Implementation of HIV drug resistance testing in Kenya

HA Duarte,[1,3] IA Beck,[3] M Levine,[3] C Kiptinness,[2] J Munyao,[2], B Chohan,[1,4] SR Sakr,[2] MH Chung,[1,2] and LM Frenkel[1,3]

[1]University of Washington, Seattle, USA; [2]Coptic Hope Center, Nairobi, Kenya, [3]Seattle Children's Research Institute, Seattle, USA; [4]Kenya Medical Research Institute, Nairobi, Kenya

Background

- Antiretroviral therapy (**ART**) that does not suppress HIV replication contributes to increased morbidity, mortality, and transmission of HIV
- Increased rates of transmitted drug-resistant HIV lead the US to recommend testing prior to ART for HIV drug resistance (**PDR**) at a cost of ~$250/person
- In Africa, testing individuals for PDR is not recommended due to the cost and insufficient laboratory infrastructure
- The prevalence of PDR in resource-limited settings was previously low, but is increasing
- An affordable and feasible strategy to diagnose PDR could increase prescription rates for effective ART in resource-limited settings
- To diagnose PDR simply and at a low-cost, an oligonucleotide ligation assay (**OLA**) was developed and validated
- Implementation of this OLA for PDR was studied in Kenya

Study Objectives

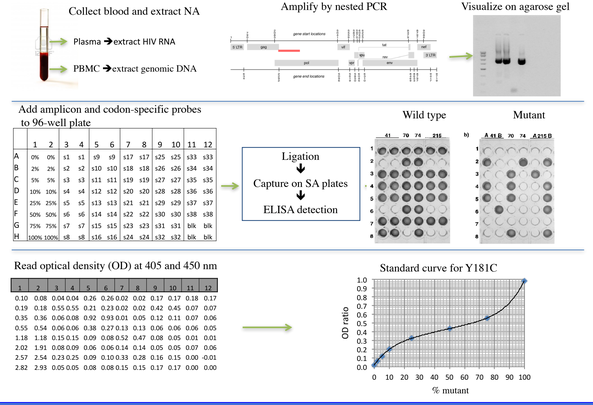
1. Describe implementation of OLA in Kenya
2. Describe the challenges encountered
3. Discuss potential strategies for large-scale use of OLA

Methods

- The OLA was transferred to the Coptic Hope Center's research lab in Nairobi, Kenya for a randomized clinical trial (**RCT**) evaluating whether detection of PDR and treatment with 2nd-line ART would increase the rate of viral suppression at month-12 of ART
- Equipment needed to perform OLA include:
  - Thermocycler, electrophoresis system, plate reader & washer
- Seattle lab manager set up laboratory, programmed instruments and ensured that all were functioning properly
- Research lab consisted of two separate rooms to prevent cross-contamination of PCR amplicon:
  - Pre-PCR Room: nucleic acid extraction and PCR set-up (space also housed study's administrative staff)
  - Post-PCR Room: PCR amplification, ligation, and detection
- Two Kenyan lab technicians were trained to perform OLA by the Seattle lab manager
- No local lab supervisor was on site, so extensive technical advice was provided via frequent emails and weekly Skype calls
- OLA results and assay interpretations were reviewed weekly for quality assurance (**QA**) by Seattle laboratory manager

Methods

Laboratory steps to perform the OLA



Results

Overview

- OLA was successfully performed on 565 patient samples
  - OLA was entirely performed by Kenyan lab technicians with time-intensive technical support from experts in Seattle
- However, some sample results were delayed due to two temporary lab shutdowns caused by difficulties with OLA:
  1. Suboptimal signal from standard curves
  2. Specimen DNA degradation

OLA Workflow*

	Monday	Tuesday	Wed	Thursday	Friday
Week 1	Enroll subjects, collect blood, isolate PBMC, and freeze samples.				
Week 2		DNA Extraction (2-3 hours)	PCR/Gel (~4 hours)	OLA (~5 hours)	Results emailed to Seattle for verification
Week 3	Final Week 1 results emailed back to Kenya	ART Initiation by Clinician			

*Samples were batched weekly (~7-10 per week) and typically processed within 1 week.

Results

General Challenges Encountered

1. The lack of an experienced, on-site lab manager with expertise in molecular biology principles and techniques precluded prompt guidance of laboratory technicians in troubleshooting problems with OLA, and remote technical support from Seattle caused delays in OLA
2. All molecular biology reagents were sent from Seattle to Kenya due to long delays and high costs of obtaining reagents from local distributors
3. Laboratory space was limited, which made it difficult to separate lab activities from administrative activities, and the crowded environment may have contributed to distractions, errors, and contamination of specimens or reagents

Specific Problems Encountered

Time Frame	Initial Problem	Actions Taken	Result
1) November to December 2013	• Standard curves had suboptimal signal intensity with decreased sensitivity for detection of mutant	• Sent new test reagents from Seattle • Changed source of distilled Kenyan H_20	• Enrollment paused from 2 Dec 13 - 1 Jan 14 • OLA working as of 26 Dec 2013
2) August to September 2014	• Specimens did not PCR-amplify as expected due to degraded DNA • Suspect cross-contamination with DNases from bacteria	• Laboratory practices extensively reviewed • Lab & equipment thoroughly cleaned • Serviced safety cabinet & air conditioning	• Enrollment paused from 2 Sept 14 - 5 Oct 14 • OLA working as of 30 Sept 14

Discussion

Potential Strategies for Improving OLA Implementation

1. Local lab supervisor with expertise in molecular techniques
2. Hire lab technicians experienced in Good Clinical Laboratory Practices, and provide further training in molecular principles and practices
3. Leverage existing infrastructure and expertise of current sophisticated clinical and university labs
4. Develop an OLA kit that simplifies the procedure (OLA-Simple)
5. A simple, point-of-care assay could allow for decentralized testing, which may simplify the logistics of specimen transport/tracking and implementation of test results

OLA-Simple POC Concept Assay

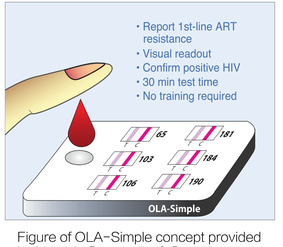


Figure of OLA-Simple concept provided by Nuttada Panpradist & Barry Lutz

Conclusions

- OLA technology was successfully transferred to a research laboratory in Kenya to guide choice of ART regimens for subjects in a clinical trial
- Key challenges identified for large-scale use of OLA:
  - shortage of technicians with sufficient education and training
  - reagent supply-chain issues
  - suboptimal laboratory infrastructure
- The current OLA requires an understanding of molecular techniques, which is infrequent among technicians in resource-limited settings, and indicates a need for a simplified assay
- Ongoing development of OLA-Simple, a pre-packaged kit, should address challenging aspects of current OLA, including the need for a high-level of technical expertise and supply chain issues
- Further research should define optimal laboratory settings, technical training, and workflow strategies to efficiently perform a large volume of OLA
- As policy leaders explore strategies to address HIV drug resistance, OLA is a clinically validated tool that could be implemented to improve clinical mangement of HIV infection

Acknowledgments

Funding for this work was provided by the National Institute of Allergy & Infectious Diseases (R01 AI091550). Dr. Duarte is supported by a Pediatric Scientist Development Program Award (5K12HD000850-30).