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BRIEF REPORT

Selection of Rilpivirine-Resistant HIV-1 in a Seroconverter From the SSAT 040 Trial Who Received the 300-mg Dose of Long-Acting Rilpivirine (TMC278LA)

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The injectable long-acting formulation of rilpivirine (TMC278LA) is a promising preexposure prophylaxis (PrEP) candidate for prevention of human immunodeficiency virus type 1 (HIV-1) infection. We evaluated HIV-1 in plasma obtained from an unexpected seroconverter in the 300-mg arm of the SSAT040 TMC278LA pharmacokinetic study for rilpivirine (RPV) resistance. Infection with wild-type HIV-1 was confirmed on day 84 after TMC278LA injection, and the K101E mutation was detected on day 115. Plasma-derived HIV-1 clones containing K101E had 4-fold increased resistance to RPV and 4–8-fold increased cross-resistance to etravirine, nevirapine, and efavirenz compared with wild type HIV-1 plasma-derived clones from the same individual. This case is a unique instance of infection with wild-type HIV-1 and subsequent selection of resistant virus by persistent exposure to long-acting PrEP.

Keywords. HIV-1; pre-exposure prophylaxis (PrEP); rilpivirine; TMC278LA; NNRTI resistance.

Rilpivirine (RPV) is a potent second-generation nonnucleoside reverse-transcriptase inhibitor (NNRTI). An injectable, long-acting formulation of rilpivirine (TMC278LA) is a promising candidate for preexposure prophylaxis (PrEP) for prevention of human immunodeficiency virus type 1 (HIV-1) infection owing to its subnanomolar 50% effective concentration, long half-life, and minimal side effects [1, 2]. Coitus-independent and monthly administration of TMC278LA could improve the adherence and effectiveness of PrEP, compared with oral and topical products. However, the long half-life of TMC278LA is concerning because of the possibility of selection of drug resistance if a person becomes infected with HIV-1 after TMC278LA receipt. The duration of TMC278LA detectability after injection has been demonstrated to exceed 150 days [3], but the levels of active drug required to prevent HIV-1 infection have not yet been determined.

The prevalence of RPV-associated mutations is only 5% in treatment-naive individuals but is as high as 59% in patients with no response to NNRTI-containing first-line antiretroviral therapy (ART) [4–8]. The July 2014 International AIDS Society USA HIV-1 drug resistance mutation update recognizes 17 mutations associated with RPV drug resistance, including L100I, K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C, and M230I/L [9], which confer up to 50-fold resistance to RPV [1, 9, 10]. During the ECHO and THRIVE trials, which evaluated RPV in a background of 2 NRTIs, E138K with M184I most commonly emerged in individuals with virologic failure. Of the samples from patients with virologic failure in these trials who exhibited phenotypic resistance to RPV, 46% had virus with cross-resistance to nevirapine (NVP), 86% had virus with cross-resistance to efavirenz (EFV), and 91% had virus with cross-resistance to etravirine (ETR) [11].

The SSAT040 study was a pharmacokinetic evaluation of the exposure and distribution of TMC278LA for use as PrEP in plasma and genital tract/rectal compartments, following a single 300-mg, 600-mg, or 1200-mg intramuscular injection. SSAT040 aimed to determine tolerability and measure detectable levels of drug in plasma and tissue at intervals ranging from 4 hours to 84 days after injection. A breakthrough HIV-1 infection unexpectedly occurred in 1 female participant in the 300-mg dose arm after a single episode of vaginal intercourse with a new male partner without the use of barrier contraception [12]. We evaluated this breakthrough infection and report here the levels of RPV in plasma and cervicovaginal fluid (CVF) and the emergence of NNRTI-resistant HIV-1, and we discuss the potential impact of this resistance on subsequent treatment with first-line NNRTIs.

