Virco®TYPE HIV-1 Phenotype

(what's inside the box?)

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Estimating fold-change from genotypic data
 Vermeiren H et al. J Virol Methods (2007); 145:47-45

Comparable results PhenosenseTM Van Houtte M et al. J Med Virol (2009); 81:1702-1709

• An example

Mixtures, mutation pairs (184V, 65R) and non-IAS mutations

Mechanics of Resistance

Change in the molecular target of a drug HIV RT enzyme: 400 codons Protease enzyme: 99 codons



Sequence

Coventional Phenotype Antivirogram[®] (Virco)



Measured Fold Change (FC) *Virtual*Phenotype[™]-LM Engine



Calculated Fold Change (FC)

October 200

3

Estimated fold change from genotypic data

Vermeiren H et al. J Virol Methods 2007; 145:47-5500



Linear Regression Model

• General Formula: $y = \beta_0 + \beta_1 x_1 + e$

- Estimating SBP from demographic factors
 y (dependent variable) = SBP (mmHg)
 B_o = mean SBP in general population
 B₁x₁ = (weight factor) x (risk factor, i.e. age, gender, etc)
- Estimating FC from mutations
 - y (dependent variable) = Fold Change (FC)
 - $B_o = FC$ of wild-type virus
 - **B**₁ x_1 = (weight factor) x (mutation)

Calculating the Fold-Change: Tipranavir Example

Protease Mutations Detected 3I, 10I, 14R, 19I, 24I, 37N, 41K, 46I, 53Y, 54V, 55R, 63P, 64V, 71V, 82T, 84V

From page 2 of the virco®TYPE HIV-1 Report

All Mutations Detected (HXB2 Reference Sequence)

PR: 3I, 10I, 14R, 19I, 24I, 37N, 41K, 46I, 53Y, 54V, 55R, 63P, 64V, 71V, 82T, 84V

RT: 20R, 35I, 41L, 74V, 103N, 169D, 181C, 184V, 200A, 214F, 215Y, 272A, 286A, 288S, 293V, 376A, 390R

*DB0802 implemented on DEC 2008; report #ASJ001979

FC Assessment: Example of Tipranavir Protease Mutation Analysis

3I, 10I, 14R, 19I, 24I, 37N, 41K, 46I, 53Y, 54V, 55R, 63P, 64V, 71V, 82T, 84V

10C & 33F	13A & 47V	20T & 73T	33I & 35G	35N	43T & 85L	48Q	54A & 84V	60E	82L
10F	13V & 15V	20T & 84V	331 & 361	35N & 43T	451	48S	54L	60E & 71V	82S
10F & 47V	13V & 34Q	20V	33I & 82A	35N & 70E	45Q	48V	54L & 76V	60E & 82A	82T
10F & 54M	13V & 36I	22V	33I & 95F	35N & 82F	45V	48V & 54A	54L & 82A	60E & 95V	83D
10F & 58E	13V & 43S	24F & 60E	33V	36A	46L	48V & 54S	54L & 82S	66V	83D & 84V
10F & 82C	13V & 71L	241	33V & 43T	36I & 83D	46L & 48M	48V & 54V	54M	69K	84A
10F & 82F	13V & 71V	24I & 33F	33V & 54V	36I & 84V	46L & 50L	48V & 71V	54M & 83D	71V & 73T	84V
10F & 82L	13V & 82F	24I & 50V	34D	36L & 53L	46L & 80I	48V & 82A	54M & 88D	71V & 74E	85V
10F & 84A	13V & 84V	24I & 82T	34D & 58E	36L & 84V	46L & 82L	48V & 84V	54S	74A	88D
10F & 84V	14T	30N	34N & 82A	36V & 82F	46L & 84V	50L	54S & 74S	74P	88G
10I & 13A	15V & 43I	30N & 50V	34Q & 36I	36V & 84V	47V	50V	54S & 82A	74P & 82S	88S
10I & 33M	15V & 95F	30N & 88D	34Q & 54V	38W	47V & 54M	50V & 58E	54S & 84V	74P & 84V	89M
10I & 82F	16A	33.1Q	35D	41K	47V & 54V	50V & 70E	54T & 82T	74S	89V & 95L
10 & 82	18H	33F	35D & 36I	41K & 54V	47V & 69K	50V & 76V	54V & 84V	76V	90M
10V	20R & 35D	33F & 36L	35D & 36V	41T & 70E	47V & 82A	53L	55R	79S	91P
10V & 34A	20R & 53L	33F & 48A	35D & 54M	43I & 55R	47V & 82I	53L & 82F	55R & 60E	82C	95F
10V & 84V	20R & 70E	33F & 50V	35D & 54V	43Q & 73T	47V & 84V	53L & 82I	55R & 95F	82C & 95F	95V
10V & 88D	20T & 33F	33F & 60E	35D & 84V	43T	48A	54A	58E	82F & 89V	
10Y & 13V	20T & 41K	33F & 66L	35D & 89V	43T & 82F	48E	54A & 82A	58E & 80I	82G	
12S & 69K	20T & 53L	33F & 82F	35G	43T & 82T	48M	54A & 82F	58E & 89V	82I & 89V	

Decrease in FC <

Increase in FC

FC Assessment: Example of Tipranavir Protease Mutation Analysis



Calculating the Fold-Change: Example of Tipranavir Mutation Analysis

PI Mutations Detected and Evaluated

3I, 10I, 14R, 19I, **24I**, 37N, **41K**, 46I, 53Y, **54V**, **55R**, 63P, 64V, 71V, **82T**, **84V**



Resistance Weight Factor: weight and direction for mutations which **impact** TPV

