Assessing Formulations of Tenofovir 1% Gel in HIV Seronegative Adults via RNA-Seq

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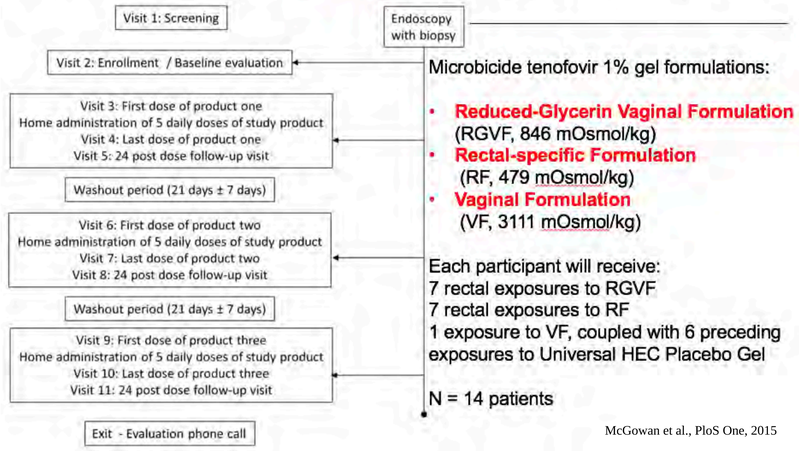
Background

Clinical trials employing rectal microbicides containing antiretroviral drugs have the goal of reducing risk of contracting HIV during sexual activity. The Combination HIV Antiretroviral Rectal Microbicide (CHARM)-01 study is a recent Phase 1, double-blinded, randomized, safety & acceptability, and pharmacokinetic study of three rectally-applied tenofovir-based microbicides in healthy adults (aged 37.7 years 14.3) completed by the Microbicide Trials Network (MTN). The three formulations included a previously used vaginal formulation (VF) gel and reduced glycerin VF (RGVF) gel, as well as a third rectal specific formulation (RF) gel unique to the CHARM-01 study.

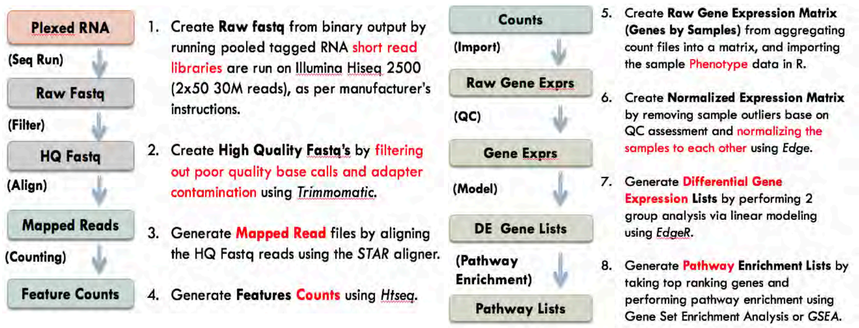
All three formulations were found to be safe and acceptable and use of all gels was associated with inhibition of *ex vivo* tissue HIV infection (McGowan et al., PLoS One 2015; 10(5): e0125363). Interestingly, higher tissue mucosal mononuclear cell levels of tenofovir diphosphate was noted with application of RF gel. With the *hypothesis that gene expression changes in the local rectal immune environment may hallmark the action of tenofovir and potentially alter risk of HIV infection*, our objective was to apply low-input RNA-Seq transcriptional analysis as a potentially more sensitive assay to uncover parallel changes in the mucosal environment caused by different gel usage.

Methods



McGowan et al., PloS One, 2015

We isolated total RNA from rectal biopsies preserved in RNAlater from participants (n=14/group) using Qiagen RNEasy Mini Plus Kits. We performed low input Illumina Truseq RNA-Seq on a HiSeq 2500 instrument. The run design was a paired-end, 50 cycle, >30x10^6 mapped reads/sample, which is capable of measuring the transcriptome with common splicing variants. Top ranking differentially expressed genes by P value (P<0.05 in T or F tests) were forwarded to Gene Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis. The R bioinformatic pipeline is below.



Results

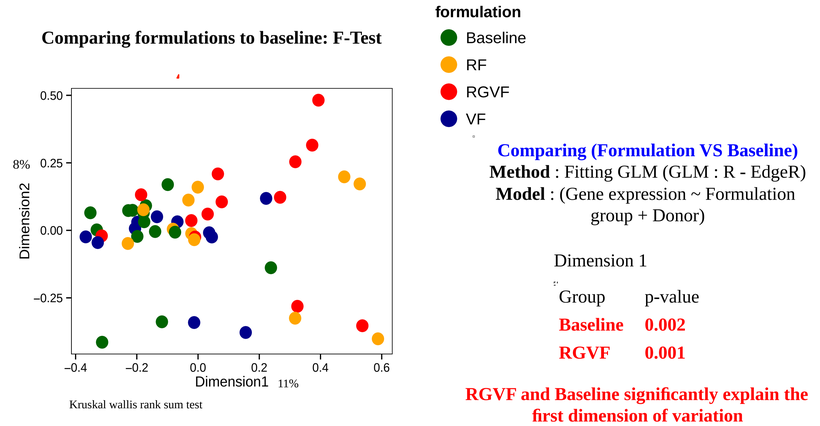


Figure 1: Effects of three microbicide tenofovir 1% gel formulations on intestinal mucosal gene expression. Multi Dimensional Scaling analysis of the top 500 F-test genes by P value shows the RGVF group has a significantly different expression profile compared to baseline. Most RF recipients cluster with the RGFVF group, however there is greater variability between current donors (n=12-14/group) in gene expression observed which may impact the RF dimensionality test.

