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Exposure-Response Relationships for Saquinavir, Zidovudine, and Zalcitabine in Combination Therapy

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The relationship of $CD4^+$ cell response, level of RNA in plasma, and quantitative peripheral blood mononuclear cell (PBMC) titer to apparent drug exposure was investigated by using data from AIDS Clinical Trial Group protocol 229, a multicenter randomized study. Patients received either saquinavir, zalcitabine, or a combination of both, along with open-label zidovudine. Approximately 100 patients were enrolled in each arm, and the primary study duration was 24 weeks. Individual drug exposure, the area under the concentration-time curve, was estimated by using population-based pharmacokinetic methods. Response was defined as the maximum increase in $CD4^+$ cell count or the maximum decrease in RNA in plasma or PBMC titer adjusted for baseline $CD4^+$ cell count, RNA in plasma, and PBMC titer, respectively. Regression of responses on exposure demonstrated an exposure effect for saquinavir which was significant for the maximum increase in $CD4^+$ cell count and the decrease in RNA in plasma. For the PBMC titer, no significant relationship could be demonstrated but the results suggested a trend similar to that of the other response variables. For all three response variables, the slope of the saquinavir exposure response was greater with the triple combination (saquinavir, zidovudine, and zalcitabine) than with the combination of saquinavir and zidovudine, suggesting possible synergism between saquinavir and zalcitabine.

Clinical studies have shown substantial individual variability in responses to identical doses of antiretroviral drugs. Sources of variability include patient characteristics such as gender, age, weight, and disease stage, baseline values of different viral and immunologic responses; and inter- and intrapatient variabilities in the pharmacokinetics of the prescribed drugs. A better understanding of the relationship between drug concentration or drug exposure and effect may prove useful for optimizing individual antiretroviral drug therapy. Instead of dose and weight-normalized dose, which do not fully take into account pharmacokinetic variation among patients, we report here on the relationship of the area under the concentration-time curve (AUC), computed as the dose divided by the quotient of individual clearance (CL) divided by bioavailability (F), of saquinavir, zidovudine, and zalcitabine to viral and immunologic responses, namely, log-transformed $CD4^+$ cell count, RNA in plasma, and peripheral blood mononuclear cell (PBMC) titer versus time. Six descriptors of these responses were assessed. We report here the results for the descriptor of each response most sensitive to exposure variation (after correcting for baseline differences), the maximum increase in $CD4^+$ cell count or the decrease in the level of RNA in plasma and PBMC titer. The relationship of descriptor to drug exposure is the subject of this report.

MATERIALS AND METHODS

Study design. AIDS Clinical Trial Group (ACTG) protocol 229 was a phase II randomized study of three treatment regimens as described in more detail in the accompanying paper (15). Briefly, about 100 patients were enrolled per treatment arm. Patients in arm 1 received saquinavir at 600 mg three times a day (TID) plus open-label zidovudine at 200 mg TID. Patients in arm 2 received saquinavir at 600 mg TID plus zalcitabine at 0.75 mg TID plus open-label zidovudine at 200 mg TID. Patients in arm 3 received zalcitabine at 0.75 mg TID plus open-label zidovudine at 200 mg TID. The initial treatment duration was 24 weeks, but all patients were offered continuation of the same blinded regimen for up to 56 weeks. For further details, see reference 3.

Laboratory procedures. $CD4^+$ cells from peripheral blood were counted by using monoclonal antibodies and flow cytometry (2). Plasma was assayed for HIV RNA by using branched DNA signal amplification (10) (Chiron, Emeryville, Calif.). The lower limit of detection was 10,000 copies/ml. PBMC were cultured for human immunodeficiency virus by a quantitative microculture technique that was previously described (5, 8). All of these assays were performed twice prior to study entry and at weeks 4, 8, 12, 16, and 24. All prebaseline observations were averaged into one baseline value. All immunology and virology laboratories performing these assays were certified by the ACTG immunology and virology quality control programs.

Pharmacokinetic study design and analysis. Intensive pharmacokinetic tests were performed with all patients at 3 of the 10 designated centers as described in the accompanying paper (15). At all of the other centers, single random blood samples were taken for drug analysis at weeks 1, 12, and 24 and every 8 weeks thereafter, resulting in an average of 2.8 drug concentrations in plasma per patient. The times when blood was drawn, when the previous three doses were administered, and when the previous meal was eaten were recorded.