Log(FC) = 0.415FC = $10^{0.415} = 2.6$

A Direct Comparison between VirtualPhenotypeTM-LM & PhenosenseTM

- Used phenotypic and genotypic data in Stanford Database to compare FC values <u>estimated</u> by Virco[™] linear model with FC values <u>measured</u> by the Monogram Phenosense[™] assay
 - HIV drugs with 287 to 902 genotype-phenotype data pairs
 Sequences exhibiting amino acid mixtures
- Pearson's correlation coefficient to compare estimated FC with measured FC

Van Houtte M, et al. J Med Virol 2009; 81:1702-1709

Pearson's Correlation: NRTI & NNRTI

ZDV 3TC DDI D4T ABC TDF NVP DLV EFV



Pearson's Correlations: Pls

IDV RTV NLF SQV APV LPV ATV



Fig. 1. (Configured)

Predicted vs. Measured FC (PhenosenseTM and Antivirogram[®])

Single FC measurements

Mean of Multiple FC measurements



Interpretation of resistance

-		Pearson's	Clinical or biolog	rical cut-offs ^a	Major	No. discordant calls
class	Drug	coefficient (R)	virco [®] TYPE HIV-1	PhenoSense™	discordances (%)	with VT/PS FC ratio $\leq 0.4 \text{ or } \geq 2.5^{\text{b}}$
NRTI	AZT	0.90	2.7	1.9	9.6	30 4
	3TC	0.97	1.0-3.4	3.5	2.4	0 9
	ddI	0.89	0.9-2.6	1.3-2.2	0.0	0 0
	d4T	0.76	0.9-2.0	1.7	6.3	18 0
	ABC	0.89	0.8-1.9	4.5-6.5	8.1	010
	TDF	0.77	0.9-2.1	1.4-4.0	1.2	0 1
NNRTI	NVP	0.94	5.5	4.5	3.1	7 12
	DLV	0.91	10.5	6.2	7.1	15 28
	EFV	0.94	3.4	3.0	4.1	6 13
PI	IDV	0.93	2.4	2.1	7.4	18 4
	IDV/r		10.6-40.1	10	8.1	38 1
	RTV	0.95	2.3	2.5	4.5	15 6
	NFV	0.93	2.2	3.6	8.0	5 14
	SQV	0.92	1.8	1.7	9.0	16 15
	SQV/r		7.1-26.5	2.3-12.0	2.7	2210
	FPV	0.91	2.2	2.0	10.3	24 9
	FPV/r		1.3-11.4	4.0-11.0	0.5	0 4
	LPV/r	0.95	9.7-56.1	9.0-55.0	0.0	0 0
	ATV/r	0.88	2.7-32.9	5.2	3.8	10 1
Overall		0.90				12

• Overall major discordances low for all drugs (<3.8%)

Higher for D4T (6.3%), ABC (8.1%), AZT (9.6%), IDV/r (8.1%)

Biologic vs. Clinical Cut-off values

 Biologic Cut-off (BCO) define what is resistant and non-resistant based on how the patient's virus responds to a drug <u>in vitro</u>

 Clinical Cut-off (CCO) values refer to the foldchange of virus susceptibility above which the drug has less activity <u>in-vivo</u> Uniform and Consistent 4-Step Methodology to Define Clinical Cut-Off Values for All Drugs

Step 1: Collect clinical outcome data and create analysis datasets (one per drug)

Step 2: Develop models (one per drug) of virologic response as a function of baseline FC as predicted by virco®TYPE HIV-1 and other variables

Step 3: Define clinical cut-offs
"Lower" — FC at which response begins to be lost
"Upper" — FC at which response is essentially gone

Step 4: Validation

Bart W et al. J Virol Method 2009; [Epub]

Case Study: Patient BH: (First-line Treatment Failure)

This case study is for discussion and education purposes only and does not constitute professional medical advice. The information provided in the case study should not be relied upon as the basis for making patient management decisions. This case study is not intended to show that any line of therapy is any more or less effective than any other therapy.

Patient 1: Treatment History

Date	Meds	CD4	HIV RNA	Comments
2002	None	441	92,526	Treatment deferred
2003	None	265	110,611	Start AZT/3TC, LPV/r; claims good adherence, tolerance; Makes most appointments; Episodes of >6 months w/o labs
Wk 24	AZT/3TC, LPV/r	>400	2,615	Pt preference: no changes; Provider: tolerate low level viremia given immunologic response
10/2006	AZT/3TC, LPV/r	>400	~2,600	
01/2007	AZT/3TC, LPV/r	>500	~2,600	Patient declines Hep C therapy; Resistance testing obtained

Patient 1: Resistance **Test Report** Page 1



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vircoTYPE HIV-1

(powered by Virtual Phenotype[™]-LM

The Complete Resistance Analysis

1		Patient/Sample Details		Physician Details
I	Patient Name	Sample ID Collection Date	318299	
DENT	Patient ID National Identifier	Received by Virco Project	101024	
CONFI	Date of birth Gender	Report Date Virco ID	VRN000857	Physician:

SUMMARY REPORT

	DRUGS		FOLD ¹ CHANGE	СП	-OFF ²	RESISTANCE ANALYSIS ³	CLINICAL NOTES (see p2 for details)
	NRTI / NtRTI mutatio	ns ⁴ : 41L, 67N, 7	'OR, 184V	, 219Q,	335D, 3	69wt/A/I/V, 371wt/V	
	Retrovir®	Zidovudine	4.6	15	11.4		
E	Epivir®	Lamivudine	48.8	1.2	4.6	MINIMAL RESPONSE	-
Ŧ	Videx®	Didanosine	1.6	0.9	2.6	REDUCED RESPONSE	
R	Zerit®	Stavudine	1.0	1.0	2.3	MAXIMAL RESPONSE	
E	Ziagen®	Abacavir	2.2	0.9	3.5	REDUCED RESPONSE	
ž	Emtriva®	Emtricitabine	44.8	3	.1	RESISTANT	
	Viread®	Tenofovir DF	1.1	1.0	2.3	REDUCED RESPONSE	

