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Authors
Dolling, David I
Dunn, David T
Sutherland, Katherine A
et al.

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Low frequency of genotypic resistance in HIV-1-infected patients failing an atazanavir-containing regimen: a clinical cohort study

David I. Dolling1*, David T. Dunn1, Katherine A. Sutherland2, Deenan Pillay3, Jean L. Mbisa2, Chris M. Parry4, Frank A. Post5, Caroline A. Sabin3 and Patricia A. Cane2 on behalf of the UK HIV Drug Resistance Database (UKHDRD) and the UK Collaborative HIV Cohort Study (UK CHIC)†

1Medical Research Council Clinical Trials Unit, London, UK; 2Virus Reference Department, Public Health England, London, UK; 3Research Department of Infection, University College London, London, UK; 4MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda; 5School of Medicine, Kings College London, London, UK

*Corresponding author. Tel: 020-7670-4933; E-mail: d.dolling@ctu.mrc.ac.uk
†For further details please see the Acknowledgements section.

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Objectives: To determine protease mutations that develop at viral failure for protease inhibitor (PI)-naive patients on a regimen containing the PI atazanavir.

Methods: Resistance tests on patients failing atazanavir, conducted as part of routine clinical care in a multicentre observational study, were randomly matched by subtype to resistance tests from PI-naive controls to account for natural polymorphisms. Mutations from the consensus B sequence across the protease region were analysed for association and defined using the IAS-USA 2011 classification list.

Results: Four hundred and five of 2528 (16%) patients failed therapy containing atazanavir as a first PI over a median (IQR) follow-up of 1.76 (0.84–3.15) years and 322 resistance tests were available for analysis. Recognized major atazanavir mutations were found in six atazanavir-experienced patients (P < 0.001), including I50L and N88S. The minor mutations most strongly associated with atazanavir experience were M36I, M46I, F53L, A71V, V82T and I85V (P < 0.05). Multiple novel mutations, I15S, L19T, K43T, L63P/V, K70Q, V77I and L89I/T/V, were also associated with atazanavir experience.

Conclusions: Viral failure on atazanavir-containing regimens was not common and major resistance mutations were rare, suggesting that adherence may be a major contributor to viral failure. Novel mutations were described that have not been previously documented.

Keywords: HIV, drug resistance mutations, naive patients, protease inhibitors, virological failure

Introduction

Since the approval of the protease inhibitor (PI) atazanavir by the FDA (and ‘positive opinion’ by the European Medicines Agency) in 2003 it has become a widely used third agent in combination antiretroviral therapy (ART) and is recommended as a possible component of first-line therapy in national and international HIV treatment guidelines.1,2 Previous research investigating the development of drug resistance to atazanavir in a non-randomized controlled trial setting is limited by its focus on patients with prior PI exposure3,4 or the development of major PI mutations.5 Randomized controlled trials6,7 have shown that the key substitutions in patients failing atazanavir-containing regimens are 150L and N88S.7 This study examines patterns of resistance to atazanavir in a large clinical cohort of patients with no prior PI exposure.

Methods

Genotypic resistance test results of population sequencing of the pol gene were obtained from the UK HIV Drug Resistance Database (UKHDRD)8 and linked to pseudo-anonymized clinical information from the UK Collaborative HIV Cohort Study (UK CHIC).9 The UKHDRD and UK CHIC were established in 2001 and collect, respectively, all resistance tests conducted at public laboratories within the UK as part of routine clinical care, and clinical information routinely collected on HIV-positive individuals aged over 16 years who have attended one of the 15 collaborating centres for care at any time from 1996.

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Patients who were PI naive, regardless of other ART experience, and who were initiated on a regimen containing atazanavir between 2001 and 2010 were included for analysis in the study. Virological failure was defined as: (i) two consecutive viral loads >1000 copies/mL after previous suppression (≤400 copies/mL); (ii) one viral load >1000 copies/mL after previous suppression followed by a treatment change; or (iii) one viral load >1000 copies/mL after 180 days on ART without suppression. Valid resistance tests were samples taken after at least 30 days of atazanavir treatment and within 30 days of discontinuing the drug. Where more than one resistance test was available for a patient, the latest test was selected for analysis. For each index resistance test we attempted to select at random 10 resistance tests from ART-experienced, PI-naive patients with the same viral subtype as controls in background polymorphisms. When fewer than 10 such tests could be identified they were supplemented by tests from ART-naive patients with the same viral subtype. For rare subtypes such as CRF11 and CRF13 (where CRF stands for circulating recombinant form), sufficient control samples to match in a 1:10 ratio were not available. Apart from these factors, control samples were selected stochastically and no other demographic information was used in the process.

