

EVOLUTION OF GENOTYPIC RESISTANCE AND PHARMACOKINETIC MEASURES OF ADHERENCE IN CHILDREN FAILING RITONAVIR/INDINAVIR (RTV/IDV) COMBINATION REGIMENS

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ABSTRACT

Background: Incomplete HIV suppression due to insufficient or erratic drug exposure is associated with evolution of genotypic resistance mutations. We evaluated pharmacokinetic (PK) data in all children receiving combination therapy with RTV/IDV and evolution of resistance mutations in the subset of patients who discontinued (d/c) RTV/IDV.

Methods: P1013 is a prospective, randomized trial of IDV with either boosting or therapeutic doses of RTV (125 mg/m² or 300 mg/m² q12h, respectively) and 2 NRTIs. Children 2-18 yrs of age failing ≥2 wks of combination therapy with HIV RNA >10,000 copies/mL and <3 primary protease gene mutations were eligible. Genotypes (GT) at study screen and RTV/IDV d/c are scored using the Stanford Algorithm. Intensive PK studies were done in each subject at wk 4 of RTV/IDV. Proximal IDV concentrations (C₀) at wks 4, 8, 12, 16, 24, and then every 12 weeks were used to assess adherence. Adherence threshold was defined as C₀ ≥ 50% of DV concentration 12 hr following an observed dose.

Results: 1051 patients enrolled sustained HIV RNA decrease >1.0 log₁₀ from baseline (median time to IDV/RTV=7 wks), despite 7 having 1-2 resistance-associated protease mutations at screening. Of 10 with virologic decrease, 6 were adherent (>80% of wks, Of 21 who d/c RTV/IDV, 6 were nonadherent (4 for RTV/IDV refusal with 5 wks and 2 w incomplete GT data) of the 15 evaluable subjects, 14 had virologic failure (VF) and 1 refused RTV/IDV at wk 80. (median time to IDV was 20-24 wks) with a median of 33% of visits with C₀ below the adherence threshold. 12/15 had ≥1 undetectable C₀ (in the visits) preceding VF. Median time to RTV/IDV d/c in 6 subjects with new resistance-associated mutations was 47 wks and in 9 subjects without such mutations 25 wks (p=0.02). Among the 15 subjects who d/c RTV/IDV, there was a significant increase in GT Stanford score for all PIs from screening (median score=4, intermediate) to d/c (median score=10-14, potentially low to intermediate) GT resistance (p=0.03), but not for NRTIs or NNRTIs.

Conclusion: PK measures of nonadherence preceded VF in 80% of subjects discontinuing RTV/IDV. In 40% (9/15) evolution of significant genotypic mutations associated with resistance to the treatment regimen may have contributed to VF. In addition, subjects failing this combination may also have increased resistance to other PIs as well as to RTV/IDV.

METHODS

Methods: P1013 Study Design

Design	Phase I/II, dose finding, open label trial
Target Sample Size	30 subjects
Population	HIV-infected children 2 years to < 18 years of age with either boosting or therapeutic doses of RTV (125 mg/m ² or 300 mg/m ² q12h, respectively) and 2 NRTIs. Children 2-18 yrs of age failing ≥2 wks of combination therapy with HIV RNA >10,000 copies/mL and <3 primary protease gene mutations were eligible. Genotypes (GT) at study screen and RTV/IDV d/c are scored using the Stanford Algorithm. Intensive PK studies were done in each subject at wk 4 of RTV/IDV. Proximal IDV concentrations (C ₀) at wks 4, 8, 12, 16, 24, and then every 12 weeks were used to assess adherence. Adherence threshold was defined as C ₀ ≥ 50% of DV concentration 12 hr following an observed dose.
Randomized Treatment Regimens	Reduced dose Arm: RTV 100mg/m ² Q12h IDV 300mg/m ² Q12h (1:1 NRTI)
	RTV boost Arm: RTV 125mg/m ² Q12h IDV 300mg/m ² Q12h (1:1 NRTI)
Virologic Failure	< 0.75 logs ₁₀ mean reduction in HIV RNA at weeks 12 and 16 Confirmed HIV RNA rebound to within 0.75 logs ₁₀ of entry level after week 12
Duration	96 weeks

* Significant resistance defined as mutations V82AF/S or I54V and/or three or more secondary/mutant mutations (M40I/S, K20M/R, L24I, V32I, L33F, M36I, I54V/L, A151V, Y175A, V177 and L96M).