NNRTI mutations⁴: 369wt/A/I/V

E	Viramune®	Nevirapine	3.6	6.0	SUSCEPTIBLE	
Ä	Sustiva®, Stocrin®	Efavirenz	2.9	3.3	SUSCEPTIBLE	
Z	Intelence™	Etravirine	1.8	1.6 27.6	REDUCED RESPONSE	Note 1

PI mutations⁴: 10F, 32I, 43T, 46I, 47V, 63P, 82A, 90M, 93L

	Crixivan®	Indinavir	24.8	1.0	5.4	MINIMAL RESPONSE		
	Crixivan ®; boosted	Indinavir/r	24.8	2.3	27.2	REDUCED RESPONSE		
	Viracept®	Nelfinavir	20.1	1.2	9.4	MINIMAL RESPONSE		
	Invirase®; boosted	Saquinavir/r	1.2	3.1	22.6	MAXIMAL RESPONSE		
H	Lexiva®,Telzir®; boosted	Fosamprenavir/r	17.4	1.5	19.5	REDUCED RESPONSE		
	Kaletra®	Lopinavir/r	26.1	6.1	51.2	REDUCED RESPONSE		
	Reyataz®; boosted	Atazanavir/r	6.2	2.5	32.5	REDUCED RESPONSE		
	Aptivus®; boosted	Tipranavir/r	0.8	1.5	7.0	MAXIMAL RESPONSE	Note 1	
	Prezista™; boosted	Darunavir/r	3.2	10.0	106.9	MAXIMAL RESPONSE		

1. Predicted Fold Change in 50% Inhibitory Concentration (IC%), relative to susceptible reference virus. 2. Cut-off values for maximal and minimal clinical response (Clinical Cut-Off) or for normal susceptibility range in vitro (Biological Cut-Off), Biological Cut-Off) are printed in Italic. See page 3 for definitions, 3. Resistance Analysis based on the magnitude of the Fold Change relative to the Clinical or the Biological Cut-Off. Biological Cut-Offs are printed in Italic. See page 1 are those reported on public lists (ANRS, Stanford, IAS-USA) or by drug development sponsors. A complete list of all differences from the reference wild type is given on page 2.

IMPORTANT: the additional clinical notes on page 2 provide important information about the specific genotype analysed and should be considered in combination with information on this Summary Page.

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VPT 4.2.01	DB0704	
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Patient 1: Resistance Test Report Page 1 (NRTI/NtRTI)

	DRUGS		FOLD ¹ CHANGE	CUT-	OFF ²	RESISTANCE ANALYSIS ³	CLINICAL NOTES (see p2 for details)
	NRTI / NtRTI mutation	ns⁴: 41L, 67N, 70	0R, 184V	, 219Q,	335D, 3	69wt/A/I/V, 371wt/V	
	Retrovir®	Zidovudine	4.6	1.5	11.4	REDUCED RESPONSE	
E	Epivir®	Lamivudine	48.8	1.2	4.6	MINIMAL RESPONSE	
tR	Videx®	Didanosine	1.6	0.9	2.6	REDUCED RESPONSE	
2	Zerit®	Stavudine	1.0	1.0	2.3	MAXIMAL RESPONSE	
E	Ziagen®	Abacavir	2.2	0.9	3.5	REDUCED RESPONSE	
N N	Emtriva®	Emtricitabine	44.8	3.	.1	RESISTANT	
	Viread®	Tenofovir DF	1.1	1.0	2.3	REDUCED RESPONSE	

