

Aplaviroc (APL, 873140), an Entry Inhibitor that Targets CCR5, Exhibits Potent In Vitro Inhibition of HIV-1 from Different Subtypes and from Individuals Failing Standard Antiretroviral Therapy (ART)

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Introduction

An estimated 40 million people are infected with HIV worldwide, with nearly 5 million new infections in 2004. For the majority of HIV infections, effective and relatively tolerable first-line treatment regimens exist. However, a variety of factors contribute to the evolution of drug-resistant HIV in many individuals. New agents with novel resistance profiles are needed for individuals failing current antiretroviral therapy (HAART) with resistant viruses. Entry inhibitors that target CCR5, such as aplaviroc (873140, APL), have been shown to effectively reduce viral load in short-term monotherapy studies in HIV-infected individuals that are either ART-naïve or have been on ART for ≥ 6 months. Here we present *in vitro* data indicating that viruses from individuals that are currently on their failing HAART regimen, including some that are highly ART-experienced, are susceptible to APL. In addition, R5-tropic virus isolates from diverse HIV-1 subtypes are susceptible to APL. GSK announced the termination of patient enrollment into Phase 2b and Phase 3 studies for the investigational HIV entry inhibitor, aplaviroc (873140), after acceptance of this abstract to EACS. Due to safety data observed in the Phase 3 and 2b studies, GSK took immediate steps to protect the safety and health of patients in these clinical studies.

Methods

APL susceptibility (IC_{50} values) of HIV-1 envelope glycoproteins (Env) derived from plasma virus was evaluated by the PhenoSense HIV Entry Assay (Monogram Biosciences) for 104 subjects failing their current ART regimen. Demographics of the patient samples are shown in Table 1. The PhenoSense HIV Entry Assay uses an engineered cell line that expresses CCR5 and CD4 (but not CXCR4) on the cell surface. Thus, only the contribution of viruses able to use CCR5 is measured in this assay. Analysis of variance was used to describe apparent IC_{50} (log_{10} transformed) in terms of other sample characteristics.

In separate experiments, HIV-1 isolates from Group M subtypes A - G and Group O were obtained from the AIDS Reference and Reagent Repository. Coreceptor tropism phenotypes of these isolates were also obtained from ARRRP. PHA-activated PBMCs were inoculated virus either alone or in the presence of increasing amounts of aplaviroc. After 7 days, virus replication was assessed by determining supernatant reverse transcriptase activity, and IC_{50} values were calculated.

Table 1. Demographics of Treatment-Experienced Sample Population

| | Median Values (%) or range) |
|---------------|-----------------------------|
| HIV-1 RNA PCR | N=113 4.98 (2.70, 5.88) |
| Tropism | N=113 |
| RSX4 | 37 (33%) |
| R5 | 76 (67%) |
| CD4+ | N=110 308 (55, 1488) |
| CD6+ | N=108 1022 (316, 4331) |
| CD4/CD8 Ratio | N=106 .301 (.037, 2.15) |
| Age | N=111 39 (64, 78) |
| Race | N=111 |
| White | 80 (72%) |
| Black | 22 (20%) |
| Other | 9 (8%) |
| Sex | N=111 |
| Female | 25 (23%) |
| Male | 86 (77%) |
| Geographic | N=111 |
| EU | 63 (57%) |
| Latin America | 0 |
| US / Canada | 48 (43%) |
| CDC Class | N=111 |
| A,B | 76 (68%) |
| C | 35 (32%) |

Figure 2. Aplaviroc is active at subnanomolar levels against R5-tropic HIV-1 isolates across virus subtypes

