**In Vitro** Antiretroviral Properties of S/GSK1349572, a Next-Generation HIV Integrase Inhibitor

Masanori Kobayashi,1 Tomokazu Yoshinaga,1 Takahiro Seki,1 Chiaki Wakasa-Morimoto,1 Kevin W. Brown,2 Robert Ferris,2 Scott A. Foster,2† Richard J. Hazen,2 Shigeru Miki,1 Akemi Suyama-Kagitani,1 Shinobu Kawauchi-Miki,1 Teruhiko Taishi,3 Takashi Kawasui,4 Brian A. Johns,2 Mark R. Underwood,2 Edward P. Garvey,2‡ Akihiko Sato,1,* and Tamio Fujiwara5

Virology, Medicinal Research Laboratories,1† Strategic Development Department,2† Medicinal Research Laboratories,2‡ Pharmaceutical Development Division,2 Shionogi & Co., Ltd., Osaka, Japan, and GlaxoSmithKline, Research Triangle Park, North Carolina5

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S/GSK1349572 is a next-generation HIV integrase (IN) inhibitor designed to deliver potent antiviral activity with a low-milligram once-daily dose requiring no pharmacokinetic (PK) booster. In addition, S/GSK1349572 demonstrates activity against clinically relevant IN mutant viruses and has potential for a high genetic barrier to resistance. S/GSK1349572 is a two-metal-binding HIV integrase strand transfer inhibitor whose mechanism of action was established through *in vitro* integrase enzyme assays, resistance passage experiments, activity against viral strains resistant to other classes of anti-HIV agents, and mechanistic cellular assays. In a variety of cellular antiviral assays, S/GSK1349572 inhibited HIV replication with low-nanomolar or subnanomolar potency and with a selectivity index of 9,400. The protein-adjusted half-maximal effective concentration (PA-EC₅₀) extrapolated to 100% human serum was 38 nM. When virus was passaged in the presence of S/GSK1349572, highly resistant mutants were not selected, but mutations that effected a low fold change (FC) in the EC₅₀ (up to 4.1-fold) were identified in the vicinity of the integrase active site. S/GSK1349572 demonstrated activity against site-directed molecular clones containing the raltegravir-resistant signature mutations Y143R, Q148K, N155H, and G140S/Q148H (FCs, 1.4, 1.1, 1.2, and 2.6, respectively), while these mutants led to a high FC in the EC₅₀ of raltegravir (11- to >130-fold). Either additive or synergistic effects were observed when S/GSK1349572 was tested in combination with representative approved antiretroviral agents; no antagonistic effects were seen. These findings demonstrate that S/GSK1349572 would be classified as a next-generation drug in the integrase inhibitor class, with a resistance profile markedly different from that of first-generation integrase inhibitors.

Twenty-three compounds are currently approved for the treatment of HIV infection. These drugs can be assigned to six classes: nucleoside (nucleotide) reverse transcriptase inhibitors [N(t)RTIs], nonnucleoside reverse transcriptase inhibitors [NNRTIs], protease inhibitors [PIs], integrase inhibitors [INis], CCR5 antagonists, and fusion inhibitors. The development of resistance to all currently marketed drugs has been observed and is a major reason for failure of therapy. Thus, the development of new, potent antiretroviral compounds with different resistance profiles and mechanisms of action is urgently needed for patients who have multidrug-resistant HIV. In addition to these characteristics, an improved side effect profile and improved dosing convenience (once-daily dosing, fixed-dose combination pills) are desirable, because they would promote high compliance, decrease the emergence of drug-resistant variants, and thus enhance the length and quality of life.