DRUG	s	FOLD ¹ CHANGE	сит	-OFF ²	RESISTANCE ANALYSIS	3 CLINIC NOTES (see p2 for c
NRTI / NtRTI mutati	ons": 41L, 67N, 7	'OR, 184V,	219Q,	335D, 3	369wt/A/I/V, 371wt/V	
Retrovir®	Zidovudine	4.6	1.5	11.4	REDUCED RESPONSE	1
Epivir®	Lamivudine	48.8	1.2	4.6	MINIMAL RESPONSE	
Videx®	Didanosine	1.6	0.9	2.6	REDUCED RESPONSE	
Zerit®	Stavudine	1.0	1.0	2.3	MAXIMAL RESPONSE	
Ziagen®	Abacavir	2.2	0.9	3.5	REDUCED RESPONSE	
Emtriva®	Emtricitabine	44.8	3	3.1	RESISTANT	
Viread®	Tenofovir DF	1.1	1.0	2.3	REDUCED RESPONSE	
NNRTI mutations ⁴ : 3	69wt/A/I/V					
Viramune®	Nevirapine	3.6	6	.0	SUSCEPTIBLE	
Sustiva®, Stocrin®	Efavirenz	2.9	3	.3	SUSCEPTIBLE	
Intelence [™]	Etravirine	1.8	1.6	27.6	REDUCED RESPONSE	Note 1
Crixivan®	Indinavir	24.8	1.0	5.4	MINIMAL RESPONSE	
	Indinavir/r	24.8	2.3	27.2	REDUCED RESPONSE	
Crixivan ®; boosted	Nolfinavir	20.1	1.2	9.4	MINIMAL RESPONSE	
Crixivan ®; boosted Viracept®	Netrinavii			00 1	MAYIMAI DECDONCE	
Crixivan ®; boosted Viracept® Invirase®; boosted	Saquinavir/r	1.2	3.1	22.0	MAXIMAL RESPONSE	
Crixivan ®; boosted Viracept® Invirase®; boosted Lexiva®,Telzir®; boosted	Saquinavir/r Fosamprenavir/r	1.2 17.4	3.1 1.5	19.5	REDUCED RESPONSE	
Crixivan ®; boosted Viracept® Invirase®; boosted Lexiva®,Telzir®; boosted Kaletra®	Saquinavir/r Fosamprenavir/r Lopinavir/r	1.2 17.4 26.1	3.1 1.5 6.1	19.5 51.2	REDUCED RESPONSE REDUCED RESPONSE	
Crixivan ©; boosted Viracept® Invirase®; boosted Lexiva®,Telzir©; boosted Kaletra® Reyataz®; boosted	Saquinavir/r Fosamprenavir/r Lopinavir/r Atazanavir/r	1.2 17.4 26.1 6.2	3.1 1.5 6.1 2.5	22.6 19.5 51.2 32.5	REDUCED RESPONSE REDUCED RESPONSE REDUCED RESPONSE	
Crixivan ©; boosted Viracept© Invirase®; boosted Lexiva©,Telzir©; boosted Kaletra® Reyataz©; boosted Aptivus©; boosted	Saquinavir/r Fosamprenavir/r Lopinavir/r Atazanavir/r Tipranavir/r	1.2 17.4 26.1 6.2 0.8	3.1 1.5 6.1 2.5 1.5	22.6 19.5 51.2 32.5 7.0	REDUCED RESPONSE REDUCED RESPONSE REDUCED RESPONSE MAXIMAL RESPONSE	Note 1
Crixivan ©; boosted Viracept© Invirase©; boosted Lexiva®,Telzir©; boosted Kaletra® Reyataz©; boosted Aptivus®; boosted Prezista [™] ; boosted	Saquinavir/r Fosamprenavir/r Lopinavir/r Atazanavir/r Tipranavir/r Darunavir/r	1.2 17.4 26.1 6.2 0.8 3.2	3.1 1.5 6.1 2.5 1.5 10.0	22.6 19.5 51.2 32.5 7.0 106.9	REDUCED RESPONSE REDUCED RESPONSE REDUCED RESPONSE MAXIMAL RESPONSE MAXIMAL RESPONSE	Note 1

Patient 1: Resistance Test Report Page 1 (NNRTI)

				🛱 virco	Reported by: Virco BVBA Generaal de Wittelaan L11 B-2800 Mechelen, Belgium	Inquiries to: Virco BVBA Generalo De Wittelaan L11B4 Mechelen, 2800 Belgium	Contact: Tel: 0032-15285605 Fax: 0032-15286346 pmckenna@vrcbe.jnj.	com
	DRUGS		FOLD ¹ CHANGE	CUT-OFF ²	RES	ISTANCE ANAL	YSIS ³	CLINICAL NOTES (see p2 for details)
	NNRTI mutations ⁴ : 36	9wt/A/I/V						
E	Viramune®	Nevirapine	3.6	6.0		SUSCEPTIBLE		
Ϋ́Υ	Sustiva®, Stocrin®	Efavirenz	2.9	3.3		SUSCEPTIBLE		
Ź	Intelence™	Etravirine	1.8	1.6 27.6	RE	DUCED RESPO	NSE	Note 1
				NRTI / NtRTI mut	tations': 41L, 67N, 2	CHANGE COTFORM RESISTAN VOR, 184V, 219Q, 335D, 369wt/A/L/	VCE ANALTSIS	a p2 for details)