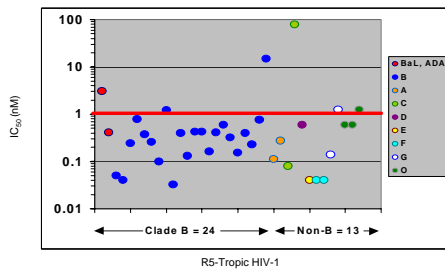


Table 3. Coreceptor Tropism and APL IC_{50} of Primary HIV-1 Isolates

| HIV-1 Subtype | Isolate ID | Coreceptor Tropism* | APL IC_{50} (nM) | HIV-1 Subtype | Isolate ID | Coreceptor Tropism* | APL IC_{50} (nM) |
|---------------|------------|---------------------|--------------------|---------------|------------|---------------------|--------------------|
| A | 92/RW/009 | RSX4 | 0.27 | B | 92/RB/003 | R5 | 0.05 |
| | 92/RB/016 | R5 | 0.11 | | 92/RB/004 | R5 | 0.04 |
| | 92/UG/029 | X4 | >320.0 | | 92/RB/017 | R5 | 0.27 |
| C | 92/BR/025 | R5 | 0.08 | 92/US/457 | R5 | 0.34 | |
| | 98/N017 | X4 | 0.54 | 92/SB/860 | R5 | 0.26 | |
| | 97ZA/003 | R5 | 79.10 | 92/US/712 | R6 | 0.10 | |
| D | 92/UG/024 | RSX4 | >320.0 | 93/SF/114 | R5 | 1.22 | |
| | 92/UG/035 | R5 | 0.60 | 92/US/727 | R5 | <0.032 | |
| | 92/UG/001 | RSX4 | >320.0 | 93/US/149 | R5 | 0.39 | |
| E | 93/THA/051 | RSX4 | >320.0 | 93/US/155 | R5 | 0.13 | |
| | 93/THA/073 | R5 | 0.04 | 93/US/157 | R5 | 0.42 | |
| | 93/BR/020 | RSX4 | >320.0 | ASJ1/05 | R5 | 0.42 | |
| F | 93/BR/019 | RSX4 | 0.04 | ASJ6/7 | R5 | 0.16 | |
| | 93/BR/029 | R5 | 0.04 | ASJ1/05 | R5 | 0.42 | |
| | 93/BR/019 | RSX4 | 0.04 | ASJ2/4 | R5 | 0.60 | |
| G | G3 | R5 | 0.14 | 93/US/072 | R5 | 0.75 | |
| | JV1083 | R5 | 1.23 | 93/US/073 | R5 | 0.19 | |
| | BCF01 | R5 | 0.59 | 93/US/074 | R5 | 0.35 | |
| O | BCF02 | R5 | 0.60 | ASJ7/9 | R5 | 0.23 | |
| | BCF03 | R5 | 1.24 | 91/US/005 | R5 | 0.15 | |
| | | | | 91/US/006 | R5 | 14.50 | |
| | | | ASJ3/4 | RSX4+ | >320.0 | | |
| | | | 92/BR/014 | RSX4 | >320.0 | | |
| | | | 92/HT/593 | RSX4 | >320.0 | | |
| | | | 92/HT/596 | RSX4 | >320.0 | | |

* Where detected by T1, coreceptor tropism was determined by infection of cell lines expressing only one co-receptor. Otherwise, coreceptor tropism was inferred from the virus 'biotype', consisting of one or more of the following: MT-2 syncytium-induction assay, replication in various immortalized T cell lines, replication phenotype (slow/low or rapid/high), syncytium induction in PBMCs.

Results

- The R5-tropic Envelope glycoproteins of viruses from treatment-experienced subjects failing their current regimen were highly susceptible to APL, with IC_{50} values similar to those previously determined for viruses from naive subjects¹
- In the PhenoSense HIV Entry Assay, the R5-tropic Env component of RSX4 patient quasipieces were slightly, but significantly, more susceptible to APL than R5-tropic viruses from R5-only patient samples (Figure 1)
- The IC_{50} to APL was not affected by the extent of resistance to current ART therapy. None of the data in Table 2 (except for tropism) was significantly associated with IC_{50} to APL.
- R5-tropic viruses of diverse subtypes showed low nM susceptibility to APL (Figure 2, Table 3)

Figure 1. IC_{50} (nM) by Tropism and ART Experience

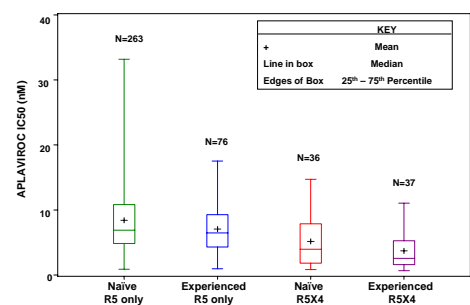


Table 2. Susceptibility of Viruses (R5-tropic only or R5-tropic component of RSX4 virus) from Treatment-Experienced Patient Samples

