Evaluating the Pharmacokinetic Profile of Darunavir Ethanolate/Ritonavir to Determine the Most Appropriate Predictor of Virologic Response in Advanced HIV-1 Infected Individuals (The PRIDE Study)

Short title: Pharmacokinetic predictors of response with darunavir

Keywords: pharmacokinetic, darunavir, HIV, virologic suppression, inhibitory quotient

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Abstract

Background: The relationship between darunavir (DRV) pharmacokinetic (PK) and pharmacodynamic (PD) parameters in predicting virologic response to antiretrovirals has yet to be determined in advanced HIV-1 infected patients.

Design: Prospective, multi-centre, observational open-label study of 48 weeks duration

Objectives: The objective of this study is to determine which week 12 PK parameter among Cmin, virtual phenotypic inhibitory quotient (vIQ), genotypic inhibitory quotient (gIQ) and normalized inhibitory quotient (NIQ) is the best predictor of virologic suppression (HIV-1 RNA < 50 copies/mL) at week 24 among patients on DRV-based regimens and to determine the target values of the best predictors. Also PK parameters will be correlated with grade 3-4 adverse events.

Methodology: Subjects enrolled will be required to give 4 blood samples to be measured for trough concentrations of darunavir at 4-, 12-, 24- and 48-weeks of therapy. HIV treatment clinics will recruit subjects whom are, triple class-experienced, starting DRV as part of their routine standard of care with an optimized background regimen, and have a history of previous virologic failure on other regimens. Patients will be excluded if it is felt that they will fail to adhere to DRV therapy, they use tipranavir 15 days prior to starting DRV, have primary HIV-1 infection, or possess conditions which compromise patient safety/adherence. Information pertaining to age, gender, race, adverse effects, CD4+, viral load, previous genotypes/phenotypes, concomitant medications, and comorbidities will be taken from the patient's medical chart. In addition to the four blood samples, patients will be required to fill out a Simplified Medication Adherence Questionnaire (SMAQ) at each study visit, to ensure adherence to DRV therapy. All

information will be de-identified and only site investigators will know the identity of the subjects involved. All sites will freeze blood samples for shipment to the main study laboratory for PK testing. Cmin, gIQ, vIQ, and NIQ will be calculated for each blood sample.

Analysis: Multivariate logistic regression models shall be used to determine which of gIQ, vIQ, IQ and Cmin (week 12) are most predictive of virologic suppression (HIV-1 RNA < 50 copies/mL) at 24 weeks, after controlling for CD4 count at baseline, viral load at baseline, years of antiretroviral therapy, number of active antiretrovirals in the antiretroviral regimen, new enfuvirtide use, etravirine use, maraviroc use, raltegravir use, and baseline genotype sensitivity score. Receiver Operating Characteristic (ROC) curves will be used to determine the best cutoffs of gIQ, vIQ, IQ and Cmin at 4, 12, 24 and 48 weeks to predict virologic suppression at 24 weeks. Once the best cutoffs for each marker at each week of follow-up are chosen, we shall determine which time point is most predictive of 24 week virologic suppression by comparing area under the ROC curve.

Clinical Significance: This study will allow for the determination of DRV PK parameters most predictive of virologic suppression.

Introduction

The introduction of protease inhibitors (PIs), and subsequent adoption of PI-based combination antiretroviral therapy (ART) for the management of human immunodeficiency virus infection (HIV) as the standard of care for HIV-infected patients resulted in profound reductions in disease related morbidity and mortality(1). However, the widespread uptake of PI-based ART for HIV-infected patients has also presented clinicians with multiple challenges, including the emergence of drug resistance and treatment-associated toxicities(2). The development of new PIs with significant antiviral activity in individuals with extensive class resistance while eliciting a minimum degree of toxicity has therefore emerged as an issue of paramount importance in the field of HIV therapeutics.

Darunavir, a new PI, addresses some of the issues unique to treatment experienced patients. Specifically, darunavir possesses excellent *in vitro* activity against both clinical and laboratory strains of HIV-1 and HIV-2. Three oxidative metabolites have been identified with potent activity against wild-type HIV(3). The combination of darunavir boosted by low dose ritonavir (DRV/r) has demonstrated a superior virologic response relative to investigator-selected ritonavir-boosted PI comparators in two large, randomized, phase III trials of treatment experienced patients(4). Based on these data, darunavir was approved by Health Canada in July 2006 for treatment-experienced HIV-1 infected patients(5).

