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Hepatotoxicity Observed in Clinical Trials of Aplaviroc (GW873140)▽

W. G. Nichols,[1]* H. M. Steel,[1] T. Bonny,[1] K. Adkison,[1] L. Curtis,[1] J. Millard,[1] K. Kabeya,[2] and N. Clumeck[2]

GlaxoSmithKline, Research Triangle Park, North Carolina,[1] and CHU-Saint-Pierre, Brussels, Belgium[2]

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Aplaviroc (APL) was a new CCR5 antagonist that was investigated in two dose-ranging studies with antiretroviral therapy-naïve, human immunodeficiency virus-infected adults: ASCENT, in which 147 subjects were randomized 2:2:1 to receive zidovudine-lamivudine (ZDV-3TC) plus APL 600 mg twice a day (BID), APL 800 mg BID, or efavirenz (EFV), respectively, and EPIC, in which 195 subjects were randomized 2:2:2:1 to receive lopinavir-ritonavir (LPV-RTV) plus APL 200 mg BID, APL 400 mg BID, APL 800 mg once a day, or ZDV-3TC BID, respectively. Both studies (and, ultimately, the clinical development of APL) were discontinued after a mean of 14 weeks of therapy because of higher than anticipated severe liver toxicity; grade 2 or higher treatment-emergent elevations in alanine aminotransferase (ALT) levels were observed in 17/281 (6.0%) APL recipients but only 2/55 (3.6%) control recipients, while grade 2 or higher elevations in total bilirubin levels occurred in 29/281 (10.3%) APL recipients but only 4/55 (7.3%) controls. Two APL recipients developed grade 3 or higher treatment-emergent elevations in both ALT and total bilirubin levels, and one of these individuals had a severe case of hepatic cytolysis that was attributed to APL. Despite the high intersubject variability in APL plasma exposures, a Pearson correlation analysis of the combined study data did not reveal any significant associations between plasma concentrations and the liver enzyme elevations observed during the study. The mechanism for the idiosyncratic hepatotoxicity observed in the clinical trials of APL is unknown but is likely intrinsic to the molecule rather than its novel mechanism of action.

Aplaviroc (APL; GW873140) is a chemokine (C-C motif) receptor 5 (CCR5) antagonist that was being developed for the treatment of patients with human immunodeficiency virus (HIV) infection/AIDS. In vitro, APL exhibited high-affinity binding to human CCR5 and had subnanomolar activity against a broad panel of laboratory and primary HIV type 1 (HIV-1) isolates (15). Preclinical studies established that after oral administration of radiolabeled APL to rats and monkeys, there was a good distribution to tissues, with 70- to 100-fold higher concentrations of radioactivity noted in the liver than in the blood, consistent with the in vitro observation that APL was both a substrate and an inhibitor of the organic anion transport protein 1B1. Radiolabeled APL studies with rats, monkeys, and humans revealed that the compound is primarily eliminated in the feces as the parent and oxidative or glucuronidated metabolites. A significant portion of the fecal excretion in intact animals was likely through the bile, based on studies with rats and monkeys. In human liver microsomes and enzyme systems that express cytochrome (CYP) P450, APL was predominantly metabolized by CYP3A, with some minor involvement of CYP2C19, and was a weak time-dependent inhibitor of CYP3A. Overall, the results from preclinical toxicology studies were consistent with further clinical development: while changes in alanine aminotransferase (ALT) and bilirubin levels were observed in long-term studies with rats treated with extremely high doses of APL (>500 mg/kg of body weight/day), no adverse effects were observed in monkeys treated with doses up to 2,000 mg/kg/day.

In healthy human subjects, APL exhibited dose-proportional pharmacokinetics (PKs) in the 200- to 800-mg twice-daily (BID) dose range and had a half-life of approximately 3 h (1). Short-term studies of APL with HIV-infected subjects revealed good antiviral responses (mean 1.66 $\log_{10}$ decline in the HIV-1 RNA level at the nadir after 10 days of monotherapy with APL 600 mg BID) and a safety profile that justified further clinical development (14). Two 96-week, phase IIb dose-ranging studies of APL with antiretroviral therapy-naïve, HIV-infected subjects were thus initiated in early 2005. ASCENT (a Study of Combivir and Entry Inhibitor in Naïve Treatment) was designed to test the activity of APL as a third antiretroviral agent in combination with zidovudine-lamivudine (ZDV-3TC); EPIC (Entry and Protease Inhibitor in Combination), was designed to investigate the novel combination of APL and lopinavir-ritonavir (LPV-RTV), given the significant boosting of plasma APL exposures observed with these drugs (2).

