High HIV Drug Resistance in Naïve, Recently Diagnosed Patients in Gondar, Ethiopia

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Introduction

Transmitted Drug Resistance:

• HIV drug resistance typically develops in patients treated with combination anti-retroviral therapy (cART).
• Drug resistance mutations can then be transmitted to treatment naïve patients and impair cART success.
• Identification of regional transmitted drug resistance trends is imperative to national HIV treatment strategies.

HIV Drug Resistance in Ethiopia :

• There are nearly 800,000 people living with HIV in Ethiopia today.
• In 2003, a free national cART program was initiated.
• Routine drug resistance testing is not available in Ethiopia, and decisions regarding cART switch are based on clinical factors and CD4 cell counts.
• A 2005 study from Addis Ababa in Ethiopia found no major HIV drug resistance mutations in cART-naïve patients (Abegaz et al, Antiviral Therapy 2008.)
• A 2003 study from Gondar in northern Ethiopia found a 3% (3/92) pre-treatment drug resistance prevalence. All mutations identified were in the reverse transcriptase (RT) (Kassu et al, AIDS Research and Human Retroviruses 2007).
• A 2008-2009 study from the same region found a slightly higher prevalence, 6% (9/160). Again, all mutations identified were in the RT (Mulu et al, BMC Infectious Diseases 2014).
• We evaluated HIV drug resistance in drug-naïve patients in Gondar, 10 years after cART availability in Ethiopia.

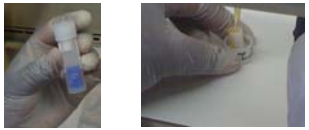

Figure 1: ViveST™ storage and transportation device

Methods

Patient Population:

• The 400-bed University of Gondar Hospital receives referrals from 4 district hospitals and provides services to over 5 million people living in the Amhara region, in northern Ethiopia (Figure 2). The prevalence of HIV in the city of Gondar (circled) is estimated to be 10%.
• The cART Clinic in Gondar follows nearly 6,500 people living with HIV.

Figure 2: Map of Ethiopia

• During April-May 2013 we enrolled HIV-infected patients at the Gondar cART Clinic.
• Inclusion criteria:
1. Adults ≥18 years
2. Newly diagnosed with HIV
3. No previous exposure to cART, based on patient self report

Laboratory methods :

•One milliliter plasma samples were mounted on ViveSTs in Ethiopia, air-dried and shipped in room temperature to the USA, 3-5 weeks after collection, for *pol* genotyping using Sanger sequencing (Figure 1).

Data Analysis:

•Analyses of drug resistance mutations and subtyping were done using Stanford Drug Resistance Database tools and the World Health Organization Surveillance Drug Resistance Mutation list (WHO-SDRM; Bennett et al PLOS One 2009).
• Phylogeny with the Bayesian Monte Carlo Markov Chain analyses within Bayesian Evolutionary Analysis of Sampling Trees (BEAST) was used to examine the relatedness of *pol* sequences of enrolled participants with 2 additional cohorts: (i) HIV positive Ethiopians who immigrated to Israel around 2003, and (ii) patients diagnosed in Gondar in 2003.
• Time of infection was estimated by a recency assay, based on ambiguous nucleic acid frequencies, with a cutoff of 0.43% per sequence suggesting infection within the last year (Maldarelli et al J Virology 2013).

Results

Drug Resistance analysis:

• Blood samples were collected from 48 drug-naïve patients diagnosed at the Gondar cART clinic, 50% females, median CD4 220 cells/μL.
• Of the 48 enrolled patients, 38 (79%) were successfully genotyped. All were HIV-1 subtype C.
• **Eleven percent** (4/38) had drug resistance mutations that are on the WHO-SDRM list. Eight additional patients had other drug resistance mutations, not counted towards resistance prevalence.
• Of 38 samples successfully genotyped, 24 belonged to patients who were diagnosed with HIV less than one year prior to enrollment.
• **Seventeen percent** (4/24) of those patients had WHO-SDRM drug resistance mutations (bold in Table 1).

Phylogenetic analysis:

• Phylogenetic analysis demonstrated that the cohorts intermingle completely (Figure 3).
• Large clusters with a common ancestor were not identified.
• We found 26 clusters of two patients, 5 clusters of three patients and one cluster of four patients.
• No linkage was evident by phylogeny among the patients with drug resistance mutations (Figure 3).

Recency analysis:

• Based on the recency analysis, only 3/24 (13%) patients diagnosed during the year prior to enrollment had an ambiguity index <0.43% and therefore were suspected to actually have been infected during that year.

	Class	Mutation	No of patients	Year of diagnosis
Major Resistance Mutations	PI	M46IM	1	2012
		V82LV	1	2012
	NNRTI	V106M	1	2013
		G190A	1	2012
Minor Resistance Mutations	PI	L10I/V	2	2005, 2012
		E35EG	1	2012
		Q58EQ	1	2012
	NRTI	A62AV	1	2013
	NNRTI	V108IV	2	<2008, 2012
		K103E	1	2013
		E138A	1	2013

Table 1: Resistance mutations in drug naïve patients in Gondar 2013

Summary & Conclusions

• We found intermediate level (11%; for all enrolled patients), and high level (17%; for patients infected in the past year) of HIV drug resistance in cART naïve patients living in Gondar, Ethiopia.
• With resistance decay over time in the absence of drug pressure, this may be an underestimation, since only 13% of the recently diagnosed patients were estimated to have actually been recently infected.
• We identified the first major protease mutations in cART naïve patients in Ethiopia.
•The observed resistance levels are higher than previously reported in Ethiopia as well as in the same clinic, and support further drug resistance surveillance and the consideration of incorporating drug resistance testing into clinical care in this setting.
•ViveSTs provided good quality sequences and should be further examined as cost effective alternative methods for drug resistance testing in resource limited settings.

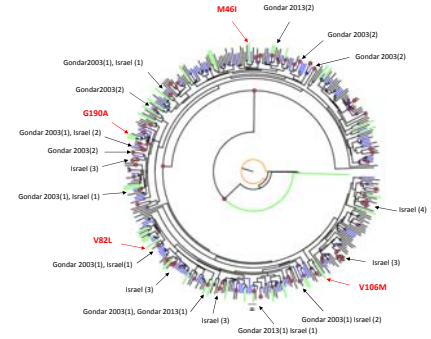

Figure 3: Phylogenetic tree of 3 Ethiopian cohorts: black branches- Ethiopian Immigrants to Israel; blue branches- patients diagnosed in Gondar 2003; green branches- current study participants, Gondar 2013. Black arrows-clusters; Number in parenthesis– number of patients in cluster. Red circles- clusters with posterior > 0.95. Red arrows- drug naïve patients with resistance mutations, Gondar 2013. Orange line– Reference B sequence.