THE CHARM-01 STUDY: ASSESSING FORMULATIONS OF TENOFOVIR 1% GEL IN HIV SERONEGATIVE ADULTS VIA TRANSCRIPTOME ANALYSIS

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CHARM-01

- Double-blinded, randomized, safety & acceptability, pharmacokinetic, and ex vivo efficacy study of rectally-applied tenofovir-based microbicide formulations.
- Dose comparison of 3 current formulations of tenofovir 1% gel
  - Vaginal formulation
  - Reduced glycerin formulation
  - Rectal specific formulation
- Endpoints
  - General and mucosal safety
  - Pharmacokinetics and pharmacodynamics
- Current status
  - Completed

McGowan I et al. PLoS ONE 2015

Transcriptomic Study Objective and Aims

Transcriptome: The complete set of coding and non-coding transcripts in a given sample and their quantity

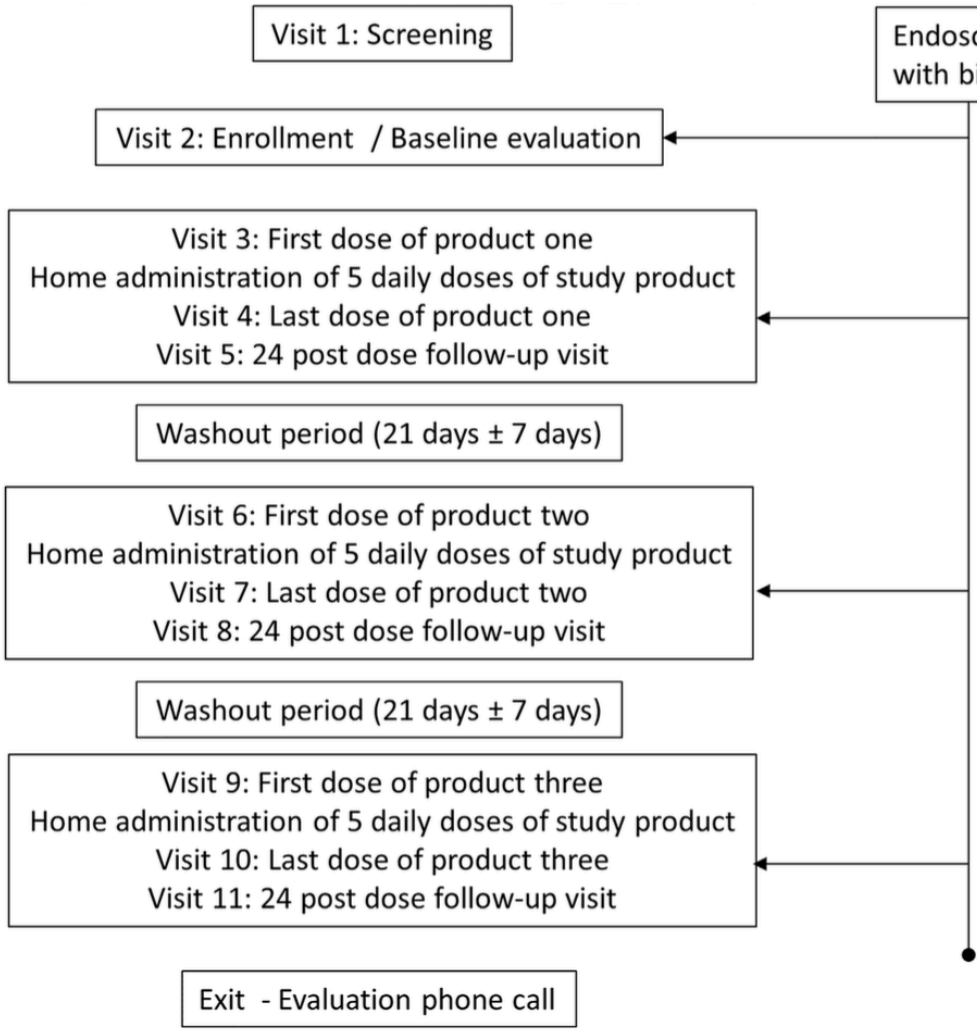
Expanded Objective: Apply low-input RNA-Seq transcriptional analysis as a sensitive assay to uncover changes to the mucosal environment caused by gel usage.

