

Supplementary Table 4: Resistance-associated aa changes in TK gene of HSV-2 including nt changes, their location, antivirals tested as well as results, methods for phenotypic testing and references.

| Amino acid change | Nucleotide change | Domain | Antivirals tested | | Drug susceptibility (EC ₅₀ ratio of resistant to sensitive viral isolate or EC ₅₀ value) | | | | | Test methods for phenotype | References |
|--------------------------|--------------------------|------------------------------------|---------------------|-----------|--|---------------------------|--------------------------|----------------------------|--------------------------|--|----------------------|
| | | | resistant | sensitive | Aciclovir (ACV) | Brivudin (BVDU) | Penciclovir (PCV) | Foscarnet (FOS) | Cidofovir (CDV) | | |
| deletion aa 1-248 | deletion nt 1-742 | conserved and non-conserved region | ACV | - | not tested | not tested | not tested | not tested | not tested | not tested | 24075863 |
| S32stop* | insertion (T) nt 9 | non-conserved region | ACV, BVDU, PCV | CDV, FOS | 161.0x | 2.6x | >133.0x | 0.4x | 0.7x | plaque reduction assay, TK functional assay | 11160674 |
| R34C | C100T | non-conserved region | ACV | FOS | EC ₅₀ 443.8 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| R51W | T153C | ATP-binding site | PCV | ACV, FOS | EC ₅₀ 2.9 µM | not tested | EC ₅₀ 16.8 µM | EC ₅₀ 58.2 µM | not tested | plaque reduction assay, in vivo test in mice, TK functional assay | 15042649 |
| Y53N | T157A | ATP-binding site | ACV, BVDU, PCV, CDV | FOS | EC ₅₀ >35.5 µM | EC ₅₀ >35.2 µM | EC ₅₀ 32.0 µM | EC ₅₀ 75.9 µM | EC ₅₀ 15.9 µM | modified dye uptake assay including formazan test | 21178503 |
| I54stop* | deletion (C) nt 25 | ATP-binding site | ACV, BVDU, PCV | CDV, FOS | >200.0x | >1.2x | >133.0x | 1.4x | 2.8x | plaque reduction assay, TK functional assay | 11160674 |
| I54stop* | deletion (C) nt 43 | ATP-binding site | ACV, BVDU, PCV | CDV, FOS | 150.0x | 3.1x | 115.0x | 0.7x | 1.7x | plaque reduction assay, TK functional assay | 11160674 |
| G59P | G175C/ G176C | ATP-binding site | ACV | FOS, CDV | EC ₅₀ 94.6 µM | not tested | not tested | EC ₅₀ 67.0 µM | EC ₅₀ 33.3 µM | plaque reduction assay, cell growth assay | 10395866 11238837 |
| G61W | G181T | ATP-binding site | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ 103.3 µM | not tested | plaque reduction assay | 24075863 |
| K62N | A186C | ATP-binding site | ACV | - | EC ₅₀ 211.7 µM | not tested | not tested | not tested | not tested | dye uptake assay | 9697707 |
| S66P | T196C | non-conserved region | ACV | FOS | EC ₅₀ 43.0 µM | not tested | not tested | EC ₅₀ <66.0 µM | not tested | plaque reduction assay, TK functional assay | 23041200 |
| L69stop | deletion (G) nt 180-183 | non-conserved region | ACV | - | EC ₅₀ 122.3 µM | not tested | not tested | not tested | not tested | plaque reduction assay, cell growth assay | 11238837 |
| A72S | G214A/ C215G | non-conserved region | ACV | FOS | EC ₅₀ 43.0 µM | not tested | not tested | EC ₅₀ <66.0 µM | not tested | plaque reduction assay, TK functional assay | 23041200 |
| M86stop | deletion (G) nt 219-222 | non-conserved region | ACV | FOS | EC ₅₀ 887.6 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| A94V | C281T | non-conserved region | ACV, FOS | - | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ >330.0 µM | not tested | plaque reduction assay | 24075863 |
| L98stop | deletion (G) nt 280 | non-conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ 256.7 µM | not tested | plaque reduction assay | 24075863 |
