



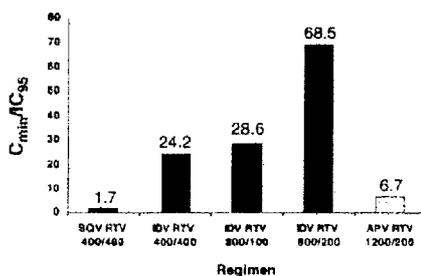
# Prediction of drug potency from C<sub>min</sub>/IC ratio: false precision?

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## Background:

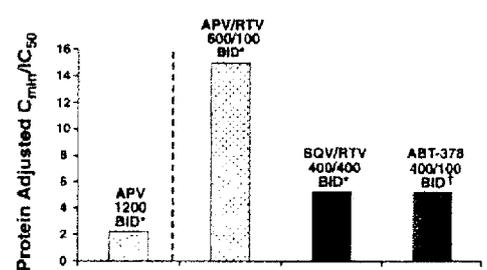
The ratio of plasma drug trough concentration (C<sub>min</sub>) to the inhibitory drug concentration (IC<sub>50</sub>, IC<sub>90</sub> or IC<sub>95</sub>) has been used to predict clinical efficacy of antiretrovirals. Low C<sub>min</sub> of saquinavir and indinavir is correlated with virological failure. However there are many different assays, assumptions and statistical methods to measure C<sub>min</sub> and antiviral endpoints. There are no standards or guidelines for how results should be reported. Currently each PI company is presenting comparisons of C<sub>min</sub>/IC<sub>50</sub> ratio's for the PI class using different methods. It is not clear which is the correct method to use, if any, for such comparisons of protease inhibitor potency. Using different analyses, it is possible to show that each of four ritonavir-boosted protease inhibitors has the greatest C<sub>min</sub>/IC<sub>50</sub> ratio (indinavir, amprenavir, ABT-378, saquinavir):

### Merck Estimates of C<sub>min</sub>/IC<sub>95</sub> Ratio



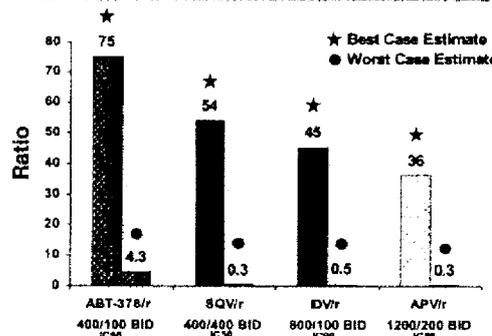
Source: Condra et al, 3rd International Workshop on Salvage Therapy for HIV Infection, March, 2000, Chicago, IL.

### Glaxo Wellcome C<sub>min</sub>/IC<sub>50</sub> comparison

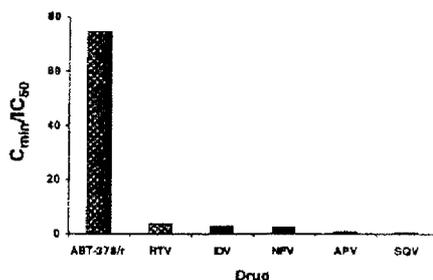


\*APV and SQV viral isolates: WT n=334.  
†ABT-378 data are for HIV IIb, adjusted for 98.5% protein binding.

### Range of potential C<sub>min</sub>/IC Ratios for RTV Boosted PI Treatments

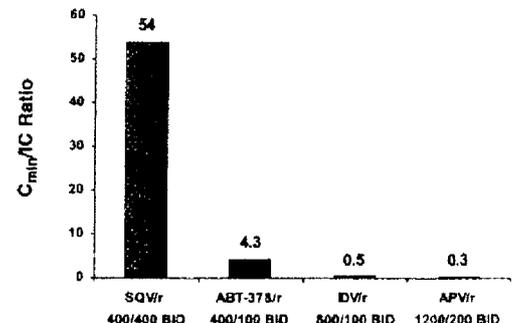


### Abbott Estimates of C<sub>min</sub>/IC<sub>50</sub> Ratio



Source: Kempf et al, 3rd Workshop on HIV resistance and treatment strategies, June, 2000, Sitges, Spain.

