Metabolic Transition During Manufacturing of Renal Autologous Cell Therapy Candidate Rilparencel

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RILPARENCEL

- Kidney cortical biopsy-derived **rilparencel is a novel investigational autologous kidney cell therapy**.[1,2]
- Rilparencel is being evaluated in a late-stage clinical trial for patients with advanced chronic kidney disease (CKD) and diabetes mellitus (DM).[3-5]
- Data from two Phase 2 studies suggest that kidney cortical injection of rilparencel may preserve kidney function in subjects with DM and advanced CKD.[1,2]

METHODS

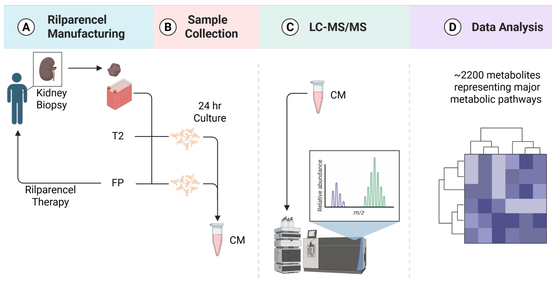

Figure 1: Flow Chart of Rilparencel Global Metabolomics Analysis.

A. **Rilparencel Manufacturing:** Rilparencel is manufactured from cells obtained from a kidney biopsy by an expansion and selection process. The cells selected by the manufacturing process are formulated into rilparencel and injected percutaneously into the kidney cortex.
B. **Sample Collection:** Conditioned media (CM) was generated from a 24-hour culture of cells isolated from manufacturing at two time points (T2 and FP).
C. **Assay design:** Global untargeted metabolomics was performed on polar and lipid fractions via LC-MS/MS (liquid chromatography-tandem mass spectrometry).
D. **Data Analysis:** Each fraction was run in positive and negative ionization modes, yielding four assays that detected 2,215 metabolites, of which 986 were confidently identified. Analysis performed by Panome Bio (St. Louis, MO).

- **Samples:** 36 conditioned media (CM) samples: 18 T2 (intermediate manufacturing), 18 FP (final product), and 3 media controls (blank media)
- **Clinical Studies:** N=5, RMCL-002; N=12, REGEN-007 (ClinicalTrials.gov NCT02836574 , NCT05018416) were Phase 2, multicenter, randomized, open-label trials of rilparencel in persons with CKD and DM [4,5]
- N=1, (CKD Stage 2) NDRI (National Disease Research Interchange)
- **Data Analysis and Statistics:**
  - Coefficient of variation (CV) <10%, <4% missing data (imputed)
  - Normalized log_2-transformed data was used for PCA and statistics
  - One-way ANOVA (T2 vs. FP) was used to identify significant metabolite levels (p-value < 0.05) with an absolute log_2FC (fold-change) greater than 1.0
  - Log_2-transformed fold changes were used for heatmaps and pathway analysis
- **Figures:** Images generated using BioRender and RStudio

RESULTS

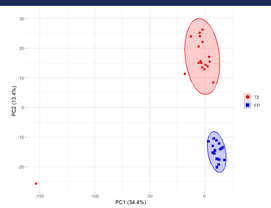

Figure 2: Principal Components Analysis (PCA) of Metabolomics Data. The combined metabolic profiles from all assays for each sample (n=5, RMCL-002; n=12, REGEN-007; n=1, NDRI) at two time points are visualized via PCA. Samples cluster into two distinct groups based on manufacturing time point (T2, blue; FP, red). Although an intermediate manufacturing sample (T2, NDRI) is separate from all other T2 samples, it clusters well with all other FP samples. This clustering suggests that manufacturing acts to normalize metabolic profiles of the cells.