MATERIALS AND METHODS

Study Samples

SSAT040 was a pharmacokinetic evaluation of the exposure and distribution of TMC278LA for use as PrEP in plasma and genital tract/rectal compartments after a single intramuscular dose of 300 mg, 600 mg or 1200 mg of RPV in 60 low-risk female HIV-negative volunteers (clinical trials registration NCT01275443). The study protocol had UK National Research Ethics Service approval, and all study participants provided

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written informed consent. Detailed design, conduct, and primary analysis of the study have been reported [12]. Participants for the SSAT040 study were verified to be HIV seronegative by the 4th Generation GS HIV Combo Ag/Ab EIA HIV-1/2+O (Bio-Rad) prior to enrollment and were deemed to be at low risk for HIV infection as indicated by self-report. Plasma samples obtained at multiple time points from the participant with breakthrough infection were retrospectively tested for HIV-1 RNA (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0, Roche Molecular Diagnostics) and for HIV-1 antibody at multiple time points, using the Bio-Rad 4th Generation GS HIV Combo Ag/Ab HIV-1/2+O EIA. Plasma and CVF were collected at regular intervals over 84 days for pharmacokinetic analysis. Resistance testing was performed on a residual sample collected following pharmacokinetic analysis. Sufficient plasma specimens for resistance testing were not available at all time points.

PK Analysis

RPV levels were measured in plasma and CVF as previously described [13].

Resistance Analysis

HIV-1 RNA from plasma samples was isolated using the ViroSeq 2.0 extraction module (Celera). In addition, RNA from plasma samples collected in heparinized tubes was precipitated in 3 M LiCl prior to amplification, to alleviate polymerase chain reaction (PCR) inhibition. Protease and full-length reverse transcriptase (amino acids 1 through 560) were amplified using in-house primers (OF1-BCD-5′-GAGGGACACCAAATGAAAG AYTG-3′ and 4232 5′-CCTGACTTTGGGGATTGTAGGGA AT-3′) in a 1-step reverse-transcription PCR, using SuperScript III One-Step (Invitrogen), followed by a second nested PCR, using in-house primers (Bcl1-5′-TAAGACAGTATGATCAAA TACTTATAGAAATTTGTGG-3′ and Xho1-5′-TAACTTTT CCCTCGAGATGTGTACAATCTAATTGCC-3′), to amplify full-length reverse transcriptase. Sequence was generated by means of 6 bidirectional primers, using Sanger sequencing methods. HIV-1 drug resistance mutations were identified using the HIVdb program v7 (Stanford University) [14]. Allele-specific PCR for K101K and K101E was performed using RNA isolated for standard genotyping. Complementary DNA species were amplified and quantified in a first-round PCR, using Phusion Hot Start II High Fidelity Polymerase (Thermo Fisher Scientific) and HIV-1 subtype C reverse transcriptase–specific primers 28F 5′-AAACAATGGCCATTGACAGAAGA-3′ and 80R 5′-GTTCATACCCCATCCAAAGAAATG-3′. A total of 1×10^6 copies of first-round PCR product were used in an allele-specific PCR reaction, using AmpliTaq Gold (Applied Biosystems) and the following patient-specific primers: forward wild-type primer 5′-GGAATACCGCACCCAGCAGGATTCA-3′, forward 101E primer 5′-GGAATACCGCACCCAGCAGGATT CG-3′, and reverse primer 5′-CTCTGGAATATTGCTGGTG ATCCTT-3′. The detection limit for K101E is 0.1%, based on testing a panel of K101:101E PCR amplicon mixtures included in each assay.

Phenotypic Analysis

Full-length reverse transcriptase amplicons created for genotyping from the day 115 sample were used to generate recombinant patient-derived virus, using a modified HIV-1_{xxLAI} vector [15]. PCR amplicons from the day 115 sample were cloned into Bcl1-Xho1 linearized xxLAI plasmids, using InFusion cloning technology (Clontech). DNA generated from single-colony bacterial isolates was selected based on having either K101 or 101E and having identical sequence for the remainder of the reverse transcriptase gene. Midiprep DNA from these isolates were used to transfect 293T cells, using Lipofectamine2000 (Invitrogen), and viral supernatants were collected 48 hours after transfection. Patient-derived viral stocks were used in drug susceptibility assays using TZM-bl cells and normalized for output of 100 relative light units in virus control wells. Fifty percent inhibitory concentrations (IC_{50}) were determined for RPV, nevirapine (NVP), efavirenz (EFV), and etravirine (ETR).

