Novel Method To Assess Antiretroviral Target Trough Concentrations Using In Vitro Susceptibility Data

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Novel Method To Assess Antiretroviral Target Trough Concentrations Using In Vitro Susceptibility Data


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Durable suppression of HIV-1 replication requires the establishment of antiretroviral drug concentrations that exceed the susceptibility of the virus strain(s) infecting the patient. Minimum plasma drug concentrations ($C_{\text{tough}}$) are correlated with response, but determination of target $C_{\text{tough}}$ values is hindered by a paucity of in vivo concentration-response data. In the absence of these data, in vitro susceptibility measurements, adjusted for serum protein binding, can provide estimations of suppressive in vivo drug concentrations. We derived serum protein binding correction factors (PBCF) for protease inhibitors, nonnucleoside reverse transcriptase inhibitors, and an integrase inhibitor by measuring the effect of a range of human serum concentrations on in vitro drug susceptibility measured with the PhenoSense HIV assay. PBCFs corresponding to 100% HS were extrapolated using linear regression and ranged from 1.4 for nevirapine to 77 for nelfinavir. Using the mean 95% inhibitory concentration ($IC_{95}$) for $\geq 1,200$ drug-susceptible viruses, we calculated protein-bound $IC_{95}$ (PBIC$_{95}$) values. PBIC$_{95}$ values were concordant with the minimum effective $C_{\text{tough}}$ values that were established in well-designed pharmacodynamic studies (e.g., indinavir, saquinavir, and amprenavir). In other cases, the PBIC$_{95}$ values were notably lower (e.g., darunavir, efavirenz, and nevirapine) or higher (nelfinavir and etravirine) than existing target recommendations. The establishment of PBIC$_{95}$ values as described here provides a convenient and standardized approach for estimation of the minimum drug exposure that is required to maintain viral suppression and prevent the emergence of drug-resistant variants, particularly when in vivo concentration-response relationships are lacking.

Fully suppressive antiretroviral therapy (ART) for human immunodeficiency virus type 1 (HIV-1) infection requires the administration of drug combinations that target multiple sites on one or more proteins required for viral replication. Approved antiretrovirals (ARVs) include nucleoside/nucleotide and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively), protease inhibitors (PIs), entry inhibitors, and integrase strand-transfer inhibitors (INSTIs). With the exception of the NRTIs, which require intracellular phosphorylation, plasma drug concentrations are correlated with drug efficacy. At the same time, high drug concentrations are associated with excess toxicity.

To durably suppress HIV replication in infected patients, ART concentrations must reach and be maintained at levels that exceed the susceptibility of the virus to that drug. Treatment response is often hampered by the failure to achieve sufficient drug exposure (i.e., poor adherence and drug interactions), reduced drug susceptibility (i.e., viral drug resistance), or both. Drug concentrations within patients vary over time and, due to ease of sampling, are generally characterized by minimum (trough) concentrations ($C_{\text{tough}}$) immediately prior to administration of the next scheduled dose. Drug concentrations also vary considerably between individual patients as a result of differences in absorption, distribution, metabolism, and excretion. In addition, each drug characteristically binds to human plasma proteins to different extents. Furthermore, the susceptibility of HIV-1 variants, even in patients not previously exposed to drug therapy, varies over a range that is unique to each drug ($23, 24, 46$).

In vivo clinical pharmacodynamic data are available for some, but not all, ARVs. Efficient collection of these data is difficult and ideally performed early in the drug development process. Alternative methods of incorporating ARV pharmacokinetics into therapeutic decision making are being explored. In vitro phenotypic drug susceptibility testing of individual patient viruses is now widely available and generates information that can be used to calculate an inhibitory quotient (IQ), defined as the ratio between the $C_{\text{tough}}$ and the drug concentration that inhibits in vitro replication by a defined percentage (e.g., 50% or 95% inhibitory concentration [$IC_{50}$ or $IC_{95}$, respectively]) ($27, 35, 43, 56$). Derivatives of the IQ, including the genotypic IQ (GIQ; $C_{\text{tough}}$ divided by the number of resistance-associated mutations for a given drug) have also been evaluated ($36$). Several studies have attempted to define the optimal IQ required to produce long-term viral suppression: in some cases, IQ has been retrospectively linked to clinical outcome ($15, 34, 41, 42, 55$), while in others, direct relationships between IQ and viral load response were not observed ($5, 12$).