Hypothesis: Local changes in the mucosal immune environment (e.g. IFNs/inflammasome) may be part-and parcel the action of microbicide treatment and alter risk of HIV infection

Aim1: To distinguish the effects of three microbicide tenofovir 1% gel formulations, administered rectally, using transcriptomics

Aim2: To compare data with other microbicide trials and validate signatures of intestinal mucosal gene expression following microbicide exposure associated with protection or risk

CHARM-01 Study Design
Blinded crossover design with Tenofovir 1% formulations in random sequence



Microbicide tenofovir 1% gel formulations:

- **Reduced-Glycerin Vaginal Formulation** (RGVF, 846 mOsmol/kg)
- **Rectal-specific Formulation** (RF, 479 mOsmol/kg)
- **Vaginal Formulation** (VF, 3111 mOsmol/kg)

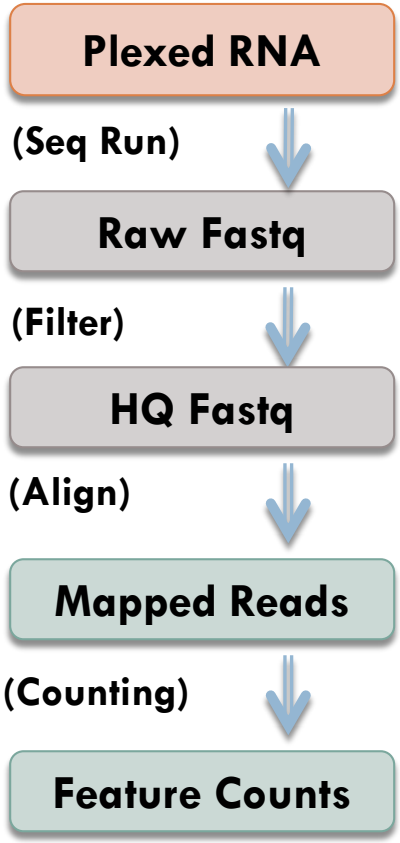
Each participant will receive:
7 rectal exposures to RGVF
7 rectal exposures to RF
1 exposure to VF, coupled with 6 preceding exposures to Universal HEC Placebo Gel

N = 14 patients

Sample Processing

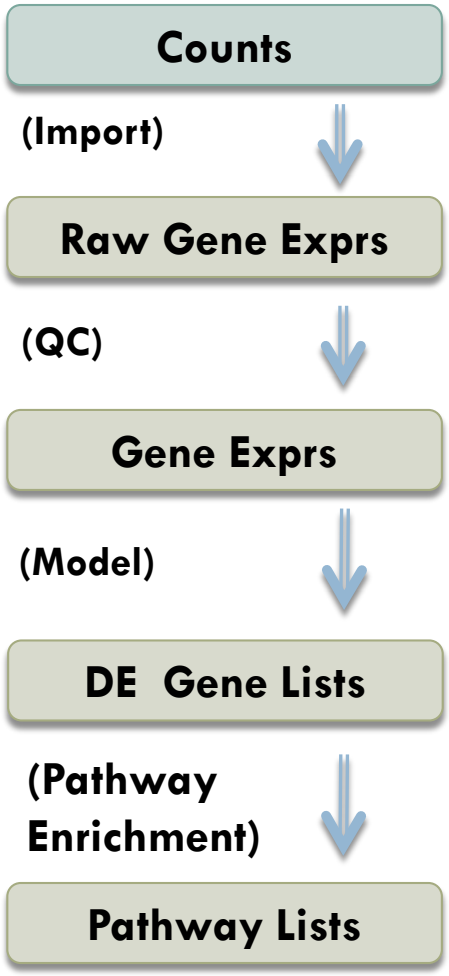
- Total RNA was isolated from 50 gut biopsies preserved in RNA*later* using Qiagen RNEasy Mini Plus Kits
- RNA-Seq data was generated using Illumina Truseq (low input SOP) kits and the Illumina HiSeq 2500
  - Paired-end, 50 cycle, $>30x10^6$ mapped reads/sample
  - Medium depth: Able to measure the transcriptome and common splicing variants.
  - RNA-Seq's advantage areas over microarray include sensitivity, specificity and quantification, as well as fielding coding (mRNA) and non-coding (e.g. small RNA) transcript counts

Preprocessing of RNA-seq: R Pipeline



1. Create **Raw fastq** from binary output by running pooled tagged RNA short read libraries are run on Illumina Hiseq 2500 (2x50 30M reads), as per manufacturer's instructions.
2. Create **High Quality Fastq's** by filtering out poor quality base calls and adapter contamination using *Trimmomatic*.
3. Generate **Mapped Read** files by aligning the HQ Fastq reads using the *STAR* aligner.
4. Generate **Features Counts** using *Htseq*.

Gene based Analysis



1. Create **Raw Gene Expression Matrix (Genes by Samples)** from aggregating count files into a matrix, and importing the sample Phenotype data in R.

