

# Prevalence of Low-Level Mutations in Primary HIV-1 Infection & Impact on Antiretroviral Therapy

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**Abstract:**  
**Objectives:** Evaluate the prevalence and impact of transmitted low-level HIV-1 drug resistance detected by the oligonucleotide ligation assay (OLA, SensiQuest™) but not by consensus sequencing (CS) in subjects infected with HIV-1 after 1996.  
**Methods:** We performed CS and OLA on the first available plasma and PBMC specimens from an observational cohort at the University of Washington Primary Infection Clinic. OLA assessed mutations in reverse transcriptase (K65R, K70R, L74V, M184V, T215F/Y, K103N, Y181C, and G190A) and protease (D30N, I50V, V82S/A/T, I84V, N88D, and L90M). We compared the number of subjects with resistance detected by each method by McNemar exact tests. The Stanford University HIV Drug Resistance Database was used to predict the impact of mutations on antiretroviral (ARV) activity. Survival analysis evaluated the time to HIV-1 RNA <50 copies/ml, among a subset of subjects treated with ARVs and were adjusted for HIV-1 RNA levels at start of ARVs.

**Results:** Specimens from 99 subjects were obtained a median of 30 days after the estimated date of HIV-1 infection. CS detected resistance in 5 subjects, and OLA detected mutations in 28 additional subjects. Compared to CS, OLA detected more subjects with mutations in plasma (p=0.001) and PBMCs (p=0.003). 89 (92%) of 97 subjects began ARV a median of 48 (IQR 24-107) days after infection. Median time to HIV-1 RNA <50 copies/ml, was 109 (IQR 63-148) days for 59 subjects without mutations, 104 (IQR 55-162) days for 12 subjects with low-level mutations treated with <3 active ARVs, and 85 (IQR 56-111) days for 12 subjects with low-level mutations treated with ≥3 active ARVs (p=7). Compared to subjects without mutations, adjusted hazard ratios for time to HIV-1 RNA <50 copies/ml, were 1.1 (95% CI 0.6-2.0, p=4) for subjects with low-level mutations treated with <3 active ARVs and 1.1 (95% CI 0.6-2.1, p=7) for subjects with low-level mutations treated with ≥3 active ARVs. Only four subjects experienced virologic failure during follow-up.

**Conclusions:** CS underestimated the prevalence of transmitted HIV-1 drug resistance in ARV-naïve subjects with primary HIV-1 infection. In this pilot project, we found no association between low-level mutations and delayed time to HIV-1 RNA <50 copies/ml, possibly due to the small sample size. With advances in ARV potency, it is also possible that low-level resistance mutations are only clinically relevant at critical codons.

**Introduction:**

- Surveys in US using consensus sequencing (CS) estimate 11-24% of persons acquire drug resistant virus.
- Consensus sequencing cannot detect viral variants <10-50% of the viral population.
- More-sensitive assays detect mutations at low concentrations ("low-level" mutations) in up to 47% of antiretroviral (ARV)-naïve subjects.
- Impact of minority variants on HIV-1 disease progression and response to ARVs remains unclear.
- The oligonucleotide ligation assay (OLA) detects mutations in as little as 2-5% of the viral quasi-species.

**Purpose/Goals:**

- Evaluate prevalence of mutations detected by CS and OLA in subjects with primary HIV-1 infection.
- Evaluate impact of minority variants on ARV therapy.

