

Tipranavir displays similar *in vitro* antiviral activity against non-subtype B clinical isolates of HIV-1 compared with isolates from subtype B

Pierre R Bonneau¹, Yolanda Lie², Richard Bethell¹

¹Boehringer Ingelheim (Canada) Ltd/Research & Development, 2100 Cunard Street, Laval, Québec, Canada, H7S 2G5; ²Monogram Biosciences, South San Francisco, CA, USA

Poster Number P3.3/02

11th European AIDS Conference (EACS), Madrid, Spain, 24-27 October 2007

Corresponding Author: Pierre R Bonneau
Boehringer Ingelheim (Canada) Ltd/Research & Development, 2100 Cunard Street, Laval, Québec, Canada, H7S 2G5
Tel: +1 450 682-4640 ext. 4372
Email: pbonneau@lav.boehringer-ingelheim.com

Abstract

Objective Tipranavir has previously been shown to have similar antiviral activity against treatment-naïve viruses from HIV-1 subtypes B, C, F, G and CRF02_AG. To confirm and extend these data, the *in vitro* antiviral activity (EC₅₀) of tipranavir (TPV) has been determined against a broad panel of wild-type HIV-1 Group M clinical isolates belonging to prevalent and emerging subtypes and circulating recombinant forms.

Method The antiviral activity of tipranavir and other marketed HIV protease inhibitors was measured using the PhenoSense™ assay for a panel of 57 HIV-1 Group M wild-type clinical isolates from antiretroviral treatment-naïve patients (subtypes A, B, C, D, F, G, and H as well as recombinant forms CRF01_AE, CRF02_AG, and CRF12_BF). EC₅₀ values were compared to a drug-susceptible reference subtype B HIV-1 NL4-3 strain.

Results TPV had the following mean EC₅₀ values (fold-change range): 180 nM (0.6–4.3) for subtype A, 99 nM (0.8–1.7) for B, 124 nM (0.8–2.1) for C, 121 nM (0.6–1.8) for D, 195 nM (1.2–2.9) for F, 103 nM (0.5–1.6) for G, 124 nM (0.9–2.0) for H, 109 nM (0.6–2.1) for CRF01_AE, 116 nM (0.9–1.8) for CRF02_AG, and 133 nM (0.5–2.8) for CRF12_BF. All subtypes had a mean fold-change at or below the PhenoSense™ lower clinical cutoff value of 2.0. Our study also shows that different TPV mutation score algorithms do not correlate well when applied to wild-type, non-subtype B isolates as evidenced by the lack of any discernible trend between mean phenotypic fold-change and mean TPV mutation scores across the subtype/CRF panel.

Conclusions Our results show that TPV maintains a level of susceptibility against wild-type HIV-1 non-subtype B isolates that is overall similar to that observed with subtype B isolates. Moreover, scoring algorithms developed predominantly with subtype B isolates are not effective at predicting the TPV phenotype among non-subtype B isolates.

Introduction

The majority of virological studies with anti-HIV agents have used subtype B viruses, the predominant HIV-1 subtype in developed countries. However other subtypes and recombinant forms are emerging or even dominating in other parts of the world. Since variations in susceptibility to antiretroviral drugs have been reported amongst the different HIV-1 subtypes, it is important to monitor drug activity against HIV-1 isolates belonging to subtypes other than subtype B.

Tipranavir (TPV) is a non-peptidic HIV-1 protease inhibitor (PI) with potent antiviral activity against a broad range of PI-resistant mutant viruses. TPV also maintains a level of antiviral activity against non-subtype B forms that is overall similar to subtype B. However, detailed analyses have revealed subtle changes in TPV susceptibility among the different subtypes, such as evidence of hypersusceptibility to subtype G [1]. Mutation score algorithms have been proposed to predict the virological response to TPV. These scores are based mainly on subtype B clinical isolates and it is still unclear whether they can be applied to other non-B subtypes.

To further explore this aspect, we have evaluated TPV susceptibility against a broad panel of prevalent and emerging HIV-1 subtypes and recombinant forms. These results were used to determine the applicability of different TPV mutation scores to adequately predict the susceptibility of HIV-1 isolates belonging to subtypes other than subtype B.

Panel selection and methodology

The clinical isolates in this study were tested for their susceptibility to TPV (along with all the other PIs currently available). The first panel of 28 isolates was tested at Virologic, Inc (San Francisco) in 2005 using the PhenoSense™ methodology which constructs recombinant viruses by incorporating the amplified PR and RT regions of the isolates into a luciferase-containing retroviral vector [2]. A second panel of 29 isolates has recently been tested at Monogram Biosciences, Inc (formerly Virologic) in August 2007 using the same PhenoSense™ assay. Results from both panels are merged in the present analysis.

