Emergence and evolution of drug resistance in the absence of viral load monitoring during 48 weeks of Combivir/Tenofovir within the DART Trial

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ISCRTN 13968779

Poster #642

ISCRTN 13968779

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ABSTRACT

Background: DART is a randomised trial comparing clinical versus clinical plus laboratory monitoring in 3316 HIV-1 infected patients initiating ART with CD4 <200 cells/mm³ in Uganda and Zimbabwe. In common with many resource-poor environments, there is no contemporaneous virological monitoring, and switch to second-line follows clinical/immunological failure. The evolution of resistance to triple ART has rarely been studied under such circumstances. We previously demonstrated that viral load (VL) suppression (<50 c/ml) at 24 and 48 weeks was 59 and 61% respectively (ITT) on CBV/TDF.

Methods: VL measurements at baseline, weeks 24 and 48 were undertaken retrospectively by Roche Amplicor 1.5 in a specific subset of 300 patients initiating Combivir/Tenofovir. Plasma virus from samples with >1000 c/ml were sequenced in the pol gene

Results: Genotype results are reported for 26 of 43 (60%) and 35 of 64 (55%) samples with VL>1000 c/ml at weeks 24 and 48 respectively, for which samples were available and sequencing was successful. The prevalence of specific mutations at 24 and 48 weeks were: M184V (65%, 77%), T215F/Y (31%, 51%), D67G/N (38%, 60%), K70R (31%, 51%) and, K65R (12%, 14%). The proportion of patients at 24 and 48 weeks with TAMS were: 0 TAMS(42%, 26%), 1-3 TAMS(54%, 37%),4-6 TAMS(4%, 37%). At 48 weeks, there were more mutations in those 12 individuals tested (of 23 in total) who had VL>1000c/ml at 24 weeks (mean 4.1 range 1-6) than in those 19 tested (of 41 in total) with VL<1000c/ml at 24 weeks (mean 3.1 range 0-6) (p=0.17). In 6 individual patients for whom resistance data were available at both 24 and 48 weeks, a mean of 2.5 (range 1-4) new NRTI mutations emerged between these time points. No clear differences were observed in patterns of emerging mutations between HIV-1 subtypes A, C and D. For 91/300 (30%) baseline samples tested, 8 (9%) demonstrated one or more key resistance mutations (5 with NNRTI resistance, 5 with M184V, 2 with M41L).

Conclusions: Virological monitoring within ART rollout in resource-limited settings will be limited. Without viral load-guided treatment switch, extensive resistance evolves over 48 weeks in those with viraemia who continue to receive 1st line CBV/TDF. This population may still benefit from two major classes of drugs. However, we note the prevalence of baseline resistance to NNRTIs and/or NRTIs, which probably reflects prior undisclosed therapy.

BACKGROUND

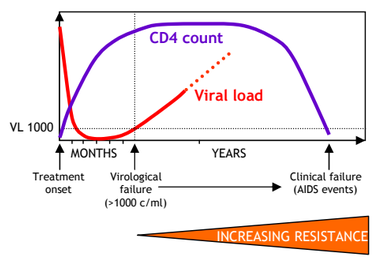
- ART rollout in the developing world will not be accompanied by intensive virological monitoring (viral load and resistance testing)
  - indeed, in many settings therapy switch will be guided by clinical failure
- Persistent viraemia without treatment switch may lead to accumulating resistance (Figure 1)
- In order to address these issues, we have studied patients from Uganda and Zimbabwe enrolled in the DART Trial Virology substudy
  - plasma samples are stored at regular intervals, but tested retrospectively
  - in total 377 participants who had initiated ART with Combivir/Tenofovir had viral load/resistance testing between ART initiation and 48 weeks
- The published data on virological and immunological response are shown in Fig 2 and demonstrate good virological efficacy

We now describe the resistance patterns emerging in those with detectable viral load at 24 and/or 48 weeks.

AIMS

1. **What spectrum of mutations emerge during virological rebound on Combivir/Tenofovir (CBV/TDF)?**
2. **How do mutations evolve over time, in those with prolonged viraemia?**
3. **What is the relationship between resistance mutations and subtype?**
4. **What are predictors of extensive resistance?**
5. **What is the prevalence and characteristics of resistance at baseline in an African population initiating 1st line therapy within a trial context?**

FIGURE 1 POTENTIAL IMPLICATIONS OF ART WITHOUT VIROLOGICAL MONITORING: FAILURE OF THERAPY

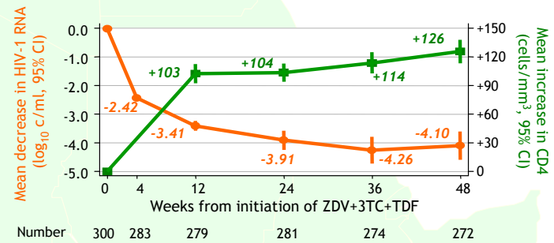


POPULATION

Originally 300 patients initiating ART with CBV/TDF were enrolled into a 24 week virology substudy: at 28 weeks 77 stopped ART for 12 weeks as part of an STI pilot study. To look at viral load responses to 48 weeks, these 77 patients were replaced with matched patients not interrupting ART. Therefore data are available on **377 patients** receiving CBV/TDF: 77 from 0-24 weeks, and 300 from 0-48 weeks.