As described in detail in the accompanying paper (15), we fitted a one-compartment model with first-order absorption to the intensive pharmacokinetic data for all three drugs by using the software program NONMEM, version IV, on either a personal computer or a Sun workstation (1). Since no differences between arms and weeks were observed in the intensively studied group, all weeks and arms were modeled together. For simplicity, no covariates were included in the model, but for zalcitabine, we included a nonrandom lag time. Subsequently, the population parameter estimates from the intensively studied group were used as a prior distribution to obtain individual empirical Bayes estimates of CL/F for patients not only in the intensively studied group but also for patients in the sparsely sampled group (1). In doing so, we discarded those occasions on which it was unlikely that the patient had taken the reported dose at the reported time by using a mixture model (6) that expresses the likelihood of the observed concentration under two mutually exclusive events: the prescribed dose was either taken or not taken at the specified time. With this method, 7.3% of the zalcitabine data, 24% of the zidovudine data, and 1% of the saquinavir data were discarded. The AUC, computed as the mean daily dose divided by CL/F, estimated as just described, was then used as a measurement of individual drug exposure to be related to individual response.

Pharmacodynamic analysis. All calculations were carried out on log-transformed $CD4^+$ cell count, PBMC titer, and RNA levels in plasma, as these transformations yielded more symmetrically distributed data. For each response

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variable, six summary measures (descriptors) of the response-versus-time curve were computed. For $CD4^+$ cell count, these descriptors were (i) time above the baseline, defined as the time until the first of two consecutive measurements, at least 21 days apart, were both less than or equal to the baseline; (ii) the normalized area above the baseline, defined as the trapezoidal-rule AUC of the difference between follow-up values and the baseline divided by the duration of follow-up; (iii) deltamax (MAX), defined as the maximum increase in $CD4^+$ cell count above the baseline (patients whose $CD4^+$ cell count did not go above the baseline were assigned a MAX of 0); (iv) intensity, defined as the slope of the line between the baseline value and the MAX; (v) slope12, defined as the slope of the linear regression (intercept forced through the baseline) on time of follow-up $CD4^+$ values up to the first 12 weeks of the study; and (vi) slope24, defined as the slope of the linear regression (intercept forced through the baseline) on time of all follow-up $CD4^+$ values. The descriptors for the plasma RNA and PBMC titers were similarly defined, except that these responses descend rather than rise with treatment, so that we speak of time under the baseline, normalized area below the baseline, and MAX as the maximum decrease (negative sign) in the level of RNA in plasma or the PBMC titer below the baseline (patients whose RNA level or PBMC titer did not go under the baseline were assigned a MAX of 0).

Statistical analysis. In a first exploratory analysis, using the full set of data from 302 patients, we sought a single response descriptor that would best reveal a relationship between response and exposure. To find this descriptor, we regressed all descriptors on treatment arm and the following independent baseline covariates: gender, diagnosis at baseline (AIDS, AIDS-related complex, or asymptomatic), weight, age, Karnofsky performance score, the duration of prior zidovudine therapy in days, the duration of prior zidovudine or zalcitabine therapy in days, the duration of prior zidovudine or miscellaneous therapy in days, exposure to zidovudine, and the log-transformed baseline $CD4^+$ cell count, level of RNA in plasma, and PBMC titer. Since all patients were exposed to zidovudine in this study, we considered zidovudine exposure to be a baseline covariate. The regression model was a generalized additive model (7), as implemented in S-PLUS (version 3.2; MathSoft, Seattle, Wash.). A stepwise procedure selected the relevant baseline covariates and the type of relationship (linear or nonlinear) between each descriptor and the relevant baseline covariate. The descriptor with the highest multiple *R*-squared value when regressing the residuals (from the previous fit of the descriptor to important baseline covariates) on exposure was then chosen as the single "best" descriptor. For $CD4^+$ cell count and level of RNA in plasma, this descriptor was the MAX. Although the slope24 was best for PBMC, the MAX was only slightly inferior and we decided to use it for PBMC, not slope24, for consistency with $CD4^+$ cell count and RNA level in plasma.

