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Evolution of drug resistance after virologic failure of a first highly active antiretroviral therapy regimen in Uganda

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Abstract

Objective—To determine the extent of viral resistance over time among non-clade B HIV-1 infected patients in Uganda maintained on first line highly active antiretroviral therapy (HAART) following virologic failure.

Methods—Genotyping was performed on sixteen patients with virologic failure who were enrolled in an open label randomized clinical trial of short-cycle treatment interruption.

Results—All patients receiving efavirenz containing HAART had at least 1 efavirenz resistance mutation develop during follow-up. The majority 13/15 (86%) developed lamivudine resistance during follow-up but no thymidine analogue mutations (TAMS) developed during a median duration of virologic failure of 325.5 days.

Conclusions—Genotypic resistance to both efavirenz and lamivudine developed early during the course of treatment after virologic failure. TAMs did not emerge early despite moderate exposure time to thymidine analogs during virologic failure.

Keywords

human immunodeficiency virus (HIV); antiretroviral drug resistance; virologic failure

Introduction

HAART has become increasingly available in resource limited settings (RLS) thanks to an increased number of international partners funding HIV treatment programs. (1;2) The goal of these programs is to achieve maximum and sustained suppression of viral replication to prolong efficacy of these first line regimens and minimize the evolution of viral resistance.(3) Second line treatment options remain limited in RLS, and some country guidelines (although not consistent with WHO guidelines) recommend continuation of zidovudine or stavudine in second line regimens based on the assumption that resistance to these agents develops slowly if at all.(4) This assumption is largely based on data from western cohorts in a predominantly clade B setting.(5;6) Recent reports from Thailand and Africa suggest that resistance to

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thymidine analogs may be emerging early in these epidemics; however, data remain extremely limited regarding the actual timing of the onset of antiretroviral resistance mutations outside in patients infected with non-clade B virus. (7-9)

This retrospective analysis was undertaken to specifically look at the impact of sustained viral replication in the setting of first-line HAART regimens on the evolution of genotypic resistance. The evolution of resistance mutations over time was evaluated among participants enrolled in a clinical trial of short-cycle treatment interruption who reached a virologic failure endpoint and were subsequently switched to continuous treatment but remained on their original regimen containing 2 nucleoside reverse transcriptase inhibitors (NRTIs).

Methods

The original clinical trial was designed to evaluate the non-inferiority of two short cycle intermittent HAART regimens compared to continuous ART. HIV-infected persons receiving HAART at the Joint Clinical Research Center (JCRC) in Kampala, Uganda, with a CD4 count equal to or greater than 125 cells/mm^3 and plasma HIV viral load below 50 copies/mL of plasma were eligible. Participants were required to be receiving at least 3 antiretroviral agents including a protease inhibitor or efavirenz. One hundred forty seven participants were randomized to either continuous HAART, 5 days on/2 days off intermittent HAART or 7 days on/7 days off intermittent HAART. Those receiving a nevirapine-based regimen were required to switch to a protease inhibitor or efavirenz if they were randomized to the interrupted arm. Participants paid for their drugs consistent with standard practice at the JCRC at the time of the trial but were offered free laboratory monitoring. At the time the study was conducted, large international programs to support the cost of antiretroviral treatment had not begun and the availability of second line treatment was extremely limited and expensive in Uganda. All participants signed informed consent prior to entry in the study. This study was reviewed and approved by the HIV/AIDS Research Committee, Uganda Council for Science and Technology and the Institutional Review Board of the National Institute of Allergy and Infectious Diseases.

Study participants were evaluated for clinical, virologic, immunologic, toxicity and adherence parameters at weeks 2 and 4 following enrollment and every 6 weeks thereafter. Adherence was measured using patient maintained medication diaries. For this analysis, virologic failure was defined by a plasma HIV RNA level greater than 400 copies/mL on at least 2 visits (not necessarily consecutive). To evaluate the evolution of resistance over time, a minimum of 12 weeks of follow-up time was required after reaching a virologic failure endpoint was required for inclusion in this sub-analysis. Resistance mutations for the purposes of this study were considered in the reverse transcriptase genome as included in the IAS-USA drug resistance mutations group and those proposed for drug resistance surveillance by Shafer *et al.*(10) Participants were switched from interrupted to continuous therapy if a virologic failure endpoint was reached. Study participants enrolled in the intermittent treatment arms who failed to achieve virologic suppression after switching to continuous treatment received ongoing clinical care, laboratory monitoring and were counseled to switch to second line therapy and were ultimately provided free second line regimens by JCRC once free second line treatment was available in Uganda.

