Survey of Brecanavir (BCV) and Other Protease Inhibitor (PI) Susceptibility to HIV-1 Variants Containing PI Resistance-Associated Amino Acid Substitutions (RAS)

C Craig[1], M StClair[2], P Yates[1], M Ait-Khaled[3]

[1] GlaxoSmithKline, Stevenage, UK; [2] GlaxoSmithKline, RTP, NC, USA; [3] GlaxoSmithKline, Greenford, UK

Abstract

Background: Brecanavir, a PI with high-affinity binding, shows >100-fold improved binding over currently approved PIs and 10-fold over TMC-114. BCV exhibits picomolar antiviral activity and a resilient resistance profile due to an adaptive thiazolylmethyl moiety. The cross-resistance profile of BCV, in Phase II development, was studied.
Methods: 94 HIV-1 clinical isolates selected according to the presence of known PI RAS (defined by IAS USA, Fall, 2005) were analysed for IC_{50} and fold change (FC) versus all approved PI except NFV using PhenoSense™.
Results: The mean number of major PI RAS per isolate was 2.6 (range: 0 – 6; median 3). The median IC_{50}s for the other PI were significantly above that for BCV (geometric mean IC_{50} nM: BCV:0.4; APV:97, IDV:99, LPV:56, ATV:20, TPV:110). The median fold change (FC) for BCV was lower than for all PIs apart from TPV (median FC: BCV:2.1, APV:8.6, IDV:15, LPV:19, ATV:15, TPV:1.3). At the clinically derived cut-offs for IDV/r and LPV/r (10-FC), ATV/r (5.2-FC) and TPV/r (4-FC), 59%, 57%, 70% and 15% of isolates were resistant to these PI respectively. For BCV, the 'resistant' proportions were 10% at 10-FC, 20% at 5.2-FC and 28% at 4-FC. The mean number of major PI RAS for isolates with FCs ≥ and < cut off were, 3.24/1.82, 3.17/1.95, 3.03/1.75 and 3.71/2.46 for IDV, LPV, ATV and TPV respectively; and 4.33/2.47, 3.53/2.42 and 3.27/2.41 for BCV at the same FCs (10, 5.2 and 4 respectively). The TPV/r mean major PI RAS ratio at 4-FC was reproduced by BCV at FC 6.5-7.0.
Discussion: BCV shows greater potency than APV, IDV, LPV, ATV and TPV and lower FC than all PIs except TPV/r, which shows relatively low intrinsic potency and low clinical cut-off. The BCV virologic profile along with early signs of good tolerability and efficacy in subjects with PI-resistant isolates further supports its on-going development.

Introduction

- In order to maintain the preliminary success of combination antiretroviral therapies (ART) against HIV-1 infection, it is important to continue to develop drugs to address the needs of highly ART-experienced individuals, whose viral infection is relatively poorly controlled due to reduced drug susceptibility. Over time, the number of these individuals and the extent of their drug resistance are increasing.
- One strategy is to develop drugs against previously validated targets such that the new drugs suffer relatively little cross-resistance to viruses containing several resistance-associated mutations selected during treatment with earlier drugs of the same class. Improved potency and selectivity of these compounds might also reduce class-associated toxicities.
- Brecanavir (BCV, GW640385) is a product of this approach.
- Brecanavir exhibits:
  - Superior binding affinity compared with previous protease inhibitors (PIs) (Ki 15x10^{-15}M) [Hazen, 2003]
  - Very high intrinsic potency *in vitro*:
    - (geometric mean IC_{50}: clinical isolates in PBMC 0.03 nM [n = 26; RT read out]; HXB2 strain in MT4-MTT assay: 0.66 nM [n = 27, data on file]; Monogram Biosciences median IC_{50} in PI-sensitive clinical isolates: 0.1 nM [n=25; range: 0.1 – 0.2 nM].
  - Robust activity versus viruses containing several PI RASs[1,2,3,4]
- The objective of the current study was to assess the cross-resistance profile for brecanavir using the combined data from two surveys presented previously[1,3] and extended to include the recently approved atazanavir (ATV) and tipranavir (TPV).

Methods

A total of 105 viruses were studied previously in the two surveys: one set[1] (n=55) comprised of 'worst case' viruses selected from a Monogram Biosciences Inc. panel based on presence of PRO mutations at codons 10, 32, 46, 47, 50, 54, 84 and/or 90 (major+minor mutation: mean=11, range 7-18). The other set[3] (n=50) included viruses selected based on the presence of single, double, triple and multiple protease RASs including mutations at residues 32, 33, 46, 47, 50 (V and L), 54, 82, 84, and 90 (median of 6, major: 2; minor: 4). Sequence analysis was performed at Monogram, Inc., South San Francisco, CA., by a thermocycling method using fluorescent dye labeled dideoxynucleotide chain terminator chemistry. RASs were classified based on the IAS USA resistance table [Johnson et al, 2005].