| I101S | T302G | non-conserved region | ACV | FOS | EC ₅₀ 42.0 µM | not tested | not tested | EC ₅₀ 145.0 µM | not tested | plaque reduction assay, TK functional assay | 23041200 |
| Q105P | A314C | non-conserved region | ACV | - | not given | not tested | not tested | not tested | not tested | plaque reduction assay, in vivo test in mice, TK functional assay | 1846499 |
| deletion (323 aa) aa 121 | deletion (969 nt) nt 363 | non-conserved region | ACV | FOS | EC ₅₀ >887.5 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 12367650 |
| T131P | A391C | non-conserved region | ACV | - | EC ₅₀ 62.6 µM | not tested | not tested | not tested | not tested | plaque reduction assay, DNA hybridization assay, dye uptake assay, cell growth assay | 9697707 11238837 |
| Y133F | A398T | non-conserved region | ACV, BVDU, PCV, CDV | FOS | EC ₅₀ >35.5 µM | EC ₅₀ >35.2 µM | EC ₅₀ 32.0 µM | EC ₅₀ 75.9 µM | EC ₅₀ 15.9 µM | plaque reduction assay including formazan test | 21178503 |

| | | | | | | | | | | | |
|-----------|---|----------------------------|------------------------|----------|---------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------|---|----------------------|
| D137stop | G409T/ C411G/A | non-conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ <66.0 µM | not tested | plaque reduction assay | 20733037 |
| L158P | T472C | non-conserved region | ACV | - | not given | not tested | not tested | not tested | not tested | plaque reduction assay, TK functional assay | 12771406 |
| R177W | C529T | nucleoside-binding site | ACV | FOS | EC ₅₀ 65.2 µM | not tested | not tested | EC ₅₀ 198.0 µM | not tested | plaque reduction assay | 8232486 |
| S182D | A544G | non-conserved region | ACV | - | not given | not tested | not tested | not tested | not tested | plaque reduction assay, in vivo test in mice, TK functional assay | 1846499 |
| S182R | G545A | non-conserved region | ACV | - | not given | not tested | not tested | not tested | not tested | plaque reduction assay, in vivo test in mice, TK functional assay | 1846499 |
| S182N | G545C | non-conserved region | ACV | - | EC ₅₀ 57.7 µM | not tested | not tested | not tested | not tested | plaque reduction assay, in vivo test in mice, TK functional assay | 8392322 |
| M183I | G549A/C/U | non-conserved region | ACV, FOS | - | EC ₅₀ 33.0 µM | not tested | not tested | EC ₅₀ <66.0 µM | not tested | plaque reduction assay, TK functional assay | 20733037 23041200 |
| M183stop | deletion (C) nt 450-452 | non-conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ 89.0 µM | not tested | plaque reduction assay | 24075863 |
| M183stop* | deletion (C) nt 463-467 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | EC ₅₀ 297.5 µM | EC ₅₀ 114.8 µM | EC ₅₀ >400.0 µM | EC ₅₀ 70.0 µM | EC ₅₀ 6.7 µM | plaque reduction assay | 11160674 |
| M183stop* | deletion (C) nt 470-472 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | 130.0x | >6.2x | >133.0x | 0.3x | 2.4x | plaque reduction assay, TK functional assay | 11160674 |
| M183stop | deletion (C) nt 519-521 | non-conserved region | ACV, BVDU | - | EC ₅₀ 100.0 µM | EC ₅₀ >150.0 µM | not tested | not tested | not tested | plaque reduction assay, TK functional assay | 1329374 |
| M183stop | deletion (G) nt 433-439 | non-conserved region | ACV | FOS | EC ₅₀ 887.5 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| M183stop* | deletion (T) nt 416-419 | non-conserved region | ACV, BVDU, PCV, FOS | CDV | 500.0x | >1.2x | 81.0x | 11.0x | 0.7x | plaque reduction assay, TK functional assay | 11160674 |