1. Non-IAS mutation

2. Mixtures

Retrovir®	Zidovudine	4.6	1.5	11.4	REDUCED RESPONSE	
Epivir®	Lamivudine	48.8	1.2	4.6	MINIMAL RESPONSE	
Videx®	Didanosine	1.6	0.9	2.6	REDUCED RESPONSE	
Zerit®	Stavudine	1.0	1.0	2.3	MAXIMAL RESPONSE	
Ziagen®	Abacavir	2.2	0.9	3.5	REDUCED RESPONSE	
Emtriva®	Emtricitabine	44.8	3	.1	RESISTANT	
Viread®	Tenofovir DF	1.1	1.0	2.3	REDUCED RESPONSE	
NNRTI mutations ⁴ : 3	69wt/A/I/V					
Viramune®	Nevirapine	3.6	6	.0	SUSCEPTIBLE	
Sustiva®, Stocrin®	Efavirenz	2.9	3	.3	SUSCEPTIBLE	
Intelence [™]	Etravirine	1.8	1.6	27.6	REDUCED RESPONSE	Note 1
PI mutations ⁴ : 10F, 3	32I, 43T, 46I, 47V	/, 63P, 8	2A, 90M	I, 93L		
PI mutations ⁴ : 10F, 3	32I, 43T, 46I, 47V	24.8	2A, 90M	5.4	MINIMAL RESPONSE	
PI mutations ⁴ : 10F, 3 Crixivan® Crixivan®; boosted	32I, 43T, 46I, 47V Indinavir Indinavir/r	24.8 24.8 24.8	2A, 90M 1.0 2.3	5.4 27.2	MINIMAL RESPONSE	
PI mutations ⁴ : 10F, 3 Crixivan® Crixivan ®; boosted Viracept®	321, 43T, 46I, 47V Indinavir Indinavir/r Netfinavir Scoulandr/r	24.8 24.8 24.8 20.1	2A, 90M 1.0 2.3 1.2 2.1	5.4 27.2 9.4 22.6	MINIMAL RESPONSE REDUCED RESPONSE MINIMAL RESPONSE	
PI mutations ⁴ : 10F, 3 Crixivan® Crixivan ®; boosted Viracept® Invirase®; boosted Lowise® Chiston boosted	321, 43T, 46I, 47V Indinavir Indinavir/r Nelfinavir Saquinavir/r	24.8 24.8 24.8 20.1 1.2	2A, 90M 1.0 2.3 1.2 3.1	5.4 5.4 27.2 9.4 22.6	MINIMAL RESPONSE REDUCED RESPONSE MINIMAL RESPONSE MAXIMAL RESPONSE DEDUCED RESPONSE	
PI mutations ⁴ : 10F, 3 Crixivan®; boosted Viracet® Invirase®; boosted Lexiv@; Telzir®; boosted Kaleta®	Indinavir Indinavir/r Netfinavir Saquinavir/r Fosamprenavir/r Loninavir/r	24.8 24.8 20.1 1.2 17.4 26.1	1.0 2.3 1.2 3.1 1.5 6.1	5.4 27.2 9.4 22.6 19.5 51.2	MINIMAL RESPONSE REDUCED RESPONSE MINIMAL RESPONSE MAXIMAL RESPONSE REDUCED RESPONSE REDUCED RESPONSE	
PI mutations ⁴ : 10F, 3 Crixivan® Crixivan®; boosted Viracet® Invirase®; boosted Lexiva®, Telzir®; boosted Kaletra® Penatar®: boosted	Indinavir Indinavir/r Nelfinavir/s Saquinavir/r Fosamprenavir/r Lopinavir/r	24.8 24.8 20.1 1.2 17.4 26.1	1.0 2.3 1.2 3.1 1.5 6.1 2.5	5.4 27.2 9.4 22.6 19.5 51.2 32 5	MINIMAL RESPONSE REDUCED RESPONSE MINIMAL RESPONSE MAXIMAL RESPONSE REDUCED RESPONSE REDUCED RESPONSE REDUCED RESPONSE	
PI mutations ⁴ : 10F, 3 Crixivan® Crixivan®; boosted Viracept® Invirace®; boosted Lexiva®; Telzir®; boosted Kaletra® Reyata%; boosted	10 Indinavir Indinavir/ Nelfinavir/ Saquínavir/r Fosamprenavir/r Lopinavir/r Atazanavir/r	24.8 24.8 20.1 1.2 17.4 26.1 6.2 0.8	1.0 2.3 1.2 3.1 1.5 6.1 2.5 1.5	5.4 27.2 9.4 22.6 19.5 51.2 32.5 7.0	MINIMAL RESPONSE REDUCED RESPONSE MINIMAL RESPONSE REDUCED RESPONSE REDUCED RESPONSE REDUCED RESPONSE REDUCED RESPONSE MAXIMAL PEEPONSE	Note 1
PI mutations ⁴ : 10F, 3 Crixivan® Crixivan®; boosted Viracept® Invirase®; boosted Lexiva®, Telzir®; boosted Kaletra® Reyata20; boosted Aptivus®; boosted Prezista®; boosted	Indinavir Indinavir/ Nelfinavir/ Saquinavir/r Saquinavir/r Lopinavir/r Atazanavir/r Darunavir/r Darunavir/r	24.8 24.8 20.1 1.2 17.4 26.1 6.2 0.8 3.2	1.0 2.3 1.2 3.1 1.5 6.1 2.5 1.5 10.0	5.4 27.2 9.4 22.6 19.5 51.2 32.5 7.0 106.9	MINIMAL RESPONSE REDUCED RESPONSE MINIMAL RESPONSE REDUCED RESPONSE REDUCED RESPONSE REDUCED RESPONSE MAXIMAL RESPONSE MAXIMAL RESPONSE	Note 1

Issue: Mixtures and Phenotype Tests

Original Virus Variant Selection Pressure Exerted by Drug X New Virus Variant



"Dilution effect"

- Mixture of susceptible and resistant strains
- IC₅₀ of pre-treatment population may be low and result in a 'sensitive' call

Time

- Selection of resistant variant by Drug X
- Viral breakthrough despite "sensitive" pre-treatment phenotype

Two Approaches to Mixtures

Previous Approach

- Provides the best correlation with a measured phenotype of the same sample
- May underestimate the clinical impact of resistance
 - genotypic interpretation could provide better warning of the potential for emerging resistance

Weighted Approach

New Approach

- Provides an estimate of the most resistant virus possible in a mixed population
- May be a less accurate prediction of the measured phenotype of the mixed virus population
- Provides a early warning of the potential for emerging resistance
- Less risk of under-estimating the clinical impact of resistance

Worst Case Scenario (WCS)

Patient 1: Resistance Test Report Page 1 (PI)

	DRUGS		FOLD ¹ CHANGE	CUT-	OFF ²	RESISTANCE ANALYSIS ³	CLINICAL NOTES (see p2 for details)
	PI mutations ⁴ : 10F, 32	1, 43T, 46I, 47V	/, 63P, 82	2A, 90M,	, 93L		
	Crixivan®	Indinavir	24.8	1.0	5.4	MINIMAL RESPONSE	
	Crixivan ®; boosted	Indinavir/r	24.8	2.3	27.2	REDUCED RESPONSE	
	Viracept®	Nelfinavir	20.1	1.2	9.4	MINIMAL RESPONSE	
	Invirase®; boosted	Saquinavir/r	1.2	3.1	22.6	MAXIMAL RESPONSE	
Ы	Lexiva®,Telzir®; boosted	Fosamprenavir/r	17.4	1.5	19.5	REDUCED RESPONSE	
	Kaletra®	Lopinavir/r	26.1	6.1	51.2	REDUCED RESPONSE	
	Reyataz®; boosted	Atazanavir/r	6.2	2.5	32.5	REDUCED RESPONSE	
	Aptivus®; boosted	Tipranavir/r	0.8	1.5	7.0	MAXIMAL RESPONSE	Note 1
	Prezista™; boosted	Darunavir/r	3.2	10.0	106.9	MAXIMAL RESPONSE	