For most ARV drugs, few or no in vivo concentration-response data have been generated, or these data are inconsistent with clinical observations. Collectively, there is insufficient agreement in the field regarding the determination of the optimal ARV target trough concentrations in the absence of concentration-response data. We conducted the present study to address this deficiency by (i) assessing the activity of PIs, NRTIs, and an INSTI in a standardized in vitro phenotypic drug susceptibility assay (PhenoSense HIV assay) on $\geq 1,200$ drug-susceptible viruses, we calculated protein-bound $IC_{95}$ (PBIC$_{95}$) values. PBIC$_{95}$ values were concordant with the minimum effective $C_{\text{tough}}$ values that were established in well-designed pharmacodynamic studies (e.g., indinavir, saquinavir, and amprenavir). In other cases, the PBIC$_{95}$ values were notably lower (e.g., darunavir, efavirenz, and nevirapine) or higher (nelfinavir and etravirine) than existing target recommendations. The establishment of PBIC$_{95}$ values as described here provides a convenient and standardized approach for estimation of the minimum drug exposure that is required to maintain viral suppression and prevent the emergence of drug-resistant variants, particularly when in vivo concentration-response relationships are lacking.

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Drug susceptibility was determined using the PhenoSense assay (47) under standard conditions (10% FBS) and in the presence of 23 to 81% HS. In some cases, because of the cytostatic effects of HS on cultured cells, infectivity (i.e., luciferase signal) was too low to accurately determine the IC$_{50}$ at 81% HS. Assays were performed using a drug-susceptible reference virus (NL4-3) and a well-characterized multidrug-resistant reference virus (MDRC-4). Representative inhibition curves generated using NL4-3 are shown in Fig. 1 for a PI (darunavir) (Fig. 1A), NNRTI (efavirenz) (Fig. 1B), and INSTI (raltegravir) (Fig. 1C). IC$_{50}$ of all drugs for both the drug-susceptible and -resistant viruses at increasing HS concentrations are plotted in Fig. 2. As expected, the inhibition curves (Fig. 1) shifted toward higher drug concentrations (i.e., to the right) as the percentage of HS increased, although the magnitude of this shift was specific for each drug. For example, the IC$_{50}$ for saquinavir and tipranavir were between 23 and 81% HS remained relatively constant, while the IC$_{50}$ for most other drugs increased incrementally. Since the relationship between IC$_{50}$ and HS concentration for most drugs was fairly linear between 23 and 81% HS, the IC$_{50}$ at 100% HS was estimated using linear regression.

The ratio between the estimated IC$_{50}$ at 100% HS (mimicking the in vivo situation) and the IC$_{50}$ under standard in vitro conditions is referred to as the PBIC$_{50}$. A PBIC$_{50}$ for 45% HS can also be calculated using the experimental data. Table 1 lists the PBIC$_{50}$ values for 100% HS and 45% HS derived here, along with the 50% HS correction factors derived in a previous study (40), as well as estimations of protein binding derived from in vitro biochemistry studies as reported by the various drug manufacturers. Correction factors for 45% HS (this work) and 50% HS (40) were highly correlated for the six drugs where both values were available. The PBIC$_{50}$ values that were independently determined in both studies also correlated with the magnitude of protein binding for each drug (Table 1). Previously reported PBIC$_{50}$s for atazanavir and amprenavir (13.4 and 7.4, respectively) are similar to the values reported here, in spite of the fact that these older studies used only purified α-1 acid glycoprotein and human serum albumin and not complete serum (14, 48).