In August 2005, a case of severe hepatic cytolysis was reported as a serious adverse event (SAE) for a subject in ASCENT; that report was followed by a report of hyperbilirubinemia in EPIC. An expedited analysis of liver enzyme abnormalities was conducted to assess the occurrence of hepatic events in the APL development program; that analysis confirmed a higher than anticipated rate of liver enzyme elevations in some recipients of APL and led to the premature discontinuation of both of these studies in September 2005. A preliminary analysis of the antiviral activity of APL and the overall safety/tolerability of APL from those studies has been presented previously (7, 24). This report presents the clinical and

* Corresponding author. Mailing address: Infectious Diseases Medicine Development Centre, GlaxoSmithKline, 881-995 Greenford Road, Greenford, Middlesex UB6 0HE, United Kingdom. Phone: 44 20 8966 4648. Fax: 44 20 8966 3674. E-mail: garrett.x.nichols@gsk.com.
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PK details for the signal of idiosyncratic hepatotoxicity that halted the clinical development of APL.

MATERIALS AND METHODS

Study design. ASCENT was a phase IIb, 96-week, randomized, partially double-blinded, multicenter study conducted to evaluate the safety, tolerability, PKs, and antiviral effects of two different dosing regimens of APL in HIV-1-infected antiretroviral therapy-naïve naive subjects. The subjects were randomized 2:2:1 to receive APL 600 mg BID, APL 800 mg BID, or efavirenz (EFV), respectively, all in combination with a fixed-dose combination of ZDV and 3TC 300/150 mg BID. EPIC was a phase IIb, 96-week, open-label, randomized, multicenter study conducted to evaluate the safety, tolerability, PKs, and antiviral effects of three different APL dosing regimens in HIV-1-infected antiretroviral therapy-naïve subjects. The subjects were randomized 2:2:2:1 to receive APL 200 mg BID, APL 400 mg BID, APL 800 mg once a day (QD), or ZDV-3TC BID, respectively, all in combination with LPV-RTV 400/100 mg BID.

The primary endpoint of both studies was the proportion of subjects with plasma HIV-1 RNA levels <400 copies/ml while remaining on their randomized treatment regimen through week 12; longer-term efficacy, in addition to PKs, safety, and tolerability, was an important secondary endpoint.

Clinical and laboratory assessments. Clinical evaluations of adverse events (AEs) and SAEs were performed at the screening, on day 1 (baseline), at weeks 2 and 4, every 4 weeks thereafter through week 24, and every 8 weeks thereafter through withdrawal. Upon the identification of the hepatic safety signal, the protocols were amended to immediately halt dosing; the subjects were encouraged to complete a withdrawal visit and follow-up visits at 2, 4, 8, and 12 weeks postwithdrawal to capture any AEs. Laboratory testing was performed at a central laboratory on the same schedule and included complete blood count with lymphocyte subset determination, a serum chemistry panel (including aspartate aminotransferase and ALT, total bilirubin, alkaline phosphatase, creatine kinase, and lipase determinations), and plasma HIV-1 RNA level determinations by PCR; the upper limit of normal (ULN) values for ALT and total bilirubin were 48 U/liter and 22 μmol/liter (1.3 mg/dl), respectively. In addition, hepatitis B surface antigen and hepatitis C serology were performed for all subjects at the baseline visit. Clinical and laboratory AEs were graded according to the 2004 toxicity grading scale of the Division of Acquired Immunodeficiency Syndrome (DAIDS), National Institute of Allergy and Infectious Diseases (20), which defines grade 2 ALT toxicity levels as 2.6 to 5.0 times the assay ULN; grade 3 and 4 ALT toxicities are defined as 5 to 10 times the ULN and >10 times the ULN, respectively. Elevations in total bilirubin levels of 1.6 to 2.5 times the ULN are defined as grade 2 toxicity, while grade 3 and 4 toxicity levels are defined as 2.6 to 5.0 times the ULN and >5 times the ULN, respectively. This report presents AE and laboratory data from the treatment phase, which includes all data collected while the subjects were receiving a randomized treatment through 30 days following treatment discontinuation, when additional antiretroviral medications were often initiated. This analysis represents a conservative approach designed to capture AEs that occurred shortly after APL withdrawal.

PK assessments. In both studies, a single sample for PK assessments was collected from each subject at weeks 4, 12, and 24. In addition, a subset of 10 to 15 subjects per dose regimen provided serial samples for PK assessments over the course of one dosing interval at week 12. APL plasma PK parameter estimates were generated by a nonlinear mixed-effects modeling approach and post-hoc analysis for all subjects who provided at least one sample for PK assessment. The relationship between individual estimates of the plasma APL area under the curve concentration (AUC) from 0 to 24 h (AUC_{0-24}) and measures of liver function (ALT or total bilirubin levels at weeks 4 and 12, maximum observed ALT level, maximum observed total bilirubin level) were explored by using Pearson's correlation analysis.

RESULTS

Baseline demographics. A total of 193 subjects were enrolled in EPIC; of these, 191 subjects received at least one dose of study medication and were included in the analysis. A total of 147 subjects were enrolled in ASCENT; of these, 145 subjects received at least one dose of study medication. The baseline characteristics for each population are presented in Table 1.

Exposure to study medications. At the time of study termination, the mean duration of exposure to APL was 14 weeks for subjects in both studies, with maximum exposures of 33 weeks in EPIC and 29 weeks in ASCENT. Five subjects (3%) receiving APL in EPIC and 17 subjects (15%) receiving APL in ASCENT had discontinued the study drugs due to AEs at the time of study termination. The most common AEs that led to the discontinuation of APL were gastrointestinal in nature (7, 24), consistent with the AEs in previous short-term studies with healthy volunteers (1) and HIV-infected subjects (14).

