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Transcriptional Changes in Peanut-Specific CD4⁺ T Cells Over the Course of Oral Immunotherapy

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Abstract

Oral immunotherapy (OIT) can successfully desensitize allergic individuals to offending foods such as peanut. Our recent clinical trial ([NCT02103270](#)) of peanut OIT allowed us to monitor peanut-specific CD4⁺ T cells, using MHC-peptide Dextramers, over the course of OIT. We used a single-cell targeted RNAseq assay to analyze these cells at 0, 12, 24, 52, and 104 weeks of OIT. We found a transient increase in TGFβ-producing cells at 52 weeks in those with successful desensitization, which lasted until 117 weeks. We also performed clustering and identified 5 major clusters of Dextramer⁺ cells, which we tracked over time. One of these clusters appeared to be anergic, while another was consistent with recently described TFH13 cells. The other 3 clusters appeared to be Th2 cells by their coordinated production of IL-4 and IL-13, but they varied in their expression of STAT signaling proteins and other markers. A cluster with high expression of STAT family members also showed a possible transient increase at week 24 in those with successful desensitization. Single cell TCRαβ repertoire sequences were too diverse to track clones over time. Together with increased TGFβ production, these changes may be mechanistic predictors of successful OIT that should be further investigated.

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Keywords

peanut allergy; food allergy; OIT; single cell; RNAseq; CD4+ T cell

1. Introduction

Food allergy is defined as an immune-mediated adverse reaction to food [1]. It has become a global public health problem, and peanut allergy (PA) is the most typical example. In the U.S., the most common cause of anaphylaxis emergency is the intake of food allergen, and the annual occurrence is estimated to be about 30,000 [2]. Unfortunately, about 200 of these events are fatal [2]. Accidental exposure to peanuts is a major contributor to these deaths, and a recent survey in the U.S. found that 1.4% of the population is allergic to peanuts [3, 4] or tree nuts [5, 6]. Previous studies indicated that of children above 13 years old, 20.2% were reported to be peanut-allergic [6], as were 0.6-1% of adults [6]. Overall, 6-8% of children in the U.S. experienced some type of IgE-mediated food allergies [3-5, 7, 8].

The most frequent symptoms/adverse events (AEs) associated with PA are gastrointestinal (e.g., abdominal pain, nausea, vomiting) followed by dermal (e.g., pruritus, angioedema, urticaria), and then by respiratory (e.g., airway inflammation, wheezing, rhinitis). Life-threatening anaphylaxis leading to death is extremely rare. These AEs, as well as other measurements (e.g., IgE and IgG levels, peanut SPT wheal size, etc.), have been assessed for cross-associations in terms of peanut allergy [9-14]. However, the findings obtained in different studies sometimes were inconsistent [15].

Oral immunotherapy (OIT) is the most studied type of immunotherapy for treating allergies to foods [1]. Previous studies have reported successful OIT trials in small cohorts, where most of the patients were children (under 18 years). In those studies, OIT achieved desensitization (i.e., clinical non-reactivity to ingestion of a known allergen, given that daily consumption of that allergen is maintained) for milk [5, 7, 8], egg [16, 17], and peanut [18-30]. However, the protocols for the reported trials varied in increases in weekly doses, or rush therapy phases, or both. In our first Phase 1 peanut OIT study [4], we focused on the safety parameters of peanut OIT in children and adults. Since the incidence and prevalence of food allergies in adults and children are rising, it is important to understand the therapeutic and mechanistic effects of food allergy OIT in both populations.

Our second Phase 1 peanut OIT study was a longitudinal study titled Peanut Oral Immunotherapy: Safety, Efficacy and Discovery (POISED), a longitudinal, randomized, double-blind, placebo-controlled peanut OIT trial [31] in allergic individuals aged 7 to 55 years. We also tested sustained unresponsiveness using standardized definitions and validated food challenges. The results reported in this article are part of the POISED study.