To derive in vivo target trough concentrations for patients infected with drug-susceptible virus, PBIC$_{50}$s were applied to mean IC$_{50}$ and IC$_{95}$ values from clinically derived HIV-1 strains. Over 6,500 results from viruses lacking detectable amino acid substitutions that are nonpolymorphic and selected under ARV drug pressure (46) were identified in a database of linked phenotypic and genotypic assay results. Fewer results were available for raltegravir (n = 1,200). The total plasma drug concentration expected to inhibit wild-type virus in vivo by 50% (PBIC$_{50}$) or 95% (PBIC$_{95}$) was calculated from these data (Table 2). Although it is not known whether the PBIC$_{50}$ or PBIC$_{95}$ most accurately reflects the optimal target trough concentration for these agents, given the consider-
The approach is to use the highest of these (i.e., the PBIC95). The corresponding optimal IQ95 (Table 1) for an effective ARV drug target should thus be 1 or greater. In fact, this is the case for all of the drugs that we examined except nelfinavir (IQ95 = 0.26). For comparison, the currently proposed target drug concentrations from the Department of Health and Human Services (DHHS) guidelines (45) are also listed in Table 2.

**DISCUSSION**

The results generated in this study add to the current body of knowledge relating ARV drug target trough concentrations to drug efficacy and more specifically demonstrate the importance of serum protein binding when relating *in vitro* drug susceptibility measurements to plasma drug concentrations. As expected, the ARV drugs that exhibit the lowest levels of protein binding (indinavir, amprenavir, atazanavir, nevirapine, and raltegravir) have the smallest PBCFs (Table 1). Notably, lopinavir also has a relatively low PBCF, despite reports of high (98 to 99%) serum protein binding. This apparent discrepancy has been reported by others and may be attributed to differential binding properties of bovine serum proteins in the tissue culture medium (26, 28).

The PBCFs reported here agree well with values previously published by Molla et al. for the subset of PIs that were tested in both studies (40). This earlier study employed a multiple-replication-cycle inhibition assay using MT4 cells, HIV-1 IIIIB, and a colorimetric readout for cytotoxic effects. In contrast, our study was conducted using a single-cycle inhibition assay, replication-defective recombinant viruses, and a luciferase reporter readout (47). Our methodology permits assessments of protein binding effects on drug susceptibility measurements at higher HS concentrations than replication-competent systems because of the shorter period of time that cells are in contact with high HS concentrations (2 to 3 days). In most cases, we were able to assess drug susceptibility in the presence of 81% HS, although the luciferase signal was considerably reduced at this high concentration.

Our results for several PIs are also consistent with previously derived *in vivo* pharmacokinetic/pharmacodynamic relationships, particularly amprenavir, indinavir, and saquinavir. In a dose-finding study of amprenavir, a fitted sigmoid maximum effect ($E_{\text{max}}$) curve between amprenavir $C_{\text{trough}}$ and HIV-1 RNA response demonstrated a significant relationship (51). The plasma concentration required to produce 50% of the maximum response ($EC_{50}$) was 87 ng/ml. Using the reported Hill coefficient, we were able to estimate the $EC_{95}$ (320 ng/ml) value as well. Current DHHS guidelines recommend an amprenavir target $C_{\text{trough}}$ of 400 ng/ml (45). Using the PBCF determined in this study, our estimate of amprenavir PBIC95 is 358 ng/ml (Table 2), which is strikingly similar to the recommended $C_{\text{trough}}$ value.

In a small subset of pediatric patients receiving indinavir, stavudine, and didanosine, a significant inhibitory $E_{\text{max}}$ relationship between the indinavir $C_{\text{trough}}$ and change in HIV-1 RNA from baseline to week 24 was described (16). The maximum reduction in HIV-1 RNA (2.0 log10 copies/ml) corresponded to an $EC_{95}$ of 80 ng/ml. In a separate study in adult patients treated with indinavir in combination with zidovudine and lamivudine, the indinavir $C_{\text{trough}}$ levels at week 4 were significantly associated with changes in HIV-1 RNA from baseline to week 4 (42). Based on an $E_{\text{max}}$ model, an $EC_{95}$ was estimated to be 110 ng/ml. Current DHHS guidelines recommend a target $C_{\text{trough}}$ of 100 ng/ml (45). We estimated the indinavir PBIC95 to be 73 ng/ml (Table 2), which again is similar to *in vivo* estimations.

