Development of a low-cost ART resistance test for Nigeria's HIV population

AS-IAS-2023-02578

Nuttada Panpradist [1,2,3], Brian Tran [1], Kyla Yamashita [1], Ingrid A Beck [4], Shane Gilligan-Steinberg [1], Enos Kline [1], Jordan Campbell [1], Jonathan Okpokwu [5], Godwin Imade [5], Oche Agbaji [5], Phyllis Kanki [6], Beth Chaplin [6], Lisa M. Frenkel [1,4], Holly Rawizza [6,7]

[1] University of Washington, Seattle, United States, [2] Chiang Mai University, Chiang Mai, Thailand, [3] Ministry of Health, National Public Health Laboratory, Nairobi, Kenya, [4] Seattle Children's Research Insitute, Seattle, United States, [5] Jos University Teaching Hospital, Jos, Nigeria, [6] Harvard T.H. Chan School of Public Health, Massachusetts, United States, [7] Brigham and Women's Hospital, Massachusetts, United States.

Presenting author: NP (nuttadap@uw.edu)

BACKGROUND

- Dolutegravir (DTG) is associated with improved antiretroviral therapy (ART) outcomes. Despite data suggesting that acquired HIV drug resistance (HIVDR) does not undermine efficacy of DTG-ART, **additional studies are needed to further evaluate the effects of extensive pre-existing nucleos(t)ide resistance on DTG-ART across subtypes**.
- In 2019, **we reported development and clinical validation of "OLA-Simple,"** an easy-to-use, point-mutation HIVDR test.[1-3]

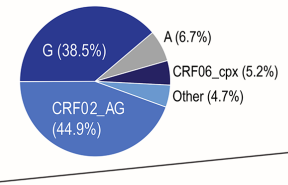
99.6% specificity
98% sensitivity
Validated in specimens from A, B, C, D, and AE subtypes

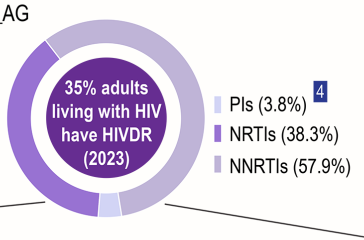
No training required
Guided by software, 1st-time users processed specimens with 97% accuracy.

~$850 equipment
<$20 reagents / sample tested
to detect DR for NRTIs (TDF/3TC) and NNRTIs (EFV/NVP) from DNA/RNA

Same-day results
<4-hour total time
<10-min hands-on time

- To expand OLA-Simple use for subtypes in West Africa, we designed and tested OLA-Simple probes for a **Nigerian** cohort
  - **2.2M PLH** in Nigeria (2021)
  - Majority subtypes in Nigeria's HIV population are G and CRF02_AG
  - HIVDR mutations against NNRTIs and NRTIs are common





[4]

Key mutations and weighted % in adults with NRTI HIVDR (n=1355)	
M41L	38 (2.1%)
K65R	25 (1.3%)
L74V/I	8 (0.7%)
Y115F	8 (0.6%)
M184V	152 (9.2%)
T215Y/F	47 (2.7%)

Key mutations and weighted % in adults with NNRTI HIVDR (n=1355) [4]	
K103N	178 (11.3%)
Y181C	50 (2.9%)
G190A	53 (3.0%)

Note: Surveillance data for pediatric population were outdated, last collected in 2012.

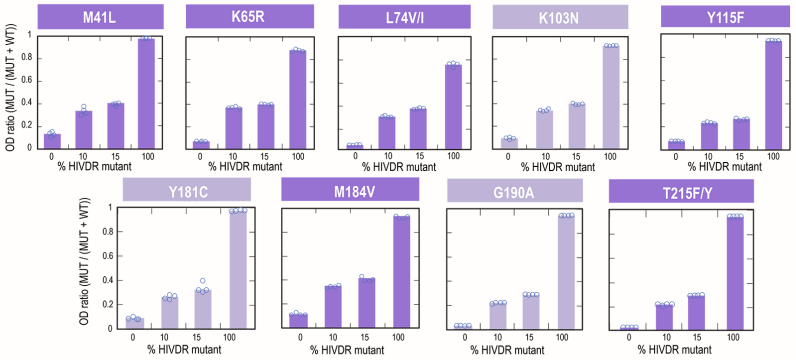
METHODS

- OLA probe sequences were designed to detect M41L, K65R, L74V/I, Y115F, K103N, Y181C, M184V, G190A, and T215F/Y based on 380 *pol* sequences from Nigerian adults' plasma from 2007–2012 with HIV-1 subtypes prevalent in West Africa
- OLA probe and ligase concentrations were optimized to detect minority frequency (10-15%) variants in each person's quasispecies, using plate-based OLA [5]
- Optimized OLA probes were evaluated on a blinded panel of 56 archived plasma RNAs extracted from specimens from multiple HIV subtypes: CRF02 (n=20), G-prime (n=16), G (n=7), A (n=7), CRF06 (n=3), C (n=2), and D (n=1). The panel was enriched for HIVDR. Of 504 codons, 144 were HIVDR
- Results were compared to previously performed Sanger sequencing [6]