To determine the relationship of exposure to response, we performed the following additional regressions. For arm 1, (i) regression of MAX on selected baseline covariates and (ii) regression of MAX on selected baseline covariates and exposure to saquinavir (AUC_{saq}). For arm 2, (i) regression of MAX on selected baseline covariates, (ii) regression of MAX on selected baseline covariates and exposure to saquinavir, (iii) regression of MAX on selected baseline covariates and exposure to zalcitabine (AUC_{ddc}), and (iv) regression of MAX on selected baseline covariates and exposure to saquinavir and zalcitabine. For arm 3, (i) regression of MAX on selected baseline covariates and (ii) regression of MAX on selected baseline covariates and exposure to zalcitabine. In all of the above regressions, the exposure effect was forced to be linear (as was suggested by an exploratory analysis). For all three arms, we used an *F* test to test the decrement in the residual sum of squares of the fit going from the model with baseline covariates only (regression 1) to a model with baseline covariates and exposure (regression 2 and for arm 2, also regression 3). A significant decrement indicates that exposure adds explanatory power to the model. For these subsequent regressions, we used reduced data sets consisting of only those patients with complete data for all of the selected baseline covariates, the descriptor of interest, and drug exposures. The reduced data sets had 273, 245, and 273 patients for $CD4^+$ cell count, RNA level in plasma, and PBMC titer, respectively. To avoid distortion by outliers, the data were "trimmed," by deleting the subjects at and beyond the lowest and highest 1% of the response descriptor.

RESULTS

Table 1 gives an overview of the baseline characteristics of the patient population. There were no significant differences between treatment arms in terms of baseline covariates or exposure to a given drug appearing in more than one arm. Although this is an indication that the different drugs do not affect each other's pharmacokinetic profiles, the large interpatient variability in drug exposure (the coefficients of variation of the computed AUCs of zalcitabine, zidovudine, and saquinavir were 30, 37, and 63%, respectively) makes it impossible to detect modest mean exposure differences between arms (see also reference 15).

TABLE 1. Baseline characteristics of the patient population in this study

Characteristic	Value for all 302 patients
Mean age in yr (range)	38 (19–75)
Mean wt in kg (range)	75 (46–121)
Gender (% female)	9
Median Karnofsky score (range)	100 (70–100)
Prior zidovudine therapy	
Median duration (days)	670
Range (days)	14–2,777
Prior zidovudine-zalcitabine therapy	
% of patients	38
Median duration (days)	205
Duration range (days)	8–746
Prior zidovudine or other antiretroviral therapy	
% of patients	20
Median duration (days)	195
Duration range (days)	10–1,000
HIV status (% of total)	
AIDS	12
ARC	51
Asymptomatic	37
$CD4^+$ cell count (no. of cells/mm^3)	
Median	156
Range	25–394
Level of RNA in plasma (no. of copies/ml)	
Median	31,500
Range	10,000–538,300
PBMC titer (no. of infectious U/10^6 cells)	
Median	27
Range	0.2–2,897

$CD4^+$ cell count versus time. The log-transformed baseline $CD4^+$ cell count (baseCD4) was selected as the only significant baseline covariate when regressing the maximum increase in $CD4^+$ cell count above the baseline (CD4MAX) on baseline covariates and predicted a linear decrease in CD4MAX as the baseline $CD4^+$ cell count increased. The statistical tests of exposure revealed a significant contribution of AUC_{saq} in arms 1 and 2 (Table 2). AUC_{ddc} did not significantly influence CD4MAX in either arm 2 or 3 (data not shown). As evident from the plots of the partial residuals of the regressions of CD4MAX on baseline $CD4^+$ versus AUC_{saq} by arm (Fig. 1), higher exposure to saquinavir corresponded to a larger maximum increase in $CD4^+$ cell count. The slope of the relationship between AUC_{saq} and CD4MAX was different in arms 1 and 2. In arm 1, there was an increase in CD4MAX of 0.04 per unit of exposure. With absolute, untransformed values, this means that an increase in exposure from mean exposure to twice that value, holding the baseline $CD4^+$ cell count fixed, was associated with a 10% increase in the maximum $CD4^+$ cell count. In arm 2, an increase in CD4MAX of 0.0677/U of exposure or a 20% increase in the maximum $CD4^+$ cell count (Table 2) was forecast for a doubling of the mean exposure at a fixed baseline $CD4^+$ cell count. Comparison of these two regression lines using a *t* test, however, showed that the difference in slope between arms was not significant. As will be discussed later, a significant difference in slope would suggest the presence of synergism.