Index case of hepatic cytolysis. The index case of severe hepatic injury occurred in a 38-year-old HIV-positive black male subject in ASCENT; his baseline CD4 count was 283 cells/mm^3, and his baseline HIV-1 RNA level was 31,000 copies/ml. He had no significant underlying medical conditions, was negative for hepatitis B and C viruses, and had normal baseline ALT and bilirubin levels. Fifty-nine days after starting APL 800 mg plus ZDV-3TC BID, he developed asymptomatic increases in ALT levels that resulted in the cessation of therapy (Fig. 1). Shortly thereafter (and coincident with further increases in ALT levels and a delayed rise in total bilirubin levels), he developed symptoms of fatigue, nausea, and memory loss. The subject denied the use of illicit drugs or concomitant medications. Serologies were negative for hepatitis A, B, C, and E viruses (including hepatitis B virus DNA and hepatitis C virus RNA), Epstein-Barr virus, cytomegalovirus, and autoimmune diseases. A liver biopsy was conducted 10 days after treatment cessation and at the time of the maximum ALT elevation. This showed portal space inflammation (Fig. 2) and fragmented discrete hepatic necrosis; the pathology was negative for alternative causes and was thus suggestive of acute drug toxicity. The investigator attributed the hepatic cytolysis to APL. Four weeks later, the subject was asymptomatic; his enzyme levels returned to normal 8 weeks after treatment discontinuation.

Treatment-emergent toxicities in ALT. Subjects were analyzed from their baseline visit through their last follow-up visit (up to 12 weeks after discontinuation of the regimen). While the occurrence of any treatment-emergent ALT toxicity (>1.25 times the ULN) was comparable between the APL and EFV arms in ASCENT (15/116 [13%] and 6/29 [21%] subjects, respectively), there was a higher frequency of grade 2 or higher ALT elevations (>2.5 times the ULN) in subjects receiving APL (8/116 [7%]) than in subjects receiving ZDV-3TC-EFV (0/29 subjects). Six subjects (10%) receiving APL 800 mg BID developed grade 2 or higher ALT elevations, whereas two subjects (3%) receiving APL 600 mg BID developed grade 2 or higher ALT elevations. Interestingly, in EPIC the proportion of subjects with ALT elevations was similar between treatment groups: grade 2 to 4 increases in ALT levels were seen in 9/165 (5.5%) APL-treated subjects and 2/26 (7.7%) of ZDV-3TC-LPV-RTV-treated subjects. There were no differences in ALT levels according to the APL dosing cohort in EPIC.

The median (interquartile range) changes in ALT levels from the baseline were plotted over time (Fig. 3) and compared according to the receipt of APL or the control by using a nonparametric Wilcoxon test. There were no significant differences detected by treatment group in either ASCENT ($P = 0.52$) or EPIC ($P = 0.23$). However, extreme outliers in the

TABLE 1. Baseline characteristics of subjects enrolled in the EPIC and ASCENT studies

Baseline characteristic	EPIC		ASCENT	
	APL + LPV-RTV (n = 165)[a]	ZDV-3TC + LPV-RTV (n = 26)	APL + ZDV-3TC (n = 116)[b]	EFV + ZDV-3TC (n = 29)
Age category (no. [%] of subjects)				
<35 yr	71 (43)	9 (35)	35 (30)	12 (41)
≥35 yr	94 (57)	17 (65)	81 (70)	17 (59)
Gender (no. [%] of subjects)				
Male	140 (85)	23 (88)	94 (81)	26 (90)
Female	25 (15)	3 (12)	22 (19)	3 (10)
Race				
African heritage	25 (15)	3 (12)	23 (20)	7 (24)
American Indian or Alaskan	3 (2)	0	4 (3)	1 (3)
Asian	2 (1)	1 (4)	1 (1)	0
Japanese/East Asian	2 (1)	1 (4)	1 (1)	0
Pacific Islander	1 (<1)	0	0	0
White	130 (79)	21 (84)	88 (76)	21 (72)
Missing	2 (1)	0	0	0
Ethnicity				
Hispanic or Latino	31 (19)	5 (19)	21 (18)	10 (34)
Not Hispanic or Latino	134 (81)	21 (81)	95 (82)	19 (66)
Baseline mean (range) $\log_{10}$ no. of plasma HIV-1 RNA copies/ml	5.2 (3.8–6.3)	5.2 (4.6–6.1)	5.0 (4.0–6.6)	5.1 (3.8–6.2)
Baseline mean (range) $CD4^+$ cell count (no. of cells/mm^3)	277 (56–821)	299 (95–529)	261 (87–663)	292 (133–633)
CDC classification (no. [%] of subjects)				
Class A	141 (85)	21 (81)	93 (80)	23 (79)
Class B	20 (12)	3 (12)	17 (15)	2 (7)
Class C	4 (2)	1 (4)	6 (5)	4 (14)
Missing	0	2 (8)	0	0
No. (%) of subjects) with the following hepatitis B virus/ hepatitis C virus infection status[c]:				
Negative/negative	142 (86)	23 (88)	106 (91)	25 (86)
Positive/negative	3 (2)	1 (4)	3 (3)	2 (7)
Negative/positive	16 (10)	0	5 (4)	2 (7)
Positive/positive	1 (<1)	1 (4)	0	0
Missing	3 (2)	1 (4)	2 (2)	0

