Prevalence of gp160 polymorphisms known to be related to decreased susceptibility to temsavir in different subtypes of HIV-1 in the Los Alamos National Laboratory HIV Sequence Database

Margaret Gartland1, Eric Arnoult2, Brian T. Foley3, Max Lataillade4, Peter Ackerman4, Cyril Llamoso4 and Mark Krystal4*

1ViiV Healthcare, Five Moore Drive, Research Triangle Park, NC 27709, USA; 2GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA; 3Los Alamos National Laboratory, T-6 Theoretical Biology and Biophysics, Mail Stop K710, Los Alamos, NM 87544, USA; 4ViiV Healthcare, 36 East Industrial Road, Branford, CT 06405, USA

*Corresponding author. E-mail: mark.r.krystal@viivhealthcare.com

Received 11 March 2021; accepted 25 June 2021

Background: Fostemsavir, a prodrug of the gp120-directed attachment inhibitor temsavir, is indicated for use in heavily treatment-experienced individuals with MDR HIV-1. Reduced susceptibility to temsavir in the clinic maps to discrete changes at amino acid positions in gp160: S375, M426, M434 and M475.

Objectives: To query the Los Alamos National Laboratory (LANL) HIV Sequence Database for the prevalence of polymorphisms at gp160 positions of interest.

Methods: Full-length gp160 sequences (N = 7560) were queried for amino acid polymorphisms relative to the subtype B consensus at positions of interest; frequencies were reported for all sequences and among subtypes/circulating recombinant forms (CRFs) with >10 isolates in the database.

Results: Among 239 subtypes in the database, the 5 most prevalent were B (n = 2651, 35.1%), C (n = 1626, 21.5%), CRF01_AE (n = 674, 8.9%), A1 (n = 273, 3.6%) and CRF02_AG (n = 199, 2.6%). Among all 7560 sequences, the most prevalent amino acids at positions of interest (S375, 73.5%; M426, 82.1%; M434, 88.2%; M475, 89.9%) were the same as the subtype B consensus. Specific polymorphisms with the potential to decrease temsavir susceptibility (S375H/I/M/N/T/Y, M426L/P, M434I/K and M475I) were found in <10% of isolates of subtypes D, G, A6, BC, F1, CRF07_BC, CRF08_BC, 02A, CRF06_cpx, F2, 02G and 02B. S375H and M475I were predominant among CRF01_AE (S375H, 99.3%; M475I, 76.3%; consistent with previously reported low temsavir susceptibility of this CRF) and 01B (S375H, 71.7%; M475I, 49.5%).

Conclusions: Analysis of the LANL HIV Sequence Database found a low prevalence of gp160 amino acid polymorphisms with the potential to reduce temsavir susceptibility overall and among most of the common subtypes.

Introduction

Fostemsavir, a prodrug of temsavir, is a first-in-class gp120-directed attachment inhibitor indicated for heavily treatment-experienced adults with MDR HIV-1 failing their current antiretroviral regimen due to resistance, intolerance or safety considerations. In vitro, temsavir is active against CCR5-, CXCR4- and dual-tropic envelopes and against almost all HIV-1 subtypes tested, except for circulating recombinant form (CRF) 01_AE and group O viruses. Preclinical and clinical studies have shown that specific substitutions at four amino acid positions in gp160, S375H/I/M/N/T, M426L/P, M434I/K and M475I, have the potential to reduce susceptibility to temsavir and these were predefined for analysis in the fostemsavir Phase 3 study (BRIGHTE). S375Y was subsequently shown to substantially decrease in vitro temsavir susceptibility in an HIV-1 LAI envelope. In vitro studies also found that L116P and A204D decrease temsavir susceptibility, although changes at these positions have not been observed in clinical studies.

Notably, in the Phase 3 BRIGHTE study, baseline polymorphisms at these sites did not preclude virological response to 8 day
### Table 1. Frequencies of HIV-1 subtypes (≥10 isolates) and most frequent (≥10%) predefined polymorphisms at amino acid positions of interest in gp160 in the LANL HIV Sequence Database