After an initial period of false starts, advances in the field of HIV integrase drug discovery since the late 1990s have been outstanding. Beginning with the discovery that molecules capable of binding two metals within the integrase active site can potently inhibit the recombinant enzyme and virus replication in cells (17), many INIs with different chemical scaffolds have proceeded from preclinical to clinical development (e.g., S-1360 [4], L-870,810 [14], S/GSK364735 [15], GS-9160 [22], raltegravir [RAL, MK-0518] [30, 31], and elvitegravir [EVG, GS-9137] [13, 43]). RAL was approved by the U.S. FDA in 2007, while EVG has progressed into phase 3 development at the time of this writing. The INI class is now recognized as among the safest and most potent anti-HIV drugs (38). However, clinical resistance to RAL and EVG has been observed, and a high degree of cross-resistance between these two agents has been demonstrated (29, 32). Furthermore, the dosing of RAL is twice daily, while once-daily administration of EVG requires a pharmacokinetic (PK) booster such as ritonavir or cobicistat (GS-9350), which raises long-term safety and/or drug-drug interaction concerns. A next-generation INI should have attributes that address these issues.

S/GSK1349572 was created by a research collaboration between Shionogi and GlaxoSmithKline (GSK) and is being developed by a joint venture, Shionogi-ViiV Healthcare.
of S/GSK1349572, RAL, and EVG are shown in Fig. 1. Search Triangle Park, NC. Efavirenz and lamivudine (3TC) were purchased from Boehringer-Ingelheim, Osaka, Japan. RAL and EVG were synthesized at GlaxoSmithKline, Research Triangle Park, NC according to the manufacturer’s instructions and were stimulated by the addition of either 20 U/ml of interleukin-2 (IL-2) or 10% natural T-cell growth factor (ZeptoMetrix, Buffalo, NY) plus 5 to 10 µg/ml of phytomagensulin (PHA). Molt-4 cells persistently infected with HIV-1 IIIB and MT-2 cells (16) were obtained from S. Harada (Kumamoto University, Kumamoto, Japan). HeLa-Cd4 cells containing an HIV-1 long terminal repeat (LTR)-driven β-galactosidase reporter gene have been described previously (20). MAGI-CR5 cells have been described previously (9). HIV-1 strain IIIB was derived from cell-free supernatants of cultures of the chronically infected cell line, H9 IIIB (H9THLV-IIIIB). HIV-1 strain Ba-L was purchased from Advanced Biotechnologies Inc. (Columbia, MD) and was expanded in PHA-activated PBMCs, while HIV-1 NL432 (1) was obtained from A. Adachi (Tokushima University, Tokushima, Japan). Plasmid pGJ3-Luci, containing a replication-defective HIV lentiviral vector expressing luciferase (21), was licensed from Christian Jassoy (University of Leipzig) and was used to create stocks of a vesicular stomatitis virus glycoprotein G (VSV-G)-pseudotyped self-inactivating pseudo-HIV (PHIV) lentiviral vector by cotransfection along with plasmid pVS-G (Clontech) into CP4 cells (a derivative of the 293T human renal epithelial cell line that expresses macrophage scavenger receptor SRA-I) to improve adherence to plastic) and harvesting of the cell-free supernatant.

In vitro strand transfer assay. The inhibitory potencies of S/GSK1349572 and other INIs were measured in a strand transfer assay using recombinant HIV integrase as previously described (5). A complex of integrase and biotinylated preprocessed donor DNA-streptavidin-coated Acintillation proximity assay (SPA) beads was formed by incubating 2 µM purified recombinant integrase with 0.66 µM biotinylated donor DNA-4 mg/ml streptavidin-coated SPA beads in 25 mM sodium morpholinopropanesulfonic acid (MOPS) (pH 7.2), 23 mM NaCl, and 10 mM MgCl2 for 5 min at 37°C. These beads were spun down and preincubated with diluted INIs for 60 min at 37°C. Then a 3H-labeled target DNA substrate was added to give a final concentration of 7 nM substrate, and the strand transfer reaction mixture was incubated at 37°C for 25 to 45 min, which allowed for a linear increase in the strand transfer of donor DNA to radiolabeled target DNA. The signal was read using a Wallac MicroBeta scintillation plate reader.