[a] All APL arms combined; APL 200 mg BID (n = 54), APL 400 mg BID (n = 56), and APL 800 mg QD (n = 55).
[b] All APL arms combined: APL 600 mg BID (n = 58) and APL 800 mg BID (n = 58).
[c] Positive or negative for hepatitis B virus surface antigen and positive or negative for hepatitis C antibody at the baseline.

measured ALT levels appeared more commonly with the APL-based regimens than with the control regimens.

Details regarding the baseline characteristics and clinical course of subjects with grade 3 and higher treatment-emergent ALT toxicities are shown in Table 2. As in the index case, increases in ALT levels were usually asymptomatic and were not accompanied by increases in alkaline phosphatase levels. In ASCENT, one subject (the index case [case 1]) developed severe hepatic cytolysis with significant elevations in both ALT and total bilirubin levels. Case 2 had normal hepatic enzyme levels at the baseline but had increases in ALT levels (56 U/liter) and total bilirubin levels (32 μmol/liter) starting at week 16. He stopped APL at week 24 with study closure, at which time his ALT level was 171 U/liter (grade 2, four times the ULN) and his total bilirubin level was normal at 22 μmol/liter. The levels of both declined 2 weeks later, but the ALT level again increased to 474 U/liter (with a total bilirubin level of 14 μmol/liter) at 4 weeks post-APL discontinuation while the subject was receiving ZDV-3TC-NVP; liver enzyme levels returned to normal 4 weeks after these medications were stopped. One additional subject had a grade 3 elevation of the ALT level (five times the ULN) at baseline, which decreased on APL 800 mg BID therapy.

In EPIC, four subjects developed treatment-emergent grade 3 or higher increases in ALT levels (Table 2). Case 9 had combined increases in ALT and total bilirubin levels; the etiology was somewhat confounded by a history of ethanol use and fatty liver, as determined by ultrasound. Cases 11 and 12 were similarly confounded by coinfection with hepatitis B or C virus and the concomitant use of ethanol; case 12 had only intermittent grade 1 ALT toxicity until after study withdrawal, when grade 4 ALT toxicity was diagnosed in the setting of ethanol use and a flare of hepatitis B virus infection (with a positive serum hepatitis B virus HBV DNA level), despite therapy with tenofovir-emtricitabine-fosamprenavir. Case 10 was not confounded by hepatitis or concomitant medications;

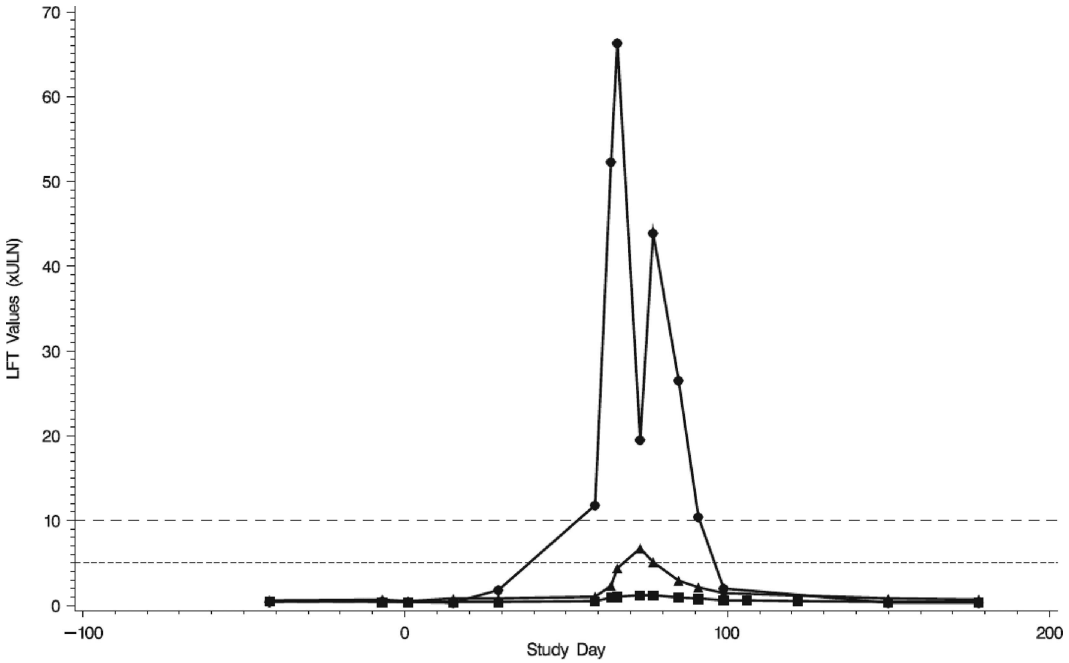

FIG. 1. Serum transaminase levels (●) in the index case increased rapidly 59 days after the initiation of APL therapy, followed by a rise in the total bilirubin level (▲) without concomitant increases in alkaline phosphatase levels (■). The abnormalities resolved 8 weeks after treatment discontinuation. LFT, liver function test; dashed and dotted lines, cutoffs for grade 4 ALT and total bilirubin toxicities, respectively (DAIDS scale; see Materials and Methods).