| Subtype       | N     | Percentage in database | No predefined polymorphisms<sup>a</sup>, n (% of subtype) | Predefined polymorphisms<sup>a</sup> amino acid, n (% of subtype) |
|---------------|-------|------------------------|----------------------------------------------------------|-------------------------------------------------------------|
| All<sup>f</sup> | 7560  | 4679 (61.9)            |                                                          | S375H 921 (12.2)                                             |
| Group M       |       |                        |                                                          |                                                             |
| B             | 2651  | 35.1                   | 1709 (64.5)                                              | S375T<sup>d</sup> 473 (17.8)                               |
| C             | 1626  | 21.5                   | 1255 (77.2)                                              | M434I 216 (13.3)                                            |
| CRF01_AE      | 674   | 8.9                    | 2 (0.3)                                                  | M475I 514 (76.3)                                            |
| A1            | 273   | 3.6                    | 156 (57.1)                                               | M434I 95 (34.8)                                             |
| CRF02_AG      | 199   | 2.6                    | 145 (72.9)                                               | M434I 36 (18.1)                                             |
| BF1           | 136   | 1.8                    | 87 (64.0)                                                | S375T<sup>f</sup> 15 (11.0)                                |
| D             | 133   | 1.8                    | 105 (78.9)                                               | M426L 18 (13.2)                                             |
| G             | 128   | 1.7                    | 114 (89.1)                                               |                                                             |
| A6            | 108   | 1.4                    | 99 (91.7)                                                |                                                             |
| BC            | 106   | 1.4                    | 92 (86.8)                                                |                                                             |
| 01B           | 99    | 1.3                    | 16 (16.2)                                                | S375H 71 (71.7)                                             |
| A1D           | 98    | 1.3                    | 64 (65.3)                                                | M434I 49 (49.5)                                             |
| A1C           | 79    | 1.0                    | 57 (72.2)                                                | M434I 20 (25.3)                                             |
| F1            | 63    | 0.8                    | 48 (76.2)                                                |                                                             |
| CRF07_BC      | 61    | 0.8                    | 57 (93.4)                                                |                                                             |
| CRF08_BC      | 41    | 0.5                    | 38 (92.7)                                                |                                                             |
| 01BC          | 39    | 0.5                    | 16 (41.0)                                                | S375H 19 (48.7)                                             |
| 02A1          | 30    | 0.4                    | 13 (43.3)                                                | M434I 7 (17.9)                                              |
| A1CD          | 30    | 0.4                    | 18 (60.0)                                                | M434I 9 (23.1)                                              |
| 0107          | 26    | 0.3                    | 11 (42.3)                                                | S375H 14 (53.8)                                             |
| CD            | 26    | 0.3                    | 17 (65.4)                                                | M434I 11 (42.3)                                             |
| CRF11_cpx     | 25    | 0.3                    | 19 (76.0)                                                | M434I 5 (19.2)                                              |
| CRF12_BF      | 18    | 0.2                    | 6 (33.3)                                                 | M426L 8 (44.4)                                              |
| CRF35_AD      | 17    | 0.2                    | 13 (76.5)                                                | M426L 2 (11.8)                                              |
| 02A           | 16    | 0.2                    | 15 (93.8)                                                |                                                             |
| A1G           | 14    | 0.2                    | 11 (78.6)                                                | M434I 2 (14.3)                                              |
| CRF06_cpx     | 13    | 0.2                    | 10 (76.9)                                                |                                                             |
| F2            | 13    | 0.2                    | 12 (92.3)                                                |                                                             |
| CRF71_BF1     | 12    | 0.2                    | 8 (66.7)                                                 | S375T<sup>e</sup> 3 (25.0)                                 |
| 02G           | 11    | 0.1                    | 10 (90.9)                                                | M434I 2 (20.0)                                              |
| 01C           | 10    | 0.1                    | 8 (80.0)                                                 | M475I 2 (20.0)                                              |
| 02B           | 10    | 0.1                    | 8 (80.0)                                                 |                                                             |
| CRF13_cpx     | 10    | 0.1                    | 4 (40.0)                                                 | M426L 2 (20.0)                                              |
| Group O       | 48    | 0.63                   | 1 (2.1)                                                  | S375H 47 (97.9)                                             |

Continued...
Supplementary data at analysed as described in the Supplementary Methods (available as html) through 31 December 2019 were obtained in FASTA format and Database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.subtypes, recombinants and CRFs included in the LANL HIV Sequence Database to probe for polymorphisms present at amino acid positions known to be associated with reduced susceptibility to temsavir.

### Methods

‘Filtered Web Alignments’ for the ENV region of the genome for all HIV-1 subtypes, recombinants and CRFs included in the LANL HIV Sequence Database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html) through 31 December 2019 were obtained in FASTA format and analysed as described in the Supplementary Methods (available as Supplementary data at JAC Online).

### Results and discussion

This analysis was conducted on 7560 full-length HIV-1 envelope sequences including 239 subtypes, recombinants and CRFs (35 with ≥10 isolates, 130 with 2–9 isolates and 74 with only 1 isolate in the database). Subtypes B and C were the most prevalent subgroups (Table 1). Sequences originated from 94 countries [2469 (33%) from Africa, 1898 (25%) from the Americas, 1670 (22%) from Asia and 1216 (16%) from Europe], extending our understanding of the potential global incidence of polymorphisms known to be associated with temsavir susceptibility beyond previous publications.