2. Create **Normalized Expression Matrix** by performing removing sample outliers base on QC assessment and normalizing the samples to each other using *Edge*.

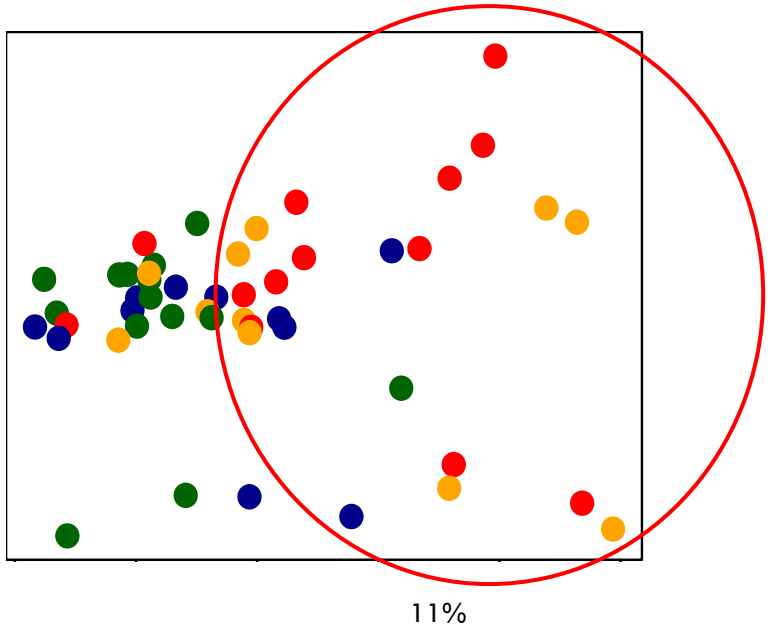
3. Generate **Differential Gene Expression Lists** by performing 2 group analysis via linear modeling using *EdgeR*.

4. Generate **Pathway Enrichment Lists** by taking top ranking genes and performing pathway enrichment using Gene Set Enrichment Analysis or *GSEA*.

Effects of three microbicide tenofovir 1% gel formulations on intestinal mucosal gene expression

Comparing formulations to baseline: F-Test

Comparing (Formulation VS Baseline)
Method : Fitting GLM (GLM : R - EdgeR)
Model : (Gene expression ~ Formulation group + Donor)



Kruskal wallis rank sum test

Multi Dimensional Scaling analysis of the top 500 F-test genes by P value shows RGVF has the most significant expression profile compared to baseline

Comments: There is a lot of variability between donors (n=12-14) in gene expression observed within the formulation groups.