A significant correlation between saquinavir $C_{\text{trough}}$ and sustained viral load suppression has been observed in children (21). The mean saquinavir $C_{\text{trough}}$ associated with durable viral load suppression was 200 ng/ml. Additional support for this target comes from the model of saquinavir pharmacokinetics/pharmacodynamics following saquinavir monotherapy in HIV-positive patients (18). Following administration of 1,200-mg saquinavir three times daily (soft gel formulation), both area under the curve (AUC) and $C_{\text{trough}}$ measures were related to peak reduction in plasma HIV-1 RNA. The median 24-h AUC (AUC24)
was 20 μg · h/ml, which corresponded to 85% of the maximum effect (EC85); the Ctrough at this dose was 216 ng/ml (18). Current DHHS guidelines recommend a target Ctrough of 100 to 250 ng/ml (45). We calculated the saquinavir PBIC95 to be 234 ng/ml (Table 2), which is highly concordant with clinically established values.

There is controversy regarding the optimum Ctrough for efavirenz. The reported therapeutic range (lower to upper exposure targets) for efavirenz is 1,000 to 4,000 ng/ml (37). These data come from a study that explored the relationships between EFV exposure, efficacy, and development of central nervous system (CNS) side effects. Since the discordance between this pharmacodynamic study and our in vitro results is considerable, it is worth noting that this was an exploratory field trial in which all patients received the same EFV dose and was not a well-controlled, broad-dose-ranging study. In addition, EFV concentrations were determined using blood samples that were collected on average 14 h postdosing (range of 8 to 20 h postdosing). Although EFV has a relatively long half-life, actual Ctrough Values were not determined or estimated. Furthermore, treatment adherence was not considered, which may account for the broad range of EFV concentrations (125 to 15,230 ng/ml) that were observed. Mutations in cytochrome P450 (CYP) 2B6 are also associated with EFV exposure (22), but in the absence of race/ethnicity data, it is not possible to infer the contribution of genetics to the interpatient variability in EFV concentrations in the study. Also, as discussed by the authors, there was considerable overlap in EFV concentrations among patients that experienced treatment failure versus those that did not. Finally, drug concentrations were determined 3 to 18 months after initiation of EFV therapy—well past the time at which EFV-associated neurologic symptoms have been correlated to plasma concentra-
TABLE 1 Protein binding correction factors

<table>
<thead>
<tr>
<th>Drug</th>
<th>PBCF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>45% HS&lt;sup&gt;b&lt;/sup&gt;</th>
<th>100% HS&lt;sup&gt;c&lt;/sup&gt;</th>
<th>50% HS&lt;sup&gt;d&lt;/sup&gt;</th>
<th>% bound&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>APV</td>
<td>4.8 ± 2.1</td>
<td>11 ± 0.6</td>
<td>8.5 ± 3.3</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>ATV</td>
<td>5.0 ± 1.1</td>
<td>9.3 ± 0.2</td>
<td>ND</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>DRV</td>
<td>6.5 ± 1.3</td>
<td>14 ± 0.2</td>
<td>ND</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>IDV</td>
<td>2.0 ± 0.7</td>
<td>3.5 ± 0.07</td>
<td>2.2 ± 1.1</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>LPV</td>
<td>5.5 ± 1.8</td>
<td>9.9 ± 1.0</td>
<td>8.8 ± 4.3</td>
<td>98–99</td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>27 ± 14</td>
<td>77 ± 8.5</td>
<td>39 ± 11</td>
<td>&gt;98</td>
<td></td>
</tr>
<tr>
<td>RAL</td>
<td>12 ± 5.9</td>
<td>20 ± 3.9</td>
<td>25 ± 10</td>
<td>98–99</td>
<td></td>
</tr>
<tr>
<td>SQV</td>
<td>14 ± 10</td>
<td>17 ± 6.8</td>
<td>34 ± 14</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>TPV</td>
<td>11 ± 4.2</td>
<td>15 ± 2.7</td>
<td>ND</td>
<td>&gt;99.9</td>
<td></td>
</tr>
<tr>
<td>ETR</td>
<td>11 ± 3.6</td>
<td>27 ± 1.9</td>
<td>12</td>
<td>99.9–75</td>
<td></td>
</tr>
<tr>
<td>NVP</td>
<td>1.3 ± 0.3</td>
<td>3 ± 0.1</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAL</td>
<td>1.5 ± 0.4</td>
<td>2.1 ± 0.03</td>
<td>ND</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> APV, amprenavir; ATV, atazanavir; DRV, darunavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; SQV, saquinavir; TPV, tipranavir; EFV, efavirenz; ETR, etravirine; NVP, nevirapine; RAL, raltegravir.