Antiviral assay in MT-4 cells. MT-4 cells growing exponentially at a density of 5 × 104 or 6 × 105/ml were infected with HIV-1 strain IIIB at a viral multiplicity of infection of 0.001 or a 50% tissue culture infective dose of 4 to 10. The cells were then aliquoted to 96-well plates in the presence of varying concentrations of compounds. After incubation for 4 or 5 days, antiviral activity was determined by a cell viability assay that either measured bioluminescence with a CellTiter-Glo luminescent reagent (Promega Corporation, Madison, WI) or measured absorbance at 560 and 690 nm using the steady-state luciferase assay [4-(2,4-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide].

Pseudo-HIV assay. The antiviral activities of compounds were measured in a single-round assay using a self-inactivating PHIV lentiviral vector. CFP cells (2 × 105/well) infected with an amount of PHIV sufficient to produce approximately 50,000 relative light units were added to 96-well black, clear-bottom plates and were incubated for 2 days with S/GSK1349572 at varying concentrations. Infected cells were measured as a function of luciferase activity in a luminometer using the steady-state luciferase assay (Promega Corporation).

Antiviral assay in PBMCs. In one 96-well culture plate, PHA- and IL-2-stimulated PBMCs (4 × 105/ml) were precultured with a compound for 1 h, while HIV-1 strain Ba-L was mixed with the same compound in a second plate. An aliquot of the Ba-L-compound mixture was then transferred to the PBMC-compound mixture and was incubated for 7 days. After this incubation, supernatants were assayed for reverse transcriptase (RT) activity by incorporation of [methyl-3H]dTTP to measure viral replication as previously described (15).

Effects of human serum and serum proteins. The effect of the presence of human serum albumin (HSA; 20 or 40 mg/ml), α1-acid glycoprotein (AAG; 2 mg/ml), or human serum (HS; up to 30% or 50% was used, and results were extrapolated up to 100%) on the antiviral activity of S/GSK1349572 was evaluated in the PHIV and MT-4 assay systems. To estimate the effects of the fold shift in protein binding, antiviral activity was tested with the addition of various concentrations of human serum to the HIV-1 IIIB replication assay mixture in MT-4 cells as previously described (15). The protein-adjusted half-maximal effective concentration (PA-EC50) was estimated by multiplying the EC50 in serum-free conditions by the percentage of compound binding to serum.

Cytotoxicity assays. In vitro growth inhibition (cytotoxicity) studies were conducted with S/GSK1349572 in proliferating human leukemia and lymphocytic cell lines (IM-9, U-937, MT-4, and Molt-4) as well as in stimulated and unstimulated human PBMCs. ATP levels were quantified by using the CellTiter-Glo luciferase reagent to measure the ability of a compound to inhibit cell growth as an indicator of the compound's potential for cytotoxicity.
Mechanistic cellular studies. To determine if S/GSK1349572 was inhibiting HIV replication in cellular assays through an integrase inhibition mechanism, the effects on the synthesis of HIV NL432 DNA species in MT-4 cells were measured in a single-round infection assay using quantitative PCR methods. Quantitative PCR analysis was performed to measure the synthesis of HIV DNA species in MT-4 cells in the presence of an INI or NNRTI as described previously, with minor modifications (15). Briefly, 293T cells were transfected with the NL432 plasmid to generate infectious virus, and the supernatant was filtered through 0.45-μm-pore-size filters and was treated with DNase I. MT-4 cells were infected with HIV-1 NL432 for 1 h, incubated with dilutions of a compound, and collected after 6 or 18 h of incubation. All cells were incubated with 0.5 μM ritonavir in order to limit HIV replication to a single cycle. Total-DNA PCR to detect late RT products was performed by incubating the samples for 6 h. Nucleo-PCR to detect integrated provirus and 2-LTR PCR to detect 2-LTR circles were performed by incubating the samples for 18 h. Reaction products were analyzed using the ABI Prism 7900HT-3 sequence detection system (Applied Biosystems, Carlsbad, CA).