RESULTS

HIV-1 Infection and RPV Levels

One female participant in the SSAT040 study acquired HIV-1 infection through heterosexual intercourse without a condom with a new male partner approximately 41 days following receipt of a 300-mg single intramuscular injection of TMC278LA. The male partner was subsequently found to have acute HIV-1 infection at the time of transmission. Retrospective testing showed that the SSAT040 participant had undetectable plasma HIV-1 RNA (level, <100 copies/mL) on the day she received the TMC278LA injection (day 0). HIV-1 RNA was first detected on day 57 (level, 370 copies/mL), followed by seroconversion on day 84. The peak viremia level (644 925 copies/mL) occurred at day 115 and declined after initiation of ART with tenofovir/emtricitabine and ritonavir-boosted darunavir (800/100 mg) on day 115 but remained detectable through day 275 (level, 125 copies/mL) until finally achieving viral suppression below the limit of quantitation (level, <40 copies/mL) on day 309 (Table 1). Both plasma and CVF drug concentrations peaked on day 15 (68.1 ng/mL and 294.9 ng/mL, respectively) and then dropped 6.5-fold and 16-fold respectively by day 41 (the suspected day of HIV exposure), but low levels of RPV were still detectable in plasma on day 226 (4.0 ng/mL). RPV drug concentrations were approximately 10.5 ng/mL and 18.3 ng/mL in plasma and CVF, respectively, at the time of HIV-1 exposure.

Selection of HIV-1 Drug Resistance

We evaluated residual plasma samples collected for pharmacokinetic analysis for the presence of selected or transmitted drug resistance (Table 1). Infection with wild-type virus, as determined by both standard population sequencing and allele-specific PCR with an assay sensitivity of 0.1% for 101E, was

Table 1. Rilpivirine (RPV) Concentration, Human Immunodeficiency Virus Type 1 (HIV-1) Infection History, and Drug Resistance Selection, by Time After Intramuscular Injection, in a Seroconverter From the SSAT040 Study

Time After TMC278LA Injection[a]	RPV Concentration, ng/mL			HIV-1 Serostatus	Plasma HIV-1 RNA Load, Copies/mL	$CD4^+$ T-Cell Count, Cells/μL (% of WBCs)	Resistance Mutation Detected[b]	K101E Frequency, % Frequency of K101E[c]
	Plasma	CVF	Vaginal Tissue					
Screening	. . .	. . .	. . .	Negative	. . .	. . .	. . .	. . .
0 h	0	. . .	. . .	. . .	<100	. . .	. . .	. . .
4 h	4.1	. . .	. . .	. . .	. . .	. . .	. . .	. . .
8 h	18.2	7.5	. . .	. . .	. . .	. . .	. . .	. . .
1 d	37.4	51.3	. . .	. . .	<100	. . .	. . .	. . .
4 d	53.6	91.8	. . .	. . .	<100	. . .	. . .	. . .
7 d	58.2	133.3	. . .	. . .	<100	. . .	. . .	. . .
11 d	42.6	. . .	. . .	. . .	<100	. . .	. . .	. . .
15 d	68.1	294.9	. . .	. . .	<100	. . .	. . .	. . .
22 d	24.3	135.3	. . .	. . .	<100	. . .	. . .	. . .
28 d	24.3	32.9	243.2	Negative	<100	. . .	. . .	. . .
44[d] d	10.5	18.3	. . .	. . .	<100	. . .	. . .	. . .
57 d	6.8	11.2	23.1	Negative	370	. . .	. . .	. . .
84 d	7.5	14.0	. . .	Positive	175 060	. . .	None	0
115[e] d	4.1	. . .	. . .	Positive	644 925	410 (22)	K101EK[f]	19.4
122 d	. . .	. . .	. . .	. . .	74 964	526 (34)	. . .	. . .
136 d	. . .	. . .	. . .	. . .	18 006	692 (44)	. . .	. . .
151 d	13.8	. . .	. . .	. . .	6204	617 (49)	K101EK	. . .
175 d	. . .	. . .	. . .	. . .	2998	605 (49)	. . .	. . .
199 d	6.0	. . .	. . .	. . .	3558	834 (53)	None	0.1
211 d	. . .	. . .	. . .	. . .	1184	. . .	. . .	. . .
226 d	4.0	. . .	. . .	. . .	769	. . .	. . .	. . .
275 d	. . .	. . .	. . .	. . .	125	. . .	. . .	. . .
309 d	. . .	. . .	. . .	. . .	<40	. . .	. . .	. . .

Abbreviations: CVF, cervicovaginal fluid; WBC, white blood cell.

[a] Participant was given a single dose of 300 mg of long-acting RPV (ie, TMC278LA).

[b] Resistance mutation detection was performed by a standard genotyping technique.

[c] Data were determined by allele-specific polymerase chain reaction analysis, with a limit of detection of 0.1%.

[d] HIV exposure self-reported to have occurred on day 41. Samples collected on day 44 were the first samples collected after reported exposure.