Until now, there has been no accurate single biological measurement to quantify underlying allergic response processes [32]. Yet it is believed that the development of regulatory T (Treg) cells is compromised during allergic responses, and is replaced by the generation of T helper 2 (Th2) cells that drive IgE class switching and the expansion of allergic effector cells [1]. Recent GWAS (genome-wide association study), epigenetic and other biomarker studies

have revealed the association of several biomarkers with peanut allergy, including MHC-class related proteins (such as HLA-DR and -DQ), transcriptional factors (such as FOXP3), and cytokines such as IL-4, IL-5, IL10, IL-13, IL-33, TNF- α and IFN- γ , which indicate Treg, Th1 and Th2 cell activity [9, 10, 33, 34]. Specifically, children with PA showed a Th2-type profile with low levels of IFN- γ and TNF- α and high levels of IL-4, IL-5, and IL-13, whereas those without PA showed a potential Th-1 skewing [1, 32, 35]. This could influence multiple organ systems, driving the responses in patients with PA to rapidly become life-threatening [36].

CD4⁺ T cells with a Th2 phenotype are known to be involved in the expression of food allergy [37]. There is evidence to suggest that, over the course of successful OIT, these cells either change phenotype (e.g., to become anergic) or disappear and are replaced by non-Th2 cells [38, 39]. Different cell populations were reported to play important roles in different patients, and on a varying time scale [40]. However, there is still considerable controversy about the clonal fate of allergen-specific T cells during OIT and which early biomarkers might indicate success of OIT. Therefore, we focused primarily on determining whether subtypes of long lived allergen-specific T cells would discern successfully vs unsuccessfully-treated individuals in our phase 2 randomized clinical trial (POISED) [31].

Based on targeted gene expression assays performed over the course of OIT, we now report on the dynamics of the peanut-responsive T cell phenotypes in those who were successfully desensitized (at week 117, 13 weeks after cessation or reduced amount of OIT) versus not desensitized to peanut. From these analyses, we suggest early T cell predictors and mechanisms of OIT success.

2. Materials and Methods

2.1. Clinical Study and Endpoints

The POISED study ([NCT02103270](#)) was a phase 2, randomized, placebo-controlled, double-blinded, longitudinal, peanut OIT study (see schematic in Figure S1). The patients in the study consisted of 100 children (with ages of 7–18 years) and 20 adults (with ages of 19–55 years). All patients had an allergy to peanut, as determined by double-blinded, placebo-controlled food challenge (DBPCFC), history, clinical symptoms, and positive skin prick test (SPT). Inclusion and exclusion criteria and clinical results are given in Chinthrajah et al. [31]. Briefly, 120 patients received either daily placebo (n=25) or peanut OIT of varying doses up to 4 g (n=95) for 104 weeks, after which they were given an oral food challenge (OFC). Those who passed a 4 g OFC at week 104 were divided to receive either no peanut or 300 mg of peanut daily after week 104, and underwent OFC again at week 117 to determine desensitization.

In this study, we only focused on 30 patients (out of the 120) who had peanut-specific T cell data from baseline to week 104. The demographic features of these 30 patients are shown in Table S1. These 30 patients had available T cell data for this longitudinal analysis due to HLA matching for our Ara h 2 dextramers expressing HLA-DRB1*1501 or HLA-DRB4. As our primary endpoint, we compared those with successful desensitization through week 117 (referred to as “success”, n=6), with those who succeeded at week 104 but failed at week

117 (referred to as “failure”, n=16), and those who received placebo (referred to as “placebo”), the only group of patients who failed at week 104 (n=8). While one placebo patient in the POISED study did not fail at week 104, that patient did not have matching HLA for this sub-study). Given the small number of patients available for analysis, we didn’t have sufficient cases to discriminate between those who had received 0 vs. 300 mg of peanut daily between week 104 and 117, either in the success group (3 received 0 and 3 received 300 mg of peanut) or in the failure group (14 received 0 and 2 received 300 mg of peanut).

2.2. Single-cell targeted RNAseq assay

CD4⁺ T cells from 30 subjects positive for HLA-DRB1*1501 or HLA-DRB4 by molecular typing were stimulated and sorted for the single-cell targeted RNAseq assay, as shown in Figure S2. CD4⁺ T cells were enriched from whole blood using RosetteSep (StemCell Technologies), at baseline and 12, 24, 52, and 104 weeks post-initiation of peanut OIT. Approximately 4×10^7 CD4⁺ T cells were stimulated for 1.5 h with 50 ng/ml phorbol myristate acetate (PMA) + 1 µg/ml ionomycin (both from Sigma, St. Louis, MO). The cells were then stained with MHC Dextramers (Immudex, Copenhagen, Denmark) comprising an Ara h 2 epitope (aa 120-139) presented by HLA-DRB1*1501 or HLA-DRB4. Dextramer-positive cells were enriched using PE-specific magnetic beads. Single Dextramer-positive (i.e., peanut-specific) CD4⁺ T cells were then sorted into wells of 96-well plates containing 5 µl RNA lysis buffer (QIAGEN OneStep RT-PCR Kit, cat.# 210212; and SUPERase from thermo-fisher, cat.# AM2694), using a FACS Aria II flow cytometer (BD Biosciences, San Jose, CA). An example of gating and analysis of a sample from the “Success” and a sample from the “Failure” group is shown in Figure S3.