The use of therapeutic drug monitoring is an emerging domain within HIV therapeutics and allows clinicians to characterize drug exposure with therapeutic response. A relationship between plasma concentrations of other PIs and virologic

efficacy has been clearly demonstrated in both clinical trials(6) and cohort studies in treatment-experienced and treatment-naïve individuals(7-10). The plasma concentration of greatest interest is the trough concentration (Cmin), taken at the end of a dosing interval. The goal of evaluating the Cmin is to maintain the Cmin at least above the inhibitory concentration at which viral replication is halted by 50% ($IC_{50,}$ also referred to as EC_{50} – effective concentration at 50%)(11). Therapeutic Cmin values have been correlated with virologic response for several protease inhibitors(12, 13). However, a patient's history of virologic failure and exposure to PIs confound the relationship between Cmin and virologic response, because of mutations in the protease gene that emerge and cause reduced drug susceptibility. Inhibition of viral replication of wildtype virus may require lower concentrations versus a mutated viral strain with decreased susceptibility to PIs(14). Thus, the utility of the Cmin is less pertinent in these treatment-experienced patients. The inhibitory quotient (IQ) overcomes this barrier for many commonly used PIs and takes the susceptibility of a patient's individual virus into account. The genotypic IQ (gIQ) is the ratio between Cmin and the number of cumulative protease mutations a patient may possess in their viral genome(15). The relationship between therapeutic gIQ values and virologic response has been demonstrated for several commonly used PIs; lopinavir(9), atazanavir(7), saquinavir(16), fosamprenavir(10), and tipranavir(8), in treatment experienced patients.

Prior to the approval of DRV, tipranavir was the only second generation protease inhibitor for the treatment of HIV-1 infection in heavily treatment experienced patients. Bonora and colleagues evaluated the relationship between tipranavir gIQ and early virologic response (viral load < 50 copies/mL) at 12 weeks follow up. This cohort was

comprised of 27 highly treatment experienced patients who had failed an average of 4 (3-5) protease inhibitors in previous ART regimens. By week 12 of follow up, 11 of 27 (40.7%) of subjects achieved a viral load < 50 copies/mL. In logistic regression analysis, tipranavir gIQ was the only significant predictor of a viral load < 50 copies/mL (OR 1.21 [1.02 – 1.43] p = 0.026). The tipranavir gIQ cut-off value associated with a viral load < 50 copies/mL was 13000 ug/mL/mutation. At week 12 follow up, 7 of 9 patients with a tipranavir gIQ > 13000 ug/mL/mutation had a viral load < 50 copies/mL versus only 4 of 18 with a gIQ < 13000 ug/mL/mutation. This was the first study to demonstrate the relationship between gIQ and early virologic response using a second generation protease inhibitor.

Alternatively, instead of considering mutations present, the phenotypic IQ (PIQ) includes the degree of drug susceptibility. The PIQ is the ratio of the Cmin and the protein-adjusted IC_{50} or fold-change for the particular drug being evaluated. Due to the high costs and cumbersome nature of true phenotypes, virtual phenotypes are performed more commonly. The resulting calculation using the virtual phenotype is known as the virtual IQ (vIQ) and is calculated by dividing the Cmin by the protein-adjusted wild-type IC_{50} multiplyied by the fold change. The variability in predicting immunologic and virologic responses between the PIQ and vIQ is negligible for lopinavir, and may be interchanged for other PIs(17). The normalized IQ (NIQ) is the ratio of the patient's vIQ to the reference vIQ. The population or reference IQ is calculated by dividing the population C_{min} by the resistance cut-off for an ARV expressed as the fold change in IC_{50}. Table 1 describes the calculation of each of the IQs. Each of the IQs described have demonstrated their ability to be predictive of virologic outcomes for several commonly

used PIs. Table 2 summarizes studies evaluating IQ predictors and virologic outcomes among treatment experienced patients.(8, 9, 11, 16, 18-26)

Clearly the relationship between pharmacokinetic parameters and virologic response exist for previously developed PIs. However, among the Cmin, gIQ, vIQ and NIQ, it is uncertain which pharmacokinetic (PK) or pharmacodynamic (PD) parameter should be used to best monitor and improve virologic and immunological responses with DRV.

During the POWER-1 and POWER-2 studies, a strong relationship was demonstrated between virologic response at week 24 and the vIQ. This relationship was primarily driven by the baseline darunavir fold-change between 4 and 40. Analysis of Covariance (ANCOVA) models demonstrated that vIQ was significantly and positively associated with $\log_{10}$ virologic response at week 24 ($p<0.001$). In the group with a baseline DRV fold-change between 4 and 40, increasing concentrations, from $C_{0h} \leq 1462$ ng/mL to $C_{0h} > 3287$ ng/mL led to greater reductions in $\log_{10}$ viral load from -1.21 copies/mL to -1.71 copies/mL. This phenomenon was not seen in the groups with fold-changes outside of the 4 – 40 range. Figure 1 displays the change, from baseline to week 24, in $\log_{10}$ viral load by DRV exposure and by baseline fold-change(27).