Note 1: The CCOs for these drugs are based on treatment responses in select populations of treatment-experienced patients participating in Phase II or III clinical trials of these new agents. The relevance of these CCOs for patients different from these study participants has not been evaluated. For more information about the datasets used to calculate virco[®]TYPE HIV-1 clinical cut-offs, please refer to www.vircotype.com

Custing Charrie	Nevirapine	3.6	6	.0	SUSCEPTIBLE	
Intelence™	Etravirine	1.8	1.6	27.6	REDUCED RESPONSE	Note 1
PI mutations ⁴ : 10	F, 32I, 43T, 46I, 47V	/, 63P, 8	2A, 90M	l, 93L		
Crixivan®	Indinavir	24.8	1.0	5.4		
Crixivan ®: boosted	Indinavir/r	24.8	2.3	27.2	REDUCED RESPONSE	
Viracept®	Nelfinavir	20.1	1.2	9.4	MINIMAL RESPONSE	
Invirase®; boosted	Saguinavir/r	1.2	3.1	22.6	MAXIMAL RESPONSE	
Lexiva®.Telzir®: booste	d Fosamprenavir/r	17.4	1.5	19.5	REDUCED RESPONSE	
Kaletra®	Lopinavir/r	26.1	6.1	51.2	REDUCED RESPONSE	
Revataz®: boosted	Atazanavir/r	6.2	2.5	32.5	REDUCED RESPONSE	
Aptivus®; boosted	Tipranavir/r	0.8	1.5	7.0	MAXIMAL RESPONSE	Note 1
Prezista™; boosted	Darunavir/r	3.2	10.0	106.9	MAXIMAL RESPONSE	
dicted Fold Change in 50% Inhibitory al susceptibility range in vitro (Biologi	Concentration (IC=), relative to susc cal Cut-Off). Biological Cut-Offs are	eptible referent printed in itali	nce virus. 2. C c. See page 3	ut-off values for for definitions.	r maximal and minimal clinical response 3. Resistance Analysis based on the magni	(Clinical Cut-Off) or for itude of the Fold Change
ve to the Clinical or the Biological C coment sponsors. A complete list of all	ut-Offs. See page 3 for definitions. differences from the reference wild	 Mutations type is given of 	printed on p page 2.	age 1 are those	e reported on public lists (ANRS, Stanfo	rd, IAS-USA) or by drug
	linial actor on page 2 area	dda import	ant inform	ation about t	the specific genetures analyzed :	and should be

Patient 01: Resistance Test Report Page 2 (PI)

Cut-off for maxi	mal virolog mal virolog	gic response ic response	(CCO1) (CCO2)	Cut-off for i	in vitro sus (BCO)	sceptibili	^{ty} C	⊐ ^{Pa} Fo	atient Sample old Change in IC ₅₀		maximal reduced minimal	virologi virologi virologi	c respor c respon c respon	nse ise ise
DRUGS		0.3	1		10		1(00	200 (>200)	FC	(95% confidence limits)	CCO 1	CCO 2	всо
Indinavir	IDV					•				24.8	(22.7-27.1)	1.0	5.4	
Indinavir/r	IDV/r									24.8	(22.7-27.1)	2.3	27.2	
Nelfinavir	NFV									20.1	(18.4-21.8)	1.2	9.4	
Saquinavir/r	SQV/r									1.2	(1.1-1.3)	3.1	22.6	
Fosamprenavir/r	FPV/r				-					17.4	(15.8-19.2)	1.5	19.5	
Lopinavir/r	LPV/r									26.1	(23.8-28.7)	6.1	51.2	
Atazanavir/r	ATV/r									6.2	(5.5-7.1)	2.5	32.5	
Tipranavir/r	TPV/r									0.8	(0.8-0.9)	1.5	7.0	
Darunavir/r	DRV/r									3.2	(2.9-3.6)	10.0	106.9	

