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-d3g3-3 autoantibodies (DSG3-CAART). The current standard of care for MPV includes broad 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-autoimmune immune cells. A chimera antigen receptor (CAR) T cell platform has been developed to specifically target and eliminate DSG3-3 expressing and T cells in patients with MPV. Currently, gene-modified autologous DSG3 specific CAR T cells (DSG3-CAART) are being evaluated in patients with MPV in a new phase I clinical trial (ClinicalTrials.gov identifier: NCT 04022957). Here, we report on the phenotypic & functional characteristics of the DSG3-CAART cell product along with phenotypic studies of 1 cell & sera from MPV patients treated with DSG3-CAART cells.

Methods
Flow cytometry analyses were performed on the infusion product or post-infusion PBMC samples to assess transduction efficiency & memory phenotype. DSG3-CAART cell cytotoxicity was performed on target cells that were pre-labeled with 51Cr in vitro.

Results

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Fig. 1. Cytotoxicity in vitro. a) Graphs depicting specific cytotoxicity (45±4.5) of DSG3-CAART in a 3 hour chromium release assay compared to naïve T cells & T cells engineered to target DSG3-3 using a CAART vector. Data are presented as mean ± SD. **p<0.005. (T) 3 x 10^4 target cells pre-labeled with 51Cr; (B) 1x10^5 DSG3-CAART cells or naïve T cells, or control wells without target cells. b) Representative dot plots depicting 51Cr release by DSG3-CAART and DSG3-3 specific control vector-transduced T cells in the presence or absence of DSG3-3 expressing Raji target cells used in the chromium release assay. (A) Control wells with 51Cr-labeled target cells. (B) Lysis of target cells by DSG3-3 specific control vector-transduced T cells. (C) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD3 antibody. (D) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD28 antibody.

Fig. 2. Persistence in vitro. a) Graphs depicting persistence of DSG3-CAART cells in the absence or presence of DSG3-3 expressing Raji target cells used in the chromium release assay. Data are presented as mean ± SD. **p<0.005. (T) 3 x 10^4 target cells pre-labeled with 51Cr; (B) 1x10^5 DSG3-CAART cells or naïve T cells or control wells without target cells. b) Representative dot plots depicting 51Cr release by DSG3-CAART and DSG3-3 specific control vector-transduced T cells in the presence or absence of DSG3-3 expressing Raji target cells used in the chromium release assay. (A) Control wells with 51Cr-labeled target cells. (B) Lysis of target cells by DSG3-3 specific control vector-transduced T cells. (C) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD3 antibody. (D) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD28 antibody.

![Image]

Fig. 3. DSG3-CAART persistence in vitro. DSG3-CAART cells persist in subjects treated at the A3 dose & at day 10 post-infusion. a) Representative dot plots depicting persistence of DSG3-CAART cells in the absence of DSG3-3 expressing Raji target cells at day 10 post-infusion. (A) Control wells with 51Cr-labeled target cells. (B) Persistence of DSG3-CAART cells in the absence of DSG3-3 expressing Raji target cells at day 10 post-infusion. b) persistence of DSG3-CAART cells in the absence of DSG3-3 expressing Raji target cells at day 10 post-infusion. (T) 3 x 10^4 target cells pre-labeled with 51Cr; (B) 1x10^5 DSG3-CAART cells or naïve T cells or control wells without target cells. c) Representative dot plots depicting 51Cr release by DSG3-CAART and DSG3-3 specific control vector-transduced T cells in the presence or absence of DSG3-3 expressing Raji target cells used in the chromium release assay. (A) Control wells with 51Cr-labeled target cells. (B) Lysis of target cells by DSG3-3 specific control vector-transduced T cells. (C) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD3 antibody. (D) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD28 antibody.

Fig. 4. Persistence in vitro. a) Graphs depicting persistence of DSG3-CAART cells in the absence of DSG3-3 expressing Raji target cells at day 10 post-infusion. Data are presented as mean ± SD. **p<0.005. (T) 3 x 10^4 target cells pre-labeled with 51Cr; (B) 1x10^5 DSG3-CAART cells or naïve T cells or control wells without target cells. b) Representative dot plots depicting 51Cr release by DSG3-3 specific control vector-transduced T cells in the presence or absence of DSG3-3 expressing Raji target cells used in the chromium release assay. (A) Control wells with 51Cr-labeled target cells. (B) Lysis of target cells by DSG3-3 specific control vector-transduced T cells. (C) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD3 antibody. (D) Lysis of target cells by DSG3-3 specific control vector-transduced T cells following treatment with anti-CD28 antibody.

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 3.5 (range 0.5–7.5) & median transduction percentage of 52% (range 38–71%).
- The infusion product is largely composed of memory cells (CD45RA&CCR7) with a broad level of DSG3-3 specific specificity.
- All infusion products manufactured to date have strong cytolytic capacity in vitro.
- DSG3-CAART T cells persist in subjects with known anti-DSG3 autoantibodies, immunity up to and including 29 days in the absence of lymphodepletion.
- To date, no immune-mediated rejection of DSG3-CAART T cells.
- Persisting DSG3-CAART T cells are predominantly CD45RA+ and CD28+. Persistence is approaching that which is correlated with efficacy in hematologic CAR T cell products.
- Subjects treated at the A3 dose (1x10^6 cells/kg) had prolonged persistence in vivo. In addition, this dose level was associated with nearly complete clinical remission in all subjects.
- There were no significant changes in persistence or persistence AUC after adminstration of corticosteroids, mycophenolate mofetil (MMF), or rituximab.
- In all subjects, clinical remission was maintained long-term (median 10 months, range 3–12 months of follow-up). This is consistent with the robust persistence following adoptive transfer.
- Immunoglobulin levels in B cells (DSG3-3 specific) were not significantly different from pre-transplant values.

* Asterisk represents an essential molecular component. ** Asterisk represents multiple introns in 3 exons across.

PACER (Pulmonary Arterial Cell Enrichment Reaction)