

G Aldrovandi¹, P Samson², T Fenton², S Schnittman³, R Rutstein⁴, and the Pediatric AIDS Clinical Trials Group P1020A Study Team.¹Univ of Alabama at Birmingham, Birmingham, AL; ²Harvard School of Public Health, Boston, MA; ³Bristol-Myers Squibb, Wallingford,CT; ⁴Univ of Penn, Philadelphia, PA.Grace M. Aldrovandi, M.D.
University of Alabama at Birmingham
845 19th St South Room 559
Birmingham, AL 35294-2170
Tel 205-934-2456
Fax 205-975-2457
Email: gracea@uab.edu

ABSTRACT

STUDY SAMPLE & PRIMARY MEASURES:

Phenotypic resistance to ATV, and other antiretrovirals (ARV), was assessed in 40 patients undergoing screening for protocol PACTG 1020A. Phenotypic resistance scores (PRS) were calculated as the \log_{10} of the ratio of patient vs. wild type IC_{50} s. 36/40 patients had genotypic testing. For analyses dealing with cross-resistance, these genotypic data were summarized into genotypic resistance scores (GRS) for each drug, using an algorithm developed by Shafer¹. An alpha level of .01 was used as the criterion for statistical significance.

ANALYTIC METHODS & RESULTS:

The geometric mean of PRS to ATV was 5.6, with 52.5% of the sample showing scores >5. Correlations between the PRS of ATV and both the PRS and GRS of the other ARV were as follows:

	ANTI-RETROVIRALS											
	NRTI's			NNRTI's			Protease Inhibitors (PI)					
	3TC	ABC	D4T	DDI	ZDV	EFV	NVP	APV	NFV	IDV	RTV	SQV
Pheno (n=40):	.03	.36	.41*	.24	.37	.14	.25	.82*	.82*	.87*	.88*	.88*
Geno (n=36):	-.05	.56*	.47*	.40	.56*	.12	.24	.77*	.78*	.80*	.80*	.77*

(*=p<.01)

A multiple regression analysis (backwards selection), with PRS to ATV as the outcome variable and the PRS of the other ARV with which it was significantly correlated as the predictors, showed that phenotypic resistance to APV, NFV, RTV and SQV all made unique and significant contributions towards predicting the extent of phenotypic resistance to ATV, ($R^2=$.94). A similar regression, with predictors consisting of the GRS which were significantly correlated with ATV, indicated that genotypic resistance to NFV and IDV made unique and significant contributions towards predicting phenotypic resistance to ATV ($R^2=$.70). Exploratory analyses testing whether ATV phenotypic resistance varied as a function of the presence or absence of mutations identified 13 significant positions ($p<$.01). A regression model with the ATV PRS as the outcome revealed that mutations at PI-54, PI-84 and RT-215 made unique and significant contributions towards predicting phenotypic resistance to ATV ($R^2=$ 0.76).

CONCLUSIONS:

These results indicate that heavily treated pediatric patients exhibited relatively high levels of resistance to ATV. Cross-resistance with other protease inhibitors (PI) was such that 94% of the variance in ATV PRS could be predicted on the basis of cross-resistance with other PIs. These findings should be interpreted with caution, since they come from exploratory analyses involving multiple comparisons, where some results may have been due to chance.

INTRODUCTION

OBJECTIVES OF THE ANALYSIS

- 1) To examine phenotypic resistance to ATV;
- 2) To test for cross resistance on the basis of phenotypic data; Specifically, to test whether the Phenotypic Resistance Scores (PRS) of other drugs predict ATV PRS:
Note: PRS = \log_{10} of the ratio of patient vs. wild type IC_{50}
- 3) To test for cross resistance on the basis of genotypic data; Specifically, to test whether Genotypic Resistance Scores (GRS) of other drugs predict ATV PRS:
Note: GRS calculated on the basis of Shafer's Algorithm.
- 4) To determine which genotypic codons correlate with the ATV PRS.

OVERALL CONCLUSIONS:

- 1) Based on the phenotype data, 37.5% of the screened patients exhibit sensitivity to ATV while 40% exhibit high resistance.
- 2) There is strong evidence of cross resistance between ATV and other PIs using both phenotypic and genotypic data.
- 3) Among the 13 codons that have significant correlations with the ATV PRS, three of them made unique contributions towards predicting ATV PRS in a regression model: PI-54, PI-84 & RT-215 (NOTE: RT-215 may be acting as a marker for overall experience.)