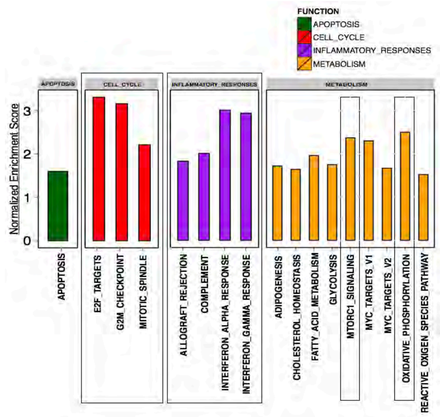


Figure 2: Gene Set Enrichment Analysis : Top pathway activities enriched in the significantly differentially expressed genes resulting from the RGVF versus Baseline contrast. Individual genes from these pathways and their expression are shown below.

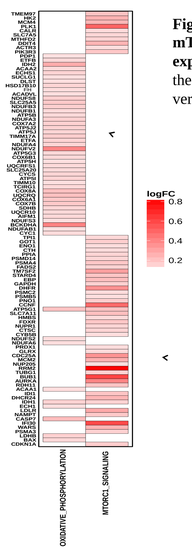
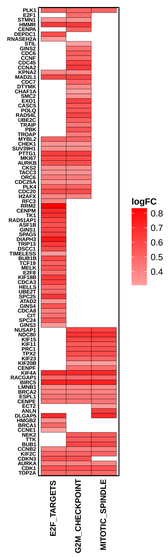
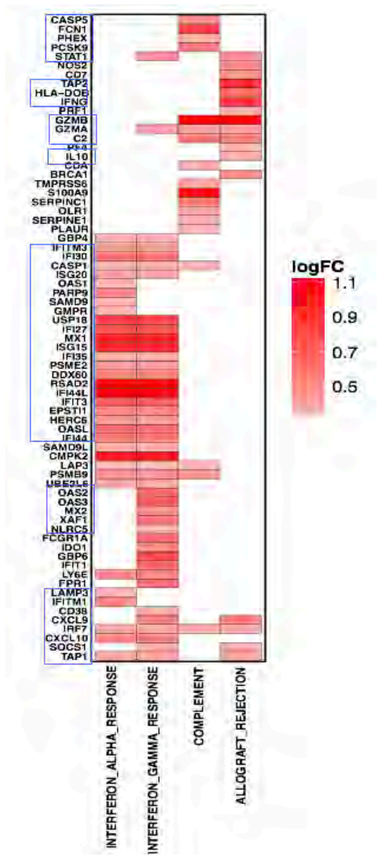


Figure 3: Gene Set Enrichment Analysis : Oxidative phosphorylation, mTORC1 signaling, and cell cycle genes are up-regulated upon exposure to RGVF formulation. These pathway activities are enriched in the significantly differentially expressed genes resulting from the RGVF versus Baseline contrast (red = upregulated).

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





- Genes up-regulated in response to IFNA
  E.g. OAS1, LAMP3, IFITM1
- Genes up-regulated in response to IFNG
  E.g. CXCL9, SOCS1, GZMA, NLRC5 (inflammasome)
- 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 in antigen presentation (allograft rejection)
  E.g. TAP1/2, IL10, IFNG, HLAs

Figure 4: Gene Set Enrichment Analysis : Interferon, proinflammatory, IL-10 and antigen presentation/MHC (allograft rejection) genes and pathways are up-regulated upon exposure to RGVF formulation. These pathway activities are enriched in the significantly differentially expressed genes resulting from the RGVF versus Baseline contrast (red = upregulated).

CONCLUSIONS

- While the N and effect sizes are small, we have begun to hone a biomarker signature associated with rectal application of tenofovir gel.
- RGVF and RF have the most unique expression profiles compared to baseline with increased pathway activities including proinflammation, IFNs, complement, antigen presentation, cell signaling and stress, hallmarking coordinated interferome and inflammasome involvement in tenofovir gel application.
- It is likely that the balance between antiviral IFNs and the greater inflammasome is likely delicate in determining infection risk and a result of multiple factors, including pH.
- While we need infection outcomes to give this balance context in determining HIV infection risk, these biomarkers may help monitor and identify mechanisms and targets of protection or infection risk in future microbicide trials.

ACKNOWLEDGEMENTS

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