Despite the wide variability observed in gp160 sequences, the most prevalent amino acids at positions known to be associated with temsavir susceptibility were the same as the subtype B consensus [L116 (99.3%), A204 (97.3%), S375 (73.5%), M426 (82.1%), M434 (88.2%) and M475 (89.9%) (Table 2)], which is consistent with their location in conserved regions of gp160. In 4679 (61.9%) isolates, there were no predefined polymorphisms known to impact temsavir susceptibility (S375H/I/M/N/T/Y, M426L/P, M434I/K and M475I) (Table 1). For 12 subtypes (D, G, A6, BC, F1, CRF07_BC, CRF08_BC, 02A, CRF06_cpx, F2, 02G and 02B), the frequency of any polymorphism at amino acid positions of interest was <10% (Table 1). In particular, L116P, A204D, M434K and S375Y were rare (<0.1% of all sequences in the database).

### Table 1. Continued

| Subtype       | N  | Percentage in database | No predefined polymorphisms\(^a\), n (% of subtype) | Predefined polymorphisms\(^a\) | amino acid | n (% of subtype) |
|---------------|----|------------------------|-----------------------------------------------|-----------------------------|------------|-----------------|
| Group N       | 11 | 0.15                   | 0 (0)                                         | S375M                       | 11 (100)   |
|               |    |                        |                                               | M426L                       | 11 (100)   |
|               |    |                        |                                               | M434I                       | 11 (100)   |

\(^a\)S375H/I/M/N/T/Y, M426L/P, M434I/K and M475I are the predefined polymorphisms. Predefined polymorphisms listed in column are only shown if they represented ≥10% of total isolates.

\(^b\)LANL database entries to 31 December 2019.

\(^c\)Including 239 subtypes, recombinants and CRFs and 135 sequences with subtype reported as unclassified or no subtype reported.

\(^d\)Other polymorphisms at positions of interest: M426R in 696 (22.5%) isolates (this polymorphism had no effect on temsavir susceptibility in an LAI background; fold change in IC\(_{50}\)=1.0).

\(^e\)Other polymorphisms at positions of interest: M426L in 29 (21.3%) isolates (this polymorphism had no effect on temsavir susceptibility in an LAI background; fold change in IC\(_{50}\)=0.86).

\(^f\)Other polymorphisms at positions of interest: M426R in 5 (16.7%) isolates (this polymorphism had no effect on temsavir susceptibility in an LAI background; fold change in IC\(_{50}\)=0.86).

\(^g\)Other polymorphisms at positions of interest: M426I in 2 (20.0%) isolates (this polymorphism had no effect on temsavir susceptibility in an LAI background; fold change in IC\(_{50}\)=3.3).

\(^h\)Other polymorphisms at positions of interest: M426V in 10 (33.3%) isolates (this polymorphism had no effect on temsavir susceptibility in an LAI background; fold change in IC\(_{50}\)=1.8).

\(^i\)Other polymorphisms at positions of interest: M426S in 44 (91.7%) isolates and M434L in 37 (77.1%) isolates.

fostemsavir treatment added to a failing antiretroviral regimen and did not affect durability of response to fostemsavir plus optimized background therapy through Week 96.\(^{1,11}\) To date, no phenotypic cut-off or genotypic algorithm has been established that can reliably predict clinical efficacy outcomes for fostemsavir-based therapy.\(^{2,3,9}\) Nevertheless, it is important to understand the natural prevalence of polymorphisms that could affect susceptibility to fostemsavir in the global population with HIV-1. Here, we analysed full-length gp160 sequences from all available isolates in the Los Alamos National Laboratory (LANL) HIV Sequence Database to probe for polymorphisms present at amino acid positions known to be associated with reduced susceptibility to temsavir.

This analysis was conducted on 7560 full-length HIV-1 envelope sequences including 239 subtypes, recombinants and CRFs (35 with ≥10 isolates, 130 with 2–9 isolates and 74 with only 1 isolate in the database). Subtypes B and C were the most prevalent subgroups (Table 1). Sequences originated from 94 countries [2469 (33%) from Africa, 1898 (25%) from the Americas, 1670 (22%) from Asia and 1216 (16%) from Europe], extending our understanding of the potential global incidence of polymorphisms known to be associated with temsavir susceptibility beyond previous publications.\(^{7,12–15}\)