The combined panel of isolates includes;

- six isolates each from the HIV-1 subtypes A, B, C, D, F, G.
- three isolates each from the HIV-1 subtype H.
- six isolates each from HIV-1 recombinant forms CRF01_AE, CRF02_AG, and CRF12_BF.
- All isolates were from treatment-naïve patients and did not contain any mutations known to confer resistance to PIs, NRTIs, and NNRTIs as shown by genotypic analysis.

Drug phenotypic susceptibility was measured and compared to that of the HIV-1 subtype B NL4-3 strain used as reference by Monogram Biosciences. The PhenoSense™ *in vitro* susceptibility values (EC₅₀) of each PI against this reference strain were: amprenavir (APV) 10.7nM, atazanavir (ATV) 1.6 nM, darunavir (DRV) 1.1 nM, indinavir (IDV) 6.8 nM, lopinavir (LPV) 2.9 nM, nelfinavir (NFV) 7.3 nM, ritonavir (RTV) 19 nM, saquinavir (SQV) 2.5 nM, and tipranavir (TPV) 89 nM (NB: these values are the mean of the *in vitro* susceptibility obtained in the 2005 and 2007 studies. These means were not used to calculate mean fold-changes).

The variation in susceptibility of the different subtypes was reported as a fold-change from the reference strain. The genotype of each isolate was also compared to the sequence of this reference strain and differences were noted as polymorphic mutations.

Several TPV-specific mutation scores (TPV unweighted score [3], TPV weighted score [4], Parkin score [5], Rega score [6], Calvez score [7]) were computed to examine the relationship between each subtype and its corresponding susceptibility to TPV.

Results

Table 1: TPV mean *in vitro* susceptibility for each subtype and CRF along with associated mean fold-change vs HIV-1 subtype B NL4-3 reference strain.

Subtype or CRF	TPV <i>in vitro</i> susceptibility (PhenoSense™ assay) EC ₅₀ (nM)		Fold-change	
	Mean*	Range	Mean*	Range
A	180.3	35–497	1.8	0.6–4.3
B	98.5	48–193	1.0	0.8–1.7
C	124.2	48–124	1.3	0.8–2.1
D	121.2	61–214	1.3	0.6–1.8
F	194.8	74–340	2.0	1.2–2.9
G	103.0	31–180	1.0	0.5–1.6
H	123.6	38–209	1.6	0.9–2.0
CRF01_AE	108.8	41–245	1.2	0.6–2.1
CRF02_AG	115.7	55–211	1.3	0.9–1.8
CRF12_BF	133.1	28–328	1.3	0.5–2.8
All non-B subtypes	133.9	–	1.4	–

* Arithmetic mean of the *in vitro* susceptibility and fold-changes obtained in the 2005 and 2007 studies.

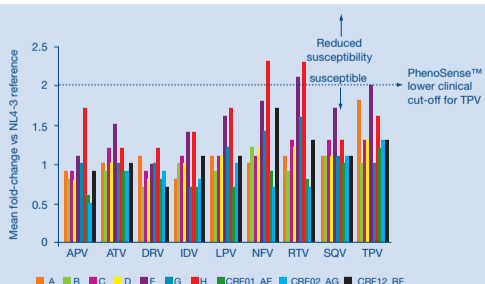


Figure 1: Mean fold-change of tested PIs (vs NL4-3 reference) for each subtype and CRF

Table 2: Median and range of TPV mutation scores for each subtype and CRF (using different algorithms)

Subtype or CRF	TPV unweighted score (TUW) ³		TPV weighted score (TW) ⁴		Parkin score ⁵	REGA score ⁶		Calvez score ⁷		
	Median	Range	Median	Range		Median	Range	Median	Range	
A	3.0	3–4	5.0	5–5	3.0	2.5–3	1.375	1.0–1.5	2.531	2.53–2.88
B	0.0	0–1	0.0	0–3	0.0	0–1	0.125	–0.25–0.75	0.0	0–2.10
C	2.0	2–4	4.0	4–5	2.0	1–3	0.875	0.75–1.25	2.091	2.08–2.09
D	1.5	1–2	3.0	1–4	1.0	0.5–2	0.625	0.5–0.75	0.651	0–2.08
F	2.0	1–3	3.0	3–5	1.5	1–2.5	0.750	0.5–1.5	1.716	1.54–2.88
G	3.0	3–3	5.0	5–5	1.5	1.5–1.5	1.625	1.25–1.75	2.091	2.09–2.53
H	4.0	4–5	5.0	5–10	3.0	3–3.5	1.500	1.25–1.5	2.091	1.64–2.98
CRF01_AE	3.0	2–4	5.0	4–7	2.5	2–3	1.500	1–1.75	2.531	2.09–2.53
CRF02_AG	3.0	3–4	5.0	5–5	2.5	2.5–3.5	1.375	1–2.25	2.311	2.09–2.53
CRF12_BF	1.0	0–1	3.0	0–3	1.0	0–1.5	0.375	0–0.75	1.224	0–1.44