this subject developed grade 3 increases in ALT levels (maximum, 266 U/liter, with a normal total bilirubin level) at week 12 that resolved with the discontinuation of APL and LPV-RTV. Serologies for acute hepatotropic viruses were negative. Rechallenge with APL plus LPV-RTV led to increases in ALT levels to 423 U/liter after 2 weeks of therapy, which again resolved after treatment discontinuation.

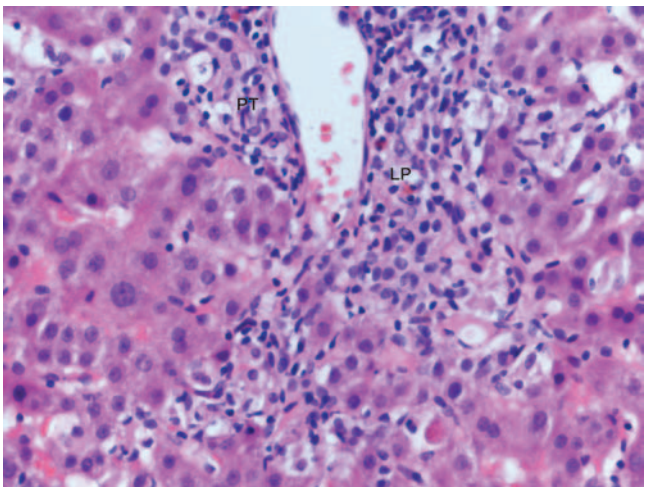

FIG. 2. A liver biopsy specimen taken from the index case 10 days after the cessation of therapy demonstrated that the portal tract (PT) was predominantly infiltrated with lymphocytes and plasma cells (LP). No lymphoid nodules, granulomas, or biliary thrombi were noted. The biopsy specimen was of adequate size, and the overall condition of the tissue was comparable to that in the micrograph shown here. Magnification, ×400.

Treatment-emergent toxicities in total bilirubin. In ASCENT, treatment-emergent toxicity in total bilirubin occurred more frequently in APL recipients (22/116 subjects [19%] experienced any toxicity grade, 13/116 [11%] subjects had grade 2 to 4 toxicities) than in those receiving EFV (1/29 [3%] subjects for either category). In EPIC, treatment-emergent toxicity in total bilirubin occurred in similar proportions of APL recipients (25/165 [15%] subjects experienced any toxicity grade, 16/165 [10%] subjects had grade 2 to 4 toxicities) and ZDV-3TC recipients (3/26 [12%] for either category).

Details regarding the baseline characteristics and clinical course of subjects with grade 3 and higher treatment-emergent toxicities in total bilirubin are shown in Table 2. In ASCENT, aside from the index case, all cases were attributed by the investigator to other causes, including baseline elevations in bilirubin levels (case 3) or the concomitant receipt of atazanavir during the follow-up period after the study was halted (cases 4 to 8). Similarly, in EPIC, baseline characteristics (cases 9 and 13) and concomitant medications (cases 14 to 17) were identified as potential confounders. Case 18 developed grade 3 bilirubin toxicity after a single dose of LPV-RTV (but the subject did not receive APL prior to withdrawal from the study).

Plasma APL PKs. High intersubject variability in the APL plasma AUC was noted, with the week 12 geometric mean APL AUC_{0-24}s being 2,101 ng · h/ml (coefficient of variation [CV], 49%) and 3,413 ng · h/ml (CV, 95%) for the 600-mg and 800-mg arms in ASCENT, respectively, and 2,006 ng · h/ml (CV, 97%), 6,065 ng · h/ml (CV, 76%), and 5,840 ng · h/ml (CV, 147%) for the 200-mg BID, 400-mg BID, and 800-mg QD arms in EPIC, respectively. The Pearson correlation analysis