Protease mutations were categorized based on the IAS-USA 2011 mutation list as either major or minor atazanavir mutations.10 The prevalence of each amino acid substitution was compared between index and control resistance tests using Fisher’s exact test. Mutations are reported if they are: (i) classified as a major atazanavir mutation or (ii) observed in at least two index resistance tests and with a significantly (P < 0.05) higher frequency compared with control tests. Subtype was inferred from the pol sequence using the REGA subtyping algorithm.11 No adjustments were made for the multiple hypothesis tests performed, on the basis that this is an exploratory analysis to identify associations to be verified in other datasets. All analyses were conducted in Stata/IC 12.1 software (StataCorp LP, College Station, TX, USA).

Results

A total of 2865 patients used atazanavir as their first PI, and of these, 2528 (88%) had sufficient viral load data for the determination of virological failure status, with a median (Q1–Q3) follow-up of 1.76 (0.84–3.15) years. Four hundred and five (16%) patients experienced virological failure, of whom 322 (80%) had one or more valid resistance tests (total of 455 tests).

Patient characteristics relating to the index and control resistance tests are shown in Table 1. Among index patients, atazanavir was typically boosted with ritonavir (n = 251; 78%) and used in combination with two or more nucleoside reverse transcriptase inhibitors (NRTIs) (n = 294; 91%). Common NRTI drugs for those on boosted atazanavir included tenofovir (n = 207; 82%), emtricitabine (n = 131; 52%), lamivudine (n = 168; 27%), abacavir (n = 55; 22%) and zidovudine (n = 25; 10%). Those patients on unboosted atazanavir typically had tenofovir (n = 46; 65%), emtricitabine (n = 26; 37%), lamivudine (n = 27; 38%), abacavir (n = 16; 23%) and zidovudine (n = 14; 20%) as part of their NRTI backbone. The median (Q1–Q3) time on atazanavir at the time of resistance sampling was 1.28 (0.49–2.73) years and the median (Q1–Q3) time on ART was 2.80 (1.00–5.87) years. Atazanavir was a component of the initial combination ART regimen for 117 (36.3%) patients; for the remainder it was first used in the second-line or subsequent regimen.

Only 6 (1.9%) index patients experiencing virological failure had a major atazanavir-associated mutation, all in isolation: 150L (n = 3), 184V (n = 2) and N88V (n = 1) (Table 2). However, 184V was also observed in 0.32% of control samples, indicating that it cannot necessarily be concluded that it was directly selected by atazanavir. Only 6 of 49 recognized minor atazanavir-associated mutations were significantly associated with atazanavir exposure, although many of these are expected to be selected only as compensatory mutations. The remaining 43 minor atazanavir mutations were either not detected in this dataset (L10C, K20V, E34Q, F53Y, IS4LM/I/A, A71L, G73C/T, V82F and I93M) or were not significantly associated with atazanavir exposure (L10I/FV, G16E, K20R/M/I/T, L24I, V32I, L33I/FV, M36L/V, M46L, G48V, I54V, D60E, I62V, I64L/M/V, A71L/T, G73S/A, V82A/I, L90M and I93L). Nine other mutations at six codons (15, 19, 43, 63, 70 and 89) not currently recognized as being associated with atazanavir were significantly more frequent in the index resistance tests compared with the control tests. This included three different substitutions (I, T and V) at codon 89. There was no evidence of a difference between ritonavir-boosted atazanavir and unboosted therapy in terms of individual mutations or in the number of mutations selected (data not shown).

Conclusive evidence that a mutation was directly selected by atazanavir in an individual patient requires demonstration of its absence in a baseline resistance test. However, a baseline test was available in only 137/322 (43%) index patients, limiting the value of this approach. Specifically, none of the patients who were observed to have I155S, K43T, IS0L, V82T, IS4V, N88S or L89V mutations at virological failure had a baseline resistance test. In almost all patients in whom a baseline resistance test was available, the common M36I and L63P polymorphisms preexisted prior to exposure to atazanavir.

