**Supplementary materials**

**Supplementary Table 1. Susceptibility of a panel of non-longitudinal virus isolates to GSK3532795, BMS MI B, LPV and ATV**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Virus #** | ***PR*-resistant viruses\*** | **PR genotype (primary PIR mutations in *PR*)** | **Maximum signal relative to WT control, %** | **FC-IC50** |
| **Major** | **Minor** | **GSK3532795** | **BMS MI B** | **LPV** | **ATV** |
| Control | Gag A364V |  |  | 97 | **>1000** | **>1000** | 0.84 | 0.92 |
| 1 | NIH Cat # 11801 | M64L, I54V, V82A, I84V, L90M | L10F, L33F, L35D, K43T, A71V | 40 | 0.23 | 0.28 | **307** | **235** |
| 2 | NIH Cat # 11803 | M36I, M46I, G48V, I50V, I54S, V82A | L10I, L33F, E35D, K43T, A71V | 54 | 0.16 | 0.25 | **355** | **189** |
| 3 | NIH Cat # 11804 | G48V, I54V, V82A, L90M | L10I, A71T | 43 | 0.17 | 0.14 | **27** | **79** |
| 4 | NIH Cat # 11805 | M46I, I84V, L90M | L10I, A71T | 61 | 0.4 | 0.68 | **15** | **11** |
| 5 | NIH Cat # 11806 | V32I, M36I, V82A, L90M | L10I, L33F, A71V | 50 | 0.28 | 0.27 | **442** | **17** |
| 6 | NIH Cat # 11807 | V32I, M36I, I54V, V82A, I84V, L90M | L10F, V11I, L33F, E35G, A71I, G73S | 84 | 0.29 | 0.34 | **123** | **415** |
| 7 | NIH Cat # 11808 | D30N, M36L, I84V, N88D, L90M | L10F, L33F, E35D, K43T | 62 | 0.68 | 0.49 | **24** | **140** |

\* Multiple-cycle assay. *Renilla* luciferase activity was measured 4 days post-transfection. FC-IC50 values >3 are bolded.

ATV, atazanavir; FC-IC50, fold-change in IC50; IC50, drug concentration leading to 50% viral inhibition; LPV, lopinavir; MI, maturation inhibitor; PIR, protease inhibitor resistance; pt, patient; WT, wild-type.

**Supplementary Table 2. GSK3532795 and PI phenotypic susceptibility of Pt04 and Pt09**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **CFB, GSK3532795** | **Single-cycle FC-IC50** | **Multiple-cycle FC-IC50** |
| **Sample name** | **Monogram assay** | **Single-cycle assay (n=2 experiments)** | **Multiple-cycle assay** | **GSK3532795** | **MI A** | **MI B** | **ATV** | **LPV** | **GSK3532795** | **MI B** | **ATV** | **LPV** |
| **Pt04 pre-PI** | 1 | 1 | 1 | 1.44(1.72, 1.16) | 0.62 | 0.59 | 0.54 | 0.73 | 2.0 | 0.92 | 0.46 | 0.43 |
| **Pt04 PTx 1** | 0.48 | 1.15(1.3, 0.98) | 0.35 | 1.7(2.26, 1.13) | 0.52 | 0.83 | **34.9** | **28.7** | 0.70 | 0.87 | **17.0** | **14.5** |
| **Pt04 PTx 2** | 7.46 | 4.17(2.3, 6.1) | 2.1 | **5.46**(3.90, 7.0) | 1.1 | 1.2 | **402** | **152** | **4.2** | 0.95 | **217** | **133** |
| **Pt09 pre-PI** | 1 | 1 | 1 | 0.64(0.70, 0.57) | 0.54 | 0.87 | 0.99 | 1.2 | 1.2 | 0.70 | 0.82 | 0.84 |
| **Pt09 PTx 1** | 27.4 | 1.53(1.40, 1.70) | 1.5 | 0.96(0.96, 0.95) | 0.31 | 0.56 | **14.8** | **9.6** | 1.8 | 0.94 | **4.4** | **5.1** |
| **Pt09 PTx 2** | 3.17 | 2.21(1.90, 2.50) | 0.79 | 1.3(1.31, 1.3) | 0.45 | 0.64 | **60.4** | **28.2** | 1.0 | 0.71 | **9.0** | **4.7** |

FC-IC50 values >3 are bolded.
ATV, atazanavir; CFB, change from baseline; FC-IC50, fold-change in IC50; IC50, drug concentration leading to 50% viral inhibition; LPV, lopinavir; MI, maturation inhibitor; PI, protease inhibitor; PT, post-PI treatment; pt, patient.

**Supplementary Figure 1. Study strategy and data summary**



ATV, atazanavir; CFB, change from baseline; DRV, darunavir; LPV, lopinavir; PIR, protease inhibitor resistance; PR, protease; PTx, post-PI treatment; PTx 1, PTx samples from first timepoint; PTx 2, PTx samples from second timepoint; pts, patients; RAM, resistance-associated mutation.

**Supplementary Figure 2.** **Additional longitudinal isolates (single-cycle assay): susceptibility to GSK3532795, BMS MI A and B, ATV and LPV\***

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\*Samples from pt10 were re-cloned and re-tested as a control for the single-cycle assay. All post-treatment samples contain ≥1 major PI RAM.

ATV, atazanavir; FC-IC50, fold-change in IC50; IC50, drug concentration leading to 50% viral inhibition; LPV, lopinavir; MI, maturation inhibitor; PI, protease inhibitor; PTx, post-PI treatment; pts, patients; RAM, resistance-associated mutation.

**Supplementary Figure 3.** **Mutations in (a) Protease (b) Gag**

**a**

**b**