TABLE 2. Relationship between exposure to saquinavir and MAX for $CD4^+$ cell count, level of RNA in plasma, and PBMC titer[a]

Response and arm	Slope	SD of slope	*P* value	Change as % of untransformed maximum (CD4) or minimum (RNA, PBMC) at mean exposure
$CD4^+$				
1	0.0402	0.0175	0.0241	+10
2	0.0677	0.0193	0.0007	+20
RNA				
1	−0.0283	0.0389	0.47	−6
2	−0.1242	0.0383	0.0017	−25
PBMC				
1	−0.0436	0.0979	0.66	−10
2	−0.1591	0.1122	0.16	−31

[a] The *P* values correspond to the *F* tests comparing the models regressing on baseline covariates alone with the models regressing on baseline covariates and exposure to saquinavir as described in Materials and Methods. The last column shows the increase or decrease per unit of mean exposure as a percentage of the untransformed maximum (CD4) or minimum (RNA and PBMC) value at mean exposure.

RNA levels in plasma versus time. The log-transformed baseline level of RNA in plasma (baseRNA) was selected as the only significant baseline covariate when regressing the maximum decrease in viral RNA from the baseline (RNA MAX) on baseline covariates. The baseline level of RNA in plasma predicted a nonlinear increase in maximum drop in the level of RNA in plasma as the baseline RNA level increased, with almost no extra gain when the natural log of the level of RNA in plasma was greater than about 5, i.e., above 100,000 copies/ml. The statistical tests of exposure revealed a significant contribution of AUC_{saq} in arm 2 but not in arm 1 (Table 2). AUC_{ddc} did not significantly influence RNAMAX in either arm 2 or 3 (data not shown). The plots of the partial residuals of the regressions on the baseline level of RNA versus AUC_{saq} by arm (Fig. 2) show that higher exposure to saquinavir corresponded to a greater drop in the level of RNA in plasma in arm 2, as well as in arm 1, although the slope in arm 1 was less steep and not significant. In arm 2, the increase in the maximum drop in the level of RNA in plasma was 0.1242/U of exposure to saquinavir or, using absolute, untransformed values, an increase in exposure from the mean exposure to twice that value, holding the baseline level of RNA in plasma fixed, would be associated with a 25% decrease in the minimum level

