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Prospective randomized HIV drug resistance testing of Kenyans before 1st-line ART

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Abstract

Background

Testing for HIV drug resistance prior to initiation of ART has been found to be cost effective in high-resource communities and is recommended by advisory panels, but due to costs is not used in resource-limited communities. The prevalence of transmitted HIV drug resistance has increased in Kenya during the past ten years. We hypothesized that a relatively simple and inexpensive point mutation assay could identify HIV drug resistance in individuals qualifying for 1st-line ART in Kenya, and that use of the test results to choose the ART regimen would improve virologic suppression after 12 months of treatment.

Methods

A randomized clinical trial tested Kenyans qualifying for 1st-line ART using a quantitative oligonucleotide ligation assay (**OLA**). Each subject randomized to OLA was tested at codons 103, 181, 184 and 190 in *pol* reverse transcriptase to detect and quantify mutations as <10% (not resistant) or ≥10% (resistant) within their quasispecies. Subjects randomized to receive pre-ART OLA with ≥1 resistance mutations were given 2nd-line ART with lopinavir/rt, whereas each individual randomized to "no OLA" received standard of care (**SOC**) non-nucleoside reverse transcriptase based ART. Plasma HIV RNA was measured pre-ART, at 12 months of ART, and if >40c/mL, again at 15 months.

Results

OLA was implemented at Coptic Hope Center in Nairobi. A total of 991 subjects enrolled into the study in 2013/14. The overall prevalence of resistance was 8.3%. The 12-month study was completed in November 2015. Testing for HIV drug resistance using a relatively simple and inexpensive point mutation OLA was feasible in Kenya. The OLA testing and initiation of 2nd-line lopinavir/rt-based ART did not significantly impact the overall rate of virologic suppression (1° outcome). Among individuals with resistance detected at ≥10%, rates of virologic suppression were improved by pre-ART OLA testing (2° outcome).

Conclusions

OLA testing for resistance did not improve rates of ART suppression at the population level. Because OLA testing improved outcomes of those with resistance, this study suggests that virologic failure was principally due to other factors. If rates of transmitted resistance continue to increase in Kenya, OLA testing may positively affect overall rates of HIV suppression, and currently, identifying individuals with resistance and starting them on 2nd-line lopinavir/rt-based ART may reduce transmission of drug resistance.

Study Schema

Enrollment visit:
- Informed consent
- Questionnaire: sociodemographic data, medical & sexual history; CD4 obtained from medical record
- Blood draw for study drug resistance testing by OLA
- Block randomization (1:1): OLA testing (OLA arm) vs. Standard of care (SOC arm)

2-3 week period:
- Training in adherence to ART by social work & pharmacists
- OLA testing on PBMC for OLA arm (K103N, Y181C, M184V, G190A), sensitive to ≥2% mutant

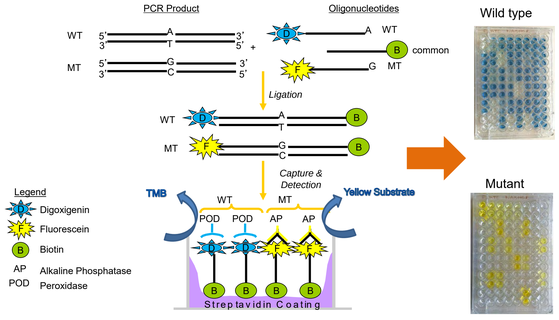
ART initiation visit:
- OLA Arm subjects with resistance ≥10% prescribed lopinavir/ritonavir (**LPV/r**)-ART
- OLA Arm subjects wild-type or resistance of 2-9% and all SOC Arm prescribed NNRTI-ART

Monthly pharmacy visits monthly or bi-monthly x 15 months:
- Adherence to ART monitored by monthly pill count and subject reports
- Current ART refilled

Study visits:
- Blood collected at 4, 8, 12 & 15 months of ART
- 12-month (and entry) plasma HIV RNA quantified (Abbott)
- 15-month plasma HIV RNA quantified if 12 month results ≥40c/mL
- OLA testing of PBMC or plasma HIV RNA ≥400c/mL