Genotype Methods

- Genotypes (GT) were performed at study screen and discontinuation of RTV/IDV
- Viral RNA was extracted from 140 µl of blood plasma using the QIAamp Viral RNA Isolation kit from Qiagen
- RT-PCR and sequencing were performed using the TRUGENE™ HIV-1 Genotyping kit from Bayer Diagnostics (formerly ViiVA Genosize Inc.)
- Genotypes were analyzed using the Bayer Diagnostics QIAseq™ system, version 1.2 software.
- Only specimens with a viral load > 500 copies/mL were processed.
- GT was scored using the Stanford Algorithm (<http://hivdb.stanford.edu/>)

PK

- PK studies were performed at week 4 of RTV/IDV
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Pharmacokinetics (PK) Methods

- An intensive PK study (8 samples over 12 hours) at week 4 was done in each subject to measure a patient specific PK reference for adherence assessment. IDV and RTV were measured using HPLC-UV.
- Proximal IDV concentrations (C₀) were done at weeks 4, 8, 12, 16, 24 and 36.
- Adherence threshold = C₀ ≥ 50% of DV concentration 12 hr following an observed dose
- Definition of adherence
 - Adherent: >80% visits had C₀ ≥ threshold
 - Partially adherent: 50%-80% visits C₀ ≥ threshold
 - Non-adherent: <50% visits C₀ ≥ threshold

RESULTS

Results

- 21 subjects enrolled, median age 9 yrs (2-17 yrs)
- 1 female/20 male
- 4 refused to take RTV/IDV, no PK data down
- 2 had incomplete GT data
- 21 evaluable subjects
- 10 had sustained HIV RNA decrease >1 log₁₀ from baseline (virologic success)
- 6 adherent, 1 partially adherent, 1 poorly adherent
- median time to RTV = 71 wks (range 11-100 wks)
- 15 discontinued (d/c) RTV/IDV
- 1 adherent, 3 partially adherent, 11 poorly adherent
- Median time to d/c 32 wks (range 20-54 wks)
- 14 had virologic failure (0-75 logs₁₀ reduction or rebound to within 0.75 logs₁₀ of entry) or d/c
- 1 refused further therapy at wk 60, beyond virologic failure at wk 72

Median GT Resistance Scores of NRTIs and NNRTIs in Virologic Failures (n=15) Before and After Treatment with RTV/IDV

Class	Median	Range	p-value
NRTI			
LVN_PyVdRT	5	(0, 6)	0.2054
ABV_PyVdRT	5	(0, 6)	0.2054
ABC_PyVdRT	30	(0, 76)	
ABC_PyVdRT	30	(0, 65)	0.0394
NNRTI			
EFV_PyVdRT	56	(15, 94)	0.0038
EFV_PyVdRT	5	(1, 94)	
DTG			
DTG_PyVdRT	37	(4, 75)	0.3072
DTG_PyVdRT	7	(4, 74)	
DDI			
DDI_PyVdRT	25	(0, 72)	0.0203
DDI_PyVdRT	30	(0, 72)	
DOB			
DOB_PyVdRT	20	(0, 62)	0.0489
DOB_PyVdRT	20	(0, 74)	
TDF			
TDF_PyVdRT	25	(18, 50)	0.7198
TDF_PyVdRT	5	(1, 50)	
NNRTI			
EVN_PyVdRT	40	(0, 120)	0.2900
EVN_PyVdRT	30	(0, 100)	0.0750
EFV			
EFV_PyVdRT	40	(0, 100)	0.0250
EFV_PyVdRT	40	(0, 100)	
RPV			
RPV_PyVdRT	40	(0, 120)	0.2900
RPV_PyVdRT	40	(0, 120)	

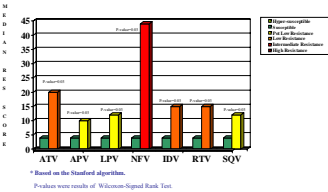
Relationship Between PK Adherence and Virologic Outcomes