Despite the wide variability observed in gp160 sequences, the most prevalent amino acids at positions known to be associated with temsavir susceptibility were the same as the subtype B consensus [L116 (99.3%), A204 (97.3%), S375 (73.5%), M426 (82.1%), M434 (88.2%) and M475 (89.9%) (Table 2)], which is consistent with their location in conserved regions of gp160.\(^{10}\) In 4679 (61.9%) isolates, there were no predefined polymorphisms known to impact temsavir susceptibility (S375H/I/M/N/T/Y, M426L/P, M434I/K and M475I) (Table 1). For 12 subtypes (D, G, A6, BC, F1, CRF07_BC, CRF08_BC, 02A, CRF06_cpx, F2, 02G and 02B), the frequency of any polymorphism at amino acid positions of interest was <10% (Table 1). In particular, L116P, A204D, M434K and S375Y were rare (<0.1% of all sequences in the database).

Although they are associated with large reductions in temsavir susceptibility in vitro, these polymorphisms were rarely observed in fostemsavir clinical studies (to date, there are no reports of L116P or A204D and only two cases of S375Y in BRIGHTE participants), suggesting a lack of viral fitness at the population level. These findings are consistent with previous analyses showing that most baseline isolates identified during fostemsavir clinical development were highly susceptible (IC\(_{50}\)<10 nM) to temsavir.\(^{6}\)

The only predefined amino acid polymorphism known to impact susceptibility to temsavir that was present in >10% of sequences in the database was S375H (12.2%) (Table 1). Most isolates with S375H (820/921, 89.0%) were CRF01_AE, 01B, 01BC, 0107 or group O (Table 2 and Table S1 (available as Supplementary data at JAC Online)). Prevalence of M475I was also high in CRF01_AE, 01B, 01BC and 0107, together making up 81.8% (583 isolates) of the 713 (9.4%) isolates with M475I in the database. Among 842 (11.1%) isolates with envelope genes containing
Table 2. Frequencies of all polymorphisms at targeted positions in gp160 in the LANL HIV Sequence Database among all isolates (N = 7560) and subtypes with ≥2% prevalence