Discussion

Our findings show that very few patients failing a therapy containing atazanavir are likely to have developed one of the major protease resistance mutations (150L, 184V and N88S) that confer high-level phenotypic resistance to this drug. However, there was an increased frequency of several other mutations, including I155S, L19T, K43T, IS0P/V, K70Q and L89I/T/V, which are not recognized to be associated with atazanavir by the most recent IAS classification. The finding of multiple amino acid substitutions at codons 63 and 89 strengthens the likelihood that mutations at these sites may have a structural impact on the protease enzyme. Two other mutations, K43T and L89V, have previously been shown to decrease susceptibility to PIs in combination with other mutations.12 The low frequency of atazanavir-associated resistance mutations in those with virological failure suggests that atazanavir-induced changes at these sites may have a structural impact on the protease enzyme. Two other mutations, K43T and L89V, have previously been shown to decrease susceptibility to PIs in combination with other mutations.12 The low frequency of atazanavir-associated resistance mutations in those with virological failure suggests that atazanavir-induced changes at these sites may have a structural impact on the protease enzyme. Two other mutations, K43T and L89V, have previously been shown to decrease susceptibility to PIs in combination with other mutations.12

There are two major approaches to examining associations between specific mutations and resistance to individual drugs using clinical data. The first is to examine the effect on clinical response of pre-existing mutations selected by prior exposure to other drugs within that class. In the current context, Van et al.16 studied 62 patients with prior PI exposure who took boosted atazanavir. They described eight mutations that had an adverse effect on viral load reduction at 3 months, but only two of these (M46I and...
and I84V) coincided with those identified in our analysis. The second approach, the one adopted in the present paper, is to identify mutations that appear to have emerged under selective drug pressure. Other cohort studies using this approach have tended to focus on highly pre-treated patients, whose patterns of resistance are likely to differ materially from those receiving the drug as the first within that class. However, several patients in the IMPACT study, which evaluated atazanavir-containing regimens, were PI naive at enrolment. Although there was a high frequency (7/39) of PI substitutions (including L33I/F and L90M, which we did not observe) in this subgroup, many patients were on NRTI-sparing dual-PI regimens and it is not possible to assess whether the substitutions observed were selected by atazanavir or by the other PI in the regimen.

Table 1. Characteristics of the study population

| Variable                        | Atazanavir-experienced (index) patients n = 322 | PI-naive (control) patients n = 3209 |
|---------------------------------|-----------------------------------------------|-------------------------------------|
| Gender                          |                                               |                                     |
| female                          | 86 (26.7)                                     | 920 (28.7)                          |
| male                            | 236 (73.3)                                    | 2289 (71.3)                         |
| Exposure source                 |                                               |                                     |
| homosexual/bisexual             | 158 (49.1)                                    | 1598 (49.8)                         |
| heterosexual                    | 132 (41.0)                                    | 1238 (38.6)                         |
| other                           | 20 (6.2)                                      | 129 (4.0)                           |
| unknown                         | 12 (3.7)                                      | 244 (7.6)                           |
| Ethnicity                       |                                               |                                     |
| white                           | 161 (50.0)                                    | 1621 (50.5)                         |
| black                           | 114 (35.4)                                    | 1022 (31.8)                         |
| other                           | 26 (8.1)                                      | 230 (7.2)                           |
| unknown                         | 21 (6.5)                                      | 336 (10.5)                          |
| Subtype                         |                                               |                                     |
| A                               | 21 (6.5)                                      | 210 (6.5)                           |
| B                               | 179 (55.6)                                    | 1790 (55.8)                         |
| C                               | 54 (16.8)                                     | 540 (16.8)                          |
| AE                              | 5 (1.6)                                       | 50 (1.6)                            |
| AG                              | 16 (5.0)                                      | 160 (5.0)                           |
| other                           | 16 (5.0)                                      | 149 (4.6)                           |
| unknown                         | 31 (9.6)                                      | 310 (9.7)                           |
| Exposure to atazanavir (years)  | Median: 1.28 Q1–Q3: 0.49–2.73 NA                | Median: NA Q1–Q3: NA                |
| Time on ART (years)\(^b\)       | 2.80 (1.00–5.87)                              | 2.63 (0.89–5.31)                    |
| Age (years)                     | 40.85 (35.73–46.01)                           | 36.94 (31.94–42.91)                 |
| Year of resistance test         | 2008 (2006–2009)                              | 2003 (2000–2006)                    |
| Baseline RNA (log10 copies/mL)  | 4.59 (3.75–5.17)                              | 4.40 (3.41–4.90)                    |
| Baseline CD4 (cells/mm\(^3\))  | 289 (121–434)                                 | 303 (170–470)                       |
| RNA at resistance sample (log10 copies/mL) | 3.36 (2.59–4.47) | 4.20 (3.41–4.90) |
| CD4 at resistance sample (cells/mm\(^3\)) | 315 (200–499) | 290 (173–438)  |