[e] Antiretroviral therapy was initiated with tenofovir/emtricitabine and ritonavir-boosted darunavir (800/100 mg).

[f] The susceptibility of patient-derived recombinant HIV-1 isolates collected on day 115 with K101E (HIV-$1_{d115/K101E}$) to RPV, nevirapine (NVP), efavirenz (EFV), and etravirine (ETR) was compared to that of wild-type HIV-1 isolates (HIV-$1_{d115/WT}$) collected on day 115. HIV-$1_{d115/K101E}$ had an RPV 50% inhibitory concentration (IC_{50}) of 0.58 ng/mL (4.3-fold greater resistance), compared with 0.13 ng/mL for HIV-$1_{d115/WT}$. HIV-$1_{d115/K101E}$ had an NVP IC_{50} of 98 ng/mL (7.9-fold greater cross-resistance), compared with 12 ng/mL for HIV-$1_{d115/WT}$. HIV-$1_{d115/K101E}$ had an EFV IC_{50} of 0.88 ng/mL (4.0-fold greater cross-resistance), compared with 0.22 ng/mL for HIV-$1_{d115/WT}$. HIV-$1_{d115/K101E}$ had an ETR IC_{50} of 1.8 ng/mL (4.0-fold greater cross-resistance), compared with 0.44 ng/mL for HIV-$1_{d115/WT}$.

. . ., Parameter not tested.

confirmed during acute infection on day 84, which was 43 days after the estimated date of HIV-1 infection. At the peak viremia level, on day 115, however, a mixed population of 101K/E was detected by both population sequencing and allele-specific PCR (19% 101E). By day 199, the predominant viral population was wild type, with only 0.1% 101E detected by allele-specific PCR (Table 1). Samples from the participant's sex partner were not available for testing.

Cross-resistance to NNRTIs

We used day 115 patient-derived recombinant viral isolates containing either 101E or 101K to evaluate phenotypic resistance to RPV and cross-resistance to NVP, EFV, and ETR. Day 115 isolates containing 101E had 4.3-fold greater resistance (IC_{50}, 0.58 ng/mL) to RPV, compared with day 115 isolates containing K101 (IC_{50}, 0.13 ng/mL). Isolates containing 101E had 7.9-fold greater cross-resistance to NVP and 4.0-fold greater cross-resistance to both EFV and ETR (IC_{50}, 98 ng/mL for NVP, 0.88 ng/mL for EFV, and 1.8 ng/mL for ETR vs 12 ng/mL for NVP, 0.22 ng/mL for EFV, and 0.44 ng/mL for ETR; Supplementary Table 1).

DISCUSSION

RPV concentrations in plasma and CVF after a single injection of 300 mg of TMC278LA were insufficient to prevent infection in 1 female participant from the SSAT040 study. The infection event occurred 41 days following TMC278LA administration, when the plasma RPV concentration was 10.5 ng/mL and the CVF RPV concentration was 18.3 ng/mL. Although the concentration of RPV required for prevention is not known, the RPV levels at the time of HIV exposure were well below the proposed

target concentration of 50 ng/mL that is the minimum needed for virologic response when RPV is used as ART [12]. Of note, the infected participant displayed higher peak concentrations of RPV in CVF and vaginal tissue, compared with other participants in the same 300-mg arm (who may or may not have had exposure to HIV), but free drug concentrations and the pharmacodynamic effect of RPV in these sites is undefined [12]. RPV levels continued to be low but detectable through the testing period of 226 days. High levels of viral replication combined with low but detectable levels of RPV led to the selection of resistant variants containing K101E. Initial infection occurred with wild-type virus, as demonstrated by both standard and sensitive resistance testing, and thus resistance was selected by TMC278 and not transmitted from the participant's partner. A mixed population of viruses, consisting of approximately 80% wild-type and 20% mutant 101E variants, emerged by day 115, coinciding with peak viremia. Phenotypic testing of the day 115 mutant showed resistance to RPV (4-fold) and cross-resistance to NVP (7-fold), EFV (4-fold), and ETR (4-fold), which indicates that the K101E mutant could negatively influence the virologic response to first-line NNRTI-containing ART. The 101E variants declined to undetectable levels in parallel with the rapid decline in viremia level after ART initiation (Figure 1).