PACTG 1020A

- Phase I/II, Open-label, Pharmacokinetic and Safety Study of a Novel Protease Inhibitor, Atazanavir (ATV), formerly known as BMS 232632, in Combination Regimens in Antiretroviral Therapy on ART Naïve and ART Experienced HIV-Infected Infants, Children and Adolescents.
- Ages 3 months and 1 day to 21 years

STUDY SAMPLE

- Heavily experienced subjects screened for potential enrollment into P1020A (Viral Load (copies/ml): 25th Percentile=16,664; Median=60,984; 75th Percentile=197,489)
- Had failed 2 or more PI containing regimens → required Phenotype Testing
- Subjects who had received at least 3 of the following: 3TC, D4T, DDI, ZDV required Genotype Testing.
- n=40 Subjects

LABORATORY METHODS/MEASUREMENTS

- **Plasma HIV RNA levels** were determined by a reverse-transcriptase-polymerase-chain reaction (Amplicor HIV-1 Monitor 1.0 Assay, Roche Molecular Systems, Branchburg, NJ) according to the manufacturer's instructions.
- **Genotypic Analysis** was performed using the Applied Biosciences ViroSeq HIV-1 Genotyping System according to the manufacturer's instructions. The resulting sequences were assembled and analyzed using the HIV-1 Genotyping System Software. For quality control, phylogenetic analysis to compare study subjects' nucleotide sequences to one another and to those of laboratory strains were employed as suggested by REF1: (Korber BT, Learn G, Mullins JI, Hahn BH, Wolinsky S. Protecting HIV databases. Nature 1995; 378:242-4). Interpretation of genotypic resistance was performed using the HIV RT and Protease Sequence Database (<http://hivdb.stanford.edu>).
- **Antiretroviral susceptibility** was determined using a rapid recombinant-vector phenotypic assay (ViroLogic PhenoSense™ HIV Assay, ViroLogic Inc., South San Francisco, California, USA). In this assay, drug susceptibility is expressed as the fold change of the drug concentration required to inhibit viral replication by 50% (IC_{50}) compared with control (wild-type virus).
→ IC_{50} Fold Change = IC_{50} patient / IC_{50} control

PHENOTYPIC RESISTANCE TO ATV

Forty patients (40) who were screened for P1020A were assessed on phenotypic resistance to ATV and other antiretrovirals. IC_{50} Fold Change was calculated as the ratio of patient vs wild-type IC_{50} s for each drug. Table 1 shows the proportion of patients exhibiting varying degrees of phenotypic resistance to ATV: note that 40% exhibit high resistance. Since none of those patients had been treated with ATV, it was hypothesized that ATV resistance was due to cross resistance resulting from experience with other antiretroviral drugs.

Table 1. Phenotypic Resistance to ATV

IC_{50} Fold Change	Percentage
Sensitive (\leq 2.5)	37.5 %
2.5 < Fold Change \leq 5.0	10.0
5.0 < Fold Change \leq 10.0	12.5
Fold Change > 10.0	40.0

Geometric Mean = 5.6, n=40

Figure 1 presents a graphical display of the proportions of patients with varying degrees of phenotypic resistance to ATV.

Figure 2, 3, 4 present similar displays depicting resistance to NRTIs (Figure 2), NNRTIs (Figure 3) and other PIs (Figure 4).

PHENOTYPIC CROSS RESISTANCE

ANALYTIC METHODS:

- **Dependent Variable:** ATV Phenotypic Resistance Score (PRS)
→ PRS = \log_{10} ratio of patient vs wild-type IC_{50}
- **Independent Variables:** PRS of other antiretrovirals (See Table 2)
- **Sample (n=40):** Patients who met the criteria for phenotypic testing
- **Identify the drugs whose PRSs exhibit significant simple correlations with ATV PRS (Pearson Correlation, $p<$.01)**
- **Enter these drugs' PRSs into a Backwards Stepwise Multiple Regression to identify which drugs make unique contributions to the prediction of ATV PRS**
- **Determine R^2**

RESULTS:

- PRSs of 6 drugs had significant correlations with ATV PRS (See Table 2).
- Phenotypic resistance to APV, NFV, RTV, SQV (all PIs) made unique contributions towards predicting the ATV PRS in a multiple regression model.
- $R^2=$.94