### Potential Roche C<sub>min</sub>/IC Ratio for RTV Enhanced PI treatments?



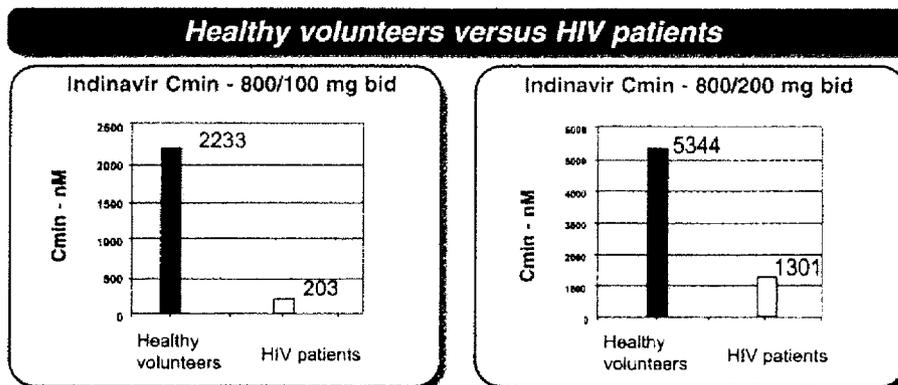
## Influences on IC50, IC90 or IC95

### 1. Statistical methods used

For most protease inhibitors, there is wide variability in the AUC and Cmin, with a minority of patients showing high levels - this "positive skew" in the data makes the arithmetic mean (Amean) greater than the geometric mean (Gmean) or the median.

### 2. Analysis of HIV patients versus healthy volunteers

HIV patients and healthy volunteers may differ in their ability to absorb drugs, and in the rate of drug clearance. The graph below shows a comparison of indinavir Cmin for two studies of indinavir/ritonavir. Cmin for indinavir was significantly higher in the study of healthy volunteers (Saah 1999) than in a study of HIV patients (O'Brien 1999):



## Influences on IC50, IC90 or IC95

### 1. IC50 versus IC90 or IC95

IC is inhibitory concentration. A higher concentration is required to inhibit viral replication by 95% (IC95) than by 50% (IC50). For the HIV protease inhibitors, IC90 or IC95 may be up to 8 times greater than IC50 (reference: Moyle, Drugs 1996).

### 2. Methods of estimation of IC50 and IC90

Inhibition can be assessed by several assays (eg. p24 antigen reduction, syncytium formation) and in different cell types (eg. activated or resting cells). For each HIV protease inhibitor, a range of IC50, IC90 or IC95 values are quoted in the drug labels, reflecting the variety of potential in vitro estimates:

**Table 1: In vitro IC50, IC90 or IC95 as quoted in US product labels:(nM)**

PI	IC50	IC90/95
Saquinavir	1-30	5-80
Indinavir	NA	25-100
Ritonavir	4-153	NA
Nelfinavir	NA	7-196
Amprenavir	80-410	NA
ABT - 378	4-27	NA

NA- not assessed

### 3. In vivo EC50 versus in vitro IC50, IC90 or IC95

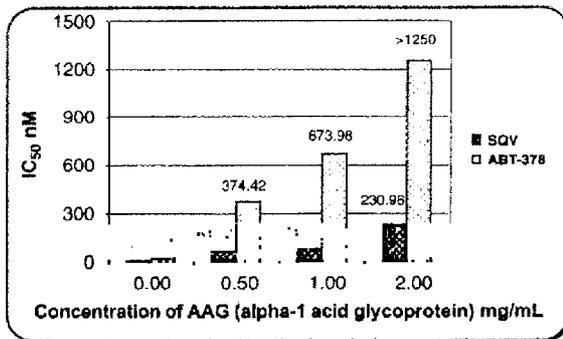
For amprenavir and saquinavir, drug levels and HIV RNA reductions have been measured in patients receiving treatment. In these trials, the "EC50" (effective concentration) is the Cmin corresponding to 50% of the maximal reduction in HIV RNA level. For saquinavir this value was 95 nM (Gieschke, CPK, 1999). This is the only reliable method to estimate in vivo EC50.