From the single cell RNA, a targeted RNAseq assay was applied, with 38 TRBV and 40 TRAV segments of human TCRs (TCRseq assay) plus 63 genes of interest as “targets” (see Table S2 for specific genes and primers). Briefly, the single-cell RNA samples were subjected to three rounds of nested cDNA amplification, of which two rounds were to amplify the target sequences and a final round to add well-specific barcodes [41]. The resulting amplified cDNA from up to 20 plates were pooled together for sequencing on a MiSeq (Illumina). A bioinformatics pipeline [41] was used to align the reads to specific wells and to TCR or phenotypic genes, and to call the dominant TCR Vβ, Jβ, Vα, Jα, and CDR3β and CDR3α sequences. The resulting spreadsheet was then subjected to data pre-processing steps below, resulting in the input data for the analysis on cellular phenotypic markers.

2.3. Data pre-processing

After all data were collected from 29 batches in total (each batch consisted of multiple 96-well plates as mentioned above), they were combined into one master dataset. The master dataset then underwent a QC process to remove batches with poor quality, duplicate runs of the same plate and patients who dropped out before week 104 (see Supplemental File 1 for details).

2.4. Longitudinal analyses on Dextramer (Dex)+ cell phenotype

After this subset was collected (6 patients in the success group, 8 in failure, 16 in placebo), markers with >5% positive call rate in at least one of the weeks of interest (i.e., week 0, 12, 24, 52 and 104) were preserved. This resulted in a marker panel of 22 markers (BCL6, CCR7, CD44, CD69, EGR2, GATA3, GPR15, IL4, IL10, IL13, IL33, PRF1, PTPRC, RUNX1, RUNX3, STAT3, STAT4, STAT5A, STAT5B, TBET, TGF β 1 and TNF). Clustering (using ward method [42]) was performed based on these markers, after removing cells that expressed none of these markers. This resulted in 3623 remaining cells, 55% of all Dex+ cells from those 30 patients). Starplots were generated using the same dataset, using the algorithm described in Holmes et al. [43], followed by statistical analysis for microarrays (SAM) [44] test to examine markers that were significantly associated with primary endpoints. Mosaic plots were generated to show individual variabilities in terms of cluster abundance, at each given timepoint.

3. Results

3.1. Participants of this study

Among the 120 participants in the POISED study, 30 (11 females and 19 males, see Table S1) were included in this single cell TCR sub-study by virtue of their expression of HLA-DRB1*1501 and/or HLA-DRB4 alleles, for which Ara h 2 peptide-MHC Dextramers were used to track their peanut-specific CD4+ T cells (example gating schemes shown in Figure S3). 8 of these were placebo subjects, while 6 were successfully desensitized through week 117 (passed peanut challenge at weeks 104 and 117) and 16 failed desensitization at week 117 (although they each passed peanut challenge at week 104).

3.2. Frequency of peanut-specific T cells over time

We first asked whether the number of peanut-specific CD4+ T cells changed over time with OIT, and whether this was significantly different between outcome groups. The numbers of collected Dex+ T cells per million CD4+ T cells over time, per outcome group, are shown in Figure S4. These ranged from 0 to 884 cells per patient time point, with a median of 39 for Success, 31.5 for Failure, and 35 for Placebo groups across all time points. T-test suggested that the frequency of Dex+ cells was not significantly different between the three groups at any time point after baseline.

3.3. Clonal persistence of peanut-specific T cells

With our single-cell TCRseq assay, we intended to track T cell clones over the course of OIT to determine whether a desensitization status was associated with persistent clones, or rather with clonal replacement. However, we observed only one CDR3 β clone that persisted within a single patient between week 0 and week 104 (Table S3). The top 5 most frequent CDR3 β sequences in Dex+ and Dex- cells at baseline are shown in Table S4, where we observed a mild bias in V-gene usage in Dex+ cells but no preference in Dex- cells. Still, the finding of only a single intra-patient persistent clone led us to conclude that our sampling rate was too low to track individual clones within a patient; so we were unable to accurately estimate the

degree of clonal persistence versus replacement. Therefore, we proceeded to analyze the phenotypic profiles of Dex+ cells.

