Development and Optimization of Oligonucleotide Ligation Assay (OLA) Probes for Detection of HIV-1 Resistance to Dolutegravir

IA Beck[1], CL Boyce[1], M Bishop[1], YL Vu[2], A Fung[2], S Styrchak[1], N Panpradist[2], BR Lutz[2], LM Frenkel[1,2]

[1] Seattle Children's Research Institute, Seattle, WA, USA; [2] University of Washington, Seattle, WA, USA

Introduction

Background:

- Dolutegravir (DTG) is the recommended drug for 1st-line antiretroviral treatment (ART) of HIV infection in resource-limited settings
- While DTG has a high barrier to resistance, following the global rollout of DTG-based ART, reports of ART failure with selection of drug resistance (DR) are emerging
- Access to timely detection of virologic failure and emerging DR is not readily available in many resource-limited settings, compromising the long-term effectiveness of ART programs

OLA-Simple:

Near-point of care HIV DR kit for detection of NNRTI and NRTI mutations associated with failure of NNRTI-based ART

- Dried reagents – **easy assay set-up**
- Custom-designed lateral flow tests – **visual readout**
- Step-by-step software guide – **minimal training**

Objective:

➢ Develop and validate OLA probes for detection of DTG resistance mutations with the goal of expanding use of OLA-Simple in resource-limited settings

Methods

Selection of DTG mutations:

- Compiled published data from clinical trials and case reports
- Identified integrase (IN) mutations prevalent in patients with virologic failure of DTG-based ART, including those occurring as single mutations

Table 1. Integrase mutations detected in individuals with virologic failure of DTG-based ART (N=58)

Mutations detected	Major Integrase Inhibitor (INSTI) Resistance Mutations (Stanford HIV Drug Resistance database)									
	T66IK	E92Q	**G118R**	E138KAT	G140SAC	Y143RCH	S147G	**Q148HRK**	N155H	**R263K**
Number	9	2	19	16	2	1	4	6	4	30
Percent	15.5	3.4	32.8	27.6	3.4	1.7	6.9	10.3	6.9	51.7

- Mutations in bold reduce DTG susceptibility or virological response
- G118R, Q148H/R/K, N155H and R263K were detected in 55/58 (95%) individuals reported to have DTG resistance at virologic failure; and as single mutations in 37/58 (64%)
- T66I/K and S147G were each detected as a single mutation in 1/58 (1.7%) patients with virologic failure; all other mutations occurred in combination with other INSTI-resistance mutations

Design of OLA probes:

- Probes were designed to detect mutations G118R, Q148H/R/K, N155H and R263K in HIV subtypes A, B, C, D and AE
- Mutation-specific ligation probes were designed using alignments from the Los Alamos National Laboratory database

OLA optimization:

- Laboratory ELISA-based OLA
- Mutation-specific standard curves were prepared with plasmid mixtures containing the mutation of interest
- OLA conditions were optimized for detection of mutant frequencies of ≥ 2% in the viral population

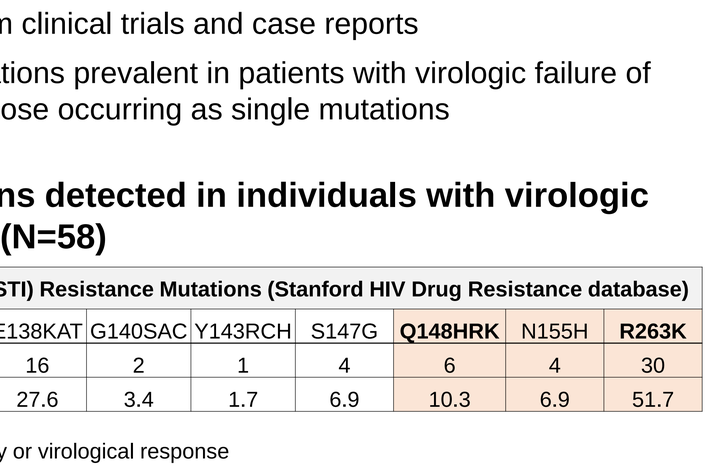


Figure 1. Oligonucleotide Ligation Assay (OLA)

Assessment of probes in clinical specimens

- HIV IN was amplified from 50 banked HIV-infected plasma specimens that included multiple subtypes (A=4, B=8, C=10, D=9 and AE=9) and tested using the laboratory-based OLA
- OLA results were compared to prior PacBio (N=13) or Sanger sequencing (N=37)