For drugs without a directly measured in vivo EC50 from patients, the in vitro IC50, IC90 or IC95 needs to be adjusted for in vivo conditions (protein binding and intracellular accumulation).

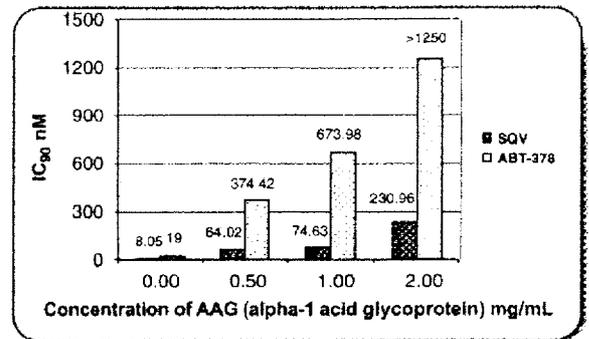
## Adjustment for protein binding

After absorption, a proportion of PI is bound to plasma proteins (principally albumin and alpha-1 acid glycoprotein, AAG) and the remainder is free drug available to inhibit viral replication. In vivo protein binding is estimated as 60% for indinavir, 97% for saquinavir, 90% for amprenavir and 98-99% for ABT-378. The figures below show geometric mean IC50 and IC90 levels of saquinavir and ABT-378 in the presence of either no alpha-1-acid glycoprotein (AAG), or rising concentrations reflecting the range of AAG plasma levels found in HIV infected patients. The IC50 of ABT-378, 100 nM (Sham et al 1998: AAC 42; 3218-3224) was previously measured in the presence of 50% human serum, which does not contain a concentration of AAG representative of HIV-1 infected patients. This new analysis shows that the IC50 of ABT-378 can be up to 6.5 times higher than previous estimates. The IC90 of ABT-378 may be over 12 times higher than previous IC50 estimates.

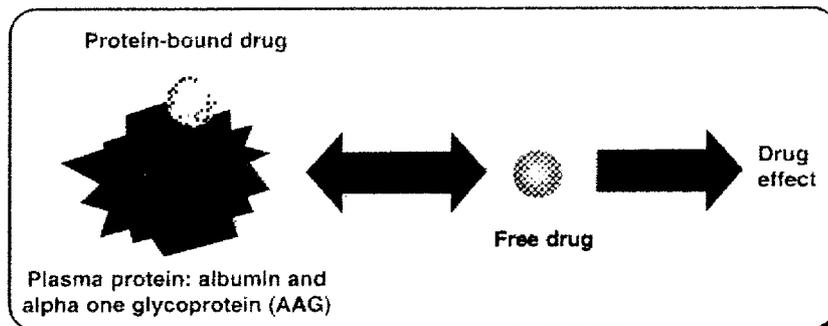
**IC<sub>90</sub> levels of saquinavir and ABT-378: Wild-type virus**



**IC<sub>50</sub> levels of saquinavir and ABT-378: Wild-type virus**



### Plasma protein binding



In the absence of an EC50 measured in treated patients, there are three methods of adjusting the in vitro IC50 for protein binding:

1. Multiplication of IC50 by percentage protein binding measured in vivo.
2. Measurement of IC50 ("EC50") in the presence of 50% human serum.
3. Multiplication of IC50 by a constant factor (often 4 is used) to control for assay variation.