| Polymorphisms, n (%) | Position | amino acid | all isolates | B, N = 2651 | C, N = 1626 | CRF01_AE, N = 674 | A1, N = 273 | CRF02_AG, N = 199 |
|----------------------|----------|------------|--------------|-------------|--------------|-------------------|-------------|-------------------|
| L116                 | L        | 7506 (99.3)| 2632 (99.3)  | 1612 (99.1) | 670 (99.4)   | 272 (99.6)        | 199 (100)   |
|                      | I        | 29 (0.4)   | 11 (0.4)     | 11 (0.7)    | 4 (0.6)      | 0                 | 0           |
|                      | X        | 8 (0.1)    | 2 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | F        | 4 (<0.1)   | 1 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | P        | 4 (<0.1)   | 0            | 1 (<0.1)    | 0           | 1 (0.4)           | 0           |
|                      | V        | 4 (<0.1)   | 1 (<0.1)     | 1 (<0.1)    | 0           | 0                 | 0           |
|                      | –        | 1 (<0.1)   | 1 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | A        | 1 (<0.1)   | 0            | 1 (<0.1)    | 0           | 0                 | 0           |
|                      | E        | 1 (<0.1)   | 0            | 0           | 0           | 0                 | 0           |
|                      | M        | 1 (<0.1)   | 1 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | R        | 1 (<0.1)   | 1 (<0.1)     | 0           | 0           | 0                 | 0           |
| A204                 | A        | 7358 (97.3)| 2564 (96.7)  | 1580 (97.2) | 666 (99.8)  | 265 (97.1)        | 193 (97.0)  |
|                      | T        | 102 (1.3)  | 52 (2.0)     | 16 (1.0)    | 5 (0.7)     | 3 (1.1)           | 5 (2.5)     |
|                      | S        | 50 (0.7)   | 18 (0.7)     | 21 (1.3)    | 0           | 1 (0.4)           | 1 (0.5)     |
|                      | X        | 14 (0.2)   | 8 (0.3)      | 1 (<0.1)    | 0           | 1 (0.4)           | 0           |
|                      | P        | 8 (0.1)    | 1 (<0.1)     | 4 (0.2)     | 1 (0.1)     | 1 (0.4)           | 0           |
|                      | G        | 7 (0.1)    | 3 (0.1)      | 1 (<0.1)    | 0           | 1 (0.4)           | 0           |
|                      | V        | 5 (<0.1)   | 2 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | –        | 4 (<0.1)   | 2 (<0.1)     | 1 (<0.1)    | 0           | 0                 | 0           |
|                      | D        | 4 (<0.1)   | 0            | 2 (0.1)     | 0           | 1 (0.4)           | 0           |
|                      | L        | 4 (<0.1)   | 1 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | E        | 1 (<0.1)   | 0            | 0           | 0           | 0                 | 0           |
| S375                 | S        | 5559 (73.5)| 1964 (74.1)  | 1492 (91.8) | 2 (0.3)     | 253 (92.7)        | 178 (89.4)  |
|                      | H        | 921 (12.2) | 18 (0.7)     | 1 (<0.1)    | 669 (99.3)  | 0                 | 0           |
|                      | T        | 701 (9.3)  | 473 (17.8)   | 75 (4.6)    | 0           | 12 (4.4)          | 11 (5.5)    |
|                      | N        | 135 (1.8)  | 102 (3.8)    | 11 (0.7)    | 1 (0.1)     | 0                 | 0           |
|                      | I        | 97 (1.3)   | 49 (1.8)     | 19 (1.2)    | 0           | 0                 | 3 (1.5)     |
|                      | M        | 93 (1.2)   | 27 (1.0)     | 14 (0.9)    | 0           | 6 (2.2)           | 4 (2.0)     |
|                      | X        | 16 (0.2)   | 10 (0.4)     | 1 (<0.1)    | 0           | 0                 | 1 (0.5)     |
|                      | R        | 8 (0.1)    | 4 (0.2)      | 2 (0.1)     | 0           | 1 (0.4)           | 0           |
|                      | F        | 7 (0.1)    | 0            | 6 (0.4)     | 0           | 0                 | 0           |
|                      | –        | 4 (<0.1)   | 2 (<0.1)     | 2 (0.1)     | 0           | 0                 | 0           |
|                      | Y        | 4 (<0.1)   | 0            | 1 (<0.1)    | 2 (0.3)     | 0                 | 0           |
|                      | A        | 3 (<0.1)   | 0            | 0           | 0           | 1 (0.4)           | 0           |
|                      | K        | 3 (<0.1)   | 1 (<0.1)     | 1 (<0.1)    | 0           | 0                 | 1 (0.5)     |
|                      | V        | 3 (<0.1)   | 1 (<0.1)     | 1 (<0.1)    | 0           | 0                 | 0           |
|                      | C        | 2 (<0.1)   | 0            | 0           | 0           | 0                 | 0           |
| M426                 | M        | 6208 (82.1)| 1777 (67.0)  | 1512 (93.0) | 648 (96.1)  | 248 (90.8)        | 188 (94.5)  |
|                      | R        | 692 (9.2)  | 596 (22.5)   | 11 (0.7)    | 2 (0.3)     | 2 (0.7)           | 0           |
|                      | L        | 434 (5.7)  | 212 (8.0)    | 73 (4.5)    | 19 (2.8)    | 7 (2.6)           | 7 (3.5)     |
|                      | S        | 52 (0.7)   | 2 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | K        | 45 (0.6)   | 36 (1.4)     | 0           | 0           | 0                 | 1 (0.5)     |
|                      | T        | 38 (0.5)   | 13 (0.5)     | 12 (0.7)    | 0           | 0                 | 1 (0.5)     |
|                      | V        | 32 (0.4)   | 3 (0.1)      | 4 (0.2)     | 1 (0.1)     | 13 (4.8)          | 0           |
|                      | I        | 21 (0.3)   | 2 (<0.1)     | 6 (0.4)     | 3 (0.4)     | 2 (0.7)           | 1 (0.5)     |
|                      | X        | 9 (0.1)    | 4 (0.2)      | 1 (<0.1)    | 0           | 0                 | 0           |
|                      | –        | 7 (0.1)    | 1 (<0.1)     | 2 (0.1)     | 0           | 0                 | 0           |
|                      | A        | 5 (<0.1)   | 1 (<0.1)     | 0           | 0           | 0                 | 0           |
|                      | W        | 5 (<0.1)   | 1 (<0.1)     | 4 (0.2)     | 0           | 0                 | 0           |
|                      | Q        | 4 (<0.1)   | 1 (<0.1)     | 0           | 0           | 1 (0.4)           | 1 (0.5)     |

Continued
two polymorphisms of interest, most (588 isolates) contained S375H and M475I and, of these, 81.5% (479 isolates) were subtype CRF01_AE (Table S2), consistent with previous observations. 