RESULTS

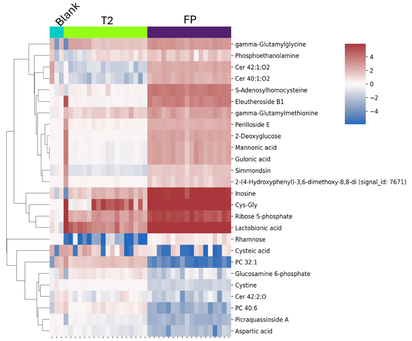

Figure 3: Heatmap of Significantly Enriched and Reduced Metabolites. The log_2FC of metabolite abundances relative to the mean Blank level is shown in the heatmap for the 18 significantly enriched (red) metabolites and 8 significantly reduced metabolites (blue). Each column represents a sample, and each row represents a metabolite. Of interest, several metabolites are significantly enriched in FP, including inosine and s-adenosylhomocysteine. Several metabolites were significantly reduced in FP, such as the phosphatidylcholines (PC), PC 32:1 and PC 40:1, and amino acids, cystine and aspartic acid, consistent with building more specialized membrane architecture.

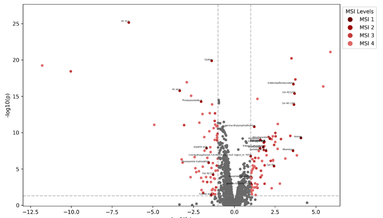

Figure 4: Volcano Plot of Metabolites. The p-value and log_2FC of the metabolite levels between T2 and FP samples are plotted against each other in the plot above. Features that meet the $p < 0.05$ threshold and have an absolute log_2 fold-change greater than 1.0 are shown as red dots. The colors of the dots reflect the Metabolomics Standards Initiative (MSI) levels, with a legend indicating the color intensity.

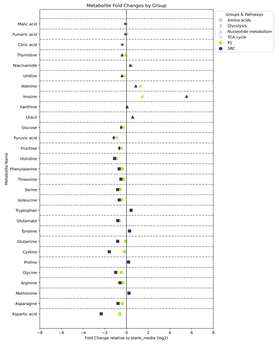

Figure 5: Fold-Change Plot of Common Metabolites Shows Changes in Amino Acid Metabolism During Manufacturing. The log_2FC in metabolite abundance visualized as a dot plot, using control media (Blank media) as a reference. Each point represents a metabolite, with color indicating their respective groups and shapes denoting different metabolic pathways. Metabolites in the plot are clustered based on their associated pathways, providing insights into the metabolic shifts among the timepoints. Of interest, Inosine is significantly enriched in FP, while aspartic acid is consumed. Inosine is suggested to have an anti-inflammatory effect, and has been linked to inhibition of the NF-κβ pathway in rats.[6] Inhibition of NF-κβ has also been observed following SRC treatment in a diseased rat model.[7]

RESULTS

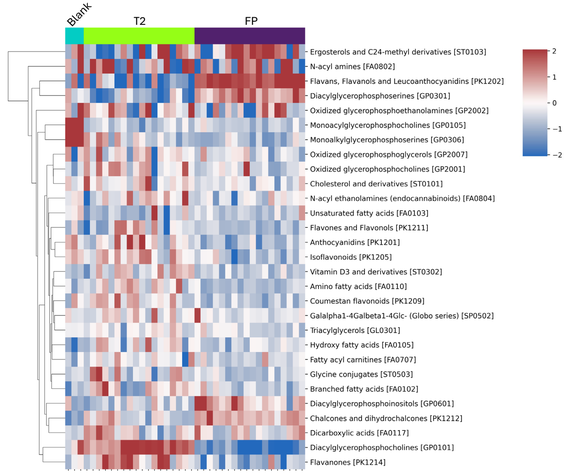

Figure 6: Lipid Enrichment Plot During Manufacturing. This heatmap presents the enrichment scores for lipid classes, organized along the y-axis, against the sample groups (Blank, T2, FP) across the x-axis. Enrichment scores are relative to the Blank group average. Distinct clustering indicates similarities in lipid classes alterations across the groups. The color intensity of each test sample corresponds to the degree of enrichment in a given pathway. Changes in phosphatidylserines and phosphatidylcholines suggest that manufacturing influences changes in cellular membranes, lipid rafts and cell signaling.