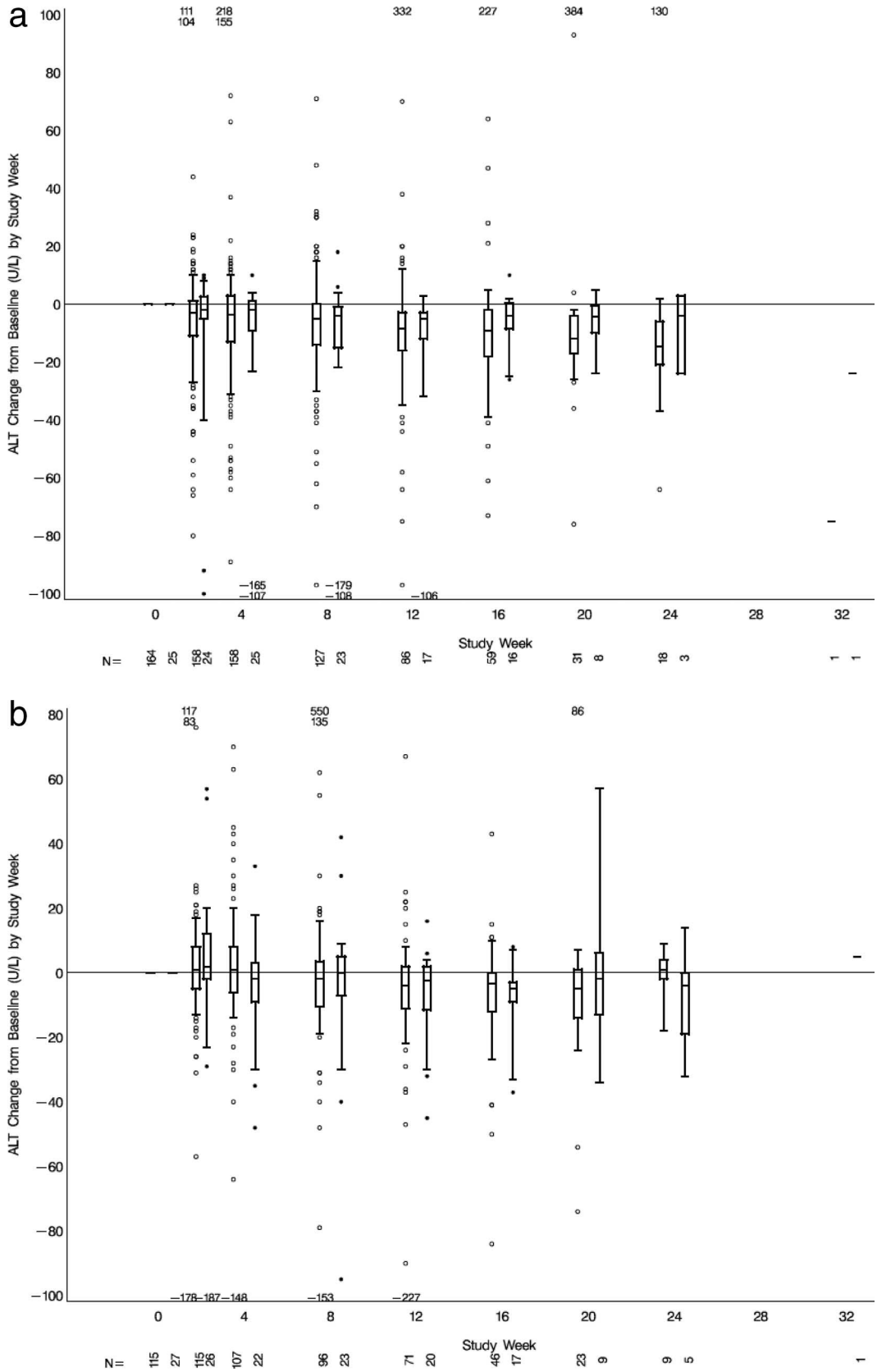

FIG. 3. Changes in ALT levels over time among the recipients of APL-containing (○) and control (●) antiretroviral regimens in EPIC (a) and ASCENT (b). The median values were similar between the treatment groups; however, extreme outliers appeared more commonly in subjects receiving the APL-based regimens than in subjects receiving the control regimens, consistent with idiosyncratic hepatotoxicity. Values outside the *y* axis are annotated with the observed measurement.

TABLE 2. Subjects with treatment-emergent grade 3 or 4 elevations in ALT or total bilirubin levels[a]