NA, not applicable.
\(^a\)Matching not possible for rare CRFs such as CRF11 and CRF13.
\(^b\)In the control group, based on the 2467 (77%) ART-experienced patients.

Our study has several limitations. Despite the large sample of patients who took atazanavir as their first PI, the fact that there were relatively few treatment failures limits the power to detect new resistance mutations. Also, resistance data were not available for all treatment failures and baseline resistance tests were not available for the majority of the subjects. Therefore, some of the mutations detected may have pre-existed as polymorphisms or transmitted mutations. Furthermore, bulk sequencing means that some mutations may have existed as an undetectable minority population at baseline. Indeed, if these pre-existing polymorphisms/mutations reduced the susceptibility to atazanavir and increased the likelihood of virological failure an association would have been induced even if atazanavir did not select for this mutation. Finally, as an exploratory analysis a large number of potential
associations were analysed and some of the significant results reported could be false positives.

Further research should continue to monitor drug resistance at virological failure in patients failing first-line atazanavir to assess whether there is an accumulation of further accessory or compensatory mutations in the absence of major resistance mutations. This could result in a re-examination of the concept that atazanavir has a distinct resistance profile with little cross-resistance to other PIs, and that these can therefore be used effectively after failure on atazanavir.6

Table 2. Mutation prevalence by atazanavir experience

| Position | Amino acid | Atazanavir-experienced (index) patients n = 322 | PI-naive (control) patients n = 3209 | P value |
|----------|------------|----------------------------------------------|-----------------------------------|---------|
|          |            | n    | %    | n    | %    |         |
| Major atazanavir mutations |            |      |      |      |      |         |
| 50       | L          | 3    | 0.9  | 0    | 0.0  | 0.001   |
| 84       | V          | 2    | 0.6  | 10   | 0.3  | 0.300   |
| 88       | S          | 1    | 0.3  | 0    | 0.0  | 0.091   |
| Minor atazanavir mutations |            |      |      |      |      |         |
| 36       | I          | 164  | 50.9 | 1436 | 44.8 | 0.035   |
| 46       | I          | 7    | 2.2  | 10   | 0.3  | <0.001  |
| 53       | L          | 3    | 0.9  | 2    | 0.1  | 0.007   |
| 71       | V          | 20   | 6.2  | 111  | 3.5  | 0.019   |
| 82       | T          | 2    | 0.6  | 0    | 0.0  | 0.008   |
| 85       | V          | 3    | 0.9  | 3    | 0.1  | 0.012   |
| Not currently recognized atazanavir mutations |            |      |      |      |      |         |
| 15       | S          | 2    | 0.6  | 2    | 0.1  | 0.044   |
| 19       | T          | 11   | 3.4  | 56   | 1.8  | 0.050   |
| 43       | T          | 2    | 0.6  | 2    | 0.1  | 0.045   |
| 63       | P          | 149  | 46.3 | 1232 | 38.4 | 0.007   |
| 63       | V          | 11   | 3.4  | 45   | 1.4  | 0.015   |
| 70       | Q          | 2    | 0.6  | 2    | 0.1  | 0.044   |
| 89       | I          | 6    | 1.9  | 22   | 0.7  | 0.037   |
| 89       | T          | 3    | 0.9  | 1    | 0.0  | 0.003   |
| 89       | V          | 2    | 0.6  | 1    | 0.0  | 0.023   |