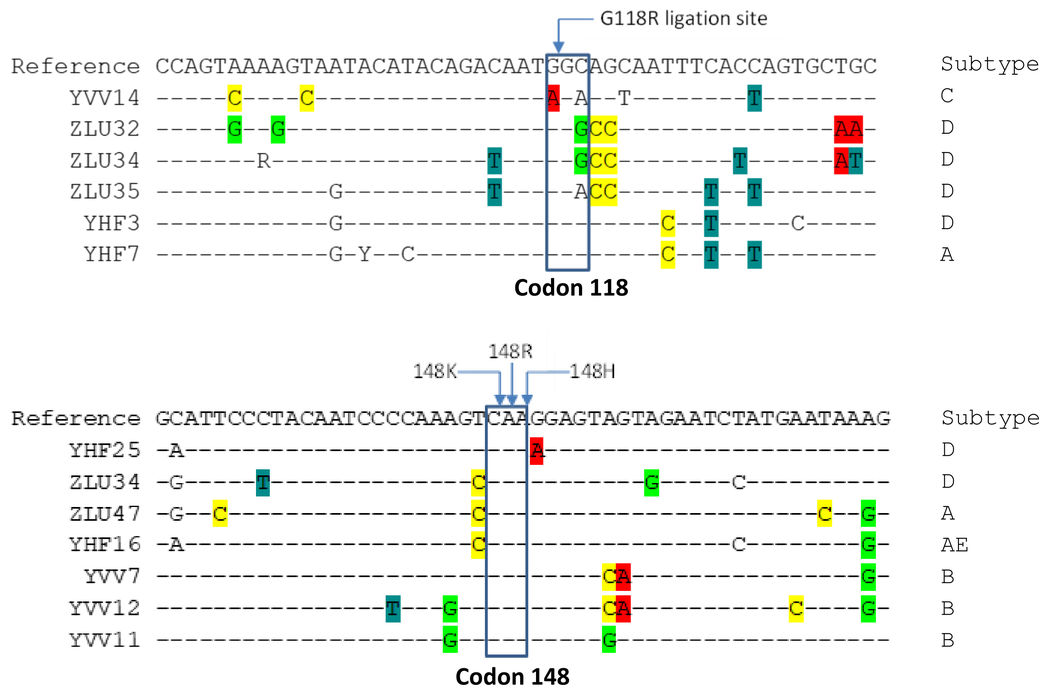
Results

Table 2. Summary of OLA genotyping results at 300 HIV IN codons compared to PacBio/Sanger sequencing

	OLA			PacBio/Sanger	
Mutation	**Mutant**	**Wildtype**	**Indeterminate**	**Mutant**	**Wildtype**
G118R	5	39	6	7	43
Q148K	0	47	3	0	50
Q148R	0	44	6	0	50
Q148H	0	48	2	0	50
N155H	5	45	0	5	45
R263K	5	45	0	5	45
Total	**15**	**268**	**17**	**17**	**283**

- OLA detected 15/17 mutations (88.2%); two G118R were missed, one had indeterminate OLA result and the other (2.8% by PacBio) was wildtype in OLA
- OLA detected minority variants quantified at 3.6%, 7.1%, 11.1%, 16.7% and 17.6% by PacBio
- Ligation failed, resulting in indeterminate OLA results (negative mutant and wildtype reactions) at 17 codons (5.7%): 6 at G118R and 11 at Q148 R, H or K

Figure 2. Sequences from samples with indeterminate OLA results at codons 118 and 148



Note: Highlighted bases indicate polymorphisms not addressed by the G118R or Q148K/R/H probes

New modified probes were designed to accommodate the following common interfering polymorphisms:

- G118R: an alternate mut-specific probe with "A" at the ligation site, and a common probe with "RCC"
- Q148R: a "Y" at the third base from the ligation site was added to the mutant and wildtype-specific probes
- Most other polymorphisms not addressed by the original probes occurred in single samples

OLA results after re-testing samples with modified probes for codons G118R and Q148R:

Mutant detection:

- 6/7 G118R mutations detected
- Final sensitivity across all DTG mutations = 16/17 (94.1%)

Indeterminate results:

- 2 at G118R; 3 at Q148R
- Final rate of indeterminates = 10/300 codons (3.3%)

Conclusions

- The OLA probes for detection of DTG resistance showed high sensitivity for mutant detection compared to PacBio and Sanger sequencing, including minority variants at frequencies >3%
- Improved performance of OLA at codons 118 and 148 will require further optimization and testing of probes located in these highly polymorphic regions of HIV

Future direction

- Further validate DTG OLA probes with a larger cohort of specimens enriched for mutant genotypes
- Include reagents for HIV integrase amplification and DTG resistance detection in OLA-Simple kits, and test performance of kits in the field

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