This case report is a unique instance of well-documented infection with wild-type HIV-1 and subsequent selection of resistant virus by continued drug exposure from a long-acting drug formulation. The 300-mg dose of TMC278LA was insufficient to prevent infection and limit viral replication but was high enough to select RPV-resistant virus, illustrating a negative consequence of a long-acting antiretroviral formulation. RPV concentrations that prevent HIV infection prevention are undefined, but the 300-mg dose of TMC278 did not achieve RPV levels above the proposed therapeutic concentration for virologic suppression (50 ng/mL). Further studies are needed to determine a target RPV concentration for HIV-1 prevention and to evaluate the risk of resistance during breakthrough infection with higher doses of TMC278. Both plasma and vaginal concentrations of RPV must be considered when selecting the appropriate dose for HIV prevention.

Our study had 2 limitations. First, resistance testing was done using residual plasma specimens obtained after pharmacokinetic testing, so all intermediate time points were not available for standard or sensitive resistance testing. Second, we were not able to collect samples from the participant's sex partner to verify that he was infected with a wild-type virus, but the founder virus in the recipient was wild type at codon 101, based on sensitive allele-specific PCR.

Nonadherence to daily or coitally dependent PrEP has emerged as the major barrier to PrEP effectiveness. Long-acting formulations have the advantage of infrequent dosing, but sustained, low drug concentrations for months after an injection is

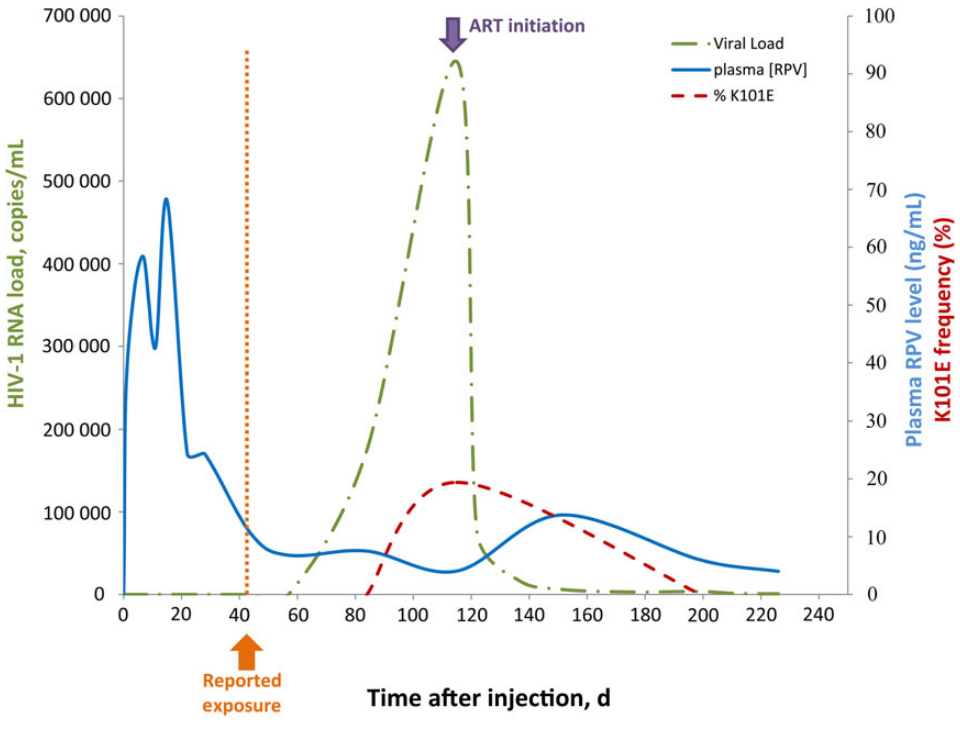

Figure 1. Timeline of human immunodeficiency virus type 1 (HIV-1) RNA load (dash-dotted line), plasma rilpivirine (RPV) concentration (solid line), and selection of the K101E mutation (dashed line) in 1 participant from the SSAT040 study. HIV-1 exposure was reported to have occurred on day 41 (dotted vertical line), and antiretroviral therapy (ART; tenofovir/emtricitabine and ritonavir-boosted darunavir [800/100 mg]) began on day 115.

problematic for the selection of drug resistance when breakthrough infection occurs. Our study highlights the importance of frequent HIV-1 testing with PrEP use and the need to develop a safe method for discontinuing long-acting products to avoid infection and drug resistance. Most importantly, the dose of injectable long-acting formulations such as TMC278LA must be high enough and delivered frequently enough to achieve drug levels that prevent HIV-1 infection.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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