<sup>b</sup> The 45% HS values (actual) represent averages ± standard deviations (SD) derived from 3 to 6 assays (this work).

<sup>c</sup> The 100% HS values (extrapolated) represent averages ± standard errors (SE) derived from linear regression (this work).

<sup>d</sup> The 45% HS values represent averages ± standard deviations (SD) derived from 3 to 6 assays (this work).

<sup>e</sup> The 100% HS values represent averages ± standard errors (SE) derived from linear regression (this work).

<sup>f</sup> Mean ± SD ratios between 50% HS and the control for all viruses listed in Table 1 from reference 48. ND, not done.

<sup>g</sup> All percent bound values are from the product prescribing information for each drug (1–3, 6–9, 17, 19, 38, 39, 53, 54).

TABLE 2 Protein-bound IC<sub>95</sub> and IQ<sub>95</sub> values

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Molar mass (g/mol)</th>
<th>C&lt;sub&gt;trough&lt;/sub&gt; (ng/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PBCF&lt;sup&gt;c&lt;/sup&gt;</th>
<th>WT IC&lt;sup&gt;d&lt;/sup&gt;</th>
<th>ng/ml</th>
<th>PBIC (ng/ml)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>DHHS target (ng/ml)&lt;sup&gt;f&lt;/sup&gt;</th>
<th>IQ&lt;sub&gt;95&lt;/sub&gt; C&lt;sub&gt;trough&lt;/sub&gt;/PBIC&lt;sub&gt;95&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>APV</td>
<td>700/100 b.i.d.</td>
<td>505</td>
<td>2,120</td>
<td>11</td>
<td>10</td>
<td>62</td>
<td>5.3</td>
<td>60</td>
<td>358</td>
</tr>
<tr>
<td>ATV</td>
<td>300/100 q.d.</td>
<td>705</td>
<td>800</td>
<td>9.3</td>
<td>2.4</td>
<td>9.2</td>
<td>1.7</td>
<td>15.9</td>
<td>60</td>
</tr>
<tr>
<td>DRV</td>
<td>600/100 b.i.d.</td>
<td>548</td>
<td>3,500</td>
<td>14</td>
<td>0.71</td>
<td>3.4</td>
<td>0.4</td>
<td>5.3</td>
<td>25</td>
</tr>
<tr>
<td>IDV</td>
<td>800/100 b.i.d.</td>
<td>614</td>
<td>1,300</td>
<td>3.5</td>
<td>7.0</td>
<td>34</td>
<td>4.3</td>
<td>15.2</td>
<td>73</td>
</tr>
<tr>
<td>LPV</td>
<td>400/100 b.i.d.</td>
<td>629</td>
<td>5,500</td>
<td>10</td>
<td>5.0</td>
<td>27</td>
<td>3.1</td>
<td>31</td>
<td>168</td>
</tr>
<tr>
<td>NFV</td>
<td>1,250 b.i.d.</td>
<td>686</td>
<td>1,000</td>
<td>77</td>
<td>16</td>
<td>75</td>
<td>11</td>
<td>819</td>
<td>3,865</td>
</tr>
<tr>
<td>SQV</td>
<td>1,000/100 b.i.d.</td>
<td>767</td>
<td>400</td>
<td>17</td>
<td>4.7</td>
<td>18</td>
<td>3.6</td>
<td>60</td>
<td>234</td>
</tr>
<tr>
<td>TPV</td>
<td>300/200 b.i.d.</td>
<td>603</td>
<td>15,670</td>
<td>15</td>
<td>88</td>
<td>433</td>
<td>53</td>
<td>796</td>
<td>3,902</td>
</tr>
<tr>
<td>ETR</td>
<td>600 q.d.</td>
<td>316</td>
<td>1,800</td>
<td>27</td>
<td>4.2</td>
<td>15</td>
<td>1.3</td>
<td>36</td>
<td>126</td>
</tr>
<tr>
<td>NVP</td>
<td>400 b.i.d.</td>
<td>266</td>
<td>4,500</td>
<td>1.4</td>
<td>120</td>
<td>952</td>
<td>32</td>
<td>46</td>
<td>366</td>
</tr>
<tr>
<td>RAL</td>
<td>400 b.i.d.</td>
<td>444</td>
<td>114</td>
<td>2.1</td>
<td>8.0</td>
<td>100</td>
<td>3.6</td>
<td>7.5</td>
<td>94</td>
</tr>
</tbody>
</table>