Etravirine Eta D 1.8 (22.6.2) 1.0 5.4 Indinavir IDV/ 24.8 (22.7271) 1.0 5.4 Indinavir IDV/ 24.8 (22.7271) 1.0 5.4 Indinavir IDV/ 20.1 (22.627271) 1.0 5.4 Indinavir NFV 20.1 (22.7271) 1.0 5.4 Indinavir NFV 20.1 (22.7271) 1.0 5.4 Seguinavir/ SQV/r 1.2 (1.7.4 (1.58.472.0) 1.3 1.2 Fosamprenavir/r FV/r 1.4 1.4 (23.8.69.2) 1.5 1.5 1.5 Copinavir/r FV/r 0.8 (0.8.0.0) 1.5 7.0 Duruavir/r Duruavir/r DV/r 0.8 (0.8.0.0) 1.5 7.0 Duruavir/r Duruavir/r DV/r 0.8 (23.7.71) 2.5 3.2 1.5 1.00 1.00 1.00 1.00 Duruavir/r <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>(2.4.3.3)</th> <th></th> <th></th> <th>-</th>									(2.4.3.3)			-
Indinavir IV 24.8 (22.727.1) 1.0 5.4 Indinavir/r IDV/r 24.8 (22.727.1) 2.3 27.2 Vetifinavir NFV 24.8 (22.727.1) 2.3 27.2 Vetifinavir NFV 24.8 (22.727.1) 2.3 27.2 Sequinavir/r SQV/r 20.1 (18.421.8) 1.3 9.4 Sequinavir/r SQV/r 1.2 4.1 1.2 4.1 Sequinavir/r FPV/r 26.1 (23.828.7) 1.5 15.1 Stazanavir/r TPV/r 26.1 (23.828.7) 1.5 15.2 Darunavir/r TPV/r 0.8 (0.80.0) 1.5 1.2 Darunavir/r DPV/r 0.8 (0.80.0) 1.5 1.0 10.0 10.6.9 AII Mutations Detected (HXB2 Reference Sequence) 3.2 (2.8.7.6) 10.0 10.6.9 R: Jl, 107, J21, 37N, 41K, 43T, 40, 47V, 63P, 62A, 90A, 91 Tt: 6.0 3.1 4.1 6.7 3	Etravirine	ETR	in the second se					1.8	(1.6-2.1)	1.6	27.6	
Indinavir/r DV/r 24.8 22.722.1 23.2 27.2 elfinavir/r DV/r 0.1 (18.27.27.1) 23.2 27.2 aquinavir/r SQVr 0.1 (18.27.27.1) 23.2 27.2 elfinavir SQVr 0.1 (18.27.27.1) 23.2 27.2 aquinavir/r SQVr 0.1 (18.27.27.1) 23.1 22.6 osamperanvir/r SQVr 0.1 (18.27.27.1) 23.1 22.6 osamperanvir/r SQVr 0.1 (12.2 (11.1.1.3) 31.2 2.6 osamperanvir/r PV/r 0 7.4 (13.6.8.0.2.0) 1.5 1.5 1.5 1.2 1.15 1.5 <th>ndinavir</th> <th>IDV</th> <th></th> <th></th> <th></th> <th></th> <th>2</th> <th>4.8</th> <th>(22.7-27.1)</th> <th>1.0</th> <th>5.4</th> <th></th>	ndinavir	IDV					2	4.8	(22.7-27.1)	1.0	5.4	
NPV 201 Re-21.6 1.2 9.4 Gragminavir/r SQV/r 1.2 (1.5.13) 1.2 5.5 Gragminavir/r LPV/r 1.2 (1.5.13) 1.2 5.5 1.2 5.5 1.2 5.5 1.2 5.5 1.2 5.5 1.2 5.5 1.2 5.5 1.2 5.1 5.1 2.5 1.2 5.1 5.1 2.5 1.2 5.1 2.5 1.2 5.7 1.2 5.1 2.5 1.2 5.2	ndinavir/r	IDV/r			-		2	4.8	(22.7-27.1)	2.3	27.2	T
aquinavir/r SQV/r 1.22 (1.1-1.3) 3.1 122.6 cosmprenavir/r FPV/r 17.4 (15.8-19.2) 1.5 19.5 copinavir/r LPV/r 2.6.1 (2.8-89.2) 1.5 19.5 copinavir/r LPV/r 0.6.2 (5.8-7.1) 2.5 12.5 12.5 pipravir/r LPV/r 0.8 (0.8-0.9) 0.8 (0.8-0.9) 10.0 10.6 pipravir/r DPV/r 0.8 (0.8-0.9) 10.0 10.6 9 pipravir/r DPV/r 0.8 (0.8-0.9) 10.0 10.6 9 pipravir/r DPV/r 0.8 (0.8-0.9) 10.0 10.6 9 All Mutations Detected (HXB2 Reference Sequence) 10.0 10.6 9 11 17.5 10.0 10.6 9 R: 10, 107, 20, 378, 41K, 437, 46, 47V, 639, 82A, 904, 91 1.7 6.33, 41L, 67N, 708, 122K, 184V, 203K-Q, 207E, 214wL-F, 219Q, 228wL-H, 277K, 286A, 293V, 297R, 301M, 335D, 357T, 369wL/A/L/V, 371wL-V, 386wL/A, 390R 10.6 10.6<	lelfinavir	NFV					2	0.1	(18.4-21.8)	1.2	9.4	T
Osamperavir/r PP/r 17.4 (15.8-19.2) 1.5 19.5 opinavir/r PP/r 26.1 (23.8-19.2) 1.5 19.5 ippanavir/r AVV/r 26.1 (23.8-19.2) 1.5 19.5 ippanavir/r AVV/r 26.2 (5.5-77.1) 2.5 2.5 ippanavir/r TPV/r 0.8 0.8-0.9 1.3 7.0 annavir/r DRVr 0.8 0.8-0.9 1.5 7.0 annavir/r DRVr 0.8 0.8-0.9 1.5 7.0 annavir/r DRVr 0.8 0.8-0.9 1.5 7.0 annavir/r DRVr 0.8 0.8 0.8 10.0 10.9 K 1.107.32 37M. 41K.437.46 AVK.639.80% 9.1 7 10.6 0.8 10.0 10.9 K 0.107.708.122K.184V.203K.02.207K.2144-07.2190.2284-01H.277K.2864.293V.297R.301M.3350.357T.369+01A/A/V.371wLV.386+01A.300R 10.0 10.9 10.0 10.9 Mote Diatorization adout the datasets used to	iaquinavir/r	SQV/r						1.2	(1.1-1.3)	3.1	22.6	Γ
Opinavir/r LPV/r 26.1 23.8-28.70 6.1 51.2 tazanawir/r TPV/r 6.2 (25.97.1) 2.5 32.5 Tipranavir/r TPV/r 6.2 (25.97.1) 2.5 32.5 Tipranavir/r DPV/r 3.2 (2.9.3.68.7) 1.5 7.0 Jarunavir/r DPV/r 3.2 (2.9.3.6) 10.0 10.9 All Mutations Detected (HXB2 Reference Sequence) 3.2 (2.9.3.6) 10.0 10.9 R: 31, 107, 321, 378, 418, 437, 404, 479, 639, 620, 900, 921 3.2 (2.9.3.6) 10.0 10.9 R: 30, 50, 51, 41L, 678, 708, 122X, 184V, 203K-Q, 207E, 214eV/F, 219Q, 228eV/H, 277K, 286A, 293V, 297R, 301M, 335D, 357T, 369eV/A/VV, 371wLV, 38eW/A, 390R Additional Clinical Notes Markin Line COS for these drugs are based on treatment responses in select populations of treatment-experienced patients participating in Phase II or III The COS for these drugs are based on calculate vircoTYPE HW-1 clinical cut-offs, please refer to www.vircotype.com	osamprenavir/r	FPV/r				1	1	7.4	(15.8-19.2)	1.5	19.5	