Study and case no.	Regimen[b]	Age (yr), race, gender	BL CD4 count (no. of cells/ mm^3)	Onset	Peak abnormality	Comment[c]
ASCENT						
1	APL 800 mg BID	38, B, M	283	Wk 8	ALT, 66× ULN; Bili, 7× ULN	Index case (see text)
2	APL 800 mg BID	33, W, M	228	Wk 28 (FU)	ALT, 10× ULN	G2 ALT toxicity while on APL (wk 24); stopped APL when study closed; G3 toxicity while on ZDV-3TC-NVP
3	APL 600 mg BID	47, W, M	378	Wk 8	Bili, 3× ULN	G2 bili at screening; small fluctuations on therapy (max G3 toxicity) until study close
4	APL 600 mg BID	36, W, M	187	Wk 24 (FU)	Bili, 3× ULN	No toxicity while on APL; WD at wk 20 with study closure; G3 Bili toxicity while on ABC-3TC-ATZ
5	APL 600 mg BID	25, B, F	283	Wk 14 (FU)	Bili, 5× ULN	No toxicity while on APL; WD at wk 12 with study closure; G3 Bili toxicity while on ABC-3TC-ATZ-RTV
6	APL 600 mg BID	43, W, M	362	Wk 20 (FU)	Bili, 5× ULN	G1 Bili toxicity while on APL; WD at wk 18 with study closure; G4 Bili toxicity while on ABC-3TC-ATZ
7	APL 800 mg BID	25, W, M	143	Wk 26 (FU)	Bili, 12× ULN	Max G2 bili toxicity while on therapy from wk 2 until study close; G4 toxicity while on TDF-FTC-ATZ-RTV
8	APL 600 mg BID	25, W, M	87	Wk 14 (FU)	Bili, 5× ULN	No toxicity while on APL; WD at wk 12 for nonresponse; G3 Bili while on TDF-3TC-ATZ-RTV
EPIC						
9	APL 400 mg BID	58, W, M	339	Wk 4	ALT, 17× ULN; Bili, 3× ULN	ALT 3× ULN and fatty liver by U/S at BL (admitted EtOH use); increase in ALT levels, with fever, diarrhea, nausea
10	APL 400 mg BID	37, W, M	443	Wk 16	ALT, 9× ULN	Positive regimen dechallenge and rechallenge
11	APL 200 mg BID	36, W, F	314	Wk 12	ALT, 8× ULN	ALT 1.6× ULN and HCV positive at BL; admitted EtOH use
12	APL 400 mg BID	38, W, M	366	Wk 20 (FU)	ALT, 16× ULN	HBsAg positive at BL; WD at wk 12 with study closure, G4 ALT toxicity while on TDF-FTC-FPV (admitted EtOH use)
13	APL 200 mg BID	38, W, M	265	Wk 4	Bili, 5× ULN	ALT 3× ULN and HBeAg positive at BL; admitted EtOH use
14	APL 400 mg BID	32, W, M	417	Wk 12	Bili, 3× ULN	Resolved with discontinuation of compazine and EtOH
15	APL 400 mg BID	27, W, M	246	Wk 20 (FU)	Bili, 3× ULN	No toxicity while on APL; WD at wk 16 with study closure; G3 Bili toxicity while on ABC-3TC-EFV
16	APL 200 mg BID	29, W, M	297	Wk 24 (FU)	Bili, 4× ULN	No toxicity while on APL; WD at wk 12 with study closure; G3 Bili toxicity while on ATZ-RTV
17	APL 400 mg BID	42, W, M	416	Wk 36 (FU)	Bili, 4× ULN	No toxicity while on APL, WD at wk 24 with study closure; G3 Bili toxicity while on ATZ-RTV
18	APL 800 mg QD	48, W, F	345	Wk 1	Bili 3× ULN	WD after one dose of LPV-RTV (exclusionary ECG at BL); never received APL

[a] Abbreviations: BL, baseline; Bili, total bilirubin; B, black; W, white; M, male; F, female; FU, follow-up (after the subject had stopped taking APL); ABC, abacavir; ATZ, atazanavir; TDF, tenofovir; FTC, emtricitabine; HCV, hepatitis C virus; HBeAg, hepatitis B e antigen; WD, withdrawn; U/S, ultrasound; EtOH, alcohol; G2, grade 2; max, maximum; ECG, electrocardiography.

[b] In the ASCENT study, all subjects received ZDV-3TC BID. In the EPIC study, all subjects received LPV-RTV BID.

[c] Unless otherwise noted, all subjects were negative for hepatitis B and C viruses at the baseline.

of the combined ASCENT and EPIC study data did not reveal any significant associations between the log-transformed APL AUC_{0-24} and the week 4 or 12 ALT levels, the week 12 total bilirubin levels, or the maximum ALT or maximum total bilirubin levels observed during the study, although there was a very weak positive association between the APL AUC_{0-24} and the week 4 total bilirubin level ($R = 0.144$ and $P = 0.049$). No significant associations between

AUC_{0-24} and measures of liver enzymes were observed in the individual study analyses.

DISCUSSION

The phase IIb studies of APL in antiretroviral therapy-naïve subjects (ASCENT and EPIC) were prematurely terminated due to treatment-emergent hepatoxicity (elevations in ALT and/or total bilirubin levels) that were observed in some subjects treated with APL-containing regimens. Two subjects developed grade 3 or higher treatment-emergent elevations in both ALT and total bilirubin levels, although one case (Table 2, case 9) was confounded by ethanol use and evidence of hepatic steatosis at the baseline. The index case, however, developed severe hepatic cytolysis that could not be attributed to alternate causes, and one other case (Table 2, case 10) demonstrated a dechallenge-rechallenge reaction in ALT levels. Because the risk/benefit ratio was not favorable for this population of subjects for whom other treatment options were available, the phase IIb studies with therapy-naïve subjects were halted. The subsequent identification of serious hepatotoxicity in a heavily treatment-experienced participant in the phase III studies of APL (22) led to the termination of the further development of APL.