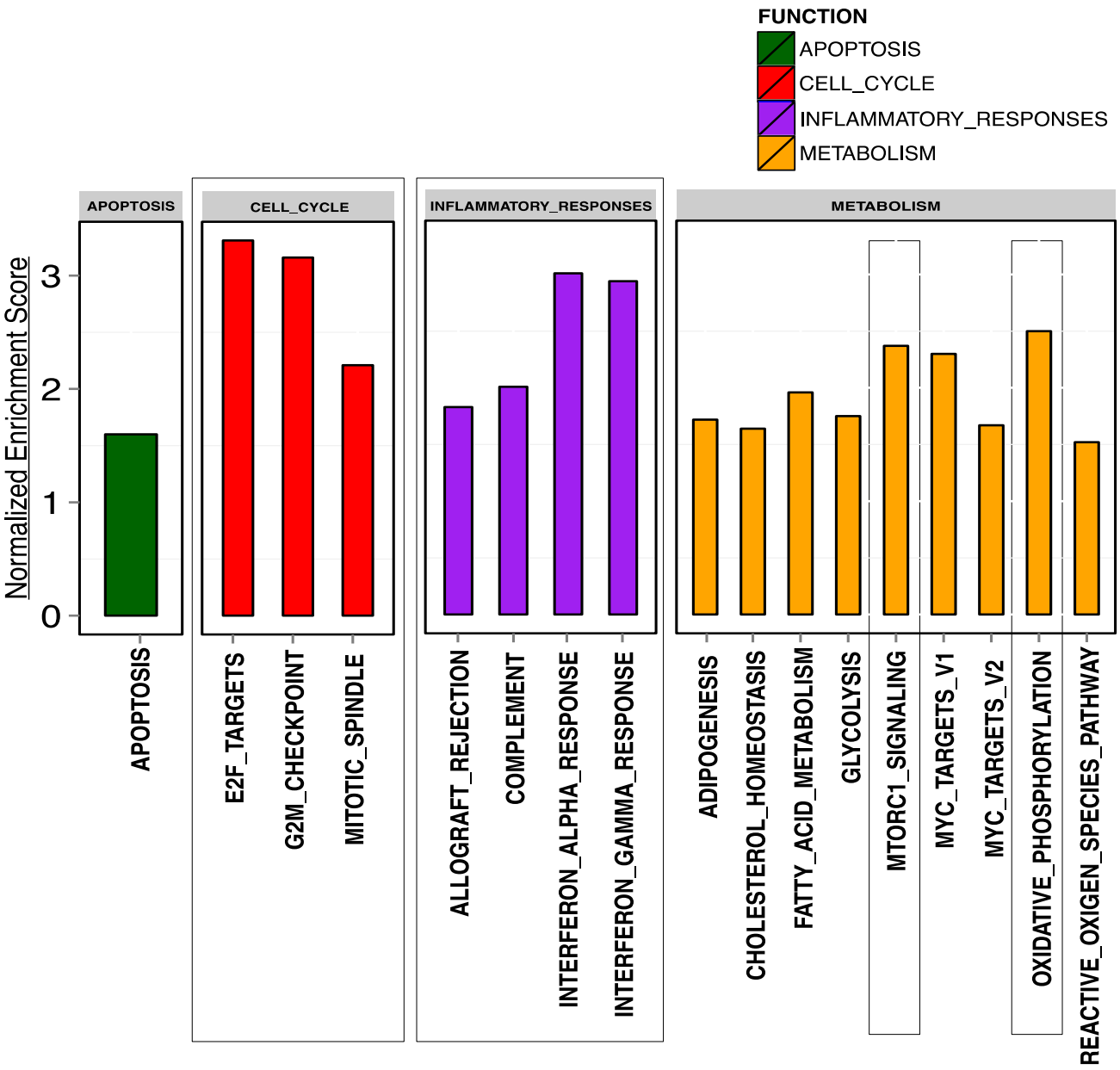
Dimension 1

Group	p-value
Baseline	**0.002**
RGVF	**0.001**

RGVF and Baseline significantly explain the first dimension of variation

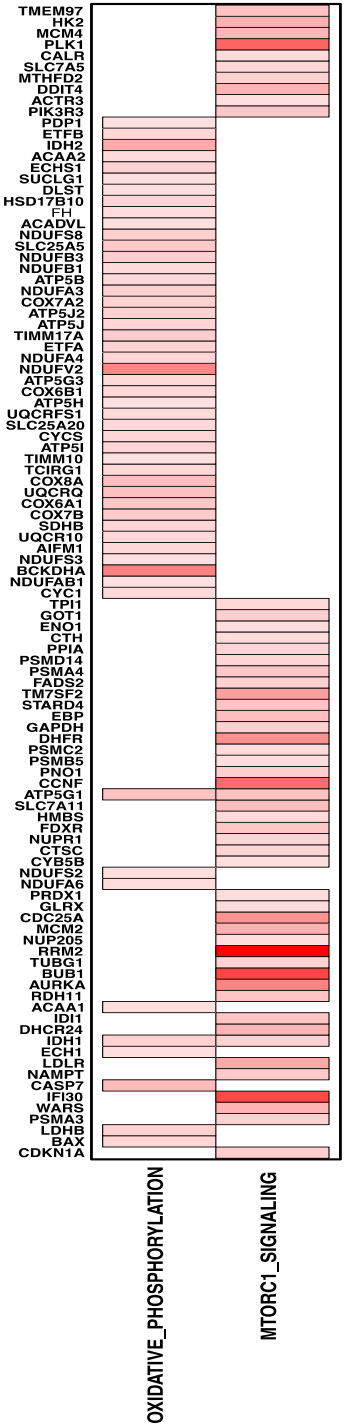
Solution: Gene Set Enrichment Analysis : RGVF – Baseline Focused

Many increased pathway activities upon exposure to RGVF



Oxidative phosphorylation, mTORC1 signaling, And cell cycle genes are up-regulated upon exposure to RGVF formulation