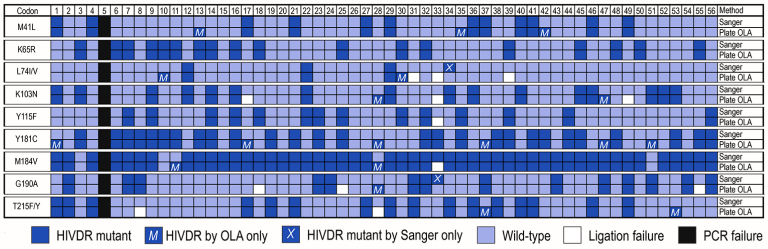
Presented at IAS 2023, the 12th IAS Conference on HIV Science

RESULTS AND DISCUSSION

- Newly developed OLA probes detected ≥10% HIVDR mutant from wildtype codons.



- RT-PCR for OLA successfully amplified 55/56 with plasma HIV RNA between 140 – 8250 copies/reaction (median: 660). The one that failed had 265 copies/reaction (CRF06 subtype)
- Of the remaining 495 codons tested by OLA, 13 failed ligation (2.6%, 95%CI:1.4-4.5), 183 had HIVDR genotypes, and 299 were wild-type



- OLA had 93% (95%CI:91-95) concordance with Sanger sequencing; including 170 HIVDR and 292 wild-type codons
- OLA detected additional 18 HIVDR codons classified as wild-type by Sanger. 13 had 10-15% HIVDR, which is below Sanger's limit-of-detection. The remaining 5 will be sequenced to confirm genotypes; OLA primers are different from those used in Sanger
- Conversely, 2 HIVDR mutant codons detected by Sanger were not detected by OLA. #33 had weak amplification potentially due to prolonged storage of RNA. #34 could be due to nearby polymorphisms as both WT signal was weak (OD = 0.4)

CONCLUSIONS AND NEXT STEPS

- OLA-Simple can be adapted for multiple HIV subtypes, including AG/G
- Our next steps include evaluation of these probes on site in Nigeria as well as designing OLA probes to detect DTG HIVDR mutations

ACKNOWLEDGMENTS

We thank Dr. Barry Lutz (PI) and collaborators: Dr. James Lai Dr. Eric Klavins, and Dr. Rena Patel at the University of Washing ton, USA. Dr.Theresa Rossouw at University of Pretoria, SA; Dr. Gonzague Jourdain and Dr. Nicole Ngo-Giang-Huong at PHPT, Thailand;Dr. Jaime Soria at Hospital Nacional Dos de Mayo, Peru.

Funding sources:
Global Health Grand Challenges Foundation 2012-13
2013-14
CFAR AI027757 2014-15
NIH 2014-24
MGH 2016-17

REFERENCES

1. N Panpradist, et. al. "**OLA-Simple: A software-guided HIV-1 drug resistance test for low-resource laboratories.**" 2019. EBiomedicine.
2. N Panpradist*, IA Beck*, et. al. **"Near point-of-care, point-mutation test to detect drug resistance in HIV-1: a validation study in a Mexican cohort."** 2020. AIDS.
3. J Vrana*, N Panpradist*, et. al. **"Implementation of an interactive mobile application to pilot a rapid assay to detect HIV drug resistance mutations in Kenya."** 2022. PLOS Global Public Health.
4. GG Aliyu, et. al. "**Prevalence of HIV drug resistance in Nigeria: results from a cross-sectional, population-based survey of Nigerian adults with unsuppressed viral load.**" 2023. AIDS.
5. IA Beck, et. al. "**Rapid and sensitive oligonucleotide ligation assay for detection of mutations in human immunodeficiency virus type 1 associated with high-level resistance to protease inhibitors.**" 2002. Clinical Microbiology.
6. ST Meloni, et. al. "**Implication of First-Line Antiretroviral Therapy Choice on Second-Line Options.**" 2017. Open Forum Infect Dis.

IAS 2023 23 – 26 July