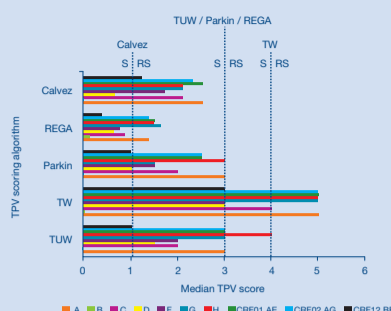


Figure 2: Median score for each subtype & CRF using different scoring algorithms

The threshold between susceptibility (S) and reduced susceptibility (RS) regions for each scoring algorithm is indicated as a vertical line on Fig. 2. For example, the threshold for the TPV weighted mutation score (TW) is set at a score of 4. According to TW, only subtypes B, C, D, and F are predicted to be in the susceptibility region

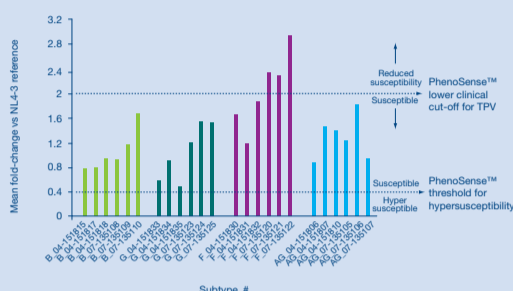


Figure 3: Phenotypic susceptibility to TPV among subtypes G & F, and CRF02_AG isolates

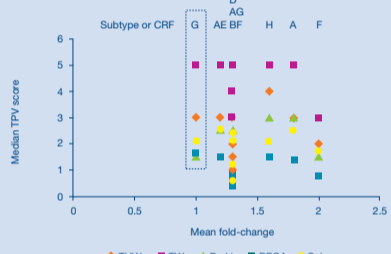


Figure 4: Lack of correlation between fold-change of non-B subtype isolates and different TPV mutation scores (each vertical line on the graph represents a subtype or CRF)

Discussion

- Tipranavir maintains a level of susceptibility against wild-type HIV-1 non-subtype B isolates that is overall similar to that observed with subtype B isolates (mean EC₅₀ of 134 nM across all 51 non-B isolates vs 99 nM for all six B isolates).
- All non-subtype B isolates have a mean phenotypic fold-change lower than 2 for TPV i.e. below the PhenoSense™ lower clinical cutoff value for TPV (determined mainly from subtype B viruses). Only subtype F isolates have a mean fold-change of 2.0 i.e. exactly at the cutoff value.
- As reported by Abecasis et al, some subtype F isolates show evidence of lower susceptibility to TPV (Fig. 3). However, unlike that group, we could not confirm any clear trends towards hypersusceptibility for subtype G and CRF02_AG isolates.
- TPV mutation scores have been developed mainly using data from subtype B isolates. Our study shows that they do not correlate well when applied to wild-type, non-subtype B isolates. For example;
 - All scoring methods (except Calvez score) predict subtype F to be well within the susceptibility area defined by each algorithm, contrasting with the evidence of lower susceptibility to TPV among some subtype F isolates.
 - Conversely, the TUW, TW, and Calvez scoring methods predict CRF02_AG to be at or above the susceptibility threshold defined by each algorithm, yet all individual CRF02_AG isolates in this study are below the PhenoSense™ lower clinical cut-off for TPV.

Conclusions

- All HIV-1 non-B subtypes included in this study had a mean fold-change at or below the TPV lower clinical cutoff value of 2 as determined by the PhenoSense™ assay.
- TPV mutation scores have been developed mainly using data from subtype B isolates. Our study shows that they do not correlate well when applied to wild-type, non-subtype B isolates as evidenced by the lack of any discernible trend between mean phenotypic fold-change and mean TPV mutation scores across the subtype/CRF panel (Fig. 4).
- By extension, it is unlikely that TPV mutation scores will accurately predict the susceptibility of TPV to non-subtype B isolates from PI-experienced patients having developed PI-resistant mutations.
- There is a need to develop superior scoring algorithms targeted more specifically toward prevalent non-subtype B strains.

References

1. Abecasis AB, et al. Antiviral Ther 2006; 11: 581-589
2. Petropoulos CJ, et al. Antimicrob Agents Chemother 2000; 44(4): 920-928
3. Baxter JD, et al. J Virol 2006; 80(21): 10794-10801
4. Scherer J, et al. Paper at 11th European AIDS Conference, Madrid, Spain, 2007
5. Parkin N, et al. 13th CROI, Denver, USA, 2006 (Abstract 637)
6. Van Laethem K, et al. Algorithm for the use of genotypic HIV resistance data, Rega v7.1.1, (July 2007, www.kuleuven.ac.be/regacev/links)
7. Flandre P, et al. Antiviral Ther 2007; 12: S83

Acknowledgements

The authors would like to thank Dr. Neil Parkin and the personnel at Monogram Biosciences, Inc. for the selection of isolates and performance of the genotype and phenotype assays.