Virologic Regimen	n	% with C ₀ ≥ Threshold	p-value
SUCCESS	10	90.0%	(0, 100)
FAILURE	15	33.3%	(0, 100)

* Kruskal-Wallis Test

n = 1513 subjects had ≥ 1 undetectable IDV level prior to virologic failure vs. 110 subjects with virologic success

Median GT Resistance Scores* of PIs in Virologic Failures (n=15) Before and After Treatment with RTV/IDV



Changes in Codon Mutations in Virologic Failures (n=15)

Mutated Amino Acid	PROTEASE GENE (BEYOND/DEL)	NRTI (BEYOND/DEL)	REVERSE TRANSCRIPTASE (NNRTI) (BEYOND/DEL)
1			R145G
2	P57(Y)		R145G
4	P59(L)		
6	P71(K)		R145G
7	P43	R145G	R145G
RTV Boost Arm			
6	P56	R145G	R145G
10	P54	R145G	R145G
11		R145G	R145G
12	P54, Y142, S2, S9	R145G	R145G
13		R145G	R145G
14	P42, S2, S9	R145G	R145G

Median Time to Treatment Discontinuation (n=15)

Group	n	Median	range	p-value
New Resistance-associated Mutations vs. No New Mutations				
With New Mutations	6	47 wks	(20, 94)	0.02
No New Mutations	9	23 wks	(0, 60)	
* Kruskal-Wallis Test				
By Treatment Arm				
Reduced RTV/IDV	3	31 wks	(0, 61)	0.17
IDV with RTV Boost	7	32 wks	(22, 49)	
* Kruskal-Wallis Test				

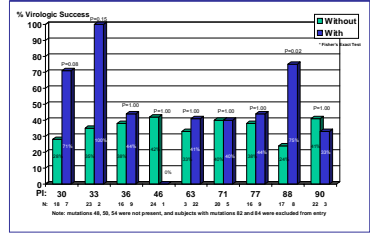
Median Percentage of Visits with C₀ ≥ Adherence Threshold in Virologic Failures with New Resistance-Associated Mutations vs. None

Group	n	% of Visits with C ₀ ≥ Threshold	range	p-value
With New Mutations	6	33%	(0, 100)	0.03
No New Mutations	9	33%	(0, 100)	
* Kruskal-Wallis Test				

Virologic Outcome and Presence of Resistance-Associated Mutations Before RTV/IDV

Virologic Outcome	n	% of Pre-treatment Mutations	range	p-value
SUCCESS	10	4	(1, 5)	0.0761
FAILURE	15	2	(0, 5)	
* Kruskal-Wallis Test				

Virologic Outcome and Presence of Specific Resistance-Associated Mutations Before RTV/IDV



CONCLUSIONS AND DISCUSSION

- Subjects with virologic success had a significantly higher percentage of visits with trough indinavir concentrations above a minimum threshold than did subjects experiencing virologic failure. Furthermore, undetectable drug concentrations were found at the visit(s) preceding virologic failure in 80% of subjects. Periodic measures of trough antiretroviral levels may allow improved surveillance of adherence and provide the clinician with the pharmacologic evidence needed to persuade patients to improve drug adherence.
- In subjects with virologic failure, genotypic resistance scores at discontinuation increased significantly for all protease inhibitors in addition to RTV and IDV. It is possible that patients who receive this drug combination with documented non-adherence after previously failing other combination regimens may be at risk for cross-resistance to multiple PIs.
- The median time to treatment discontinuation was significantly longer for subjects who developed new resistance-associated mutations than for those who did not; viral replication in the presence of low or erratic drug levels for a longer time period may have allowed the emergence of these mutations.
- Time to treatment discontinuation was similar for both treatment arms (IDV + Balanced RTV or RTV boost) which suggests that tolerability was not different for either regimen.
- Subjects with virologic success had a marginal trend toward a higher number of pre-existing protease mutations than subjects who failed therapy. This may reflect greater pre-entry antiretroviral drug adherence with selective genetic pressure for resistance mutations.