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GSK1265744 Demonstrates Robust *in Vitro* Activity Against Various Clades of HIV-1

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To the editors:

INTRODUCTION

Antiretroviral agents have been approved by the U.S. Food and Drug Administration for use as pre-exposure prophylaxis (PrEP) against acquisition of HIV-1 in high-risk populations.¹ Studies of daily oral emtricitabine and tenofovir disoproxil (FTC/TDF) have demonstrated variable efficacy in preventing incident infections.² This variability is thought due in large part to issues surrounding adherence to the prescribed daily oral medication regimen.³ Disappointingly, two trials, FEM-PrEP and VOICE, were stopped prematurely due to futility, and measurements of plasma drug concentrations revealed that fewer than 30 percent of participants were taking the drug appropriately.^{4,5} It has been hypothesized that the use of long acting antiretroviral agents given by intramuscular injection in the clinic may overcome some obstacles to adherence.

GSK1265744 (GSK744), an analog of the integrase inhibitor dolutegravir, is formulated as a long-acting (LA) injectable nanosuspension.⁶ When given intramuscularly, it has demonstrated a prolonged half-life in plasma of 21 to 50 days. When dosed at 800 mg, levels of drug have remained greater than 4 times the 90% protein-adjusted inhibitory concentration (PAIC₉₀) for up to three months, supporting dosing every 12 weeks.⁷ GSK744

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LA has shown 90 to 100% efficacy in preventing SHIV162P3 transmission intrarectally and intravaginally in animal models.^{8,9} Given this pharmacokinetic profile and its activity in the macaque/SHIV model, this compound has entered the clinic for testing as a next generation PrEP agent.

It is critical that a next generation global PrEP agent be active against a variety of HIV-1 clades. To this end, we determined the relative effective concentrations of GSK744 against a panel of recombinant viruses containing integrase coding regions derived from various clades of HIV-1

METHODS

We assembled a panel of recombinant viruses derived from 5 prevalent clades: clade B viruses from the Primary Infection Cohort at the Aaron Diamond AIDS Research Center in New York;¹⁰ clade A1 and D viruses collected from patients in Uganda and Rwanda;¹¹ clade AE viruses collected from Thai patients¹² and clade C viruses collected from patients in Zambia.¹³ All viral specimens were obtained from newly infected, untreated patients and were de-identified.

The integrase coding region (867 bp) was amplified from viral cDNA, proviral DNA or plasmid DNA using nested PCR as previously described.¹⁴ Each integrase coding region was recombined into a plasmid, R7/3(X/S)Bsd, which was derived from HXB-2, and carries inactivated envelope.¹⁵ Pseudotyped viruses were prepared by co-transfecting proviral DNA and pCI-VSV-G into HEK293T using jetPRIME transfection reagent (PolyPlus, Illkirch, France). The cells were fed the next day and viral supernatant was harvested after 48 hours and saved at -80°C until use. Viral concentrations were measured by p24 ELISA.

The 50% and 90% effective concentrations (EC₅₀ and EC₉₀) of GSK1265744 for each integrase were determined by a single-cycle *in vitro* infectivity assay. As described previously,¹⁴ TZM-bl cells were plated at 8000 cells per well with 150 µL of Dulbecco's modified Eagles's medium (DMEM) with 10% fetal bovine serum (FBS). After incubation for 24 hours at 37°C, culture media was aspirated and TZM-bl cells were incubated for 30 minutes in DMEM/10% FBS with 20 µg Polybrene (Abbott Laboratories, Abbott Park, IL). A GSK744 stock solution was serially diluted to multiple concentrations. Polybrene-containing media was replaced by 150 µL of DMEM/10% FCS and 50 µL of each of these preparations was added. After another 30 minutes of incubation, pseudotyped virus (5 ng of p24 in 50 µL media) was added to the appropriate wells. After a 48h incubation, the cells were processed to measure β-galactosidase using the Galacto-Star (Applied Biosystems, Framingham, MA) protocol and the light signal was measured on a MLX microplate luminometer (Dynex Technologies, Inc., Chantilly, VA).

All assays were done in triplicate and repeated to verify the results. The luminometer results were analyzed using Grafit (Erithacus Software, Horley, UK) and Prism (GraphPad Software, San Diego, CA) software to create dose-response curves and to calculate the EC₅₀ and EC₉₀ for each drug/virus combination (including the reference virus R7/3). We then expressed each combination as the ratio of n-fold change EC₅₀ and EC₉₀ over R7/3.