All drug susceptibility assays were performed by scientists at Monogram, Inc., South San Francisco, CA [Petropoulos, 2000]. The mean percent inhibition for each drug concentration was determined and used to calculate the IC_{50}. The FC in drug susceptibility was determined by comparing the IC_{50} for the subject virus to the IC_{50} for the drug-sensitive reference virus containing the PRO and RT sequences of the NL4-3 strain of HIV-1.

Conclusions

- Even in the presence of PI RASs, brecanavir showed a high intrinsic in vitro potency (median IC_{50}: 300 pM).
  - Brecanavir shows potency resilience in the face of multiple PI RASs.
- Preliminary assessments suggest increased numbers of PI RASs are required to generate higher-level fold changes (>2.1-fold).
- The presence of amino acid substitutions at protease residues 32, 47, 48 and 50 were associated with highest geometric mean fold changes (≥5.5-fold) but also with higher numbers of PI RASs.
- Overall, brecanavir shows characteristics suitable for use in a highly PI-experienced population and warrants further study in clinical trials.

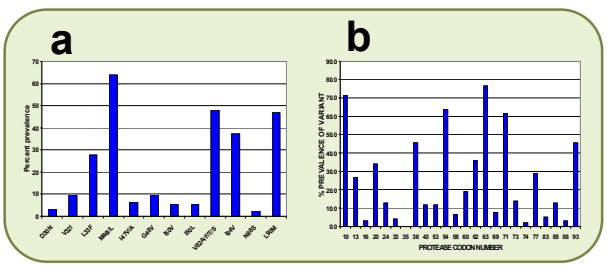
Discussion

- The set of 94 viruses studied represent a cross-section of variants from those containing a moderate number of PI RASs to 'worst-case' PI-resistant viruses. However, the viral population analyzed here does not represent an unbiased cross-section of currently circulating viruses. There was positive selection within the population (e.g. to include viruses containing I50V and I50L) and also for variants containing I84V in the context of both several and moderate numbers of PI RASs. Detailed analysis of the gag region was not undertaken.
- Despite the presence of significant proportions of viruses containing major PI RASs, the median IC_{50} for BCV remained sub-nanomolar. Thus intrinsic *in vitro* potency of BCV was retained. The next most potent PI observed in this analysis was ATV, with median IC_{50} 78.5-fold higher than that for BCV. The median fold change observed with BCV was also less than those found with other PIs except for TPV, which gave a slightly lower fold change.
- The analysis of the distribution of numbers of PI RASs above and below the median BCV fold change showed that there were more of both major and total PI RASs at the higher fold changes (>2.1-fold).
- In terms of the presence or absence of particular mutations, the presence of mutations at protease residues 32, 47, 48 and 50 gave the highest geometric mean fold changes (5.5 – 9.5-fold) to BCV, while the presence of the I84V substitution showed a modest overall geometric mean increase (4.4-fold). A high number of PI RASs again appears to be a feature of higher fold change.
- The clinical cut-off for BCV has not yet been derived. However, using the cut-off values established for other PIs, and comparing the proportions of viruses in the population that had fold changes below these values, it is clear that BCV shows considerable advantages over the other PIs apart from TPV, which shows a slight advantage at the lower TPV cut-off value (2-fold) but this is not so marked at the higher cut-off value (8-fold).
- In another study of current circulating viruses from the HPR20001 clinical trial[4], the I84V mutation was observed as a significant factor for increased FC. Despite the high prevalence of I84V in the current study (n=35), this finding was not repeated. The number of total PI RASs in the current study was less than that found in the HPR20001 viral population, therefore it's possible that I84V is relevant to BCV resistance only in the context of additional resistance-associated mutations.

Results

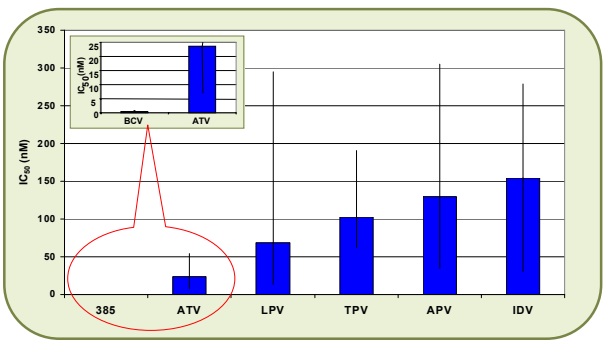
Of the 105 viruses included in the two original surveys, 11 could not be recultured for ATV and TPV analysis, therefore unless otherwise stated, analyses were carried out using the 94 complete data sets.