The occurrence of drug-associated hepatotoxicity is a major problem in all phases of clinical drug development and the most frequent cause of postmarketing warnings and withdrawals (12, 21). Although asymptomatic increases in serum transaminase levels are common in this patient population (23), the decision to halt the clinical development of APL was made in light of the seminal observations by Hyman Zimmerman, who noted that the combination of elevations in serum transaminase levels and jaundice (indicative of serious elevations in serum bilirubin levels) in the setting of drug-induced toxicity was associated with a mortality rate of 10 to 50% (25). Two recent cohort studies have confirmed that drug-induced liver injury is associated with high rates of mortality and/or liver transplantation (4, 6). These observations have been informally adopted as "Hy's Law" by the U.S. Food and Drug Administration (FDA), which considers a cutoff of more than three times the ULN for ALT levels in combination with elevations of total bilirubin levels over 2 mg/dl as being of particular clinical concern (8).

As is the case for many drug candidates (5), liver toxicity was observed at very high doses (500 mg/kg/day) in preclinical toxicology studies with rats but was not observed in repeat-dose studies with monkeys. The mean plasma exposures at which ALT level elevations were detected after repeat dosing in rats, however, were 14-fold higher than the highest mean concentrations observed in the APL 400-mg BID dosing arm in EPIC. Furthermore, high plasma concentrations of APL did not appear to be associated with liver enzyme level elevations, despite the high intersubject variability in APL plasma exposure within and between the two studies. High liver concentrations of APL were observed in some preclinical species; although the concentrations of APL in the human liver are unknown, the observation that APL is both a substrate and an inhibitor of human organic anion transport protein 1B1 suggests that the compound is efficiently taken up by the liver in humans as well. The reason that hepatotoxicity would manifest in only some human subjects treated with the compound, however, is unclear.

For a novel drug class (particularly one that targets the immune system, like CCR5 antagonists do), the primary question is whether the toxicity is compound specific or foreshadows a toxicity for other compounds that share its mechanism of action. In support of the latter hypothesis, two reports recently suggested that CCR5-knockout mice are more susceptible to concanavalin A-induced, immune-mediated hepatic injury (3, 18). The mechanism for this finding appears to be the resistance of CCR5-positive NK T cells to apoptosis; these surviving cells produce high levels of interleukin-4, which appears to mediate the liver damage (3). Studies with HIV-infected and -uninfected humans do not support this animal model, however; the circulating cytokine and chemokine levels (including interleukin-4) in these patients and healthy volunteers showed little change from the baseline after treatment with APL (13). Preclinical toxicology studies also contradict a mechanism-based toxicity. Like other CCR5 antagonists (19), APL binds to macaque CCR5 but not to CCR5 from other species in preclinical studies; the fact that hepatotoxicity was detected in rats but not monkeys supports the theory that hepatotoxicity was more likely compound related rather than due to the effects of CCR5 antagonism.

The clinical experience with other CCR5 antagonists is also informative, particularly since patients have been treated with maraviroc and vicriviroc for longer periods of time. A single case of serious hepatotoxicity was observed in a subject in the treatment-naïve study of maraviroc; that case, however, was confounded by the concomitant receipt of isoniazid, trimethoprim-sulfamethoxazole, and high-dose acetaminophen, drugs that have been associated with hepatotoxicity (16). The combined analysis of the pivotal phase III trials of maraviroc in treatment-experienced patients revealed an exposure-adjusted incidence of grade 3 and 4 adverse liver events of 1.2, 3.5, and 5.3 events per 100 patient-years in the maraviroc daily, twice-daily, and placebo groups, respectively, suggesting no signal for hepatotoxicity (17). Likewise, differences in liver enzyme levels were not reported in phase II studies of vicriviroc (11). An FDA advisory panel concluded that the hepatoxicity observed in the trials of APL does not appear to be a class effect (9).

Indeed, most cases of drug-induced hepatotoxicity are ultimately classified as compound specific and idiosyncratic (12). Idiosyncratic drug reactions are defined by the fact that only certain individuals appear to be susceptible to the drug-induced toxicity. Although the reason that only some individuals experience drug-induced hepatotoxicity remains unknown, it is likely that some combination of genetic predisposition and environmental factors is accountable (12). In the case of APL, the relatively low proportion of affected individuals among those treated is consistent with idiosyncratic drug hepatotoxicity. Indeed, there were no statistically significant differences in the median change in ALT levels from the baseline between the APL-treated and the control arms in either study. This observation suggests one of two possibilities: APL toxicity may occur in only some patients with an as yet undefined cofactor, or the treatment duration was not sufficient to allow hepatotoxicity to emerge in the majority of APL-treated subjects. The former explanation is consistent with the definition of idiosyncratic hepatotoxicity. It appears to be clear that hepatitis B or

C virus coinfection and/or the use of concomitant medications cannot explain our findings in isolation, particularly since the index case (with the most severe hepatic cytolysis) had no such confounding factors. The genetic predictors of toxicity (including factors that may predispose an individual to altered drug metabolism and distribution, polymorphisms in CCR5 and/or its ligands, and other immune response variants) are currently undergoing investigation and will be the subject of a separate report.

In summary, the clinical development of APL was discontinued due to an idiosyncratic toxicity that appeared to be related to the compound itself rather than to its mechanism of action. Indeed, maraviroc has now been approved by the FDA for use by treatment-experienced patients with HIV infection/AIDS (10).

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