

Adding a PI for 6 Months to a Standard NNRTI-based Regimen Reduces the Risk of Virological Failure without Inducing Resistance to the PI: The FORTE Virology Analysis

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on behalf of the Forte Virology Group and Trial Steering Committee

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BACKGROUND

The FORTE trial was the first randomised control trial to demonstrate a decreased risk of virological failure with an induction strategy of 4 drugs (3 classes) followed by a 3 drug (2 class) compared to a standard 3 drug (2 class) regimen in antiretroviral naive patients. Exploratory analyses provided no evidence that the observed difference between the treatment strategies was the effect of a particular drug or combination. Further analyses were undertaken to investigate additional virological outcomes to help explain the mechanism for the observed treatment benefit.

OBJECTIVES OF VIROLOGY SUBSTUDIES

- To investigate the rate of HIV RNA decay in the first 2 weeks of therapy
- To investigate the prevalence and pattern of genotypic resistance at baseline and at treatment failure
- To describe the viral subtypes and distribution across study arms
- To investigate the change in pro-viral DNA over time

TRIAL DESIGN

Antiretroviral naive patients (CD4 count >25 x10⁹/l) randomised (1:1) to receive open label:
1. Standard therapy (ST). 2NRTIs + 1NNRTI for at least 48 weeks
2. Induction Maintenance therapy (IM) 2NRTIs + 1NNRTI + 1PI for 24-32 weeks, then if 2 consecutive HIV RNA <50 copies/ml drop the PI and continue 2NRTIs + 1NNRTI to at least 48 weeks

Main study Primary Endpoint

Definition time to Virological Failure:
Viral Load >50 at 32 weeks or Viral Load >400 after 32 weeks if ≤50 at 32 weeks

METHODS

Quantification of Plasma Viral Load: HIV-1 plasma viral load was quantified from plasma viral RNA using a bDNA-based assay (Bayer Diagnostics Ltd, version 3, cut-off <50c/ml). The assay was quality assured with internal controls in every assay run and externally assured by contributing to the UK National External Quality Assessment Service (NEQAS).

Genotypic Resistance: Genotypic resistance was determined from HIV-1 plasma RNA using consensus sequencing ('Truegene', Bayer Diagnostics Ltd) and analysed for mutations associated with resistance using 'GeneLibrarian' (Ver. 8). The assay was quality assured using an internal control in every assay run and externally assured by contributing to the ACTG resistance quality assurance program and the ENVA panel evaluations (www.icvc.org.uk).

Subtype Diversity: Consensus subtype analyses were performed using *pol* gene sequences submitted to 3 comparative databases: Los Alamos (www.hiv.lanl.gov/content/index), Stanford (<http://hivdb.stanford.edu>), and NCBI (<http://www.ncbi.nlm.nih.gov>).

HIV-1 Pro-viral DNA detection: Cell pellets were resuspended in 250 µl PBS and extracted using the Qiagen BioRobot 9604 QIAamp 96 Virus protocol. HIV-1 proviral DNA was amplified in an Applied Biosystems Prism 7000 using TaqMan Universal master mix and TaqMan primers and probe in the 5'LTR region of HIV-1. The results were quantified against a standard curve generated from a 10 fold serial dilution of DNA extracted from 8E5 cells which contain a single integrated copy of HIV-1 per cell. Cell numbers were quantified against a human DNA standard from Applied Biosystems using TaqMan primers and probe to the pyruvate dehydrogenase (PDH) gene. The PDH also acted as an extraction and amplification control. Thus the results were expressed in HIV-1 copies per million cells.

Statistical Analysis:

Viral Load Decline: Extra viral load measurements were taken in the first 2 weeks. We analysed change of log₁₀ RNA viral load with measurements at baseline and at least once before day 15 using a multilevel model with random intercept and slope.

Genotypic Resistance and Subtype: The proportion of patients with non B subtype, those with at least one major resistance mutation at baseline, and at failure, were compared by trial arm and failure outcome at 32 weeks using a chi-squared test.

Pro-viral DNA: The effect of log₁₀ pro-viral DNA at baseline, week 24 or its change on virological failure in the first 32 weeks was analysed by proportional-hazards model, adjusted for baseline RNA, baseline CD4, and treatment. The association between baseline pro-viral DNA and HIV RNA suppression ≤50 copies/ml at week 24 was analysed by logistic regression.