Isolation of drug-resistant viruses. Drug-resistant viruses were isolated according to a previously described protocol (24). Briefly, the virus used for initiating passage work was prepared by coculturing MT-2 cells with Molt-4 cells persistently infected with HIV-1 strain IIIB for 3 days. Fresh MT-2 cells were dispensed into each well of a 24-well tissue culture plate. Three wells of each culture containing several concentrations of a compound were used initially, and the virus prepared as described above was added to each well. Every 3 or 4 days, the cells were passaged with or without the addition of fresh MT-2 cells. When a cytopathic effect was observed, the supernatants were used to infect fresh MT-2 cells, and the concentration of the compound was held constant and/or increased 5-fold. Even 2 weeks, when virus replication was ascertained by observation of a cytopathic effect, the infected cells were collected and used for genotypic and phenotypic analyses. For the analysis of mutations, DNA was extracted from infected cells using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany), and the integrase region of HIV proviral DNA was amplified by PCR with specific primers (M-poli7, AACAAGTAGATAAATTAGTCAGT; M-poli8, TATGTGGGATGTGTACTTCTGAAC). The products were sequenced by Operon Biotechnologies' sequencing service. The sequence of the integrase region determined from isolated viruses was compared with that of wild-type IIIB, and amino acid substitutions were identified.

Construction of integrase region-recombinant HIV-1 molecular clones. The Xbal-EcoRI fragment from pln-IN301 (the Xbal site was inserted into the 5′ end of the integrase region of pNL432 [1] and termed pln-IN301) was cloned into the XbaI-EcoRI site of cloning vector pUC18. In vitro mutagenesis was performed with the QuikChange site-directed mutagenesis kit (Stratagene, a division of Agilent Technologies, Santa Clara, CA) using pUC18 cloned with the integrase region as a template. The mutated Xbal-EcoRI fragment was amplified and ligated into pln-IN301 to construct a recombinant HIV-1 molecular clone. Plasmids were subsequently transfected into 293T cells to generate infectious virus. Supernatants were harvested after 2 days of culture and were stored as cell-free culture supernatants at −80°C.

Cross-resistance profiling of S/GSK1349572. S/GSK1349572 was evaluated against molecular clones with mutations in the integrase-, RT-, and protease-coding regions. INI-, NRTI-, and NNRTI-resistant mutants were analyzed by the reporter assay based on HeLa-C4D cells, while PI-resistant mutants were analyzed by infectivity in MT-4 cells, with monitoring of RT activity as described previously (15). The HIV-1 wild-type infectious molecular clone pNL432 was used for site-directed mutagenesis to generate HIV clones containing mutations. Fifty INI-resistant mutants were constructed. The molecular clones with a K103N or Y188L mutation (2, 3) within the RT-coding region were used as NNRTI-resistant viruses; those with M184V (6, 40), D67N/K70R/T215Y (7), or K103N or Y188L mutation (2, 3) within the RT-coding region were used as PI-resistant viruses. 293T cells were subsequently transfected with the plasmids to generate infectious virus, and the supernatant was filtered through a 0.45-μm-pore-size filter and treated with DNase I. MT-4 cells were infected with HIV-1 NL432 for 1 h, incubated with dilutions of a compound, and collected after 6 or 18 h of incubation. All cells were incubated with 0.5 μM ritonavir in order to limit HIV replication to a single cycle. Total-DNA PCR to detect late RT products was performed by incubating the samples for 6 h. Nucleo-PCR to detect integrated provirus and 2-LTR PCR to detect 2-LTR circles were performed by incubating the samples for 18 h. Reaction products were analyzed using the ABI Prism 7900HT-3 sequence detection system (Applied Biosystems, Carlsbad, CA).