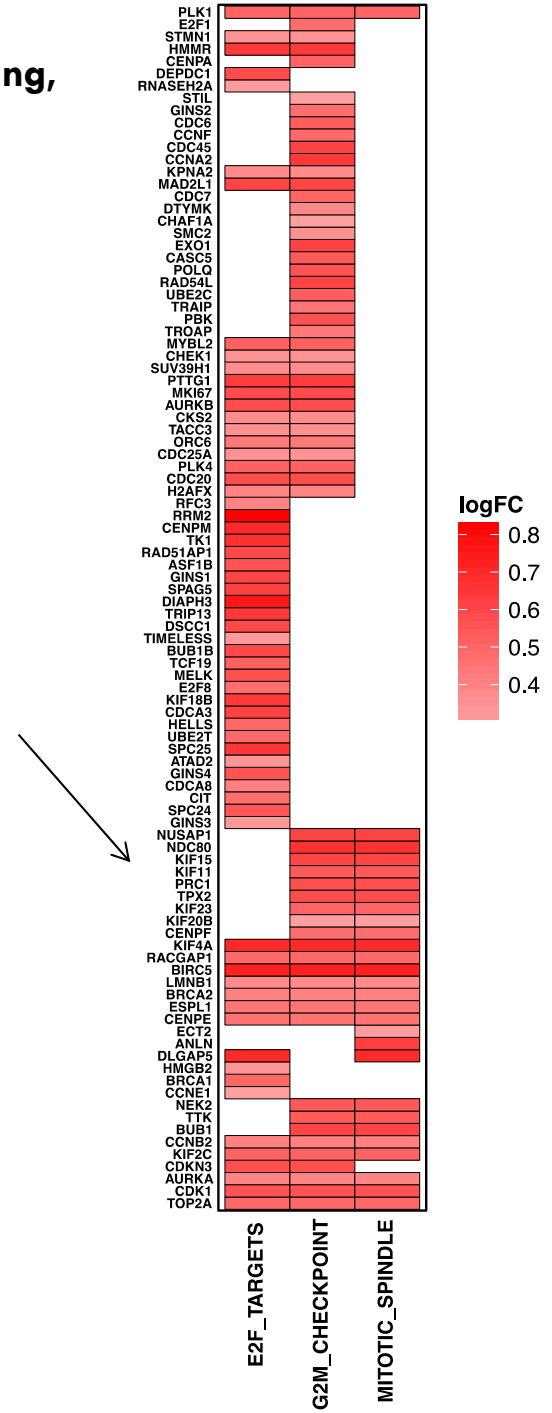
- Genes encoding proteins involved in oxidative phosphorylation



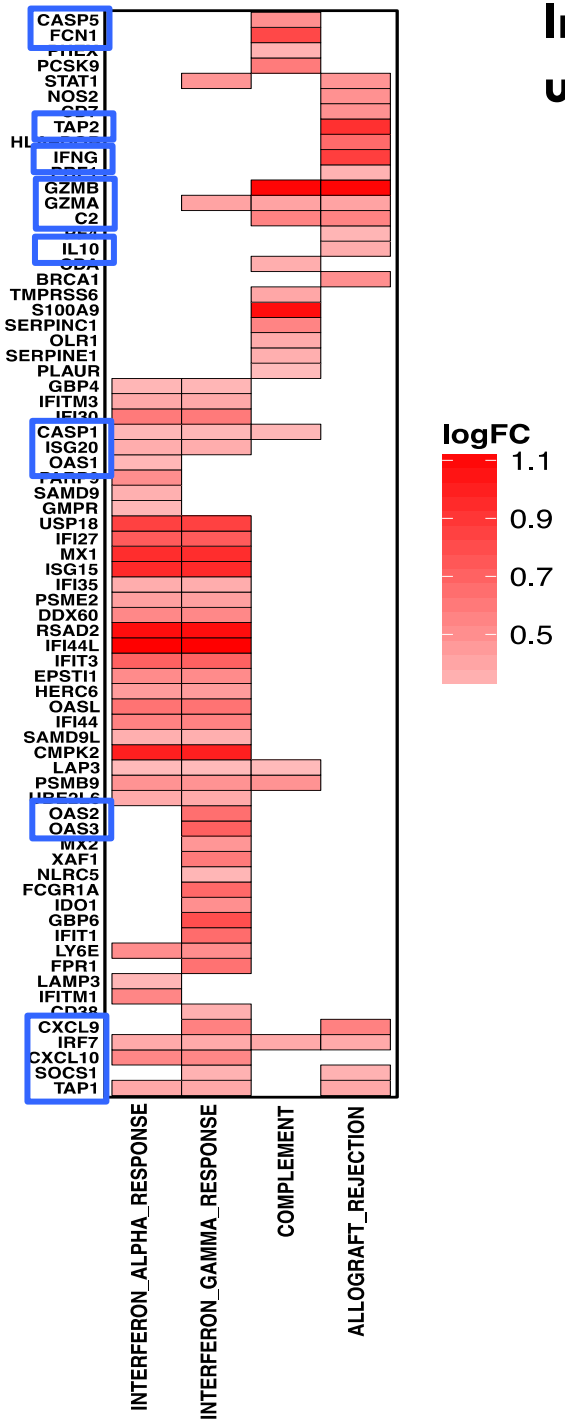
- Genes encoding cell cycle and related targets of E2F transcription factors
- Genes important for mitotic spindle assembly
- Impact on cell proliferation, stress response

Ph related?