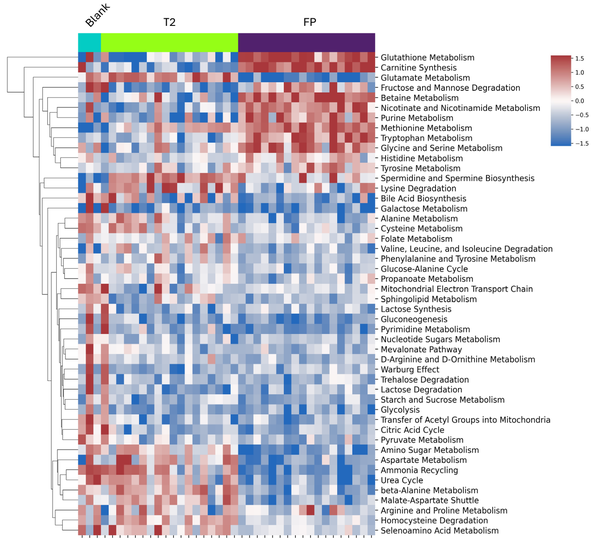

Figure 7: Pathway Enrichment Plot During Manufacturing. This heatmap presents the enrichment scores for metabolic components of intracellular pathways, organized along the y-axis, against the sample groups (Blank, T2, FP) across the x-axis. Enrichment scores are relative to the Blank group average. Distinct clustering indicates similarities in metabolic pathway alterations across the groups. The color intensity of each test sample corresponds to the degree of enrichment in a given pathway, with the sample group colors denoted in the legend. For example, the glutathione metabolism pathway appears more enriched in FP compared to T2, suggesting a shift in the cells' redox balance during manufacturing.

RESULTS

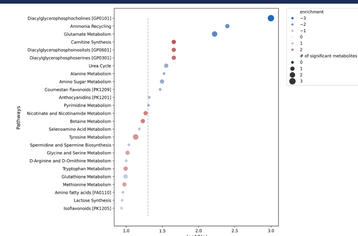

Figure 8: Pathway Analysis of Significant Metabolites During Manufacturing. The dot plot displays the enriched pathways identified in the analysis, with their corresponding significance levels represented on the x-axis. The significance threshold ($p = 0.05$) is denoted by the grey dashed line. The size of the dots reflects the number of metabolites that showed statistical differences between the sample groups (T2, FP). The color of the dots represents the enrichment, which indicates the extent to which the metabolites in the pathway were clustered amongst the compounds with the most positive fold-changes (red, positive enrichments), the most negative fold-changes (blue, negative enrichments), or uniformly distributed (white, enrichment values near 0.0) across high and low fold-change compounds. Notably, diacylglycerophosphoserines, a class of phospholipids involved in cell signaling, and carnitine synthesis were enriched in CM, suggesting either more consumption, or less secretion, by the T2 cells than FP cells.

STUDY LIMITATIONS

- Study size was relatively small (n=18 at two time points). Samples (retains) were obtained from manufacturing runs for participants in Phase 2 clinical trials with slightly differing enrollment criteria to Phase 3. NDRI source did not match trial eGFR inclusion.
- The study focuses on metabolites secreted from cells from two stages of product manufacture. Results represent metabolites secreted in an *in vitro* environment. Follow up studies (with cells) will better inform this metabolic interplay during manufacturing.

SUMMARY AND CONCLUSIONS

- Cell metabolic requirements evolve during rilparencel manufacturing and show distinct metabolic signatures based on manufacturing time point **(Figures 2 & 7)**.
  - Cells with more variable metabolic expression in intermediate manufacturing (T2) show less variability at the end of manufacturing (FP), as shown by PCA clustering **(Figure 2)**.
- FP cells demonstrate a partial epithelial re-differentiated state **(SA-PO0014)** .[10,11] Rilparencel metabolic profile may support this cellular epithelial plasticity phenotype, as shown by altered methylation activity **(Figure 3)**.
- Changes in purine metabolism, as indicated by the increased secretion of inosine in FP cells relative to T2 cells **(Figures 3-5)**, support data from secretome and phenotype analysis. As previously reported, we observed a reduction in secretion of inflammatory markers in FP relative to earlier stages in manufacturing **(SA-PO0015)**. [6-10]
- Compared to T2 cells, FP cells undergo reproducible metabolic maturation and may be better primed to survive potentially hostile environments via altered redox balance, as shown by upregulation of glutathione metabolism **(Figures 7 & 8)**.

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