3.4. Phenotype of peanut-specific T cells

Figure S5 shows numerous differences in marker expression between Dex+ and Dex- CD4+ T cells. These included increased expression of the canonical Th2 transcription factor GATA3 in dextramer-positive cells. There was also reduced expression of STAT family members and TNF among the Dex+ cells. Interestingly, we did not observe much difference in the rate of IL-4 and IL-13 gene expression between Dex+ and Dex- cells. This could be due to generalized Th2 skewing of these allergic individuals, and/or some level of false positive signals for these genes.

3.5 Longitudinal analysis of markers on peanut-specific T cells

To study how markers on Dex+ cells changed with OIT, we checked the longitudinal marker profile on each of the 22 selected markers. Figure 1 shows the trend of each marker, stratified by the desensitization outcome at week 117. CD69 was always high before and throughout OIT, indicating successful in vitro stimulation. CCR7, GATA3, RUNX3 and TBET showed a declining trend. EGR2 showed a slight ascending trend in the failure group, but not in the success group. However, the baseline values of those markers were also different in the different endpoint groups.

Principal components analysis did not show a clear segregation based on desensitization status. However, we saw a reasonable group separation using a starplot algorithm [43]. As shown in Figure 2, there was little segregation between the 3 groups at baseline and week 24. However, at week 52, a better segregation was achieved between the desensitization failure and success group. This was mediated in large part by high expression of TGF β in the success group (see starplot vectors in Figure 2). Interestingly, at week 104, this segregation was less obvious than at week 52. The SAM test suggested that the following markers were significantly increased in the success group:

- Baseline: CD69, IL-13 and TNF
- Week 12: RUNX1
- Week 24: TGF β , RUNX3
- Week 52: TGF β

No marker was shown to be significantly increased in the failure group.

3.6. Phenotypic clustering of peanut-specific T cells

Because the data from single cells is connected across markers, we performed clustering of Dex+ cells as well as single-marker analysis. To determine the optimum cluster number for this subset of phenotypic markers, we performed a hierarchical clustering analysis (Figure 3) and divided the cells into 5 clusters based on empirical analysis of the hierarchy (clustering algorithms yielded optimal cluster numbers between 3 and 8). The profile of the markers in each cluster is shown in Figure 4. All clusters expressed IL-4 and IL-13 in a coordinated manner. Since our samples were stimulated with PMA+ionomycin, CD69 was expected to

be expressed, thus cluster 1 (which expressed little CD69 or other functional markers) could be seen as anergic or exhausted. Cluster 2 was high in CD69, IL-13 and IL-4, indicating a Th2 phenotype, although it was low in GATA3. Interestingly, GATA3 was low in all the clusters except Cluster 5. Also, all clusters expressed reasonably high IL-4 and IL-13 (except cluster 1), but only Cluster 5 co-expressed these with GATA3.

Clusters 3 and 4 were largely similar, except that the latter had higher GPR15, IL-13 and IL-4, and lower CD44, EGR2, RUNX family, TBET and TNF. In terms of the STAT family, STAT3 and STAT4 were similarly expressed in Cluster 3 and 4, but STAT5A and 5B were less expressed in the latter. This was in sharp contrast to Cluster 5, where STAT family members were almost absent. But Cluster 5 was rich in a number of markers that were not highly expressed in other clusters, including BCL4, CCR7, PRF1, PTPRC, TBET, RUNX family and TGF β . These observations together indicate that Th2 cells, defined as those expressing IL-4, IL-13, and/or GATA3, can encompass subpopulations, as exemplified by these clusters, with differential expression of transcription factors and signaling molecules.

3.7. Cluster changes over time

To examine how cluster abundance changed over the course of OIT, we plotted the relative proportions of each cluster at each time point, for the desensitization success and failure groups, as well as for placebo and all groups combined (Figure 5). From this analysis, we found the general pattern over time in the success group to be somewhat different from the failure group. In addition to a different baseline cluster composition, there appeared to be a transient increase in cluster 3 at week 24 in the success group, though this was not statistically significant compared to other groups. Since this cluster seemed to represent activated Th2 cells (i.e., expressing high levels of IL-4, IL-13, and STAT family members), this might signal that a transient increase in activated STAT⁺ Th2 cells precedes successful OIT. In addition, cluster 5 can be seen to decrease over time, but this was not limited to the success group. The individual variabilities in terms of cluster abundance are shown in Figure S6. Overall, there appears to be a decrease in cluster 1, increase in clusters 2, 3, and 4, and disappearance of cluster 5 over time.