- 253/377 (67%) were women, median 37 years at ART initiation [IQR 33-43]
- 26% WHO stage 4 (AIDS), 51% WHO stage 3, 23% WHO stage 2
- median CD4 108 cells/mm³ [52-155], median HIV-1 RNA 319,300 c/ml [106,500-663,900]

VL and CD4 OUTCOMES TO 48 WEEKS



Note: restricted to 300 patients not interrupting ART at week 28

AIDS 2006; 20(10):1391-9

GENOTYPIC RESISTANCE TESTING*

- Overall, 63% of individuals had VL<1000 c/ml at weeks 24 and 48
- Virus from around 50% of samples at baseline or with VL>1000 c/ml at weeks 24 or 48 have been sequenced (see below)
  - baseline tests from those with VL>1000 c/ml at week 24 or 48 were prioritised: therefore baseline testing is not strictly representative
  - of 94 patients with VL>1000 c/ml at either week 24 or week 48, 74 (79%) have been tested at baseline
  - of the remaining 283 patients (either suppressed at 24 and 48 weeks or missing data), 84 (30%) have been tested at baseline
- Reasons for incomplete sequence data include exhaustion of available samples, and failure to PCR amplify and/or sequence
- There were no significant viral load differences between samples with and without sequence

* updated from abstract

Sample	Number of patients	Genotypes analysed	Not analysable	Results pending
Week 0 (ART initiation)	377	**158 (42%)**	0 (0%)	219 (58%)
Week 24: VL>1000 c/ml	53	**26 (50%)**	10 (25%)	10 (25%)
Week 48: VL>1000 c/ml	64	**44 (69%)**	8 (12.5%)	12 (20%)

BASELINE RESISTANCE

10% of baseline samples had evidence of drug resistant virus:

- 16 (10%) had any IAS USA major resistance mutations
- 9 (6%) had NRTI resistance
- 7 (4%) had NNRTI resistance
- 4 (3%) had PI resistance/polymorphisms

RT mutation patterns were, for individuals patients, (108I) (103N) (103N 190A) (103N 181C 184V) (103N 184I) (103N 184V) (181C 184V 67N) 2x(41L) (210W) (184V 215F 41L) (62V)

PR mutation patterns were (46L) 3x(33F)

No patient had both RT and PR mutations at baseline

RESISTANCE AT 24 & 48 WEEKS

Overall, prevalence of resistance was higher at 48 compared to 24 weeks. The proportion of samples with 4-6 TAMS at 24 and 48 weeks was 4% and 39% respectively, all with co-existing M184V (**Table 1**)

Although there was a small excess of TAM-1 pathway mutations at 24 weeks compared to 48 weeks, the most notable finding was that most viruses with TAMS at week 48 did not fit neatly into either of the two pathways

In 7 patients with sequence available at 24 and 48 weeks, continuing evolution of resistance was observed (**Table 2**)

No significant differences in mutational patterns were observed between the major HIV-1 subtypes (A, C and D) represented in DART. Of note, there was a low incidence of K65R emergence amongst all subtypes (**Table 3**)

The co-existing mutations with K65R in eight viruses were; alone (3), 115F (1), 184V (1), 115F+184V (1), 67N (1), 215Y (1)

TABLE 1: PREVALENCE OF MUTATIONS AT 24 AND 48 WEEKS

Mutation	Week 24 (n=24)	Week 48 (n=41*)
M184V	15 (62%)	32 (78%)
K65R	3 (12%)	6 (15%)
M41L	7 (29%)	17 41%)
D67NG	9 (38%)	23 (56%)
K70R	8 (33%)	23 (56%)
L210W	0 (0%)	3 (7%)
T215FY	7 (29%)	17 (41%)
K219QEN	1 (4%)	9 (22%)
Total TAMs: 0	10 (42%)	11 (27%)
1-3	13 (54%)	18 (44%)
4-6	1 (4%)	12 (39%)
TAM Group** I	5 (36%)	2 (7%)
II	4 (11%)	11 (37%)
I and II	5 (36%)	17 (57%)