Table 2. Pearson Correlation Coefficients Based on PRS

	ANTI-RETROVIRALS											
	NRTI's			NNRTI's			Protease Inhibitors (PI)					
	3TC	ABC	D4T	DDI	ZDV	EFV	NVP	APV	NFV	IDV	RTV	SQV
ATV (n=40):	.03	.36	.41*	.24	.37	.14	.25	.82*	.82*	.87*	.88*	.88*

* p<.01

CONCLUSIONS:

- There is a strong evidence of cross resistance between ATV and other drugs (PIs) on the basis of phenotypic resistance, such that 94% of the variability in ATV PRSs scores are explained by the PRSs of APV, NFV, RTV, SQV.

GENOTYPIC CROSS RESISTANCE

ANALYTIC METHODS:

- **Dependent Variable:** ATV Phenotypic Resistance Score (PRS)
- **Independent Variables:** Genotypic Resistance Scores (GRS) of other antiretrovirals (See Table 3)
- **Sample (n=36):** Out of 40 patients, 36 met the criteria for genotypic testing
- **Identify the drugs whose GRSs exhibit significant simple correlations with ATV PRS (Pearson Correlation, $p<$.01)**
- **Enter these drugs' GRSs into a Backwards Stepwise Multiple Regression to identify which drugs make unique contributions to the prediction of ATV PRS**
- **Determine R^2**

RESULTS:

- GRSs of 8 drugs had significant correlation with ATV PRS.
- Genotypic resistance to NFV and IDV (both PIs) made unique contributions towards predicting ATV PRS in a multiple regression model.
- $R^2=$.70

Table 3. Pearson Correlation Coefficients Based on GRS

	ANTI-RETROVIRALS											
	NRTI's			NNRTI's			Protease Inhibitors (PI)					
	3TC	ABC	D4T	DDI	ZDV	EFV	NVP	APV	NFV	IDV	RTV	SQV
ATV (n=36):	-.05	.56*	.47*	.40	.56*	.12	.24	.77*	.78*	.80*	.80*	.77*

*p<.01

CONCLUSIONS:

- Strong evidence of cross resistance between ATV and other drugs (PIs) on the basis of genotypic scores, such that 70% of the variability in ATV phenotypic resistance scores is explained by the GRS of NFV and IDV.

GENOTYPIC MUTATIONS ASSOCIATED WITH PHENOTYPIC RESISTANCE TO ATV

ANALYTIC METHODS:

- **Dependent Variable:** ATV PRS
- **Independent Variables:** 99 PI Codons, 324 RT Codons
→ Codon data coded as: 1=MUTATION, 0=WILD-TYPE
- **Sample (n=36):** Patients with both genotypic and phenotypic data
- **Identify the codons which exhibit significant correlations with ATV PRS using a series of t-tests comparing the mean ATV PRS for patients having Mutations vs. those having Wild-Type at a given codon, $p<$.01**
- **Enter these codons as dummy variables into a Backwards Stepwise Multiple Regression to identify those which make unique contributions to the prediction of ATV PRS**
- **Determine R^2**

RESULTS:

- 13 codons had significant association with ATV PRS ($p<$.01).
PI Codons: 10, 33, 54, 57, 63, 82, 84, 92
RT Codons: 100, 208, 210, 215, 288
- Mutations on PI-54, PI-84, RT-215 made unique contributions towards the prediction of ATV PRS in the multiple regression model.
- $R^2=$.76.

CONCLUSIONS:

- Strong evidence of genotypic and phenotypic association, such that 76% of the variability in ATV phenotypic resistance is explained by genotypic mutation data at codons PI-54, PI-84 and RT-215.
- RT-215 may be a marker for overall drug experience, given that it is associated with ZDV resistance.