RESULTS

Inhibition of recombinant HIV integrase and HIV replication by S/GSK1349572. S/GSK1349572 inhibited HIV-1 integrase-catalyzed strand transfer with a 50% inhibitory concentration (IC50) of 2.7 nM. The EC50 against HIV-1 was 0.51 nM in PBMCs, 0.71 nM in MT-4 cells, and 2.2 nM in the PHIV assay, which uses a pseudotyped self-inactivating virus. Measurements of the fold shift from EC50 to EC90 ranged from 3-fold to a maximum of 4-fold; as a conservative measure, an EC90 4-fold higher than the EC50 was used. This low-nanomolar potency was similar to the potencies of RAL and EVG (Table 1). In the MT-4 antiviral assay, the estimated potency shift was 75-fold when the results were extrapolated to 100% human serum and 32-fold in the presence of 20 mg/ml HSA. In the PHIV assay, the potency shift was 11-fold with 40 mg/ml HSA and 2.1-fold in the presence of 2 mg/ml AAG. The extrapolated potency shift of 75-fold for S/GSK1349572 in the presence of 100% human serum was also applied to EC50 and EC90 in PBMCs, resulting in PA-EC50 and PA-EC90 values of 38 nM and 152 nM, respectively. These values were similar to those of EVG and 4.1-fold less potent than those of RAL.

The 50% cytotoxic concentrations (CC50) for S/GSK1349572 in proliferating IM-9, U-937, MT-4, and Molt-4 cells were 4.8, 7.0, 14, and 15 μM, respectively. In unstimulated and stimulated PBMCs (both from the same 4 donors), the CC50 were 189 μM and 52 μM, respectively. Based on the EC50 of S/GSK1349572 against HIV-1 in PBMCs (i.e., 0.51 nM), this translates to a cell-based therapeutic index of at least 9,400.

<table>
<thead>
<tr>
<th>INI</th>
<th>IC50 (nM) for strand transfer by enzyme assay</th>
<th>EC50 (nM) in PBMCs</th>
<th>Fold potency shift with 100% HSA</th>
<th>PA-EC50 (nM)</th>
<th>PA-EC90 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/GSK1349572</td>
<td>2.7 ± 0.05a</td>
<td>0.51 ± 0.25a</td>
<td>75</td>
<td>38</td>
<td>152</td>
</tr>
<tr>
<td>Raltegravir</td>
<td>3.3 ± 0.6</td>
<td>2 ± 1</td>
<td>4.7</td>
<td>5.6</td>
<td>23</td>
</tr>
<tr>
<td>Eltgravir</td>
<td>6 ± 1</td>
<td>2 ± 1</td>
<td>22</td>
<td>20</td>
<td>78</td>
</tr>
</tbody>
</table>

a The strand transfer IC50 and HIV replication EC50 of RAL and EVG are from reference 15.

b The fold shift in protein binding was estimated from antiviral activity with various concentrations of human serum.

c Mean from at least 3 experiments.

d Mean from 12 experiments with HIV-1 strain Ba-L.
Cellular mechanistic studies. As shown in Fig. 2, S/GSK1349572 inhibited the integration of viral DNA (Fig. 2b), with a concomitant increase in 2-LTR circles (Fig. 2c) and no effect on viral DNA production (Fig. 2a). Thus, S/GSK1349572 demonstrated the expected effects of an INI. Furthermore, the concentration dependency of the effects was within the range of error for the potency observed in the inhibition of viral replication in PBMCs and MT-4 cells (11). These effects were similar to the effects observed with RAL and S/GSK364735 in a previous study (15) and contrasted with the effects of the NNRTI efavirenz.

Cross-resistance profiling of S/GSK1349572. When tested against HIV strains resistant to marketed NNRTIs or NRTIs, S/GSK1349572 showed efficacy against five different NNRTI-resistant or NRTI-resistant viruses, with activity equivalent to that against wild-type virus ($EC_{50}$, 1.3 to 2.1 nM). Likewise, S/GSK1349572 showed efficacy against two different PI-resistant viruses, with activity equivalent to that against wild-type virus ($EC_{50}$, 0.36 and 0.37 nM).