Results

One hundred forty-seven participants were enrolled in the original clinical trial from 2002-2006. Sixteen of these participants were included in this sub analysis and are reported here. Three of the participants were originally randomized to the continuous treatment arm and 13 were randomized to the interrupted treatment arm (9 to the 7/7 arm and 4 to the 5/2 arm). Table 1 shows the failure times, CD4 and viral loads of these participants. Treatment regimens

and genotypic results are listed in table 2. One participant had genotyping done on their last follow-up visit only. A majority of participants (15/16; 94%) were on an efavirenz-based regimen with 2 nucleoside reverse transcriptase inhibitors (NRTIs), with 1 participant receiving indinavir with 2 NRTIs. The median time on HAART before virologic failure (including time before study entry) was 315.5 days (range 79-936 days) with the median total ART exposure time (pre and post virologic failure) of 654 days (range 205-1293 days). The median exposure time of virologic failure was 325.5 days (range 126-483 days). The median viral load overall during this exposure period was 5800 copies/ml (range 329-89 372 copies/ml). The median change in CD4 during the time of virologic failure was an increase of 33 cells/ul. None of the participants would qualify as having either clinical of immunologic failure criteria by the WHO failure definition despite this period of prolonged virologic failure.

Among the 14 participants who were receiving efavirenz containing HAART and had a baseline genotype performed near the beginning of virologic failure, 13/14 (93%) had evidence of resistance to efavirenz. 14/15 (93%) participants on efavirenz had evidence of resistance at the last follow-up visit with all 15 participants receiving efavirenz having 2 or more NNRTI mutations at some point during follow-up. Lamivudine resistance was present among 9/15 (60%) of participants during early virologic failure and among 13/16 (81.25%) by the last follow-up visit. None of the participants developed any evidence of TAMs during follow-up. All participants in this sub-study reported >95% adherence to their antiretroviral medications during study follow-up.

Discussion

This is the one of the first reports of the chronology of resistance mutations from African patients receiving HAART regimens consisting of 2 NRTIs plus either efavirenz or a PI. Knowledge of the chronology of resistance mutations in this predominantly clade A, D and recombinant setting is particularly important to guide policy makers on the choices of second line therapy since there is a virtually no access to genotyping outside of research settings in Africa.(11) Currently, some country guidelines including Uganda maintain zidovudine or stavudine as components of second line regimens based on the assumptions that TAMs will emerge slowly, leaving these compounds active despite failure of first line regimens.(4) The data from our study are encouraging, as we did not see any resistance emerge to these compounds despite a median period of virologic failure of approximately 1 year. A particularly concerning ancillary finding from this study is the lack of any evidence of clinical or immunologic failure despite a median follow-up during viral rebound of 325.5 days. Research centers participating in the current antiretroviral treatment scale up urgently need to examine the sensitivity and specificity of the current WHO immunologic and clinical failure criteria compared to virologic criteria.(12)

Our findings must be taken with caution for a number of reasons. Of particular note, none of the participants in this analysis would have been identified as having failure in the absence of virologic monitoring. This is concerning since most centers in Africa providing HAART do not have access to routine virologic monitoring. The participants in our study would have been assumed to be still responding to first line HAART by most centers and would not have been considered for switch to second line therapy. The lack of emergence of TAMs may be due to the period of follow-up; TAMS may have emerged had these patients continued to have virologic rebound while remaining on first line regimens until they experienced immunologic or clinical failure. The sample size for this sub-analysis was small and may have limited our power to truly detect the rate of TAM accumulation. The study inclusion criteria (VL<50 copies/ml) may not be representative of the broader treatment population in Africa where full virologic suppression may not have occurred following treatment initiation. Finally, the genotyping methods used in this study were not sensitive to detecting minority mutations.

Our findings differ from those of some other reports from non-clade B settings where TAMs were seen to emerge relatively early during the course of treatment among patients experiencing failure to first line HAART.(7-9) The DART trial reported a higher incidence of TAMs among failing patients but differed from our study in that most participants were treated with a triple NRTI regimen, which may affect the threshold for the emergence of TAMs. Participants in our study also paid for their medication and therefore had no incentive to falsely claim that they had not received treatment elsewhere prior to initiation on HAART. Many free treatment programs during the early ART scale focused on treatment naive individuals, which may have provided an incentive for treatment experienced individuals not to disclose prior antiretroviral exposure during periods of self-pay. (13) Under-reporting of prior antiretroviral exposure could potentially bias surveillance studies investigating antiretroviral resistance.

Prolonged virologic failure prior to changing therapy may also limit future treatment options if the newer generation NNRTI compounds become available for treatment programs in RLS. All of the participants in our study developed at least 2 NNRTI resistance mutations during follow-up. Preliminary data from the DUET trial of etravirine suggest that individuals with multiple genotypic resistance mutations (defined as 3 or more etravirine resistance associated mutations) may achieve suboptimal virologic outcomes compared to individuals with 2 or fewer mutations.(14)

As the current scale up of antiretroviral treatment continues in RLS, prudent surveillance of the rates of genotypic resistance to antiretroviral drugs from sentinel sites will be critical to guide rationale choices for second line treatment options. The potential emergence of TAMs could greatly limit the currently available second line options in RLS, most of which maintain a nucleoside backbone. Alternative first line regimens using non-thymidine analog NRTIs such as tenofovir may offer an alternative treatment approach to limit the development of TAMs and class wide nucleoside resistance ultimately preserving stavudine and zidovudine for use in second line regimens. Sustainability of the early success of antiretroviral treatment scale up will depend on a combination of sustained donor funding, adherence counseling and prudent monitoring to identify those at risk for failure and the development of genotypic resistance.