**Methods: Patient Population:**

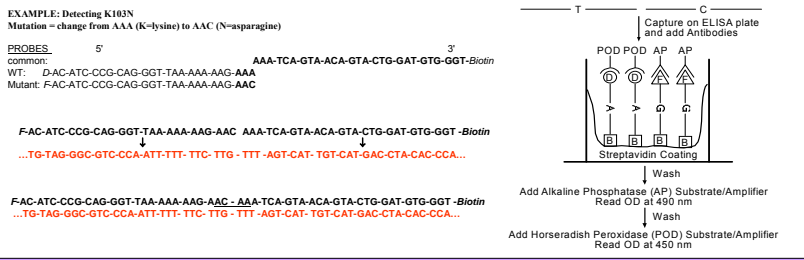
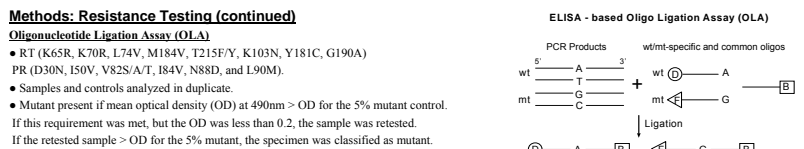
- Since 1992, >300 enrolees in observational cohort at the University of Washington Primary Infection Clinic (PIC).
- Selected a subgroup of 99 subjects who had primary HIV-1 infection, met criteria for enrollment in the PIC, and acquired HIV-1 after 1996 and who:
  - enrolled within one month of HIV-1 infection, or
  - had baseline consensus sequencing previously performed, or
  - initiated ARV therapy within 6 months of study enrollment.
- Resistance testing was performed on the first available plasma and peripheral blood mononuclear cell (PBMC) specimens collected no more than 7 days after start of ARVs.
- Approved by University of Washington Institutional Review Board. All subjects gave written consent.

**Methods: Resistance Testing**  
**RT-PCR and PCR for genotyping of HIV-1 pol**

- RNA extracted and reverse transcribed using GeneAmp RNA PCR Core kit.
- DNA extracted from PBMCs using Puregene Cell and Tissue kit.
- Nested PCR: 1<sup>st</sup>-round PCR of cDNA or DNA in 50 µl reaction mixture with 10 µl of cDNA1 µg DNA. 2<sup>nd</sup>-round PCR contained 2µl 1<sup>st</sup>-round product.
- Amplicon = 1,193-bp DNA fragment encoding all of PR and RT to amino acid 220.

**Consensus sequencing**

- Bidirectional sequencing of amplicons performed using fluorescence-labeled dideoxynucleotide chain terminators.
- Sequences analyzed with Sequencer, version 3.0. Genotypes were aligned in ClustalX and Stanford University HIV Drug Resistance Database used to identify mutations.



**Methods: Statistical Analysis**

- McNemar's exact tests compared number of subjects with mutations detected by OLA and consensus sequencing in plasma and in PBMCs.
- Survival analyses compared the time to virologic suppression (defined as HIV-1 RNA <50 copies/mL), adjusted for HIV-1 RNA level at start of ARVs.
- Stanford University HIV Drug Resistance Database used to predict number of active agents (without intermediate or high-level resistance) in the regimen.
- Subjects were divided into three groups:
  - Subjects without detectable HIV-1 drug resistance mutations,
  - Subjects with low-level mutations who were treated with ARV regimens with fewer than three active ARV agents, and
  - Subjects with low-level mutations who were treated with ARV regimens with three or more active agents.

**Results: Demographic & baseline characteristics**

- All subjects were men 98% were men who have sex with men (MSM)
- All subjects acquired HIV-1 subtype B infection

	No mutations (n=66)	Low-level mutations only (n=28)	Mutations by CS (n=5)	All subjects (n=99)	
Age (Median, IQR)	35 (31-39)	34 (29-42)	33 (32-41)	34 (30-40)	NS
Caucasian	91%	96%	100%	93%	NS
Days from infection to study screening	22 (13-56)	29 (23-59)	54 (34-80)	27 (15-63)	p=07
Screening CD4	508 (395-646)	507 (389-697)	421 (396-550)	504 (393-694)	NS
Screening HIV RNA	5.3 (4.5-6.2)	5.1 (4.6-5.5)	5.2 (5.0-6.0)	5.2 (4.5-6.0)	NS
Received ART	80%	82%	100%	87%	NS
Initial regimen					
PI	25 (40%)	7 (25%)	2 (40%)	34 (40%)	NS
NNRTI	20 (34%)	11 (40%)	2 (40%)	33 (38%)	
PNNRTI	12 (21%)	4 (17%)	1 (20%)	17 (20%)	
NNRTI only	3 (5%)	1 (4%)	0	2 (2%)	