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UKHDRD Steering Committee

Celia Aitken, Gartnavel General Hospital, Glasgow; David Asboe, Anton Pozniak, Chelsea and Westminster Hospital, London; Daniel Webster, Royal Free NHS Trust, London; Patricia Cane, Public Health England, Porton Down; Hannah Castro, David Dunn (Co-Chair), David Dolling; Esther Fearhull, Kholoud Porter, MRC Clinical Trials Unit, London; David Chadwick, South Tees Hospitals NHS Trust, Middlesbrough; Duncan Churchill, Brighton and Sussex University Hospitals NHS Trust; Duncan Clark, St Bartholomew’s and The London NHS Trust; Simon Collins, HIV i-Base, London; Valerie Delpech, Public Health England, Centre for Infections, London; Anna Maria Geretti, Institute of Infection and Global Health, University of Liverpool; David Goldberg, Health Protection Scotland, Glasgow; Antony Hale, Leeds Teaching Hospitals NHS Trust; Stéphane Hué, University College London; Steve Kaye, Imperial College London; Paul Kellam, Wellcome Trust Sanger Institute and UCL Medical School; Linda Lazarus, Expert Advisory Group on AIDS Secretariat, Public Health England, London; Andrew Leigh-Brown, University of Edinburgh; Nicola Mackie, Imperial NHS Trust; Chloe Orkin, St Bartholomew’s Hospital, London; Philip Rice, St George’s Healthcare Trust, London; Deenan Pillay (Co-Chair), Andrew Phillips, Caroline Sabin, University College London Medical School; Erasmus Smit, Public Health England, Birmingham Heartlands Hospital; Kate Templeton, Royal Infirmary of Edinburgh; Peter Tilston, Manchester Royal Infirmary; William Tong, Guy’s and St Thomas’ NHS Foundation Trust, London; Ian Williams, Martimer Market Centre, London; Hongyi Zhang, Addenbrooke’s Hospital, Cambridge; Mark Zuckerman, King’s College Hospital, London.

Centres contributing data to the UKHDRD

Clinical Microbiology and Public Health Laboratory, Addenbrooke’s Hospital, Cambridge (Jane Greatorex); HIV/GUM Research Laboratory, Chelsea and Westminster Hospital, London (Adrian Wildfire); Virus Reference Department, Public Health England Public Health Laboratory, Birmingham Heartlands Hospital, Birmingham (Erasmus Smit); Public Health England London (Siobhan O’Shea, Jane Mullen); Virus Reference Department, Public Health England Public Health Laboratory, Birmingham Heartlands Hospital, Birmingham (Erasmus Smit); Public Health England London (Tamyo Mbisa); Imperial College Health NHS Trust, London (Alison Cox); King’s College Hospital, London (Richard Tandy); Medical Microbiology Laboratory, Leeds Teaching Hospitals NHS Trust (Tony Hale, Tracy Fawcett); Specialist Virology Centre, Liverpool (Mark Hopkins, Lynn Ashton); Department of Clinical Virology, Manchester Royal Infirmary, Manchester (Peter Tilston); Department of Virology, Royal Free Hospital, London (Claire Booth, Ana Garcia-Diaz); Edinburgh Specialist Virology Centre, Royal Infirmary of Edinburgh (Jill Shepherd); Department of Infection and Tropical Medicine, Royal Victoria Infirmary, Newcastle (Matthias L. Schmid, Brendan Payne); South Tees Hospitals NHS Trust, Middlesbrough (David Chadwick); St George’s Hospital, London (Philip Hay, Philip Rice, Mary Paynter); Department of Virology, St Bartholomew’s
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**Coordinating Centre for the UKHDRE**

Medical Research Council Clinical Trials Unit (MRC CTU), London (Kate Coughlin, David Dolling, David Dunn, Esther Fearnhill, Lorraine Fradette, Kholoud Porter).

**UK CHIC Steering Committee**

Jonathan Ainsworth, Jane Anderson, Abdel Babiker, David Chadwick, Valerie Delpech, David Dunn, Martin Fisher, Brian Gazzard, Richard Gilson, Mark Gompels, Phillip Hay, Teresa Hill, Margaret Johnson, Stephen Kegg, Clifford Leen, Mark Nelson, Chloe Orkin, Adrian Palfreeman, Andrew Phillips, Deenan Pillay, Frank Post, Caroline Sabin (PI), Memory Sachikonye, Achim Schwenk, John Walsh.

**Central coordination for UK CHIC**

UCL Research Department of Infection and Population Health, Royal Free Campus, London (Teresa Hill, Susie Huntington, Sophie Jose, Andrew Phillips, Caroline Sabin, Alicia Thornton); Medical Research Council Clinical Trials Unit (MRC CTU), London (David Dunn, Adam Glabay).

**Participating centres in UK CHIC**

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**Transparency declarations**

None to declare.

**Disclaimer**

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