**Methods**

- Site-directed mutants (SDM) containing combinations of TAMs (M41L, D67N, K70R, L210W, T215Y/F) with or without M184V were generated.
- Multi-cycle assays were performed in MT-2 cells using either a two-step Single-Cycle (SC) assay or a 4-day Multi-Cycle (MC) assay (Table 1).
- Single-cycle HIV-1 was generated by co-transfection of the replication-competent proviral plasmid and the wild-type proviral plasmid into HEK293T cells.
- Multi-cycle HIV-1 was generated by transduction of the replication-competent proviral plasmid into MT-2 cells.
- Additional patient-derived (PD) mutants with TAMs were tested using the MC assay.

**Results**

- High level of resistance to TAMs in the presence of TAMs (up to 315 fold).
- Intermediate level of TAM resistance seen in presence of TAMs (up to 5-fold).
- Presence of TAMs increases the severity to both TAF and TFV.

**Conclusions**

- Low and gradual reduction in TAF susceptibility was observed with increasing number of TAMs (Table 1 and Figure 5).
- In the presence of M184V, the antiviral activity of TAF was increased in HIV-1 mutants harboring TAMs, similarly to TFV (Table 1 and Table 2).
- M184V effect was more pronounced in mutants with ≥3 TAMs.
- In viral breakthrough assays, TAMs inhibited the breakthrough of most TAMs-containing HIV-1 (Table 3).
- Breakthrough with TAF observed in ≤3 mutants compared to ≥5 mutants with TFV (Table 3).
- Viruses that showed breakthrough with TAF also had breakthrough with TFV.

**References**