We also asked whether cluster profiles themselves changed over the course of OIT. As shown in Figure 6, most markers were stably expressed in most clusters, with a few shifts in expression at one or two time points. An exception was cluster 5, which showed steady decreases in GATA3, PRF1, and (to a lesser extent) IL-13 expression over time. However, it should be noted that this cluster itself decreased in abundance over time, such that there was only one cell in Cluster 5 at week 104.

4. Discussion

In this study, we profiled peanut-specific CD4⁺ T cells over time in subjects undergoing OIT or on placebo. We then compared the profiles of those undergoing OIT between those who passed or failed desensitization at week 117, after they all had been successfully desensitized by week 104. At the single marker level, our major finding was a transient increase in TGF β expression at week 52 in those who ultimately had successful desensitization, as determined on week 117. The SAM test confirmed this significant association of TGF β with success at

weeks 24 and 52, along with other significantly up-regulated markers in the success group (CD69, IL-13, and TNF at baseline, and RUNX1 and RUNX3 at week 12 and 24, respectively). At the level of cell clusters, our major observation was a transient increase in Th2 cells with high STAT expression (clusters 3), at week 24, in the success group.

The frequency of epitope-specific T cells in our sub-study ranged from 0 to 884 cells per million CD4⁺ T cells at all patient time points, with a median of 31.5 (Failure group) to 39 (Success group) across time points. This is similar to other reported studies. For example, Renand et al. [45] reported a frequency of memory CD4⁺ T cells specific to Arah2-derived peptides of close to 100 per million CD4⁺ T cells. Since we assayed only a single peptide epitope (aa 120-139) in our study, it would be expected that our frequencies would be slightly lower.

Our phenotyping of epitope-specific CD4⁺ T cells was limited to CD45RA vs. CRTH2 during sorting, as shown in Figure S3. Similar to Renand et al. [45], we found the epitope-specific CD4⁺ T cells to be primarily memory (CD45RA⁻) in phenotype. However, we found a lower proportion of these (10%) to be CRTH2⁺. This could be due to the epitope in question, or could be a technical issue of our staining. Additional phenotyping with regard to gut-homing receptors would also be of interest, and is currently being evaluated by our group using CyTOF. The homing receptors included in our single-cell RNAseq panel, however, did not provide sufficient reads to pass our initial quality control steps.

Cluster 1 expressed very few cytokines or activation markers, and could be considered anergic. The other four clusters could be considered Th2 (by having at least some expression of IL-4 and IL-13). Cluster 2 expressed considerably high levels of IL-13, IL-4 and CD69, and reasonable levels of EGR3, RUNX1, STAT4 and STAT5A. Only clusters 3 and 4 expressed high levels of STAT3, STAT4, STAT5A, and STAT5B, and they both expressed CD44, EGR2, GPR15, and IL-33. Only cluster 5 expressed significant GATA3. These distinctions highlight the existence of subpopulations of Th2 cells in our patients, as defined by differential gene expression.

In our study, the Th2 cells with high expression of STAT family members seemed to dynamically change with successful OIT, more so than the other clusters. For example, within the success group at week 24, we observed a transient increase in Cluster 3, although this was not statistically significant. In contrast, the failure group showed a constantly increasing activated Th2 cell population up until week 104. Thus, the early appearance of these activated Th2 cells in the success group may play an important role in achieving successful desensitization.

We also saw, in all groups, a disappearance over time of cluster 5, the most multifunctional cluster, and the only one with high levels of GATA3. This cluster, with combined expression of GATA3, IL-4, IL-13, and BCL6, resembles the recently described TFH13 cells [46]. These cells are thought to drive anaphylactic IgE production [46]. While their decrease during OIT is interesting, it was not specific to those in the success group, as defined here.