FIGURES

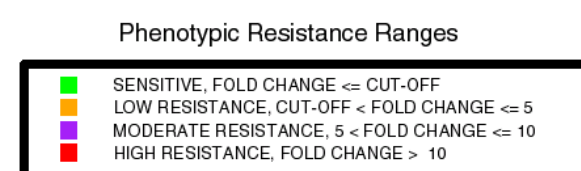
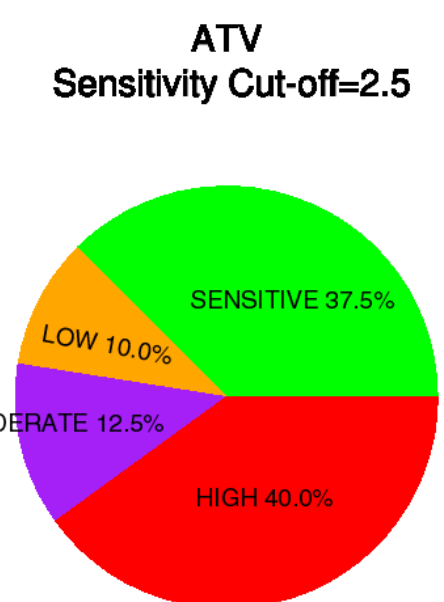
Figure 1. PHENOTYPIC RESISTANCE TO ATV
Proportion of Patients Within Various Ranges of Patient vs Wild-Type IC_{50} 

Figure 2. PHENOTYPIC RESISTANCE TO NRTIs

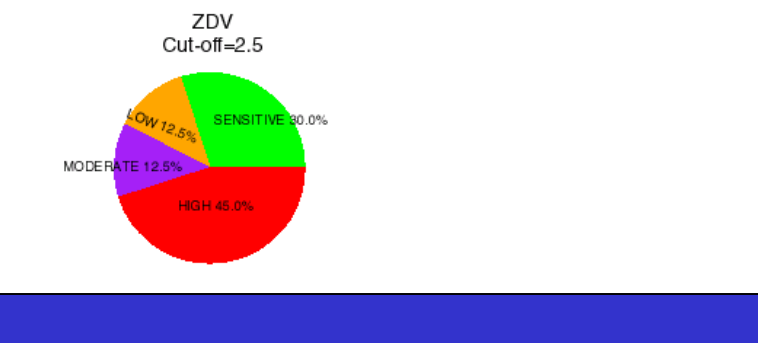
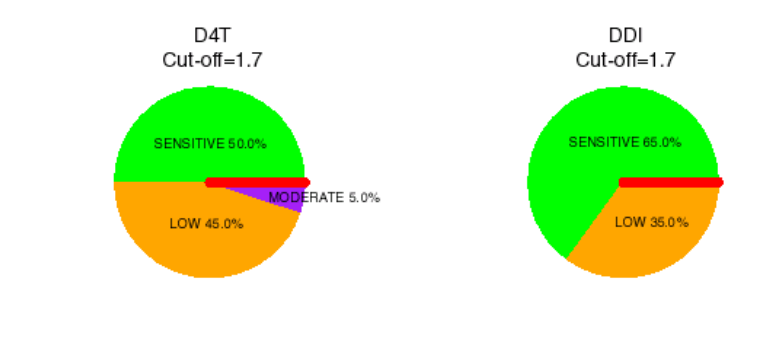
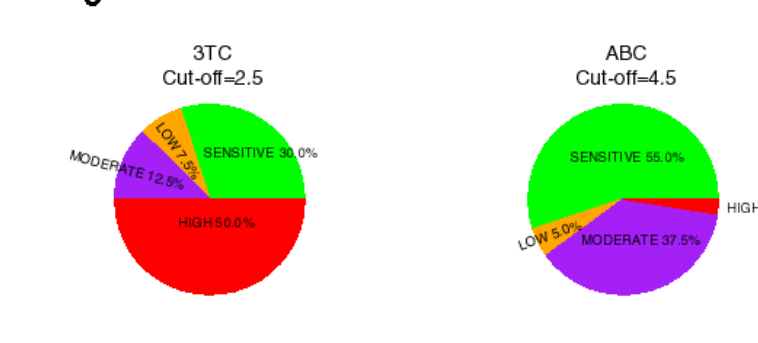