Outcomes:
- Rates of virologic failure (plasma HIV RNA ≥400c/mL) by randomization arm
- Rates of virologic failure in those with resistance
- Data Safety and Monitoring Board evaluated outcomes when 300 & 600 completed 12-months of ART

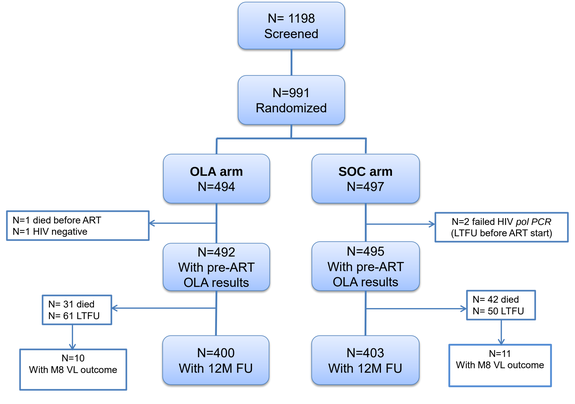
Oligonucleotide Ligation Assay (OLA)



Study Sample Size Assumptions

- Rate of pre-ART drug resistance 7.5%
- Rates of VF: HIV drug resistance detected, 85%; OLA-guided-ART and wild-type, 8%
- Rates of death and loss-to-follow-up: 10%
- Sample size = 949 to detect difference in VF of 8% in OLA vs. 14% in SOC with 80% power at P=0.05

Enrollment, Lost-to-Follow-up & Deaths



Results

Table 1. Demographic characteristics at enrollment by randomization arm

Characteristics	Total (N=991)	OLA (n=494)	SOC (n=497)	P-value
Female	644 (65.0%)	321 (65.0%)	323 (65.0%)	0.997
Age in years, mean (min-max)	38.1 (2.9-84.7)	38.9 (2.9-84.7)	37.3 (3.3-71.1)	0.068
2-13 14-17 18-29 30-39 40-49 ≥50	24 (2.4%) 13 (1.3%) 179 (18.1%) 382 (38.5%) 244 (24.6%) 149 (15.0%)	9 (1.8%) 3 (0.6%) 82 (16.6%) 196 (39.7%) 121 (24.5%) 83 (16.8%)	15 (3.0%) 10 (2.0%) 97 (19.5%) 186 (37.4%) 123 (24.7%) 66 (13.3%)	0.120
Employment, adults only	769 (80.6%)	388 (80.5%)	381 (80.7%)	0.811
CD4 (cells/uL), median (min-max)*	231 (2-2245)	233 (3-1560)	231 (2-2245)	0.918
Plasma HIV RNA (copies/ml), median (min-max)*	45,805 (20-5,091,744)	48,172 (20-4,260,021)	42,728 (20-5,091,744)	0.399
% resistance in HIV population* ≥10% 2-9% wild-type	82 (8.3%) 33 (3.3%) 872 (88.4%)	46 (9.3%) 16 (3.3%) 430 (87.4%)	36 (7.3%) 17 (3.4%) 442 (89.3%)	0.495

* N=987 with OLA results (OLA =492; SOC=495); CD4 counts missing for 3 subjects, 2 in OLA and 1 in SOC; plasma HIV RNA available for 412 in OLA and 416 in SOC

Table 2. Time from enrollment to ART initiation (days)

Arm	Mean	Min-Max	Median*	IQR
OLA (n=467)	21.5	11 to 159	**18**	**14-23**
SOC (n=473)	20.8	4 to 238	**15**	**14-21**
Total (n=940)	21.2	5 to 238	16	14-22

* p=0.0001, time from enrollment to ART initiation of OLA vs. SOC arms by Mann-Whitney