- Activation of the mTORC1 complex
- Impact on senescence and T cell fate

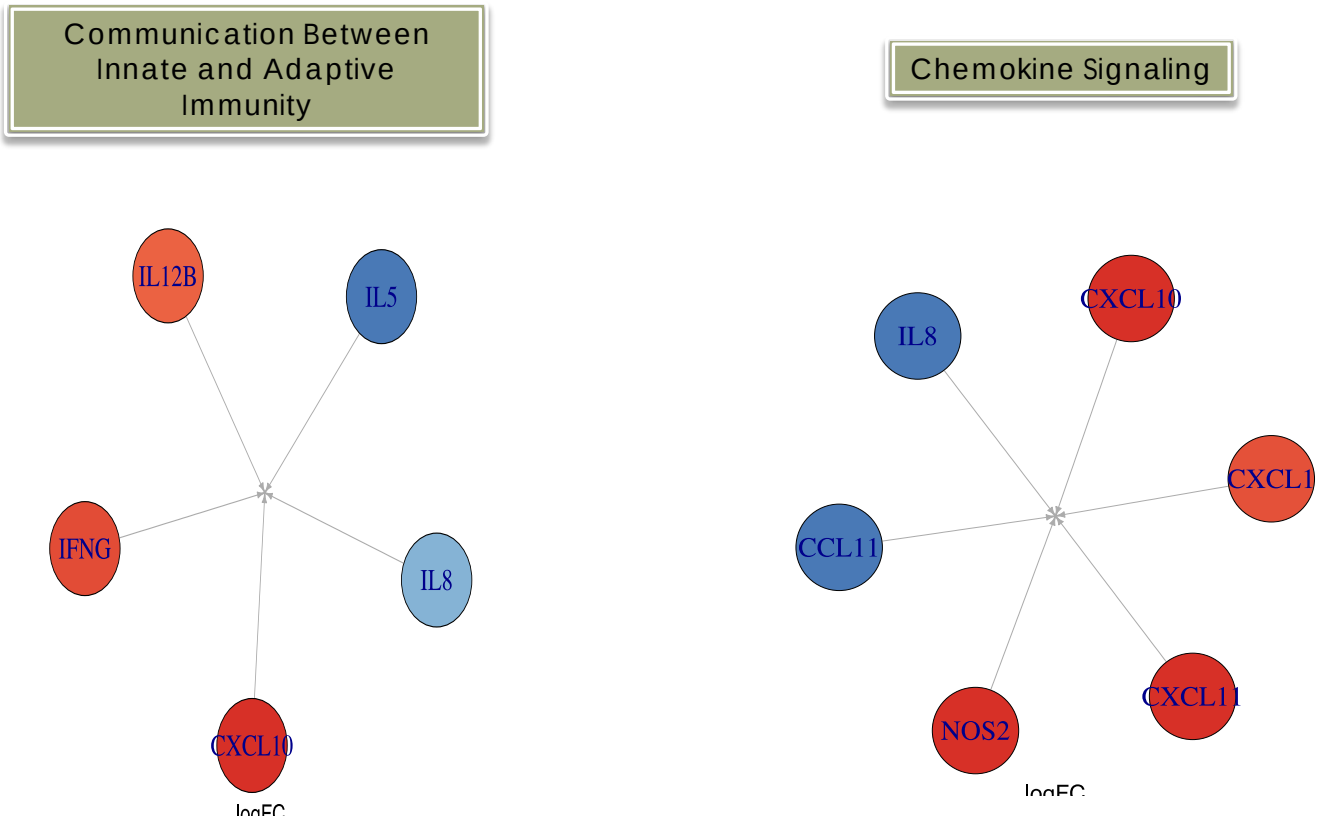


Inflammasome-related pathways are up-regulated upon exposure to RGVF formulation



- Genes up-regulated in response to IFNA
  E.g. OAS1, LAMP3, IFITM1
- Genes up-regulated in response to IFNG
  E.g. CXCL9, SOCS1, GZMA
- General IFN response genes
  E.g. IRF7, CXCL10, IFIs/IFITs, MX1, ISG20
- Genes associated with the complement system
  E.g. SERPINs, FCN1, C2, CASP1/5
- Genes up-regulated during allograft rejection
  E.g. TAP1/2, IL10, IFNG, HLAs