Isolation of viruses resistant to S/GSK1349572 and other INIs. In vitro passage experiments were performed with S/GSK1349572 and RAL, with the NRTI lamivudine as a reference, starting with wild-type HIV-1 IIIB (Table 2), itself a quasispecies. In the culture with S/GSK1349572, the T124A substitution was observed first on day 14 (notably, IIIB contains ~40% T124A at baseline). The T124A/S153F substitution was observed on day 28, followed by the T124A/S153Y and L101I/T124A/S153F substitutions on day 70; these substitutions persisted throughout the remaining passages. Viruses with the S153Y substitution were isolated on days 84 and 112. Only four viruses with substitutions were observed on day 112.

Mutations that reduced susceptibility more than 5-fold, as measured by phenotypic assays, are shown in boldface in Table 2. This level of resistance was observed on day 14 with lamivudine and on day 28 with RAL. Highly resistant mutants with a high fold change (FC) ($>$100-fold) in the $EC_{50}$ were isolated in the presence of RAL; viruses with many of these mutations have also been isolated in the clinic from patients failing RAL-based regimens (11). In contrast, no highly resistant mutants were isolated in the presence of S/GSK1349572 through day 112. Multiple substitutions in integrase observed during S/GSK1349572 passage conferred only a low FC (maximum, 4.1-fold). Since these viruses had relatively low FCs and these substitutions remained unchanged from day 70 to day 112, they should not significantly affect resistance to S/GSK1349572.

The dose escalation patterns of S/GSK1349572, RAL, and EVG were also compared (Fig. 3); the in vitro passage experiment with EVG has been described previously (24). Each passage was started with 0.26, 1.3, 6.4, and 32 nM each INI, and compound concentrations were increased in a step-wise manner. Figure 3 shows the dose escalation patterns, starting from 1.3 nM, that permitted viral replication to continue. The concentration of RAL was increased to 160 nM on day 35, followed by 800 nM on day 77. The concentration of EVG was increased to 160 nM on day 56. In contrast, no replication was observed at S/GSK1349572 concentrations of 32 nM or greater throughout the passage experiment. Thus, the maximum concentration of S/GSK1349572 allowing replication was 6.4 nM.

Sensitivities of drug-resistant molecular clones to INIs. A panel of 50 INI-resistant site-directed mutants was constructed and tested for susceptibility to S/GSK1349572 and other INIs (Table 3). Most of these mutants were isolated during in vitro passage studies and/or clinical trials of RAL, while a few were derived from the literature. Efavirenz was used as a control, and the FC in its $EC_{50}$ against the INI-resistant mutants was as high as 2.9-fold. Therefore, the viruses for which the $EC_{50}$ was $\geq$3-fold that for the wild type were considered to be resistant in this study.
Although substitutions involving S153 (i.e., S153F, S153Y, L101I/S153F, T124A/S153Y, and L101I/T124A/S153F) were isolated during the in vitro selection study with S/GSK1349572, these mutant viruses remained susceptible to S/GSK1349572. L101I and T124A are polymorphic, and all INIs showed wild-type potency against these site-directed mutants. S/GSK1349572 showed a moderate reduction in potency (FC, 3- to 10-fold) against five mutant viruses (the I151L, T66K/L74M, E138K/Q148R, G140C/Q148R, and G140S/Q148R mutants), with FCs from 3.5- to 8.4-fold. Ten RAL- and seven EVG-resistant mutants had FCs within the 3- to 10-fold range. However, except for one mutant (I151L), all of these moderately RAL and EVG resistant mutants were susceptible to S/GSK1349572. Only two mutant viruses, the E138K/Q148K and Q148R/N155H mutants (the latter of which has not been demonstrated on the same genome), were shown to lead to an FC of 10-fold for S/GSK1349572; these mutants also shared a highly resistant phenotype against RAL (FCs, 330- and 140-fold, respectively) and EVG (FCs, 371- and 390-fold, respectively). In contrast, RAL and EVG showed FCs of 10-fold in their EC50s against 23 and 32 mutant viruses, respectively. Notably, S/GSK1349572 is potent against all single mutants examined in our panel. Furthermore, limited cross-resistance against RAL- and/or EVG-resistant viruses was observed; S/GSK1349572 was active against most INI-resistant viruses with double or more mutations, including clinical isolates from patients with RAL treatment failure (42).