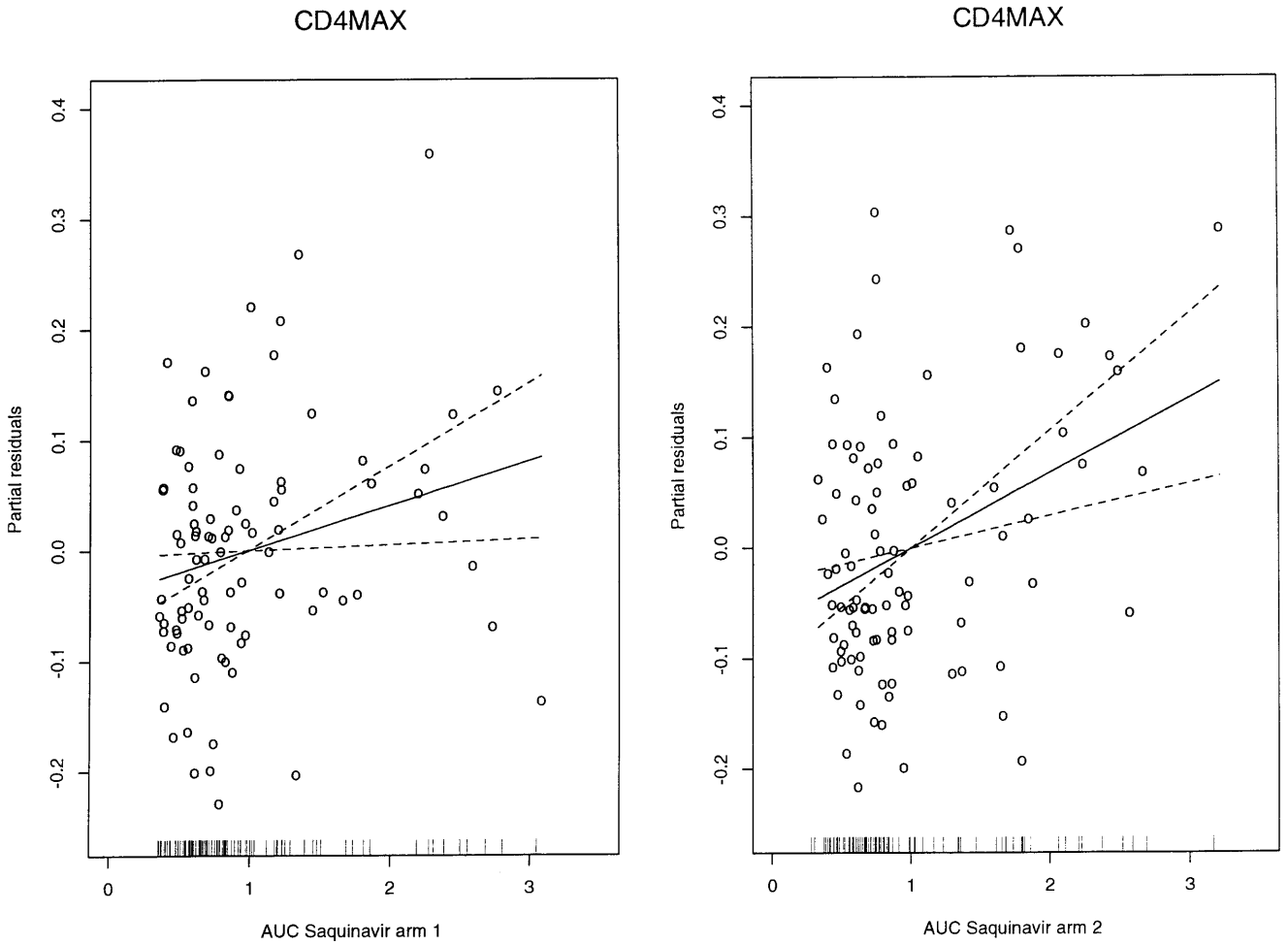

FIG. 1. Partial residuals of the regression of CD4MAX on baseline $CD4^+$ versus AUC_{saq} by arm. (Left) Partial residuals versus AUC_{saq} in arm 1. (Right) Partial residuals versus AUC_{saq} in arm 2. The solid line represent the fit, and the dashed line represents the ±2 standard-error curves. Partial residuals are residuals corrected for all other variables in the regression (here, the baseline $CD4^+$ cell count). Exposure is expressed as the individual AUC divided by the mean AUC of the corresponding arm. Ticks along the horizontal axis represent the *x* values of the data points.