| M183stop* | deletion (T) nt 482-485 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | 130.0x | >6.2x | >133.0x | 0.3x | 2.4x | plaque reduction assay, TK functional assay | 11160674 |
| T184stop | insertion (GG) nt 433-439 | non-conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ <66.0 µM | not tested | plaque reduction assay | 20733037 |
| G201D | G602A | non-conserved region | ACV | FOS | not tested | not tested | not tested | not tested | not tested | TK functional assay, in vivo test in mice | 12771406 |
| R217H | G649A | conserved region | ACV | FOS | EC ₅₀ 42.2 µM | not tested | not tested | EC ₅₀ 81.2 µM | not tested | plaque reduction assay | 9094663 |
| R221C | A658T | conserved region | ACV | FOS | EC ₅₀ 20.0 µM | not tested | not tested | EC ₅₀ 66.6 µM | not tested | plaque reduction assay | 24075863 |
| R221H | A658C | conserved region | ACV, BVDU, PCV, CDV | FOS | EC ₅₀ >35.5 µM | EC ₅₀ >35.2 µM | EC ₅₀ 32.0 µM | EC ₅₀ 75.9 µM | EC ₅₀ 15.9 µM | modified dye uptake assay including formazan test | 21178503 |
| Q222stop | C664T | conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ <66.0 µM | not tested | plaque reduction assay | 20733037 |
| R223H | G669A | conserved region | ACV | - | EC ₅₀ 7.1 µM | not tested | not tested | not tested | not tested | plaque reduction assay, TK functional assay | 2829709 |
| R223stop | deletion (CTCCGGA GCCCGC GGGG) nt 111-127 | non-conserved region | ACV | - | EC ₅₀ 42.2 µM | not tested | not tested | not tested | not tested | plaque reduction assay | 9094663 |
| D229stop* | insertion (T) nt 366 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | 500.0x | >1.2x | >133.0x | 0.3x | 0.4x | plaque reduction assay, TK functional assay | 11160674 |
| D229stop* | deletion (C) nt 551-556 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | >200.0x | 2.0x | >133.0x | 0.5x | 1.6x | plaque reduction assay, TK functional assay | 11160674 |

| | | | | | | | | | | | |
|---------------------|--|-------------------------|-------------------|----------|---------------------------|------------|---------------------------|-------------------------------|-------------------------|---|--|
| D229stop | insertion (C) nt 551-555 | non-conserved region | ACV | - | EC ₅₀ 887.6 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| D229stop | insertion (C) nt 586-590 | non-conserved region | ACV | FOS | EC ₅₀ 443.8 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| D229stop | deletion (G) nt 433-439 | non-conserved region | ACV | FOS | EC ₅₀ 43.1 µM | not tested | not tested | EC ₅₀ 149.5 µM | not tested | plaque reduction assay | 24075863 |
| D229stop | insertion (G) nt 433-439 | non-conserved region | ACV | FOS | EC ₅₀ 443.8 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay, DNA hybridization assay, dye uptake assay, cell growth assay | 14670581 9697707 9094663 11238837 |
| D229stop | insertion (G) nt 440-442 | non-conserved region | ACV | FOS | EC ₅₀ 43.1 µM | not tested | not tested | EC ₅₀ 149.5 µM | not tested | plaque reduction assay | 24075863 |
| D229stop | insertion (T) nt 626-627 | non-conserved region | ACV | FOS | EC ₅₀ 887.6 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| Y239stop | T720A/G | non-conserved region | ACV | FOS | EC ₅₀ 887.6 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| D259 frameshift* | insertion (G) nt 774-775 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | >200.0x | 1.5x | >133.0x | 0.8x | 1.0x | plaque reduction assay, TK functional assay | 11160674 |
