Utility of Dried Blood Spot-Derived ARV Biomarkers as an Objective Measure of Treatment Adherence in South Africa

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HIV Center, NYS Psychiatric Institute and Columbia University; University of Cape Town; University of Colorado
Objective measures of ART adherence should:

- impose minimal burden on patients/research participants and on healthcare systems;
- be usable in a range of settings;
- be valid and reliable;
- provide information to facilitate timely intervention and clinical management.
MONITORING ARVs IN BLOOD

Strengths:
- Direct evidence of drug ingestion
- Acceptability

Limitations:
- Short plasma $t_{1/2}$ of parent drugs → snapshot of very recent ingestion
- Sampling and processing constraints

Tenofovir-diphosphate (TFV-DP)
- $t_{1/2}$ in RBCs = 17 days → drug ingestion over a longer period
MONITORING ARVs IN BLOOD

- Assay of TVF-DP from dried blood spots (DBS)
  - Sampling advantages
  - Characterized in “high-resource” clinical research settings in the US among HIV– research participants and among HIV+ patients.*


We explored the utility of this assay as a measure of ART adherence in a real-world, low-resource clinic setting in South Africa.

How do DBS-derived TFV-DP levels relate to

- ARV adherence as determined by an EMD (i.e., Wisepill openings)?
- ARV adherence as determined by self-report?
PARTICIPANTS (N=29)

- HIV+ patients enrolled in an RCT of Masivukeni, a multimedia adherence intervention being evaluated in public HIV care clinics in Cape Town.

- Initiated a once-daily ART regimen containing tenofovir (TFV) in the past 1-2 months (e.g., Atroiza, Odimune).
5 monthly visits

Blood draw by venipuncture for DBS.

Self-reported adherence in past month: 4 questions adapted from Wilson et al.

Daily Wisepill output (as part of parent study)

R300/study visit
METHODS: DBS AND ASSAYS

- Blood samples (25 μl) from venipuncture pipetted onto Whatman 903 ProteinSaver cards, air dried, and stored at -80°C.

- Shipped to University of Colorado on dry ice for assay in the Anderson laboratory.

- 3 mm punches extracted and assayed for TFV-DP by LC/MS/MS.*

METHODS: DATA ANALYSIS

- % Wisepill adherence (openings) in 28 days prior to DBS sampling:
  \[
  \frac{\text{# days device opened}}{\text{# days device detected as active}}
  \]

- Pre-steady-state TFV-DP levels (fmol/punch) were adjusted to steady-state (TFV-DP\text{adj}) assuming a 17-day half-life in RBCs.

- TFV-DP\text{adj} levels were log-transformed, and Pearson correlations were calculated.
RESULTS: SAMPLE CHARACTERISTICS (1)

- **Demographics:**
  - 90% women, 100% Black African
  - Mean age: 30 years (SD±5.25)

- **Time on ART** at pilot study Visit 1:
  - Mean: 32 days; Median: 29 days;
  - Range: 23-52 days

- % Wisepill openings over *entire study*:
  - Mean: 76%; Median: 84%; SD: 25%

- % Wisepill openings in the *4 weeks previous to each study visit*:
  - Mean: 74%; Median: 90%; SD: 34%
### RESULTS: SAMPLE CHARACTERISTICS (2)

**DBS tenofovir-diphosphate (TFV-DP) levels (fmol/punch)**

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<thead>
<tr>
<th></th>
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<th>Median</th>
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<td>HIV+ women on ART (US)</td>
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RESULTS:
SAMPLE CHARACTERISTICS (2)

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<tr>
<td>TFV-DP_{adj} all time points</td>
<td>1,013</td>
<td>939</td>
<td>0-3,623</td>
<td>489</td>
</tr>
</tbody>
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RESULTS: SELF-REPORTED ADHERENCE

At least 98% of the time, participants reported excellent – if not “perfect” – adherence to their HIV meds in the past month:

- 0 days missing one or more doses
- Always or almost always taking meds “as supposed to”
- Excellent or very good job taking meds “as supposed to”
- Taking their meds as “about the same as usual”
RESULTS: TFV-DP and WISEPILL

Correlation of $TFV-DP_{adj}$ with % Wisepill openings in previous 28 days:

**ALL PARTICIPANTS**

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<td>All visits</td>
<td>.348</td>
<td>&lt;.001</td>
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<td>(142 data points)</td>
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RESULTS:
TFV-DP and WISEPILL (cont’d.)

BR434

TVF-DP_{adj} (fmol/punch)

- OPENED
- NOT OPENED

ART/WP start
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5

HIV CENTER for Clinical and Behavioral Studies
at the New York State Psychiatric Institute and Columbia University
Correlation of $\text{TFV-DP}_{\text{adj}}$ with % Wisepill openings in previous 28 days:

Exclude 6 participants with significant $\text{TFV-DP}_{\text{adj}}$ but sustained absence of Wisepill openings

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<th>Participants excluded (N=23)</th>
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Determining associations between DBS-derived TFV-DP levels and ART adherence was hampered by problems in Wisepill use in at least 20% of our sample.

These device-use problems might not have been recognized without the TFV-DP assay results.
Larger studies are needed to understand the strengths and limitations of DBS ARV anabolite assays and EMDs as clinically meaningful objective measures of adherence.

- Are moderate correlations between TFV-DP levels and % Wisepill openings due to use of the device, intra-individual PK variability, or both?

- Can drug anabolite assays overcome the confounder of EMD adherence – i.e., by measuring actual medication ingestion rather than device use?
Our sample (which was, overall, relatively adherent to Wisepill use) has a lower median TFV-DP level than the US sample. Is this due to

- Genetic variation among patients/populations?
- Exposure to other medical conditions/drugs?
- Use of generic medications?

Does the assay need to be “calibrated” for different populations?
A DBS-based assay of TFV-DP has potential as a tool for monitoring adherence and helping patients manage their HIV disease.

- How will providers interpret/use assay results?
- Can assay results be used to motivate adherence among patients?
- How should results be framed? How will patients understand/act on feedback?
- How would point-of-care or home-testing versions of the assay be used?
CONCLUSIONS and AREAS FOR FUTURE RESEARCH

Could DBS ARV anabolite assays have advantages over standard clinical markers (e.g., CD4+ cell counts, viral load) in detecting adherence problems?

- Can the assay predict the development of viral breakthrough/viremia due to non-adherence?

- How much advanced warning of viral breakthrough/viremia could regular (e.g., monthly) use of this assay give?
ACKNOWLEDGMENTS

HIV Center, NYSPI and Columbia University
Robert H. Remien, Ph.D.
Patricia Warne, Ph.D.
Reuben Robbins, Ph.D.
Claude Ann Mellins, Ph.D.
Javier López-Rios, B.A.
Cheng-Shiun Leu, Ph.D.
Bruce Levin, Ph.D.

University of Cape Town
John Joska, M.B.Ch.B.
Hetta Gouse, Ph.D.
Michelle Henry
Yoliswa Mtigeni

University of Colorado
Peter L. Anderson, Pharm.D.
José Castillo-Mancilla, M.D.

National Institute of Mental Health
Administrative Supplement to “Masivukeni: A Multimedia ART Adherence Intervention for Resource-Limited Settings (R01-MH95576-03S1; PI: Robert H. Remien, Ph.D.)

NIMH Program Officer: Michael Stirratt, Ph.D.

HIV Center for Clinical and Behavioral Studies
(P30-MH43520; Center Director and PI: Robert H. Remien, Ph.D.)