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Table 1

CD4 Change and Viral Load during Failure

Participant	CD4 at first VL>400 copies/ml	CD4 at last VL>400 copies/ml	CD4 Change	Failure week (First VL>400)	Failure time (weeks) since first VL>400 copies/ml	Median VL Copies/ml[1]
6	298	297	−1	12	44	4842
9	304	309	+5	18	51	2854
20	117	170	+53	12	54	329
31	227	274	+47	18	34	1108
35	701	608	−93	4	69	87335
48	312	219	−93	2	49	33276
66	187	178	−9	36	34	56515
82	391	431	+40	18	49	51167
87	102	71	−31	11	18	89372
88	95	277	+182	18	55	16389
90	154	319	+165	24	39	497
91	182	215	+33	0	58	5050
98	209	174	−35	24	43	56489
109	24	103	+79	6	65	3986
147	128	206	+78	6	23	3277
248	298	ND	ND	4	19	6551

Median CD4 change (missing data on JCH-248MS): +33

Median Failure Time: 46.5 weeks

Median VL copies/ml: 5800.5 copies/ml

[1]Median VL based on all VL measurements after first VL>400 copies/ml

Table 2

Viral load and Reverse Transcriptase Genotypes over Time

Patient (visit)	Therapy	Days since VL>400 copies/ml	VL copies/ml	Mutations
6 (1)	D4T/3TC/EFV	182	50130	K103N, M184V
6 (2)	D4T/3TC/EFV	238	74635	K103N, M184V
6 (3)	D4T/3TC/EFV	308	26129	K103N, V108I, M184V,
9 (1)	AZT/3TC/EFV	0	24410	K103N
9 (2)	AZT/3TC/EFV	210	5463	Non amplifiable
9 (3)	AZT/3TC/EFV	357	23031	A62V, K103N, V108I, M184V, P225H
20 (1)	AZT/3TC/IDV	84	199	M184V
20 (2)	AZT/3TC/IDV	378	8064	M184V
31 (1)	D4T/3TC/EFV	0	1787	K103N, M184V
31 (2)	D4T/3TC/EFV	238	6266	V75A/V, K103N, M184V, P225H
35 (1)	D4T/3TC/EFV	483	43836	K101Q, K103N, M184V
48 (1)	D4T/3TC/EFV	63	15755	K103N, M184V, M230L
48 (2)	D4T/3TC/EFV	343	163448	K103N, V108I
66 (1)	D4T/3TC/EFV	0	81451	K101E, K103N, P236L
66 (2)	D4T/3TC/EFV	28	>100000	K101E, K103N, M184V
66 (3)	D4T/3TC/EFV	238	31578	K101E, K103N, M184V
82 (1)	D4T/3TC/EFV	77	175000	K103N, M184V
82 (2)	D4T/3TC/EFV	161	51167	K103N, M184V
82 (3)	D4T/3TC/EFV	343	77669	A98G, K103N, M184V
87 (1)	D4T/3TC/EFV	0	121866	K103N
87 (2)	D4T/3TC/EFV	126	4600	K103N, V108I/V, Y188H/Y
88 (1)	D4T/3TC/EFV	0	124590	K103N, M184M/V, M230M/L
88 (2)	D4T/3TC/EFV	91	31108	K103N, M184V, M230L
88 (3)	D4T/3TC/EFV	385	26651	WT
90 (1)	D4T/3TC/EFV	0	100446	K103N
90 (2)	D4T/3TC/EFV	273	4566	K103N, V108I, M184V, P225H
91 (1)	AZT/3TC/EFV	315	4374	K103N, M184V, M230L
91 (2)	AZT/3TC/EFV	406	2860	T69N/T, K103K/N, M184I/M/V, M230M/L
98 (1)	AZT/3TC/EFV	0	56800	K103N, M184I/M,
98 (2)	AZT/3TC/EFV	49	22202	K103N, M184I/M/V
98 (3)	AZT/3TC/EFV	126	148937	K103N, M184I
98 (4)	AZT/3TC/EFV	301	56178	K103N, V108I, M184V
109 (1)	D4T/3TC/EFV	0	>100000	WT
109 (2)	D4T/3TC/EFV	126	286900	WT
109 (3)	D4T/3TC/EFV	371	21266	K103N, M184V
109 (4)	D4T/3TC/EFV	455	20614	K103N, M184V
147 (1)	D4T/3TC/EFV	0	94200	K103N, V106I
147 (2)	D4T/3TC/EFV	161	8791	V75I, L100I, Y181C, M184I
248 (1)	AZT/3TC/EFV	0	1885	K101P, K103N, M184V

Patient (visit)	Therapy	Days since VL>400 copies/ml	VL copies/ml	Mutations
248 (2)	AZT/3TC/EFV	56	14398	A62V, K101P, K103N, M184V
248 (3)	AZT/3TC/EFV	133	62903	A62A/V, K101P, K103N, M184V, M230M/L