In vitro testing of S375H and M475I polymorphisms in an HIV-1 LAI background revealed a profound impact on temsavir susceptibility (IC50 >16 000-fold higher than WT) (Table S2), which is consistent with results obtained with the PhenoSense Entry assay6 and likely to impact the response to fostemsavir-based therapy. In BRIGHTE, two participants in the randomized cohort had HIV-1 CRF01_AE virus at screening; both virologically suppressed at Week 96.1 One with S375H and M475I at baseline did not respond to 8 days of fostemsavir functional monotherapy. The other with S375N at baseline received placebo during the blinded period. These findings will be most relevant for people living with HIV in Southeast Asia, East Asia and Australia, where HIV-1 CRF01_AE is predominant or increasing.16–18 Frequency of other double polymorphisms at gp160 amino acid positions of interest was low among group M sequences. Only S375T and M434I was present in >1% of the database; this combination was associated with a 5-fold reduction in temsavir susceptibility in vitro (Table S2).

Some of the polymorphisms known to impact temsavir susceptibility were present at a frequency of >10% in certain subtypes, recombinants or CRFs. Frequency of M434I was >10% in 12 group M subtypes, recombinants or CRFs and >20% in 6: A1, A1D, A1C, A1CD, 02A1 and CRF13_cpx (Table 1). Although it has been shown to have a minor impact on temsavir susceptibility in vitro, M434I

| Table 2. Continued |
|--------------------|
| **Polymorphisms, n (%)** | **Subtypes** |
| Position | amino acid | all isolates | B, N = 2651 | C, N = 1626 | CRF01_AE, N = 674 | A1, N = 273 | CRF02_AG, N = 199 |
| M434 | M | 6669 (88.2) | 2492 (94.0) | 1382 (85.0) | 640 (95.0) | 170 (62.3) | 162 (81.4) |
| L | 731 (9.7) | 134 (5.1) | 216 (13.3) | 27 (4.0) | 95 (34.8) | 36 (18.1) |
| T | 32 (0.4) | 16 (0.6) | 5 (0.3) | 0 | 3 (1.1) | 0 |
| V | 27 (0.4) | 2 (0.1) | 11 (0.7) | 0 | 2 (0.7) | 0 |
| X | 12 (0.2) | 1 (0.1) | 2 (0.1) | 0 | 1 (0.4) | 0 |
| K | 6 (0.1) | 0 | 1 (0.1) | 0 | 0 |
| F | 4 (0.1) | 0 | 0 | 0 | 0 |
| A | 3 (0.1) | 1 (0.1) | 0 | 1 (0.1) | 0 | 1 (0.5) |
| P | 3 (0.1) | 0 | 2 (0.1) | 0 | 0 |
| R | 3 (0.1) | 1 (0.1) | 1 (0.1) | 0 | 0 |
| G | 2 (0.1) | 1 (0.1) | 1 (0.1) | 0 | 0 |
| N | 2 (0.1) | 0 | 1 (0.1) | 0 | 0 |
| Y | 2 (0.1) | 0 | 2 (0.1) | 0 | 0 |
| Q | 1 (0.1) | 0 | 0 | 0 | 0 |
| S | 1 (0.1) | 0 | 1 (0.1) | 0 | 0 |

| M475 | M | 6800 (89.9) | 2609 (98.4) | 1605 (98.7) | 147 (21.8) | 265 (97.1) | 196 (98.5) |
| L | 713 (9.4) | 33 (1.2) | 11 (0.7) | 514 (76.3) | 5 (1.8) | 2 (1.0) |
| T | 13 (0.2) | 0 | 2 (0.1) | 6 (0.9) | 1 (0.4) | 0 |
| L | 8 (0.1) | 2 (0.1) | 1 (0.1) | 3 (0.4) | 0 |
| V | 7 (0.1) | 1 (0.1) | 2 (0.1) | 2 (0.3) | 1 (0.4) | 0 |
| D | 4 (0.1) | 1 (0.1) | 1 (0.1) | 1 (0.1) | 0 |
| R | 4 (0.1) | 2 (0.1) | 1 (0.1) | 0 | 0 |
| X | 4 (0.1) | 1 (0.1) | 0 | 1 (0.1) | 1 (0.4) | 0 |
| K | 3 (0.1) | 2 (0.1) | 0 | 0 | 0 | 1 (0.5) |
| – | 1 (0.1) | 0 | 0 | 0 | 0 | 0 |
| G | 1 (0.1) | 0 | 1 (0.1) | 0 | 0 |
| N | 1 (0.1) | 0 | 1 (0.1) | 0 | 0 |
| W | 1 (0.1) | 0 | 1 (0.1) | 0 | 0 |

The top row for all amino acid positions shows the reference HXB2 sequence. Rows in bold show a frequency of at least 10%. Rows in italic identify specific amino acid polymorphisms known to be associated with reduced susceptibility to temsavir.1,3,8–10

aAmino acid positions 116, 204, 375, 426, 434 and 475.