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MAIN TRIAL RESULTS

Trial Population

- 124 patients recruited between October 1999 and July 2002 from 14 clinics
- 2 withdrew consent on the day of randomisation and are excluded from all analyses.
- Data to 30 June 2003 are presented when participants had at least 48 weeks follow up.

Baseline characteristics	IM	ST	Total
Patients Randomised	62	60	122
Male	56 (90%)	51 (85%)	107 (88%)
Age (years): mean (sd)	37.8 (7.9)	38.1 (8.9)	37.9 (8.4)
Risk Factor:			
sex between men	46 (74%)	41 (68%)	87 (71%)
sex between men & women	12 (19%)	17 (28%)	29 (24%)
Stage: Asymptomatic	30 (48%)	33 (55%)	63 (52%)
CD4 median (IQR) 10⁶/l	80 (104-260)	145 (73-235)	160 (92-260)
Viral load: mean log₁₀ RNA (sd)	4.92 (0.64)	4.96 (0.63)	4.94 (0.63)

Follow up

- median length of follow-up was 79.6 weeks (range 0- 190.6) IM arm, 82.0 weeks (range 0 - 189.0) ST arm
- only five patients (4%) were lost to follow-up (defined as no data received within the 4 months prior to trial closure)

Treatment Received

- At 24 weeks, 83% of patients in the IM arm and 89% of patients in the ST arm were on the allocated treatment strategy.
- By 48 weeks, 84% (IM) and 83% (ST) of patients were on allocated treatment strategy.

INITIAL REGIMEN	IM (n=62)	ST (n=60)
NNRTI: ddI/d4T	31	32
AZT/3TC	28	23
other 2NRTI	3	3
NNRTI: NVP	40	37
EFV	22	22
PI: NFV	44	-
LPV/r	17	-
IDV	1	-

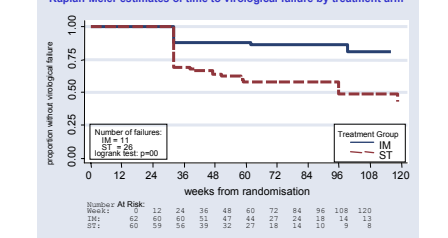
*1 patient never started ART and died at week 4.

•MAIN STUDY PRIMARY ENDPOINT

Virological failure:	IM n (%)	ST n (%)	Total n (%)
Failure to achieve HIV RNA <50 by 32 weeks	7	17	24
HIV RNA <50 at 32 weeks, >400 after 32 weeks	4	9	13
Total	11 (18)	26 (43)	37 (30)

Logrank $\chi^2_{(1)} = 10.06, p = 0.002$
HR* 0.34 (95% CI: 0.17- 0.70)

*Adjusted for baseline RNA and CD4



VIROLOGY SUBSTUDY RESULTS

VIRAL SUBTYPES

HIV-1 subtype was characterized by 3 comparative databases in 108 of 122 patients studied (IM 55 : ST 53). The number of subjects with subtype B were 41 (75%) and 43 (81%) in the IM and ST arms respectively. No significant difference between study arms in the proportion with non B subtype (25% IM, 19% ST) was observed (p=0.4).

Subtype	A	B	C	D	AE	G	AB
IM	2 (4%)	41 (75%)	7 (13%)	1 (2%)	1 (2%)	2 (4%)	1 (2%)
ST	0 (0%)	43 (81%)	6 (11%)	1 (2%)	3 (6%)	0 (0%)	0 (0%)

Significantly more patients with subtype B failed at week 32 than those with non-B subtype. (36% B vs 8% non-B, p=0.01).

PLASMA HIV-1 GENOTYPIC RESISTANCE

Inclusive lists of major and minor mutations associated with resistance ('GeneLibrarian', version 8) in RT and then PR described by trial arm. Those patients with polymorphic changes alone have not been included.