Cellular combination studies. S/GSK1349572 was tested in combination studies with 8 HIV antiretrovirals representing each class approved at the time, as well as adefovir and ribavirin, which are likely to be coadministered to HIV patients coinfected with HBV or HCV (Table 4). No antagonism was observed with any combination, and no enhanced cytotoxicity was observed within the concentrations tested for antiviral activity. As expected, S/GSK1349572 was additive with itself. In combination with nucleoside RT inhibitors, S/GSK1349572 was synergistic with stavudine and abacavir. In combination with nonnucleoside RT inhibitors, S/GSK1349572 was synergistic with elvitegravir.
All truth is split into two halves. The first half is the known truth, and the second half is the unknown truth. The unknown truth is where we need to keep looking for the answer. The known truth is where we have already found the solution. The unknown truth is where we have not yet found the solution. The known truth is where we have already found the solution. The unknown truth is where we have not yet found the solution. The known truth is where we have already found the solution. The unknown truth is where we have not yet found the solution.
Table 4. Deviation from additivity for S/GSK1349572 with marketed HIV drugs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Deviation from additivb</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>STV</td>
<td>0.473 0.083 0.001</td>
<td>Synergy</td>
</tr>
<tr>
<td>ABC</td>
<td>0.641 0.095 0.001</td>
<td>Synergy</td>
</tr>
<tr>
<td>EFV</td>
<td>0.356 0.102 0.005</td>
<td>Synergy</td>
</tr>
<tr>
<td>NVP</td>
<td>0.246 0.087 0.015</td>
<td>Synergy</td>
</tr>
<tr>
<td>LPV</td>
<td>0.310 0.082 0.005</td>
<td>Synergy</td>
</tr>
<tr>
<td>APV</td>
<td>0.332 0.085 0.003</td>
<td>Synergy</td>
</tr>
<tr>
<td>ENF</td>
<td>0.245 0.110 0.034</td>
<td>Synergy</td>
</tr>
<tr>
<td>ADV</td>
<td>-0.179 0.168 0.168</td>
<td>Additive</td>
</tr>
<tr>
<td>S/GSK1349572</td>
<td>0.031 0.151 0.423</td>
<td>Synergy</td>
</tr>
</tbody>
</table>

*STV, stavudine; ABC, abacavir; EFV, efavirenz; NVP, nevirapine; LPV, lopinavir; APV, amprenavir; ENF, enfuvirtide; ADV, adefovir.

Values in the range of 0.1 to 0.2 indicate weak synergy; values that approach −0.5 indicate strong synergy; and positive values of 0.1 to 0.2 indicate weak antagonism.
poorer than those of the wild type (34). The Q148R and N155H mutations were not observed in the same genome in clinical isolates but were found as mixtures (28), and the replication capacity of the Q148R/N155H mutant molecular clone was poor in PBMCs (unpublished data). It is thus noteworthy that S/GSK1349572 was poor in PBMCs (unpublished data). It is thus noteworthy that S/GSK1349572 as a candidate for clinical development, preclinical findings formed part of the rationale for the selection of S/GSK1349572 as a candidate for clinical development, and they provide a strong foundation for its ongoing clinical investigation.

ACKNOWLEDGMENTS

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REFERENCES


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