bLANL database entries to 31 December 2019. Accessed October 2020.
Prevalence of polymorphisms in subtype B isolates was consistent with Bouba et al. and BRIGHTE study baseline data. Polymorphisms present in ≥10% of subtype B isolates included S375T (17.8%) and M426R (22.5%) (Table 2). S375T had no measurable effect on in vitro temsavir susceptibility in an LAI background and has also been previously shown to have less of an impact on temsavir susceptibility than S375M and S375H in clinical isolates derived from participants in the proof-of-concept fostemsavir monotherapy study. Also consistent with Bouba et al., a particularly high prevalence of M426R in subtype B was observed (22.5%). This amino acid polymorphism, also common in subtype B recombinants (BF1, 21.3%; 01B, 7.1%) but not in non-B subtypes, has not previously been associated with reduced temsavir susceptibility and did not affect in vitro temsavir susceptibility in an LAI background.

Data on the small number of non-M HIV-1 groups showed the consensus for group N isolates to be 375M, 426L and 434I (Table 1). Similarly, 47 of 48 group O isolates carried S375H and 7 of these also had M434I, consistent with previously reported data. The impact of these amino acids on temsavir susceptibility in a group N or O background has not been confirmed in vitro; however, it seems likely that these viruses would have reduced temsavir susceptibility. Indeed, two group O clinical isolates tested in vitro showed no phenotypic susceptibility to temsavir concentrations up to 2000 nM.

This analysis is limited by the fact that sequences in the LANL database are a selected set with a number of unknown ascertainment biases. Subtypes, recombinants and CRFs were based on classification reported by sequence contributors and are thus not consistent and were not verified in our study. Furthermore, we focused only on polymorphisms at amino acid positions that have previously been shown to be associated with reduced temsavir susceptibility. It is possible that polymorphisms at other positions may also play a role in the response to fostemsavir-based therapy.

In conclusion, the overall frequency of polymorphisms previously associated with the potential to reduce susceptibility to temsavir was low. Patterns of gp160 polymorphisms did vary across different subtypes, consistent with the previously observed wide range of phenotypic temsavir susceptibilities. CRF01_AE, group O and group N isolates had a prevalence of multiple relevant polymorphisms likely to impact responses to fostemsavir-based therapy. Similar patterns of baseline gp160 polymorphisms and phenotypic temsavir susceptibility were seen in the Phase 3 BRIGHTE study, but these factors were not reliably predictive of response to short-term fostemsavir functional monotherapy and did not affect durability of response to fostemsavir plus optimized background therapy through 96 weeks of treatment. Thus, although the HIV-1 envelope gp160 sequence is variable, the overall prevalence of specific temsavir-relevant amino acid polymorphisms is low and should not pose a barrier to successful treatment with fostemsavir-based regimens for most heavily treatment-experienced people with MDR HIV-1.

Acknowledgements
We would like to thank the patients, scientists and clinicians who contributed to the development of the LANL database resource.

Funding
This work was supported by Viiv Healthcare. In addition, editorial assistance (see below) was funded by Viiv Healthcare.

Transparency declarations
M.G., E.A., M.L., P.A. and M.K. are employees of Viiv Healthcare or GlaxoSmithKline, which markets fostemsavir, and as such receive salary, grants of GlaxoSmithKline stock and additional benefits from the companies. At the time of this work, C.L. was an employee of Viiv Healthcare. B.T.F.: none to declare.

The funder of the study had a role in the study design, data collection, data analysis, data interpretation and writing the report.

Editorial assistance was provided under the direction of the authors by Esther Race, PhD, Race Editorial Ltd, and Jennifer Rossi, MA, ELS, MedThink SciCom.

All authors had full access to the data and are responsible for the accuracy and completeness of this report. The corresponding author had final responsibility for the decision to submit for publication.

Author contributions
M.G. contributed to the conception and design of the study, the analysis and interpretation of data, drafting the manuscript and critically revising the manuscript for important intellectual content. E.A. contributed to the design of the study, the analysis of data and critically revising the manuscript for important intellectual content. B.T.F. contributed to the acquisition of data and critically revising the manuscript for important intellectual content. M.L., P.A. and C.L. contributed to the conception and design of the study, the interpretation of data and critically revising the manuscript for important intellectual content. M.K. contributed to the conception and design of the study, the analysis and interpretation of data, drafting the manuscript and critically revising the manuscript for important intellectual content. All authors approved the submitted manuscript for publication.

Supplementary data
Supplementary Methods and Tables S1 and S2 are available as Supplementary data at JAC Online.