Figure 1. Prevalence of IAS USA[5] (a) major and (b) minor RASs



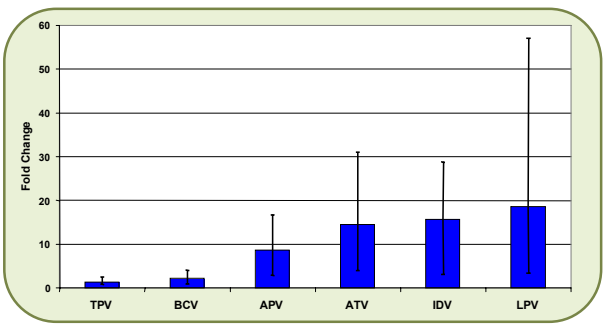
The profile of the genotypes indicates a typical population of PI-experienced persons. There was a median of 3 (IQR 2-3) major PI RASs and 9 (IQR 6-11) total RASs.

Figure 2. Median IC_{50} (nM) with interquartile ranges.



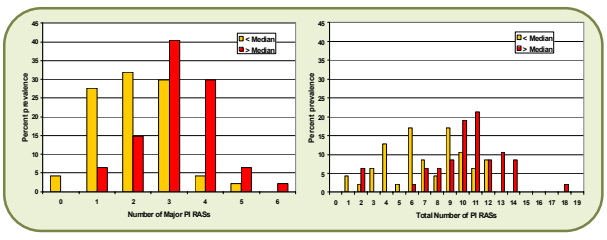
A high level of intrinsic *in vitro* potency was preserved with BCV (median IC_{50}: 0.3 nM), despite the presence of multiple PI RASs. Among the PIs tested, BCV showed the lowest IC_{50} values with a narrow range of activity.

Figure 3. Median fold change with interquartile ranges



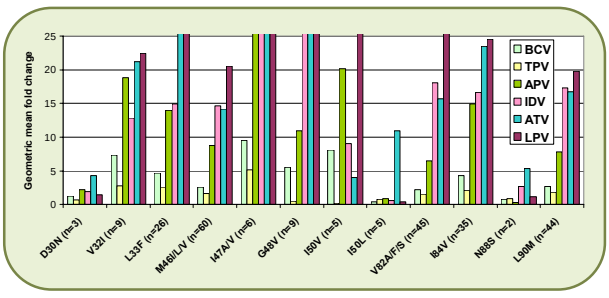
The median fold-change for BCV was lower than for the other PIs apart from TPV.

Figure 4. Distribution of numbers of major and total PI RASs in viruses with fold changes above and below the median fold change.



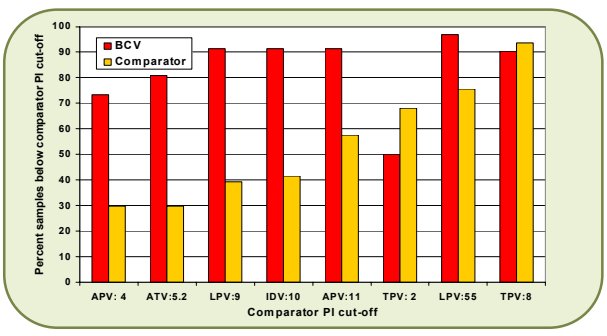
The median fold change to BCV for this set of viruses was found to be 2.1-fold. The number of major and total PI RASs is higher in viruses with BCV-susceptibilities above the median fold change (2.1) compared with those with values below the median fold change (median major PI RASs: below median BCV FC: 2; above median BCV FC: 3; t-test p<0.05; median total PI RASs below median BCV FC: 7; above median BCV FC: 11; t-test p<0.05).

Figure 5. Association between the presence of specified mutations and geometric mean fold change



The highest levels of resistance to BCV were observed in viruses with V32I, I47A/V, G48V and I50V, with geometric mean fold changes 7.4, 9.5, 5.5 and 8.2 respectively. These were in the context of median 4, 4.5, 4 and 3 major PI RASs respectively. Hypersusceptibility to TPV (geometric mean fold change 0.22) was observed with I50V.

Figure 6. Proportion of viruses with susceptibilities below comparator PI clinical cut-off values



Clinical data are not available to determine a clinical cut-off for BCV. However, BCV showed robust retention of activity below values over a range of clinical cut-offs established for other boosted PIs. Apart from TPV, more viruses retained susceptibility to BCV at levels below the fold changes analysed compared with the relevant comparator PI.

References

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