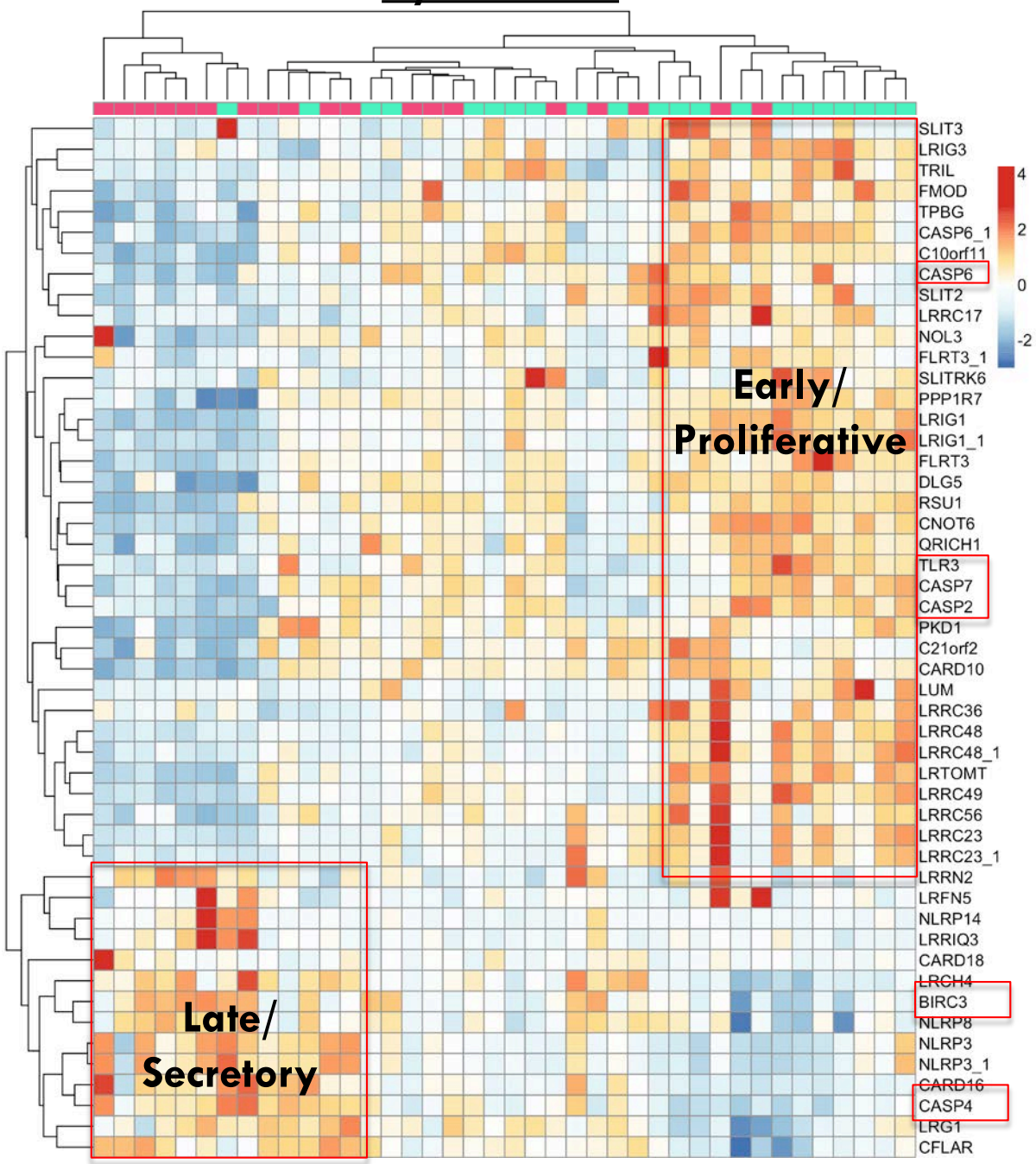
Comparison: Project Gel GSEA: (RG Tenofovir Day1)



IFNG-mediated pathway signatures do not persist to D7 in Project Gel, however…

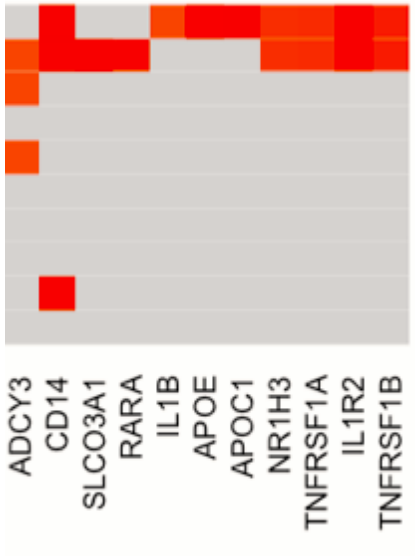
Cytobrushes

Inflammasome signatures are associated with early/late menstrual cycle phases



Inflammasome-filtered (Gaucher et al., 2008)