<sup>a</sup> APV, amprenavir; ATV, atazanavir; DRV, darunavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; SQV, saquinavir; TPV, tipranavir; EFV, efavirenz; ETR, etravirine; NVP, nevirapine; RAL, raltegravir.

<sup>b</sup> Shown are the prescribed dose and frequency (b.i.d., twice daily; q.d., once daily). For protease inhibitors other than NFV, the amount coadministered with ritonavir is indicated after the slash.

<sup>c</sup> All C<sub>trough</sub> values are taken from the individual drug manufacturer’s prescribing information (1–3, 6–9, 17, 19, 38, 39, 53, 54), except for those for raltegravir, which were from reference 50.

<sup>d</sup> Protein binding correction factor at 100% HS, extrapolated from the experimental data described above.

<sup>e</sup> 50% and 90% IC<sub>50</sub> and IC<sub>90</sub>, respectively.

<sup>f</sup> The wild-type (WT) virus IC<sub>50</sub> and IC<sub>90</sub> data are the means of a large collection of independent determinations from clinical samples lacking drug-selected mutations in PR or RT (for PIs and NNRTIs; n = 6,537) or in IN (for RAL; n = 1,200).

<sup>g</sup> 50% and 95%, PBIC<sub>95</sub> and PBIC<sub>95</sub>, respectively.

<sup>h</sup> For details, see reference 45. NA, not applicable because no target drug concentration has been determined for PI-naïve patients. For PI-experienced patients, the recommended C<sub>trough</sub> values for darunavir, tipranavir, and etravirine are 550, 20, 300, and 52 ng/ml, respectively (45).
virologic failure, suggesting that a nevirapine $C_{trough}$ of 3,000 ng/ml or greater should be maintained to obtain optimum virologic outcomes. Using similar approaches, target $C_{trough}$ values of 150 ng/ml and 20,500 ng/ml, respectively, have been proposed for ritonavir-boosted atazanavir (atazanavir/rit) and tipranavir (tipranavir/rit) (20, 44); however, neither study was sufficiently powered to define threshold therapeutic concentrations. These values are higher than the PBIC$_{95}$ measures that we determined in this study (60 ng/ml and 3,902 ng/ml, respectively) (Table 2). One possible explanation is related to the use of cohort studies, which are likely to include intermittently adherent patients, to define minimum effective $C_{trough}$ values for antiretroviral drugs. Unlike carefully conducted, wide-dose-ranging pharmacokinetic/pharmacodynamic studies, target drug concentrations estimated from cohort studies are based on variations in interpatient concentrations for a given dose administered to many individuals. Consequently, this approach generates insufficient data to accurately populate the low end of a true concentration-response curve and will tend to overestimate the threshold drug concentrations.

