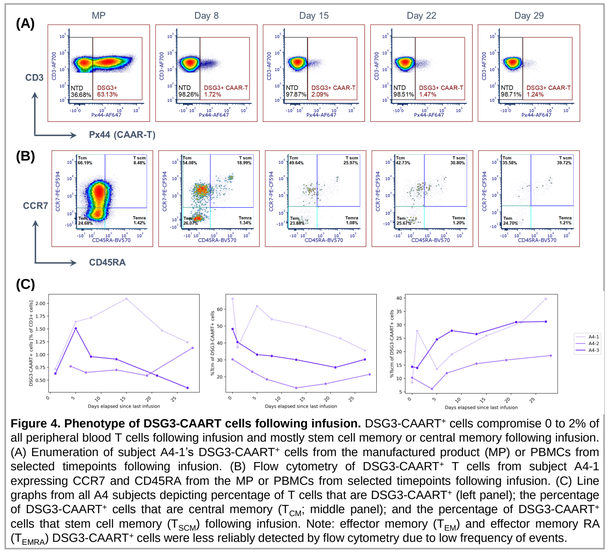


Figure 4. Phenotype of DSG3-CAART cells following infusion. DSG3-CAART+ cells compromise 0 to 2% of all peripheral blood T cells following infusion and mostly stem cell memory or central memory following infusion. (A) Enumeration of subject A4-1's DSG3-CAART+ cells from the manufactured product (MP) 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 central memory (T_{CM}; middle panel); and the percentage of DSG3-CAART+ cells that stem cell memory (T_{SCM}) following infusion. Note: effector memory (T_{EM}) and effector memory RA (T_{EMRA}) DSG3-CAART+ cells were less reliably detected by flow cytometry due to low frequency of events.

Conclusions

- A 100% manufacturing success rate has been achieved to date across the 12 subjects treated in cohorts A1 to A4 in CAB-101
  - 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 cells (T_{CM}, T_{SCM}, & T_{EMRA})
  - All infusion products manufactured to date have strong cytolytic capacity *in vitro*
- DSG3 CAAR-T cells persist in subjects with known anti-DSG3 auto-immunity up to and including 29 days in the absence of lymphodepletion
  - To date, no immune mediated rejection of DSG3 CAAR-T cells
  - Persisting DSG3 CAAR-T cells are predominantly T_{CM} and T_{SCM}
- There is a clear dose dependent increase in persistence and persistence AUC_{29d} across 12 subjects (in the absence of lymphodepletion)
  - Persistence is approaching that which is correlated with efficacy in hematologic CAR trials (>1000 copies / ug DNA)
- To date, in the low dose cohorts A1 to A3, there is no clear pattern of changes in anti-DSG3 auto-antibody levels
  - A3 dose (5 x 10^8 DSG3 CAAR-T cells) is ~ 7 to 10% of A5 dose (5-7.5 x 10^9 DSG3 CAAR-T cells)
- Initial results warrant further exploration at higher doses of DSG3-CAART