Table 3. HIV drug resistance mutations detected at enrollment

	OLA (n=492)				SOC (n=495)			
Codon	Wild-type n (%)	2-9% mutant n (%)	≥10% mutant n (%)	Median % mutant within a subject's HIV population (range)	Wild-type n (%)	2-9% mutant n (%)	≥10% mutant n (%)	Median % mutant within a subject's HIV population (range)
K103N	450 (91.5%)	7 (1.4%)	**35 (7.1%)**	70.5% (3-100)	455 (92.3%)	12 (2.4%)	**26 (5.3%)**	56.0% (2-100)
Y181C	474 (96.7%)	8 (1.6%)	**8 (1.6%)**	8.5% (2-100)	475 (96.9%)	6 (1.2%)	**9 (1.8%)**	14.0% (2-100)
M184V	481 (97.8%)	4 (0.8%)	**7 (1.4%)**	12.0% (2-90)	484 (98.0%)	6 (1.2%)	**4 (0.8%)**	6.5% (2-91)
G190A	472 (98.3%)	4 (0.8%)	**4 (0.8%)**	41.5% (3-100)	483 (98.6%)	3 (0.6%)	**4 (0.8%)**	14.0% (3-100)

Table 4. Intent-to-treat & as-treated virologic failure (VF) at 1-year of ART

	OLA (n=400)		SOC (n=403)		Chi² p-value
	N Tested	N (%) VF	N Tested	N (%) VF	
Intent-to-treat, Total N=803	400	34 (8.5%)	403	39* (9.7%)	0.562
- With No Previous ART exposure	372	33 (8.9%)	376	39* (10.4%)	0.486
- With Previous ART exposure	28	1 (3.6%)	27	0 (0%)	0.322
As-treated, Total N=743 (those who remained on initial randomized ART)	368	29 (7.9%)	375	28* (7.5%)	0.832
- With No Previous ART exposure	342	29 (8.5%)	349	28* (8.0%)	0.827
- With Previous ART exposure	26	0 (0%)	26	0 (0%)	-

* Includes one subject with VF at 8 months who switched from NNRTI to PI-based ART before 12 months.

Table 5. Virologic failure (VF) at 1-year of ART by amount of resistance at enrollment

	OLA (n=400)		SOC (n=403)		Chi² p-value
OLA results	#Tested	# (%) VF	# Tested	# (%) VF	
Wild-type	351	26 (7.4%)	363	22 (6.1%)	0.472
Mutation 2-9%	13	3 (23.1%)	14	5 (35.7%)	0.472
Mutation ≥10%	36	5 (13.9%)	26	12* (46.2%)	0.005

* Includes one subject with VF at 8 months who switched from NNRTI to PI-based ART before 12 months.

Table 6. Virologic failure (VF) at 1-year of nevirapine vs. efavirenz-ART by amount of resistance at enrollment

	Nevirapine-based-ART		Efavirenz-based-ART		
Amount of mutant	# subjects	# (%) VF	# subjects	# (%) VF	p-value
Total	268	38 (14.2%)	490	29 (5.7%)	<0.001
Wild type	252	27 (10.7%)	453	20 (4.2%)	0.001
2-9%	8	5 (62.5%)	19	3 (15.8%)	0.015
≥10%	10	6 (60.0%)	21	6 (28.6%)	0.093

Table 7. Virologic failure (VF) at one-year of NNRTI-ART by amount of resistance at enrollment

Variable	# Subjects	VF (HIV RNA >400 c/mL)	No VF	P-value
No of subjects	758	66 (8.7%)	692 (91.3%)	
Wild-type	705	46 (6.5%)	659 (93.5%)	<0.001
2-9% mutant	27	8 (29.6%)	19 (70.4%)	
≥10% mutant	26	12 (46.2%)	14 (53.9%)	
Median pre-ART HIV RNA (range), c/mL				
Wild-type	705	142,309 (857-3,661,629)	41,710 (20-5,091,744)	0.0002
2-9% mutant	27	38,841 (1,040-569,291)	13,174 (227-2,315,991)	0.490
≥10% mutant	26	74,175 (4,534-2,354,785)	7,301 (<40-324,938)	0.035
Median pre-ART CD4 count (range)				
Wild-type	705	189 (3-612)	237 (2-1,327)	0.059
2-9% mutant	27	300 (27-483)	238 (13-466)	0.633
≥10% mutant	26	135 (2-425)	255 (137-480)	0.018
Median mutant % (range)				
2-9% mutant	27	3.5% (2-7)	3.0% (2-6)	0.489
≥10% mutant	26	84.5% (16-100)	83.5% (14-100)	0.897
Median mutant load* (range)				
2-9% mutant	27	1,425 (48-22,772)	552 (9-46,320)	0.457
≥10% mutant	26	26,821 (3,763-2,001,567)	1,762 (17-321,689)	0.027
K103N only, # subjects (%)				
2-9% mutant	10	2 (25.0%)	8 (42.1%)	0.401
≥10% mutant	13	3 (25.0%)	10 (71.4%)	0.018
Median % K103N (range)				
2-9% mutant	10	4.5% (4-5)	3.0% (2-5)	0.225
≥10% mutant	13	25.0% (16-100)	85.5% (14-100)	0.610
Median K103N load* (range), c/mL				
2-9% mutant	10	20,280 (17,789-22,772)	645 (13-5,998)	0.037
≥10% mutant	13	26,126 (6,473- 557,452)	7,586 (17-321,689)	0.311