*excluding 3 patients with baseline NRTI resistance ** TAM I=41L, 67NG, 210W, 215Y; TAM II= 67N, 70R, 215F, 219QEN

Note: no MDR mutations /insertions/deletions were observed

TABLE 2: EVOLUTION OF RESISTANCE 24-48 WEEKS (n=7)

Patient	Mutations at week 24	**Additional** mutations by week 48
A	184V	67N,70R,215F
B	41L,67N,70R,184V,215Y	210W
C	184V	41L,67N,210W,215Y
D	67N,70R,184V	41L,215Y,219Q
E	67N,70R,215F	184V,219E
F	67N,70R,184V,215N	41L,215Y
G*	41L,67N,181I,184V,215Y	70R

* had 67N,181C,184V at baseline. Lost 181I by week 48.

TABLE 3: IMPACT OF VIRAL SUBTYPE ON RESISTANCE MUTATIONS*

Mutation	Subtype A(A1) (n=33)	Subtype C (n=14)	Subtype D (n=12)	P-value
M184V/I	24 (73%)	9 (64%)	9 (75%)	0.8
K65R	5 (15%)	3 (21%)	1 (8%)	0.7
# TAMs				0.9
0	10 (30%)	4 (29%)	5 (42%)	
1-3	17 (52%)	6 (43%)	5 (42%)	
4-6	6 (18%)	4 (29%)	2 (17%)	
TAM Group*				0.5
I	2 (9%)	3 (30%)	2 (29%)	
II	8 (35%)	2 (20%)	2 (29%)	
I and II	13 (57%)	5 (50%)	3 (43%)	

* Note: based on last genotype per patient. p-values from on chi-squared tests.

TABLE 4: UNIVARIATE PREDICTORS OF RESISTANCE AT WEEK 48

Mutations at week 48	Mean (SD) $\log_{10}$(VL) at week: 0	4	12	24	ΔVL (0-4)	AUC (0-24)
# TAMs						
0 (n=11)	5.4 (0.8)	2.8 (0.8)	2.1 (0.6)	2.4 (0.7)	-2.7 (0.5)	2.7 (0.5)
1-3 (n=18)	5.4 (0.5)	2.8 (0.7)	2.3 (0.6)	2.7 (0.7)	-2.6 (0.7)	2.8 (0.4)
4-6 (n=12)	5.5 (0.8)	2.8 (0.8)	2.9 (1.2)	3.3 (1.4)	-2.7 (0.8)	3.2 (0.9)
P-value	0.9	0.9	0.07	0.09	0.9	0.18
K65R						
No (n=35)	5.4 (0.7)	2.8 (0.7)	2.5 (0.9)	2.8 (1.1)	-2.6 (0.7)	2.9 (0.7)
Yes (n=6)	5.2 (0.8)	2.5 (0.8)	2.1 (0.5)	2.6 (0.7)	-2.7 (0.4)	2.6 (0.3)
P-value	0.5	0.3	0.3	0.5	0.7	0.2
M184V/I						
No (n=9)	5.3 (0.9)	2.6 (0.9)	2.2 (0.7)	2.1 (0.6)	-2.8 (0.4)	2.6 (0.5)
Yes (n=32)	5.4 (0.6)	2.8 (0.7)	2.5 (0.9)	3.0 (1.0)	-2.6 (0.7)	2.9 (0.7)
P-value	0.6	0.5	0.4	0.02	0.6	0.2

P-values < 0.1 highlighted. All p-values are based on t-tests or 1-way ANOVA. Sample sizes vary slightly due to missing RNA values

PREDICTORS OF RESISTANCE

In an attempt to identify specific predictors of the **EXTENT OF RESISTANCE**, we studied the association between viral load at baseline, weeks, 4, 12 and 24, and the numbers of resistance mutations at week 48. For this analysis we only used viral loads for patients with week 48 resistance data. In addition we considered the 0-4 week change in VL, and the viral load area under the curve (0-24 weeks) as a possible correlates (**Table 4**).

- higher viral load at 12 and 24 weeks was associated with an increasing number of TAMS at week 48 (p=0.07 and 0.09 respectively)
- the AUC 0-24 correlated with number of TAMs, but did not reach statistical significance
- M184V at week 48 was associated with a higher viral load at week 24 (p=0.02)
- By contrast, we did not identify any predictors of K65R at week 48