References
1. Rukobia (Package Insert). Viiv Healthcare, 2020.
2. Kozal M, Aberg J, Pialoux G et al. Fostemsavir in adults with multidrug-resistant HIV-1 infection. N Engl J Med 2020; 382: 1232–43.
3. Lataillade M, Lalezari JP, Kozal M et al. Safety and efficacy of the HIV-1 attachment inhibitor produg fostemsavir in heavily treatment-experienced individuals: week 96 results of the phase 3 BRIGHTE study. Lancet HIV 2020; 7: e740–51.
Li Z, Zhou N, Sun Y et al. Activity of the HIV-1 attachment inhibitor BMS-626529, the active component of the prodrug BMS-663068, against CD4-independent viruses and HIV-1 envelopes resistant to other entry inhibitors. *Antimicrob Agents Chemother* 2013; 57: 4172–80.

5 Nowicka-Sans B, Gong Y-F, McAuliffe B et al. In vitro antiviral characteristics of HIV-1 attachment inhibitor BMS-626529, the active component of the prodrug BMS-663068. *Antimicrob Agents Chemother* 2012; 56: 3498–507.

6 Gartland M, Zhou N, Stewart E et al. Susceptibility of global HIV-1 clinical isolates to fostemsavir using the PhenoSense® Entry assay. *J Antimicrob Chemother* 2021; 76: 648–52.

7 Saladini F, Giannini A, Giammarino F et al. In vitro antiviral characteristics of HIV-1 attachment inhibitor BMS-626529, the active component of the prodrug BMS-663068. *Antimicrob Agents Chemother* 2012; 56: 3498–507.

8 Gartland M, Zhou N, Joshi SR et al. Viral drug resistance through 48 weeks, in a phase 2b, randomized, controlled trial of the HIV-1 attachment inhibitor prodrug, fostemsavir. *J Acquir Immune Defic Syndr* 2018; 77: 299–307.

9 Ray N, Hwang C, Healy MD et al. Prediction of virological response and assessment of resistance emergence to the HIV-1 attachment inhibitor BMS-626529 during 8-day monotherapy with its prodrug BMS-663068. *J Acquir Immune Defic Syndr* 2013; 64: 7–15.

10 Zhou N, Nowicka-Sans B, McAuliffe B et al. Genotypic correlates of susceptibility to HIV-1 attachment inhibitor BMS-626529, the active agent of the prodrug BMS-663068. *J Antimicrob Chemother* 2014; 69: 573–81.

11 Gartland M, Ackerman P, Mannino F et al. Clinical significance of gp120 polymorphisms, TMR IC50 FC, and HIV-1 subtype in BRIGHTE. *Conference on Retroviruses and Opportunistic Infections*, Boston, MA, USA, 2020. Abstract 503.

12 Lepore L, Fabrizio C, Bavaro DF et al. Gp120 substitutions at positions associated with resistance to fostemsavir in treatment-naive HIV-1-positive individuals. *J Antimicrob Chemother* 2020; 75: 1578–80.

13 Soulé C, Lambert-Niclot S, Fofana DB et al. Frequency of amino acid changes associated with resistance to attachment inhibitor BMS-626529 in R5- and X4-tropic HIV-1 subtype B. *J Antimicrob Chemother* 2013; 68: 1243–5.

14 Fofana DB, Charpentier C, Maiga Al et al. Genetic barrier for attachment inhibitor BMS-626529 resistance in HIV-1 B and non-B subtypes. *J Antimicrob Chemother* 2015; 70: 130–5.

15 Booba Y, Berno G, Fabeni L et al. Identification of gp120 polymorphisms in HIV-1 B subtype potentially associated with resistance to fostemsavir. *J Antimicrob Chemother* 2020; 75: 1778–86.

16 Hemelaar J, Elangovan R, Yun J et al. Global and regional molecular epidemiology of HIV-1, 1990-2015: a systematic review, global survey, and trend analysis. *Lancet Infect Dis* 2019; 19: 143–55.

17 Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. *Curr Opin HIV AIDS* 2019; 14: 153–60.

18 Castley A, Sawleshwarkar S, Varma R et al. A national study of the molecular epidemiology of HIV-1 in Australia 2005-2012. *PLoS One* 2017; 12: e0170601.

19 Nettles RE, Schurmann D, Zhu L et al. Pharmacodynamics, safety, and pharmacokinetics of BMS-663068, an oral HIV-1 attachment inhibitor in HIV-1-infected subjects. *J Infect Dis* 2012; 206: 1002–11.

20 Alessandri-Gradt E, Charpentier C, Leoz M et al. Impact of natural polymorphisms of HIV-1 non-group M on genotypic susceptibility to the attachment inhibitor fostemsavir. *J Antimicrob Chemother* 2018; 73: 2716–20.