* Mutant load = % mutant x plasma HIV RNA

Table 8. Multivariable analysis evaluating risk of virologic failure (VF) at one-year of ART

Baseline characteristics	Univariable Regression		Multivariable Regression	
	RR (95% CI)	P-value	Adjusted RR (95% CI)	P-value
Gender (male vs. female)	1.03 (0.65, 1.63)	0.91	-	-
Age (years)	0.97 (0.95, 0.99)	0.001	0.97 (0.95, 0.99)	0.002
CD4 count (cells/uL)	0.99 (0.99, 1.00)	0.064	-	-
Plasma HIV RNA (log_{10})	1.18 (1.06, 1.32)	0.003	1.20 (1.08, 1.35)	0.001
% Pre-ART drug resistance				
≥10%	4.08 (2.40, 6.65)	<0.001	3.98 (2.41, 6.56)	<0.001
2-9%	4.41 (2.32, 8.38)	<0.001	4.95 (2.61, 9.41)	<0.001
wild-type	Reference	-	Reference	-
Randomization Arm (SOC vs. OLA)	1.14 (0.73, 1.77)	0.562	1.23 (0.79, 1.93)	0.362

* Univariable and multivariable Poisson regression with robust standard errors was used to calculate risk ratios for VF at one-year of ART by potential enrollment correlates including gender, age, pre-ART drug resistance, plasma HIV RNA, CD4 count, and randomization arm. Multivariable model did not include CD4 count at baseline due to collinearity with plasma HIV RNA at ART.

Summary & Conclusions

Summary

- OLA & SOC Arms initiated ART in similar timeframe
- Overall "as-treated" virologic failure (**VF**) was similar in OLA (7.9%) vs. SOC (7.5%) Arms
- Among those with resistance, OLA management reduced VF (13.9% vs. 46.2%: p<0.005)
- VF was reduced to a greater degree with efavirenz- vs. nevirapine-ART
- Within groups on nevirapine- or efavirenz-ART, subjects with 2-9% and ≥10% had similar rates of VF
- After adjusting for age, plasma HIV RNA and pre-ART drug resistance, there was no difference in risk of VF between OLA and SOC arms (aRR=1.23, 95% CI 0.79-1.93, p=0.362)
- Individuals with ≥10% and 2-9% resistance were 4 and 5-times at increased risk of VF compared with wild-type (aRR=4.95, CI 2,61-9.41, p<0.001; aRR=3.98, CI 2.41-6.56, p<0.001, respectively).

Conclusions

In this randomized trial of OLA testing for HIV drug resistance coupled with administration of LPV/r-ART when resistance was ≥10% of an individual's HIV population, did not reduce the overall rate of VF.

Given that OLA markedly reduced VF in subjects with ≥10% resistance, the study was underpowered due to higher death and loss-to-follow-up rates, lower VF rates (EFV > NVP), and slightly fewer individuals with ≥10% resistance than anticipated.

Given that low levels (2-9%) of resistance conferred a greater risk of VF than anticipated, this suggests that If rates of transmitted resistance increase in Kenya, OLA testing could reduce rates of VF at the population level, and thus could reduce HIV transmission. Currently, death and loss-to-follow-up challenge effective ART.