For methods 1 and 2, the protein binding adjustment factors would be as follows for the major protease inhibitors:

**Table 2:**

PI	Method 1	Method 2	Method 3
Saquinavir	133	5-26	4
Indinavir	2.5	2	4
Amprenavir	10	1.5-36	4
ABT-378	50-99	NA	4
Nelfinavir	33	37	4

*note for saquinavir and amprenavir, different assays have generated different estimates of the adjustment for protein binding by method 2. Human serum from healthy volunteers and HIV patients may differ in concentration of plasma proteins such as AAG, which can then affect the results from these assays.*

## Adjustments for intracellular accumulation

In vitro data suggests substantial differences in the degree of intracellular accumulation between protease inhibitors. However there is no accepted method to adjust the IC50 or IC90 for the PI's with low cellular accumulation.

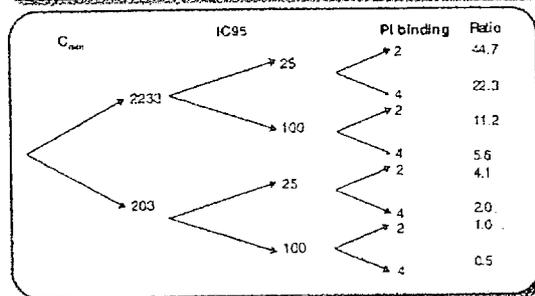
## Combined analysis

For indinavir/ritonavir (800/100 mg bid) and saquinavir/ritonavir (400/400 mg bid), all available estimates of C<sub>min</sub>, IC95 and protein binding were used to calculate the range of possible C<sub>min</sub>/IC95 ratios. Figure 1 and Figure 2 show the range of estimates which can be calculated, by using the highest and lowest estimates of C<sub>min</sub>, IC95 and protein binding adjustment. All data shown is in nM units.

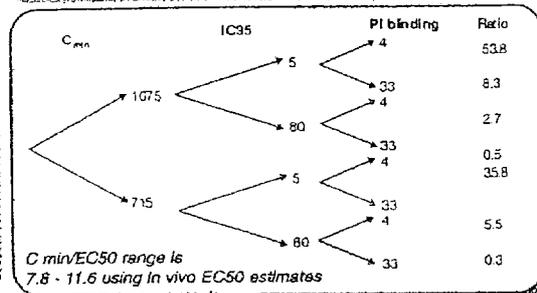
Data sources for indinavir are as above. For saquinavir C<sub>min</sub> the data sources were the product label and Cameron AIDS 1999. IC95 for indinavir and IC90 for saquinavir are from the US product labels.

For indinavir/ritonavir, the C<sub>min</sub>/IC95 ratio was significantly lower when based on analysis of HIV infected people rather than healthy volunteers. For saquinavir/ritonavir the ratio was most sensitive to estimates of protein binding. However use of the in vivo EC50 (93 nM) reduces the range of possible C<sub>min</sub>/EC50 ratios to 7.8 - 11.6 for ritonavir/saquinavir.

**Figure 1: Range of C<sub>min</sub>/IC95 ratio estimates: indinavir/ritonavir (800/100mg bid) 0.5 to 44.7**



**Figure 2: Range of C<sub>min</sub>/IC95 ratio estimates: saquinavir/ritonavir (400/400mg bid) 0.3 to 53.8**



## Conclusions

A wide range of possible C<sub>min</sub>/IC95 or C<sub>min</sub>/EC50 ratios can be calculated for any protease inhibitor at a particular dosage and regimen and therefore estimates of intracellular accumulation should be treated with caution.

There is an urgent need for standardisation in how this data is reported:

1. Standardised units should be used (eg. nM)
2. C<sub>min</sub> should be based on Intent to Treat PK analysis in HIV infected people treated with the PI under assessment using median or geometric mean levels.
3. In vivo EC50 measured directly in treated patients should be used wherever possible.
4. Differences in intracellular accumulation need to be accounted for
5. Where there is a range of possible estimates of C<sub>min</sub>/IC90 ratio, the range of estimates should be presented, rather than single values.