Finally, we observed a transient increase in TGFβ-producing cells in the success group at week 52, suggesting a potential role of TGFβ in the desensitization process. TGFβ is known

to be a pleiotropic cytokine involved in both suppressive and inflammatory immune responses [47–49]. It has been reported that TGF β inhibits Th1-, and Th2-cell differentiation, and plays a role in maintaining peripheral tolerance against food allergies, but it can also promote expression of inflammatory mediators, such as IL-6 and lymphotactin [50]. In the success group we saw the increase in TGF β -producing cells after the increase in activated Th2 cells, suggesting that the role of TGF β here might be suppressive. However, there was also some increase in TGF β in the placebo group at week 24, which could reflect technical or biological noise in the assay, since this group was not undergoing OIT.

A limitation of this study is the small number of FILA-matched patients, and the fact that it was focused on T cells specific for a single epitope of Ara h 2 (aa 120-139). As such, these findings may not reflect the changes seen in all peanut-specific CD4+ T cells. Furthermore, the number of Dextramer+ cells was often quite limited, creating a sampling bias whereby some patients provided more Dextramer+ T cells for analysis than others. This was shown in Figure S6, where the width of each bar in the mosaic plots was proportional to the cell number of each individual. Finally, it was impossible to know whether the targeted RNAseq method was fully sensitive for detecting expression of each target gene in each individual cell; as such, false negatives could occur. Because of this, we prefiltered data to exclude cells with little or no expression across all genes, and to exclude genes with no expression across all cells. Fortunately, from our assay qualification data (manuscript in preparation), we know that false positive signals are essentially absent in this assay, so gene expression, where seen, can be considered valid.

All patients in this sub-study who received peanut OIT (i.e., not placebo) passed the desensitization tests at week 104. That said, we defined successful desensitization as those that maintained their clinical non-reactivity at week 117 (instead of week 104). This meant that the distinctions between the success and failure groups may have been subtle, since even the patients in the failure group were initially desensitized (at week 104). We suggest that biomarkers of OIT success might be more apparent if we had been able to compare to patients who failed to achieve even initial desensitization. Additionally, the active OIT participants either received 0 mg or 300 mg peanut daily between week 104 and week 117. Given the sample size limitation in this study, stratification by these study arms was not possible. We therefore had to analyze success vs failure groups at week 117, independently of the treatment for the final 13 weeks. This has to be considered when interpreting our results, and the possible stratification based on low vs. no OIT maintenance dose should be studied in larger trials.

Overall, our results suggest two potential biomarkers of successful peanut OIT, both occurring transiently, at weeks 24 and 52, respectively. The first is an increase in Th2 clusters that express STAT3, 4, 5A, and 5B, as seen in Figure 5. The increase in these “activated” Th2 cells might presage their further differentiation and/or anergy. The second is an increase in TGF β production, as seen in the starplots of Figure 2. We also saw a decrease in potential TFFI13 cells, though this was not specific to those in the desensitization success group. We suggest that these potential biomarkers be further evaluated in additional trials that include larger numbers of patients and cells with additional specificities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Wang et al. Highlights

- Single-cell targeted RNAseq tracks peanut-specific T cells during immunotherapy.
- Peanut-specific T cells transiently increase TGFP during successful desensitization.
- Different clusters of Th2 cells can be identified based on STATs and other markers.

Figure 1.

Longitudinal trend of Dex+ cells per marker, grouped by OFC results. Data (mean values with standard errors) were obtained from single cell RNAseq assay of 30 patients (11 females and 19 males), over five time points (week 0 to week 104).

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Figure 3.

Clustering (with Ward's minimum variance method, Euclidean distance) on longitudinal cellular phenotypes of Dex+ cells only. Clusters 1 – 5 are color highlighted from top to bottom. Data were obtained from single cell RNAseq assay of 30 patients (11 females and 19 males).

Figure 4.

Cluster signatures (week 0, 12, 24, 52, 104 combined). The bar length represents positive call rate (percentage of cells expressing the marker). Data were obtained from single cell RNAseq assay of 30 patients (11 females and 19 males).

Figure 5.

Proportions of Clusters over time in all patients (top left), or in the outcome groups as listed. Note the transient expansion in the “success” group of cluster 3 at week 24, relative to the “failure” group. Data were obtained from single cell RNAseq assay of 30 patients (11 females and 19 males).

Figure 6.

Positive marker call rates exhibiting distinguishing features of 5 clusters, with change over time (mean values with standard errors). Data were obtained from single cell RNAseq assay of 30 patients (11 females and 19 males).