| Sample Grouping | N | Median IC_{50} | Range |
|---|-----|------------------|--------------|
| Naïve samples | 263 | 6.70 | 0.83 - 33.19 |
| Experienced Samples | 104 | 5.83 | 0.67 - 17.53 |
| R5-tropism only samples | 75 | 6.50 | 0.98 - 17.53 |
| RSX4 samples (tested on R5-only cells) | 29 | 2.58 | 0.67 - 11.06 |
| Samples with RTI resistance mutations* | 80 | 5.99 | 0.67 - 17.53 |
| Samples without RTI resistance mutations* | 18 | 6.18 | 1.86 - 13.82 |
| Samples with PI resistance mutations | 41 | 5.37 | 0.67 - 17.53 |
| Samples without PI resistance mutations | 67 | 6.23 | 0.98 - 16.58 |
| Primary resistance mutations†: | | | |
| 1 class | 1 | 1.66 | |
| 2 classes | 40 | 6.56 | 0.98 - 16.58 |
| 3 classes | 40 | 5.38 | 0.67 - 17.53 |
| no mutations | 17 | 6.23 | 1.74 - 13.82 |
| Number of primary resistance mutations: | | | |
| 1 | 14 | 7.43 | 1.13 - 13.46 |
| 2 | 23 | 4.50 | 0.87 - 16.28 |
| 3+ | 4 | 5.84 | 0.67 - 17.53 |
| None | 17 | 6.23 | 1.74 - 13.82 |
| Number of prior ART drugs: | | | |
| 3 | 64 | 6.06 | 0.67 - 17.53 |
| 4 | 19 | 6.14 | 0.86 - 18.31 |
| 5+ | 19 | 4.64 | 1.13 - 16.28 |

*All samples with NRTI mutations also had NNRTI mutations
†Primary resistance mutations as defined by IAS, April 2005

Discussion

Here we show that Envs from viruses resistant to currently used RT and PRO inhibitors are effectively inhibited by APL. Additional data presented previously showed that APL is also highly active against viruses resistant to enfuvirtide.² This is not unexpected, as APL inhibits HIV by a novel mechanism, inhibiting a step in the virus lifecycle not targeted by current therapeutic agents.

The data presented here also show that APL effectively inhibited *in vitro* replication of R5-tropic HIV strains of diverse subtypes using PHA-activated normal human PBMCs as virus target cells. These cells express both CCR5 and CXCR4, making the inhibition of four reportedly RSX4- or X4-tropic virus strains surprising (92RW009, 93BR019, 98N017, and 93THA073). Interestingly, the majority of the subtyped virus strains used were initially characterized for virus 'biotype' using methods that predated identification of CCR5 and CXCR4 as HIV coreceptors.³ While some correlation between 'biotype' and coreceptor tropism exists, the assays used were not designed to directly assess coreceptor usage and the results are subject to over-interpretation. In fact, subsequent publications and testing have shown that two of the four viruses (92RW009 and 93BR019) use CCR5 only^{4,5} although another source lists 92RW009 as using both CCR5 and CXCR4.⁶ Use of CCR5 and not CXCR4 by the subtype A virus 92RW009, which showed sub-nanomolar susceptibility to aplaviroc, is consistent with the subnanomolar susceptibility of this virus isolate to the β -chemokine analog AOP-RANTES.⁵ The 93BR019 isolate was shown to use only CCR5 in the U87 cell system (E. Arts, personal communication). The results of specific coreceptor utilization testing for 98N017 and 93THA073 have not been ascertained to date.

Conclusions

- R5-tropic viruses and the R5-tropic component of RSX4 virus quasipieces from patients failing their current ART regimen were highly susceptible (low nM IC_{50}) to aplaviroc.
- R5-tropic viruses from patients with RSX4 virus were slightly, but significantly, more susceptible to aplaviroc than were viruses from patients with R5-only virus.
- The extent of ART-experience/number or type of resistance mutation did not influence susceptibility to aplaviroc.
- Care should be exercised in accepting reported tropism of primary and clinical isolates. With older isolates, particularly those characterized at the population level, the methods used to determine biological phenotype may have been less precise and the extrapolation to coreceptor tropism may not reflect the variety of viruses present in the isolate population.

References

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