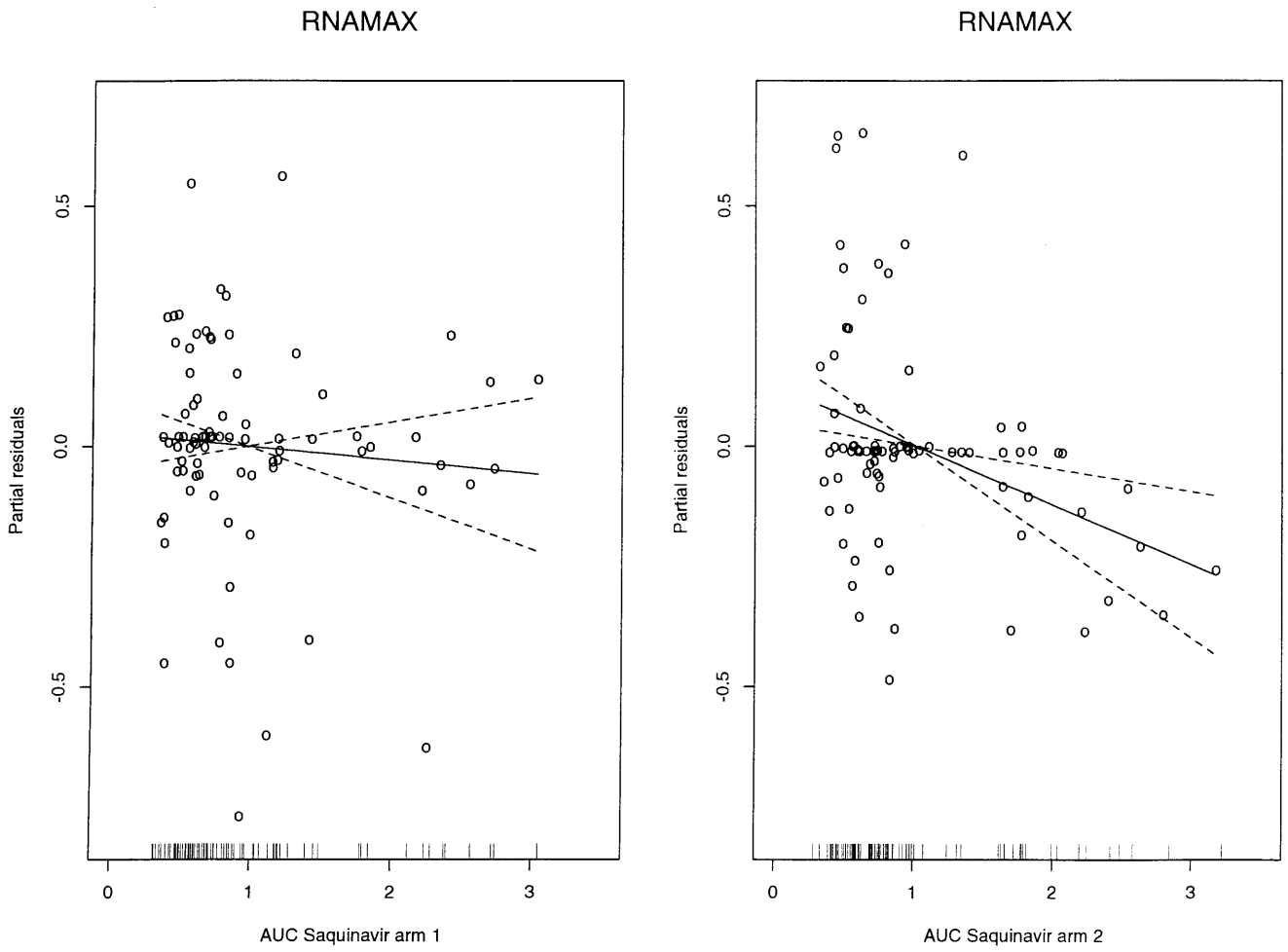

FIG. 2. Partial residuals of the regression of RNAMAX on baseline RNA versus AUC_{saq} by arm. (Left) Partial residuals versus AUC_{saq} in arm 1. (Right) Partial residuals versus AUC_{saq} in arm 2. The solid line represent the fit, and the dashed line represents the ±2 standard-error curves. Partial residuals are residuals corrected for all other variables in the regression (here, the baseline level of RNA in plasma). Exposure is expressed as the individual AUC divided by the mean AUC of the corresponding arm. Ticks along the horizontal axis represent the *x* values of the data points.

of RNA in plasma (Table 2). Comparison of these two regression lines using a *t* test showed that there was no significant difference between the slope of the relationship between AUC_{saq} and RNAMAX in arms 1 and 2. As before, a significant difference would suggest synergism.

PBMC titer versus time. The log-transformed baseline PBMC titer was selected as the only significant baseline covariate when regressing the maximum decrease in PBMC titer from the baseline (PBMCMAX) on baseline covariates and predicted a linear increase in the maximum drop in the PBMC titer as the baseline PBMC titer increased. The tests for exposure effect revealed that neither AUC_{saq} (Table 2) nor AUC_{ddc} (data not shown) significantly influenced PBMCMAX. The plots of the partial residuals of the regressions on the baseline PBMC titer versus AUC_{saq} per arm, however, show a small slope in arm 1 and a steeper slope in arm 2 (Fig. 3). Although neither of these slopes reached significance, probably due to the high variability (Table 2), they nevertheless suggested a trend that, as for the other response variables, higher exposure to saquinavir was associated with a larger maximum drop in the PBMC titer, especially in arm 2.

DISCUSSION

The results above show a significant relationship between exposure to saquinavir and the maximum increase in $CD4^+$ cell count in both arms exposed to saquinavir, as well as a significant relationship between exposure to saquinavir in the triple combination arm (arm 2) and the maximum decrease in the level of RNA in plasma after correction for differences in the baseline values. For the PBMC titer, no significant relationship could be demonstrated but the trend is similar. Although we present results for only one descriptor (deltamax), the results are similar for most of the other descriptors (data not shown). For all three response variables, the relationship is stronger (i.e., the slope of the regression is steeper) in arm 2 than in arm 1, suggesting an influence of the presence of zalcitabine on the saquinavir exposure-response relationship, although the difference does not reach statistical significance. Given no exposure effect of zalcitabine, an increase in the response per unit of saquinavir exposure is synergism by the usual definition, namely, that the response to the combination exceeds the sum of the responses to the single agents. Practically speaking, these results suggest that antiviral effects can be