CVL Samples at Early Phase

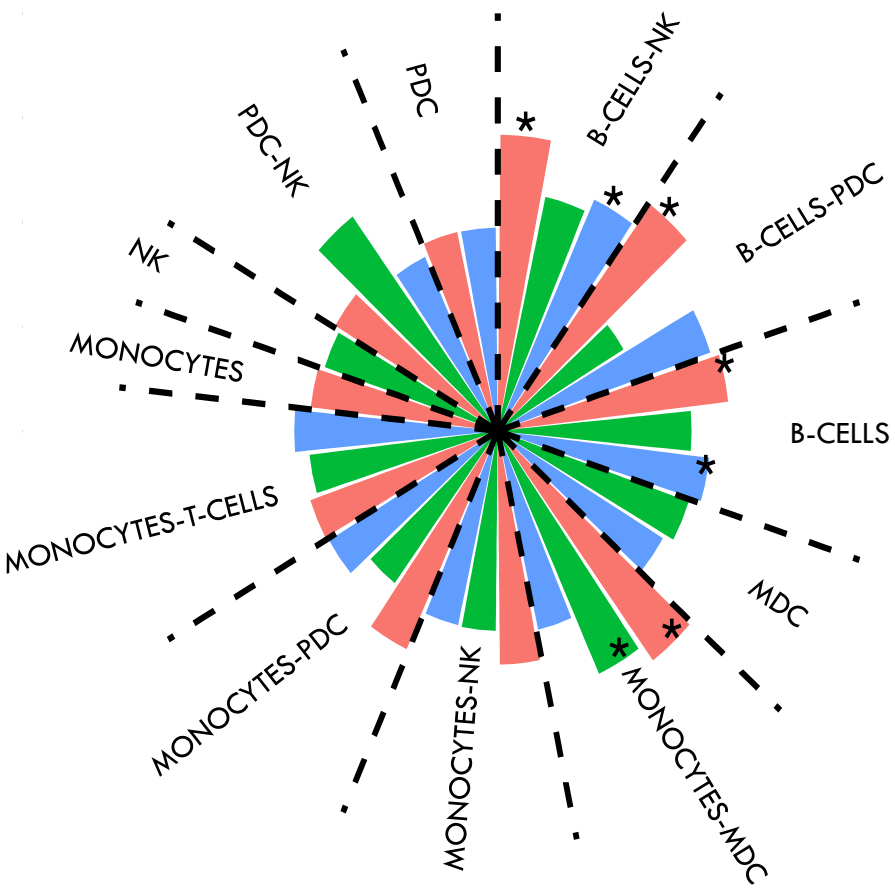


BIRC3: regulates caspase cascades inflammatory gene signaling

HIV Mucosal Immunology Group

Enrichment in B cell and monocyte-related genes describes the D1 and D7 Project Gel gene expression signature

Deconvolution: OMIC Module-Based Filtering
Nakaya et al. (*Nat Immunol* **12**(8): 786, 2011)



Conclusions:

- While the N and effect sizes are small, we have begun to hone a *signature* associated with rectal application of tenofovir gel
- RGVF has the most unique expression profile compared to baseline
  - Increased pathway activities include proinflammatory pathways, IFNs, cell signaling, stress and cell cycle.
  - Coordinated inflammasome involvement in RGVF formulation
  - Balance between antiviral IFNs and the greater inflammasome is likely delicate in determining an outcome and a result of many factors
  - Need outcomes to give this balance context in risk
- CHARM inflammation signature can be seen early in Project Gel
  - IFNG/IFNG-response genes, SERPINs, SOCS, chemokines are up-regulated
- Nakaya filtering (a deconvolution method) indicate that gene activity in Project Gel may stem from alterations in B cell and Monocytes/MDC.

A lot of work remains:

Perform additional contrasts to pair an individual subject with their baseline and formulation sequence and map their overall responses in the MDS and inflammasome

- hampered by lower subject numbers and resulting bioinformatic approaches (e.g. ranking DGE by nominal P)

Increase subject numbers, integrate different data types (flow data, proteome) and *outcomes,* train and test signatures in similar human trials, or meta-analysis across very different studies (human, NHP)

Develop and validate new flow panel and/or Fluidigm PCR panel to probe these signatures at a finely sorted and in titration down to the single cell level

Identify and target mechanisms and biomarkers of protection or infection risk in future trials

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