Effective concentrations were compared using analysis of variance (ANOVA) and pairwise with the Mann-Whitney U test.

RESULTS

In vitro infectivity assays were performed on 23 pseudoviruses containing the integrase genes from various clades of HIV-1 from newly infected, untreated patients (Table 1). In our assay, GSK744 had an EC₅₀ of 0.28nM and EC₉₀ of 0.85nM against R7/3, and it demonstrated potent activity *in vitro* against pseudotyped viruses with integrase genes from all 5 clades of HIV-1. The mean EC₅₀ fold-change was 0.91 (range 0.17 – 1.38); the mean EC₉₀ fold-change was 0.97 (range 0.28 – 3.16). ANOVA revealed statistically significant differences between viruses in drug susceptibility relative to R7/3 ($p=0.0007$). Notably, viral sample R462F, from a clade A1 virus, was found to be hyper-susceptible to GSK744, with an EC₅₀ fold-change of 0.17, and this value is lower (more susceptible) than those of several other samples. All other patient-derived samples demonstrated consistent susceptibility to GSK744. As controls, we used two viruses with integrase mutations known to confer resistance to first-generation integrase inhibitors: p8070 (G140S, Q148H) and p4736 (E92Q, N155H).¹⁶ The fold-change for EC₅₀ and EC₉₀ for raltegravir against p8070 and p4736 was 5307 and 194 and 444 and 64, respectively when compared to the reference strain R7/3. In contrast, the EC₅₀ for GSK744 was about 11-fold higher against p8070 and about 4-fold greater against p4736 when similarly compared to R7/3. These results suggest that similar to dolutegravir, GSK744 demonstrates activity against viral variants highly resistant to first generation strand transfer inhibitors of HIV-1 integrase.

DISCUSSION

To conclude, GSK744, an analog of dolutegravir, is an integrase inhibitor that is amenable to quarterly or every 12-week dosing.⁶ Our results demonstrate that this drug displays activity against a broad range of transmitted viruses derived from various clades across geographical locations. Given its pharmacokinetic and resistance profile as well as its broad activity, it appears to be a promising candidate as a next-generation PrEP agent. This being the case, intramuscular injections of GSK744 are currently undergoing Phase II safety and tolerability testing in anticipation of large Phase III efficacy trials.

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EC50 and EC90 of HIV-1 pseudovirus stocks containing integrase genes from various clades, relative to the reference virus, R7/3

Table 1

All viruses from clades A1, AE, B, C and D are as susceptible to GSK1265744 as the reference virus. The viruses with known resistance mutations to raltegravir show a modest, but statistically significant increase in the EC of GSK1265744.

Clade	ID	EC ₅₀ F.C.	s.d.	ED ₉₀ F.C.	s.d.
A1	p191084	1.38	0.13	0.99	0.21
	p9004SDM	0.90	0.90	0.78	0.12
	R462F	0.12	0.10	0.23	0.27
	R880F	0.62	0.54	0.62	0.24
AE	AA081a	0.79	0.29	0.77	0.05
	AA114a	0.85	0.21	1.11	0.11
	AA116a	0.94	0.44	0.61	0.15
	AA117b	0.95	0.22	0.88	0.30
	AA118a	0.78	0.10	1.05	0.14
	AA120b	1.17	0.18	0.94	0.42
B	610 1-1	1.07	0.03	1.15	0.11
	610 10-1	1.35	0.07	1.07	0.10
	610 21-1	0.70	0.21	0.81	0.19
	610 30-1	0.94	0.51	0.90	0.50
	610 32-1	0.67	0.38	0.70	0.41
C	ZM246F	0.78	0.11	0.99	0.07
	ZM249M	0.91	0.12	1.32	0.10
D	p190049	0.83	0.10	0.60	0.14
	p191647	0.55	0.02	0.97	0.23
	p191859	1.17	0.03	0.73	0.10
	p191882	0.76	0.24	1.64	1.20
	SC191727	0.52	0.40	0.57	0.21
RAL-R	p8070	11.17	8.40	14.71	8.29
	p4736	3.88	1.37	8.03	3.50

RAL-R = raltegravir resistant. EC50 F.C. = 50% effective concentration fold-change relative to the reference virus. EC90 F.C. = 90% effective concentration fold-change. s.d. = standard deviation.

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