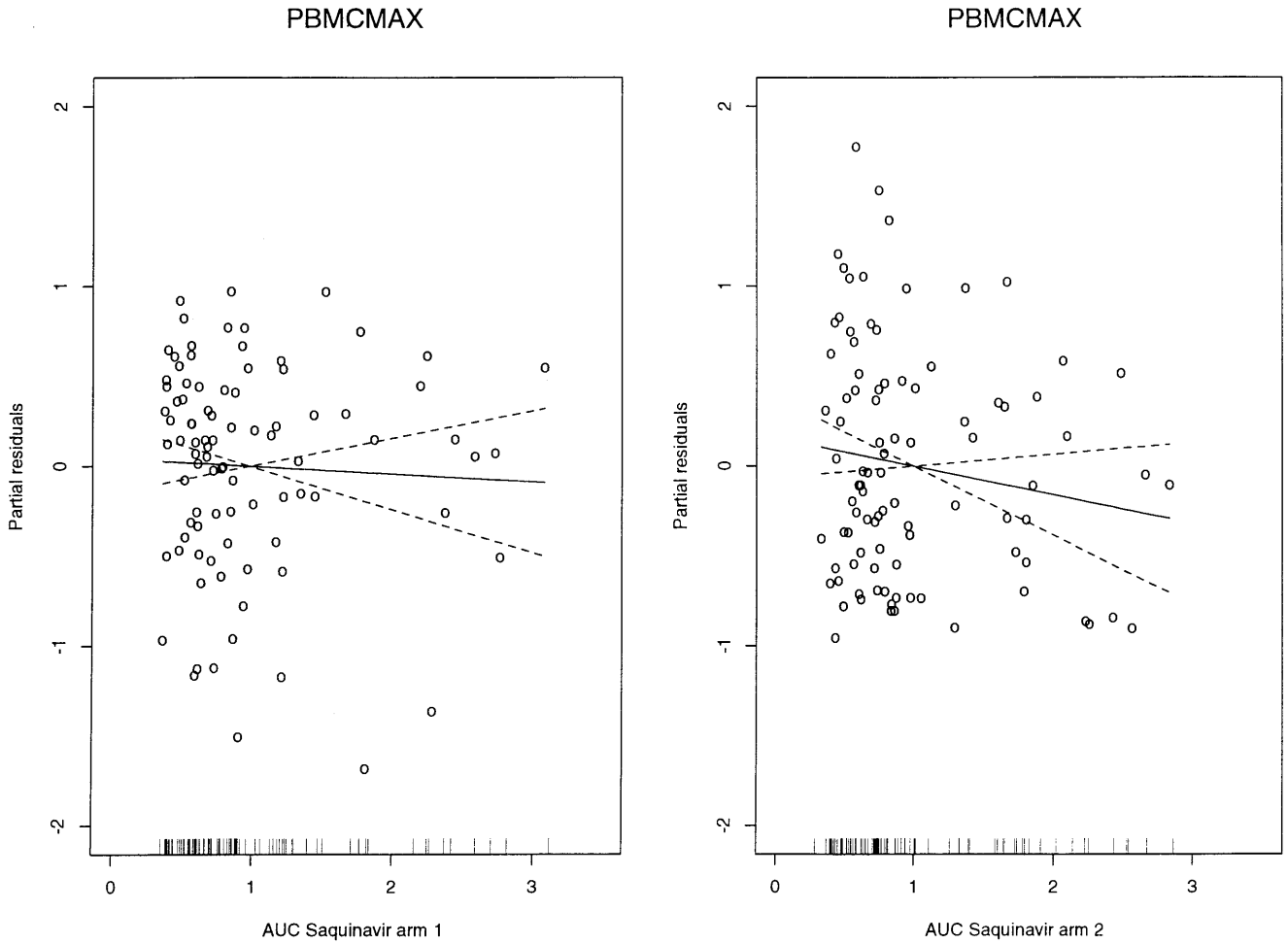

FIG. 3. Partial residuals of the regression of PBMCMAX on baseline PBMC versus AUC_{saq} by arm. (Left) Partial residuals versus AUC_{saq} in arm 1. (Right) Partial residuals versus AUC_{saq} in arm 2. The solid line represent the fit, and the dashed line represents the ±2 standard-error curves. Partial residuals are residuals corrected for all other variables in the regression (here, the baseline PBMC titer). Exposure is expressed as the individual AUC divided by the mean AUC of the corresponding arm. Ticks along the horizontal axis represent the *x* values of the data points.

increased by increasing doses if they are tolerated by the patient. The apparent synergism suggests that this is especially likely if saquinavir is combined with zalcitabine.