DISCUSSION

- **36% of those with VL>1000 c/ml at 48 weeks of therapy had 4-6 TAMS, severely limiting future drug options within the NRTI class. However, in this population initiating ART with 3NRTI, this must be balanced against the potential benefit of maintaining 2 further active classes, including boosted PIs**
- The majority of patients enrolled into the virology substudy had undetectable viral load at weeks 24 and 48. We assume this reflects the lack of resistance at these timepoints
- 10% of this DART population have evidence of drug resistance prior to initiating 1st line trial therapy. This likely reflects prior therapy with NNRTI and NRTI classes of drugs, although we cannot exclude transmitted resistance. Pre-existing drug resistance should be considered as a potential cause of drug failure in ART-rollout populations
- The viral load at 12 weeks predicted the extent of TAMS at week 48 (p=0.07). This is probably explained by more prolonged viraemia, thus facilitating continual evolution of more complex resistance patterns. Indeed, the AUC viral load did correlate with numbers of mutations , but did not reach statistical significance. TAM pattern may also be determined in part by specific drug combinations
- Despite recent observations of more rapid emergence of K65R in subtype C HIV-1, we found no evidence that CBV/TDF selected different sets of mutations between subtypes A, C and D, nor that emergence was quicker in some subtypes than others, although numbers were small. This does not exclude differences being observed for other drug combinations, or over longer follow up
- Of interest, we find that the traditional classification of nucleoside analogue drug resistance mutations into TAM-I and TAM-II pathways loses relevance during prolonged viraemia. This suggests that resistance studies in the context of ART rollout with little virological monitoring will identify novel sets of resistance mutations which are rarely observed in the resource-rich setting
- HIV sequences were obtained in only approximately half of eligible samples from 24 and 48 weeks, due to sample exhaustion and/or technical problems. There were no significant differences between the viral loads in samples which yielded sequences compared to those which did not, and therefore we are confident that our results are representative of the population with detectable viral loads at these time points

We thank all the patients and staff from all the centres participating in the DART trial.
MRC Programme on AIDS/Uganda Virus Research Institute, Entebbe, Uganda: H Grosskurth, P Munderi, K Wangati, D Kajungu, B Amuron, D Nsibambi; R Kasirye, E Zalwango, M Nakazibwe, B Kikaire, G Nassuna, R Massa, K Fadhiru, M Namyalo, A Zalwango, L Generous, P Khauka, N Rutikarayo, W Nakahima, A Mugisha, J Nakiyingi, P Hughes. **Joint Clinical Research Centre, Kampala, Uganda:** P Mugyenyi, C Kityo, D Tumukunde, F Ssali, D Atwine, G Mulindwa, G Kabuye, R Byaruhanga, T Bakeimyaga-Grace, H Katabira, E Nimwesiga, G Barungi, S Atwiine, F Ahimbisibwe, S Tugume, T Otim, J Takubwa, M Mulindwa, S Murungi, J Tukamushaba, D Muebesa, H Kyomugisha, J Kagina, L Namale. **University of Zimbabwe, Harare, Zimbabwe:** A Latif, J Hakim, V Robertson, A Reid, A Jamu, S Makota, T Mupudzi, G Musoro, N Ngorima, M Pascoe, F Taziwa, L Chakonza, E Chidziva, H Chirairo, S Chitsungo, F Mapinge, A Mawora, C Muvirimi, G Tinago, J Chimanzi, J Machingura, C Maweni, S Mutsai, R Warara, M Matongo, N Mdege, S Mudzingwa, M Jangano, I Machingura, K Moyo, L Vere, E Chigwedere, M Phiri. **Academic Alliance, Mulago Hospital, Uganda:** E Katabira, J Oyugi, A Ronald, A Kambungu, J Martin, R Nalumenya, R Nairubi, E Bulume, M Teopista, C Twijukye, F Sematala, H Byakwaga. **The AIDS Support Organisation (TASO), Uganda:** A Costantino, B Etukoit. **Imperial College , London, UK:** C Gilks, K Boocock, C Puddephatt, D Winogron. **MRC Clinical Trials Unit, London, UK:** J Darbyshire, DM Gibb, A Burke, D Bray, A Babiker, AS Walker, H Wilkes, M Rauchenberger, S Sheehan, L Peto, K Taylor. **Trial Steering Committee:** I Weller (Chair), A Babiker (Trial Statistician), S Bahendeka, M Bassett, A Chogo Wapakhabulo, J Darbyshire, B Gazzard, C Gilks, H Grosskurth, J Hakim, A Latif, C Mapuchere, O Mugurungi, P Mugyenyi; Observers: S Jones, E Loeliger, P Naidoo, M Palmer, J Rooney, J-M Steens. **Data and Safety Monitoring Committee:** A McLaren (Chair), C Hill, J Matenga, A Pozniak, D Serwadda. **Endpoint Review Committee:** T Peto (Chair), A Palfreeman, M Borok, E Katabira. **Funding:** DART is funded by the **UK Medical Research Council**, the **UK Department for International Development (DFID)**, and the **Rockefeller Foundation**. **GlaxoSmithKline**, **Gilead** and **Boehringer-Ingelheim** donated first-line drugs for DART.