BASELINE

PATIENT	RT	PR	SUBTYPE
IM ARM (n=55)			
A	K103N	V17A	B
B	Y181C	DEL	B
C	K103N	DEL	B
D	V17A	DEL	AE
E	ASG	DEL	C
F	ASG	DEL	C
G	DEL	DEL	DEL
H	DEL	DEL	DEL
ST ARM (n=53)			
J	V17A	DEL	B
K	V17A	DEL	B
L	V17A	DEL	B
M	DEL	DEL	DEL
N	DEL	DEL	DEL
O	DEL	DEL	DEL
P	DEL	DEL	DEL
Q	DEL	DEL	DEL
R	DEL	DEL	DEL

FAILURE

PATIENT	RT	PR	SUBTYPE
IM ARM (n=11)			
A	DEL	DEL	DEL
B	DEL	DEL	DEL
C	K103N	DEL	B
D	K103N	DEL	B
E	DEL	DEL	DEL
ST ARM (n=26)			
F	DEL	DEL	DEL
G	K103N	DEL	B
H	K103N	DEL	B
I	DEL	DEL	DEL
J	DEL	DEL	DEL
K	DEL	DEL	DEL
L	DEL	DEL	DEL
M	DEL	DEL	DEL
N	DEL	DEL	DEL
O	DEL	DEL	DEL
P	DEL	DEL	DEL
Q	DEL	DEL	DEL
R	DEL	DEL	DEL
S	DEL	DEL	DEL
T	DEL	DEL	DEL
U	DEL	DEL	DEL
V	DEL	DEL	DEL
W	DEL	DEL	DEL
X	DEL	DEL	DEL
Y	DEL	DEL	DEL
Z	DEL	DEL	DEL

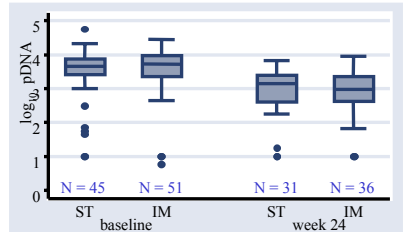
GENOTYPIC RESISTANCE: Summary

	IM	ST	Comparison
No. randomised	62	60	-
Rate of Baseline resistance	16.3% (9/55)	14.3% (8/56)	p = 0.8
No. with virological failure at or after 32 weeks	11	26	p = 0.002
No. with VL>400 at failure	5	17	-
Genotype available at failure	5	15	-
Overall Resistance:	40% (2/5)	85% (11/13)	p = 0.1
NNRTI Resistance:	40% (2/5)	85% (11/13)	p = 0.1
NRTI Resistance:	20% (1/5)	46% (6/13)	p = 0.6
PI Resistance:	0% (0/5)	NA	-
Rate with virological failure and detectable resistance	3% (2/62)	18% (11/60)	p = 0.01

CHANGES IN PRO-VIRAL DNA

Pro-viral DNA (pDNA) was available at baseline in 96 (79%), at week 24 in 67 (55%), and at both time points in 53 (43%) patients. Patients with pDNA results had higher CD4 at baseline than patients without; Other baseline characteristics were similar, including the proportion of patients who subsequently experienced virological failure.

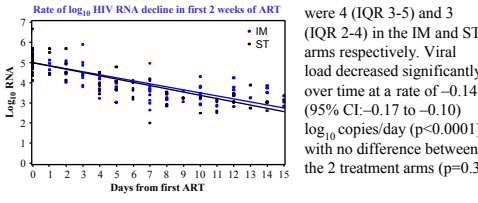
Distribution of pDNA measurements at baseline and week 24, by Arm



There was no significant difference in mean (SD) fall in log₁₀ pDNA between the treatment arms: IM (n:28):-0.69(0.63), ST (n:25):-0.64(0.38). Neither baseline pDNA nor change from baseline to 24 weeks predicted failure, and there was no influence of pDNA on RNA suppression ≤50 copies/ml at week 24.

EARLY VIRAL LOAD DECLINE

34 patients (20 IM, 14 ST) had repeated measurements of viral load during the first 15 days from starting ART. The average number of measurements in each arm were 4 (IQR 3-5) and 3 (IQR 2-4) in the IM and ST arms respectively. Viral load decreased significantly over time at a rate of -0.14 log₁₀ copies/day (p<0.0001), with no difference between the 2 treatment arms (p=0.3).



CONCLUSIONS

- The use of a PI in a 3 class induction regimen did not result in the emergence of PI resistance at failure.
- Overall the incidence of genotypic resistance was lower with an IM strategy.
- The improved virological efficacy of the IM strategy was not explained by differences between the groups in viral sub-type, baseline resistance, change in pro-viral DNA at 6 months or initial rate of viral RNA decay.
- Further studies of an induction maintenance strategy using a 4 drug (3 class) regimen are warranted.