Our finding that increasing saquinavir exposure increases the effect is consistent with a recent study with saquinavir doses which were two and four times as high as the dose used in ACTG protocol 229. This study demonstrated a strong correlation between the AUC of saquinavir at week 4 and the drop in viral load (12).

Previous efforts to demonstrate a relationship between zidovudine exposure and $CD4^+$ cell response (11, 13) have failed, and no reports have been published previously on the relationship between exposure to zidovudine or zalcitabine and the effect on the level of RNA in plasma or on the PBMC titer. This study also fails to demonstrate a significant exposure effect for zidovudine or zalcitabine after correction for baseline values and demonstrates only a shallow slope for saquinavir exposure response. The reason for this is most likely a combination of a weak signal and considerable noise. Noise, in particular, differences between estimated and "true" values of an explanatory (exposure) or response ($CD4^+$ count, RNA levels in plasma, or PBMC titer) variable always attenuates the apparent strength of a relationship.

For all three drugs, there are a number of likely contributors to noise in our exposure measurement which might have contributed to the attenuation of an exposure effect. A first contributor is noncompliance. Exposure is proportional to both the actual dose and the pharmacokinetic disposition of that dose. Our AUC estimate, computed as the mean daily assigned dose divided by the estimated CL/*F* only reflects pharmacokinetic individuality but fails to reflect individual differences between the assigned and actual mean doses. Since no compliance monitoring was performed in this study, interpatient variability in compliance could not be corrected for and the AUC thereby becomes a noisy measurement of true drug exposure. Second, errors in the recorded times of immediate previous dose administration and blood sample collection for pharmacokinetic measurement for drugs with short half-lives, such as all those used in this study, can introduce considerable noise into the estimates of CL/*F*. Equally, variability in measurements of the different responses might have contributed to noise in the response variable and thus might have attenuated an exposure effect. The larger variability associated with the PBMC titer might explain the lack of a significant exposure-response relationship for this variable.

That a relationship is seen, despite this noise, with saquina-

vir but not with zidovudine and zalcitabine may be due to factors increasing the noise or attenuating the signal that apply to the latter two drugs but not to the former. A factor increasing the noise for zidovudine and zalcitabine but not for saquinavir is that nucleoside analogs such as zidovudine and zalcitabine must undergo intracellular phosphorylation before they are effective. As it has not been possible to demonstrate a relationship between intracellular phosphorylated zidovudine and zidovudine concentrations in plasma (14), the AUC, used as a measurement of drug exposure in our study, may only poorly reflect the real exposure to the phosphorylated active drug. Protease inhibitors, in contrast, do not have to undergo phosphorylation or other metabolic activation before they are effective. A factor attenuating the response signal for zidovudine and zalcitabine but not for saquinavir is that the doses used in ACTG protocol 229 are at the high, flat end of the dose-response curve for zidovudine and zalcitabine but apparently are not in this region for saquinavir. Also, it should be noted that finding an exposure-response relationship when patients are receiving fixed doses requires a large variability in exposure. Due to a low *F*, the largest variability in exposure was present in patients receiving saquinavir (the coefficients of variation of the computed AUCs of zalcitabine, zidovudine, and saquinavir were 30, 37, and 63%, respectively), which makes it easier to find an exposure-response relationship for saquinavir than for zidovudine and zalcitabine.

Saquinavir has been demonstrated to be synergistic in vitro with both zidovudine and zalcitabine (4, 9). Since recent monotherapy studies show a significant antiviral effect with saquinavir at higher doses than those used in this study with only mild and reversible adverse reactions (12) and since our results are consistent with both possible synergism and not being at the top of the saquinavir dose-response curve, further investigation of the combination of saquinavir and zalcitabine, using a higher dose for saquinavir, is indicated.

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