| R262stop | deletion (CCCC) nt 551-556 | non-conserved region | ACV | FOS | EC ₅₀ 887.6 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| L263stop | deletion (C) nt 551-555 | non-conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ 66.0 µM | not tested | plaque reduction assay | 24075863 |
| L263stop | deletion (C) nt 586-590 | non-conserved region | ACV, PCV, CDV | FOS | EC ₅₀ >35.5 µM | not tested | EC ₅₀ >32.0 µM | EC ₅₀ 78.2 µM | EC ₅₀ 4.8 µM | modified dye uptake assay including formazan test | 22155911 |
| L263stop | deletion (CTTG) nt 651-654 | non-conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ 66.0 µM | not tested | plaque reduction assay | 24075863 |
| L263stop | deletion (G) nt 779-782 | non-conserved region | ACV | FOS | EC ₅₀ >50.0 µM | not tested | not tested | EC ₅₀ 66.0 µM | not tested | plaque reduction assay, cell growth assay | 24075863 11238837 |
| R271V | C814G/ G815T | non-conserved region | ACV | - | EC ₅₀ 89.3 µM | not tested | not tested | not tested | not tested | dye uptake assay, DNA hybridization assay | 9697707 |
| P272S | C814T | non-conserved region | ACV | - | EC ₅₀ 89.3 µM | not tested | not tested | not tested | not tested | dye uptake assay, DNA hybridization assay | 9697707 |
| D273R | G820A/ C821G | non-conserved region | ACV | - | EC ₅₀ 89.3 µM | not tested | not tested | not tested | not tested | dye uptake assay, DNA hybridization assay | 9697707 |
| T288M | C863T | conserved region | ACV | - | EC ₅₀ 198.0 µM | not tested | not tested | not tested | not tested | plaque reduction assay, dye uptake assay | 9697707 |
| T288 frameshift* | deletion (AC) nt 862-863 | conserved region | ACV, BVDU, PCV | FOS, CDV | >200.0x | 1.5x | >133.0x | 0.8x | 1.0x | plaque reduction assay, TK functional assay | 11160674 |
| I308 frameshift* | insertion (A) nt 922 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | 60.0x | 5.6x | 133.0x | 0.9x | 4.3x | plaque reduction assay, TK functional assay | 11160674 |
| V335 frameshift* | insertion (C) nt 1000-1002 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | >200.0x | 6.2x | >133.0x | 1.2x | 1.3x | plaque reduction assay, TK functional assay | 11160674 |
| C337Y | G1007A | non-conserved region | ACV | - | EC ₅₀ 39.6 µM | not tested | not tested | not tested | not tested | plaque reduction assay, DNA hybridization assay, dye uptake assay | 9697707 9094663 |
| M348stop | deletion (ACCCGA GGACGG) nt 821-833 | non-conserved region | ACV | - | not tested | not tested | not tested | not tested | not tested | not tested | 24075863 |
| M348stop | deletion (C) nt 809-812 | non-conserved region | ACV | FOS | EC ₅₀ 887.6 µM | not tested | not tested | EC ₅₀ <660.0 µM | not tested | plaque reduction assay | 14670581 |
| M348stop | deletion (C) nt 816-819 | non-conserved region | ACV | FOS | EC ₅₀ 131.6 µM | not tested | not tested | EC ₅₀ 148.3 µM | not tested | plaque reduction assay, TK functional assay | 17854045 |

| | | | | | | | | | | | |
|----------|--|-------------------------|-------------------|----------|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|---|----------------------|
| M348stop | deletion (CGCCCCG ACCCCCG) nt 814-826 | non-conserved region | ACV | - | not tested | not tested | not tested | not tested | not tested | not tested | 24075863 |
| M348stop | deletion (G) nt 856 | non-conserved region | ACV | FOS | EC ₅₀ 16.0 µM | not tested | not tested | EC ₅₀ <66.0 µM | not tested | plaque reduction assay | 20733037 |
| M348stop | deletion (T) nt 923-927 | non-conserved region | ACV, BVDU, PCV | FOS, CDV | EC ₅₀ 114.4 µM | EC ₅₀ 80.1 µM | EC ₅₀ 252.0 µM | EC ₅₀ 80.9 µM | EC ₅₀ 10.7 µM | plaque reduction assay, in vivo test in mice, TK functional assay | 11160674 10762577 |