The PBIC$_{95}$ calculated for nelfinavir (3,865 ng/ml) in this study is considerably higher than the measured (1,000 ng/ml) or recommended target (800 ng/ml) $C_{trough}$ (45). This discrepancy may be at least partly explained by the observation that nelfinavir is metabolized in the liver, producing metabolites, including M8 (32), which itself exhibits antiviral activity comparable to that of the parent drug (57). M8 concentrations approach 30% of the parent drug concentration, and this metabolite exhibits slightly less serum protein binding than nelfinavir (57). Since nelfinavir is not metabolized to M8 in vivo, our estimate of the nelfinavir PBIC$_{95}$ in this study is likely higher than the true in vivo value, and thus the effective IQ$_{95}$ is likely to be underestimated.

The IQ$_{95}$ for raltegravir (1.2) is lower than expected for a highly efficacious drug. A recent presentation reported a median plasma $C_{trough}$ in healthy volunteers of 82 ng/ml ($n = 45$), which agrees well with our estimates of PBIC$_{95}$ but is 30% higher than the value reported on the drug label (33). In vitro studies have shown that dissociation of raltegravir from the preintegration complex occurs more slowly than dissociation of the preintegration complex itself, meaning that raltegravir binding is essentially irreversible (25). Results from a 48-week once-daily (800-mg) versus twice-daily (400-mg) raltegravir study (50) showed that the once-daily arm was virologically inferior to the twice-daily arm. The average 12-h concentration ($C_{12h}$), derived from intensive pharmacokinetic assessments, was 257 nM (114 ng/ml) in the 400-mg twice daily group versus 40 nM (17.8 ng/ml) in the 800-mg once-daily group. Our results indicate that the raltegravir PBIC$_{95}$ is 94 ng/ml (211 nM), which may in part explain why the once-daily group was inferior. Relationships between $C_{trough}$ and HIV-1 RNA response for two additional INSTI candidates have been demonstrated in wide-dose-ranging early phase monotherapy trials (11, 52).

The application of in vitro susceptibility data to in vivo PBIC$_{95}$ predictions has several limitations. The HS conditions used in the infectivity assays described here may not accurately mimic the situation in vivo (i.e., drug binding in human blood). Inter- and intraindividual variabilities in binding protein concentration encountered in vivo are not taken into account using this approach. However, our study was performed using two lots of HS, each pooled from multiple donors, to minimize the possibility of lot-specific artifacts. In addition, in vitro determinations of IC$_{50}$ or IC$_{95}$ values for individual drugs do not capture synergistic, antagonistic, or additive effects among different drugs that may occur in vivo. Furthermore, the use of a simple linear regression model to extrapolate PBCFs for 100% HS from the experimentally determined IC$_{50}$ in the presence of 23 to 81% HS may not be equally applicable to all drugs. Most importantly, there is a paucity of in vivo pharmacodynamic data with which our results can be validated.

Collectively, the available in vivo concentration-response data, which are considered the “gold standard” from the pharmacodynamic perspective, correlate strikingly well with the PBIC$_{95}$ values we have derived from in vitro susceptibility and PBCF measurements, with the exceptions of the cohort studies described above. Consequently, we propose that this parameter is an appropriate surrogate measure of target $C_{trough}$ for antiretroviral drugs (excluding those that require intracelular activity [i.e., NRTI]) in lieu of formal early phase pharmacodynamic assessments. Ideally, concentration-response relationships for ARVs should be defined early in the development process. Phase III trials (or phase IV postmarketing studies) are not the appropriate time to define drug target concentrations because their design requires that all participants receive either one given dosage or one of several dosages within a narrow range. Late phase I or early phase II trials should incorporate wide dose-finding designs encompassing dosages that exceed 10-fold differences (preferably larger) in order to adequately populate concentration-response curves. In the absence of clearly defined concentration-response relationships, identification of precise target drug exposure is not possible.

The derivation of PBCFs that can be applied to IC$_{50}$ data generated by routine phenotypic assays such as PhenoSense HIV also provides a useful way to relate these in vitro concentrations to total in vivo drug exposure for applications that include therapeutic drug monitoring and calculation of IQ values for viruses with reduced drug susceptibility.

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