Figure 3. PHENOTYPIC RESISTANCE TO NNRTIs

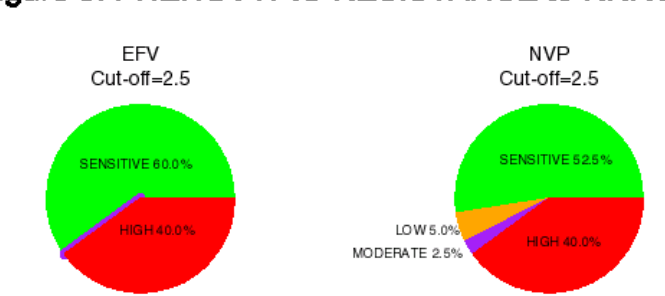
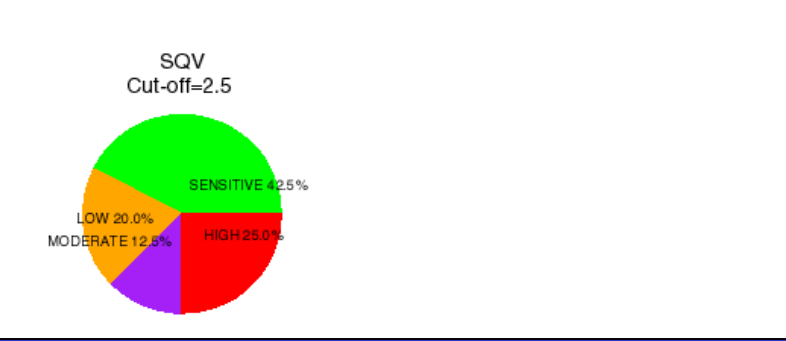
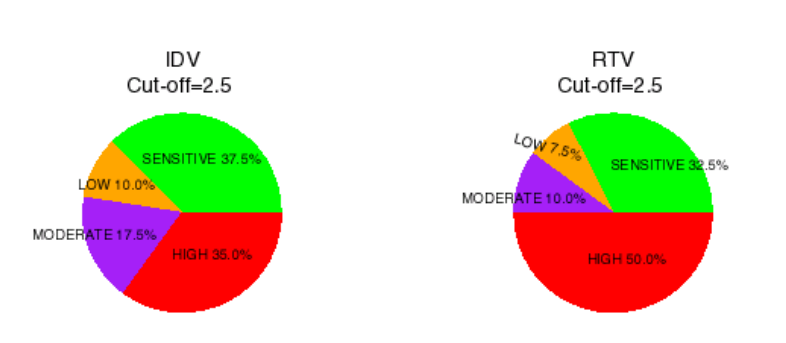
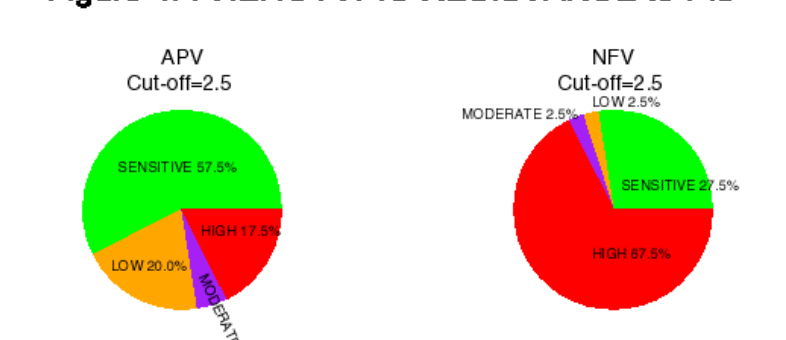


Figure 4. PHENOTYPIC RESISTANCE TO PIs



DISCUSSION

- The subjects in this analysis represent a group of heavily treated HIV-infected infants, children and adolescents, failing their present therapy.
- Resistance to multiple medications, from all classes of antiretroviral HIV medications was noted.
- 40% of the screened subjects were ineligible for enrollment to P1020A, based on the ATV IC_{50} Fold Change > 10. (12.5% of subjects had moderately increased ATV IC_{50} Fold Change of 5-10).
- Resistance to the study drug, ATV, was related to phenotypic resistance to other PIs and the presence of multiple PI-related mutations noted from genotypic resistance assays, in particular mutations at codons 54 and 84.

FUTURE DIRECTIONS

- Examine phenotypic resistance to other new PIs, including LPV and TFV;
- Test for cross resistance on the bases of phenotypic/genotypic data from standard antiretroviral drugs:
→ predict LPV PRS
→ predict TFV PRS
- Determine which genotypic codons correlate with LPV PRS & TFV PRS.

ACKNOWLEDGEMENTS

The authors would like to thank the children, their families, Bobbie Graham, Heather King, Michael Wantman and the PACTG 1020A Study Team for their support and contribution to this research.