Matanawi/r ATV/r 0 6.2 0.5.9.7.11 2.5 3.2.5 Tiponawi/r/r DRV/r 0.8 0.8.40.9.9 1.5 7.0 1.6 7.0 1.6 7.0 1.0 106.9 Darunavir/r DRV/r 0 0.8 0.8.40.9.9 1.0 106.9 All Mutations Detected (HXB2 Reference Sequence) 0.3.2 (2.9.3.6) 10.0 106.9 B: 31, 107, 321, 37N, 41K, 43T, 46I, 47V, 63P, 82A, 50M, 93L 1.6 0.35I, 41L, 67N, 70R, 122K, 184V, 203K/Q, 207E, 214wL/F, 219Q, 228wL/H, 277K, 286A, 293V, 297R, 301M, 335D, 357T, 369wL/A/LV, 371wL/V, 386wL/A, 390R Mote 1 Additional Clinical Notes Example Example <td>.opinavir/r</td> <td>LPV/r</td> <td></td> <td></td> <td></td> <td></td> <td>2</td> <td>6.1</td> <td>(23.8-28.7)</td> <td>6.1</td> <td>51.2</td> <td></td>	.opinavir/r	LPV/r					2	6.1	(23.8-28.7)	6.1	51.2	
Tippranuvir/r TPV/r 0.8 0.8-0.9) 1.5 7.0 Jarunavir/r DRV/r 3.2 (2.9-3.6) 10.0 106.9 All Mutations Detected (HXB2 Reference Sequence) 3.2 (2.9-3.6) 10.0 106.9 R: 31, 10F, 321, 37N, 41K, 43T, 44, 7Y, 64P, 82A, 50N, 9.1 T 60, 351, 41L, 67N, 70R, 122K, 184Y, 203K, Q, 207E, 214w.07, 219Q, 228w.0/H, 277K, 286A, 293V, 297R, 301M, 3350, 357T, 369w.C/A/VV, 371wLVV, 386w.C/A, 390R Mathematical Clinical Notes C Additional Clinical Notes The CCOS for these drugs are based on treatment responses in select populations of treatment-experienced patients participating in Phase II or III The CCOS for these drugs are based on treatment responses in select populations of treatment experienced patients participating in Phase II or III The circle of these drugs are based on treatment experience of these drugs participating in Phase II or III The circle of these drugs are based on treatment experience of these drugs participating in Phase II or III The circle of these drugs are based on treatment experience of these drugs participating in Phase II or III The circle of these drugs are based on treatment experience of these drugs participating in Phase II or III The circle of these drugs are based on treatment experience of these drugs participating in Phase II or III The circle of these drugs are based on treatment experience of these drugs participating in these are lin or III Th	Atazanavir/r	ATV/r						6.2	(5.5-7.1)	2.5	32.5	
Description Classical Control of Cont	fipranavir/r	TPV/r				-		0.8	(0.8-0.9)	1.5	7.0	
All Mutations Detected (HXB2 Reference Sequence) R: 31, 107, 321, 37N, 41K, 43T, 461, 47Y, 63P, 82A, 90K, 91L T: 60, 351, 41L, 67N, 70R, 122K, 164V, 203K, Q, 207E, 214wL/F, 219Q, 228wL/H, 277K, 286A, 293V, 297R, 301M, 335D, 357T, 36%wL/A/LVV, 371wL/V, 386wL/A, 390R Additional Clinical Notes Image: Sequence of these drugs are based on treatment responses in select populations of treatment-experienced patients participating in Phase II or III clinical Linical Table of these close for calculate vircol 1972 HrV-1 clinical cut-offs, phase refer to www.vircotype.com)arunavir/r	DRV/r		0				3.2	(2.9-3.6)	10.0	106.9	
Additional Clinical Notes The CC0s for these days are based on treatment responses in select populations of treatment-experienced patients participating in Phase II or III Clinical triats of these new agents. The evenance of these CC0s for patients different from these study participants has not been evaluated. For more information about the datasets used to calculate vircoTVPE HV-1 clinical cut-offs, please refer to www.vircotype.com												
Note 1 The CCOs for these drugs are based on treatment responses in select populations of treatment-experienced patients participating in Phase II or III initial trials of these may agents. The relevance of these CCOS for patients different from these study participants han and been evaluated. For more information about the datasets used to calculate vircoTYPE HV-1 clinical cut-offs, please refer to www.vircotype.com												
				Addi	tional Clinical	Notes						



Questions/Comment??