**Results: Prevalence of transmitted drug resistance**

- Plasma and PBMC specimens from median of 29 (IQR 19-66) and 31 (IQR 19-66) days after HIV-1 infection.
- CS detected mutations in 5 (5%) subjects
- OLA detected mutations in 33 (33%) subjects.
- No trend in incidence of transmitted drug resistance over time.

**Table: Number of subjects with mutations detected**

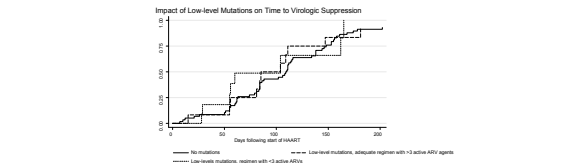
p<0.001	OLA versus CS in plasma			OLA versus CS in PBMCs		
	OLA			OLA		
	-	+		-	+	
CS	-	73	22	-	78	16
	+	0	4	+	1	4

**Table: Drug resistance mutations detected and their impact on ARVs**

Subject	Mutation	Plasma		PBMCs		Time to HIV-1 RNA <50 copies/ml (days)	Virologic failure
		CS	OLA	CS	OLA		
1						109	
2						104	
3						85	
4						109	
5						104	
6						85	
7						109	
8						104	
9						85	
10						109	
11						104	
12						85	
13						109	
14						104	
15						85	
16						109	
17						104	
18						85	
19						109	
20						104	
21						85	
22						109	
23						104	
24						85	
25						109	
26						104	
27						85	
28						109	
29						104	
30						85	
31						109	
32						104	
33						85	
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44						104	
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85						109	
86						104	
87						85	
88						109	
89						104	
90						85	
91						109	
92						104	
93						85	
94						109	
95						104	
96						85	
97						109	
98						104	
99						85	

VF: virologic failure; NA: not applicable (subjects did not initiate ARV); DNS: subjects discontinued ARVs (eg due to ADR, ISR) or were censored prior to virologic suppression. No subjects had the L74V, T219V, D30N, or V82S mutations.

**Figure: Time to virologic suppression among subjects with/without low-level drug resistance**



- 89 subjects began ARVs a median of 48 (IQR 24-107) days after HIV-1 infection.
- Median time to virologic suppression (HIV-1 RNA <50 copies/mL) was:
  - 109 (IQR 63-148) days for 59 treated subjects without detectable mutations
  - 104 (IQR 55-162) days for 12 subjects with low-level mutations treated with <3 active ARVs, and
  - 85 (IQR 56-111) days for 12 subjects with low-level mutations treated with ≥3 active ARVs (p=7).
- Compared to subjects without mutations, time to suppression was not significantly different either for subjects with low-level mutations treated with <3 active ARVs (aHR 1.3, 95% CI 0.6-2.9, p=4) or subjects with low-level mutations treated with ≥3 active ARVs (aHR 1.1, 95% CI 0.6-2.1, p=7).

**Limitations:**

- Only 4 subjects were observed to have virologic failure.
- Study was underpowered to detect differences in clinical outcomes or impact of specific mutations.
- OLA may not have detected mutations that may have been present at even lower levels.

**Conclusions:**

- Transmitted drug resistance was detected in 33% of ARV-naïve subjects with primary HIV-1 infection.
- Consensus sequencing significantly underestimated the prevalence of drug resistance.
- We found no association between low-level mutations and delayed time to HIV-1 RNA <50 copies/mL.
- Impact of transmitted drug resistance may not be uniform and could be modified by level of resistance, use of specific ARVs, or other factors.