Another predictor of response was the overall number of active agents in the optimized background regimen(4). The mutations present help to determine the number of active agents and would suggest that the gIQ may be of value in examining PK predictors of response to DRV. Because the NIQ has been correlated with outcomes with other PIs(20), it may be of importance when examining predictors of virologic suppression to DRV/r therapy.

The PRIDE study will evaluate the pharmacokinetic determinants of virologic suppression in patients receiving DRV/r based therapy during 2007 – 2008 in highly treatment experienced, HIV-1 infected patients. The PRIDE study will evaluate which week 12 pharmacokinetic parameter of, C_{min}, vIQ, GIQ, and NIQ is most predictive of virologic suppression at 24 weeks of treatment with DRV.

Hypothesis

We hypothesize a relationship exists between the DRV gIQ and virologic suppression. We predict that higher week 12 gIQ values will lead to increased likelihood of achieving virologic suppression at week 24.

Methods

Study Design

The PRIDE study is a prospective open-label observational pharmacokinetic study of 48 weeks duration of HIV-1 infected adults who received ritonavir boosted darunavir (DRV/r) plus an optimized background regimen containing other antiretrovirals.

The objectives of the PRIDE study are threefold. The primary objective of the PRIDE study will be to evaluate which week 12 PK parameter of C_{min}, vIQ, gIQ, and NIQ is most predicitive of virologic suppression (HIV-RNA < 50 copies/mL) at 24 weeks of DRV therapy. The secondary objective will be to determine the target values associated with the PK parameters most predictive of virologic suppression. The tertiary objective is to correlate reported toxicities of DRV with the collected PK parameters.

This study is multi-centered and conducted at the University Health Network – Toronto General Hospital, Maple Leaf Medical Clinic (Toronto), McGill University Health Centre – Montreal Chest Institute (Montréal), University of Torino (Italy), and Radboud University Medical Center Nijmegen (the Netherlands). The enrollment period began Feb 8, 2007 and is presently ongoing. This study was reviewed and approved by local ethics boards at all participating sites and conducted in accordance with the Declaration of Helsinki.

Study Population

All patients enrolled in the study were identified and screened for eligibility by the individual site study research nurse. Inclusion criteria for the PRIDE study were age > 18 years with documented HIV infection, triple antiretroviral class experienced with limited treatment options due to virologic failure or intolerance, detectable viral load, failing their last treatment regimen and were prescribed a DRV/r based regimen with an optimized background regimen as part of their routine standard of care. Patient excluded from the PRIDE study were those with primary HIV infection or conditions compromising subject's safety or ability to adhere to study protocol. Any active or clinically significant disease had to be resolved or stabilized for 30 days prior to the screening phase of the study. The use of tipranavir within 15 days of initiating DRV/r therapy was prohibited because of tipranavir's inductive effects on CYP3A4 isoenzyme. Antiretroviral agents used in conjunction with DRV/r were selected based on treatment history and genotypic resistance testing at the discretion of the treating physician. Hypersensitivity to any of the excipients of DRV was also a reason for exclusion. DRV

is a sulfonamide derivative, but patients with previous sulfonamide allergy were permitted to enter the study, as there have been no reported cases of cross-allergenicity.

Sample and Data collection (Appendix A and B)

A physical examination was performed by the patient's physician at the screening visit. Information collected from the physical exam included height, weight, oral temperature, supine blood pressure, pulse, respiratory rate, and review of systems. Demographic information was obtained from the patient's medical chart. Demographic information collected included age, sex, race and smoking status. Baseline laboratory analysis included CD4 cell count and HIV-RNA. The results of baseline genotype and virtual phenotype (Virco®) were also recorded. If a genotype and virtual phenotype had been done within two months of enrollment, a baseline genotype and virtual phenotype was not performed. A list of cumulative protease and reverse transcriptase mutations was determined from the patient's previous genotypes and collected from the patient's medical chart. This was used to calculate the genotypic sensitivity score (GSS) and determine the number of active agents in the patient's regimen. Zero points were assigned for mutation(s) present for each agent in the regimen. One point was assigned for absence of mutation(s) for each agent in the regimen. The sum of the points will determine the GSS(28). The number of active agents was determined by using the International AIDS Society – USA (IAS-USA) mutation algorithm(29) and was interpreted by a panel of 3 investigators. The name, doses, and indication for use of concomitant medications, including antiretroviral agents, co-administered with DRV/r were documented. Follow-up laboratory evaluations were performed at week 4, 12, 24

and 48. Laboratory parameters evaluated at each follow-up visit were CD4, HIV-RNA, and DRV concentration at the end of the dosing interval (Cmin). Adverse events, reported by the patient, were recorded at each follow-up visit. The Simplified Medication Adherence Questionnaire(30) (Appendix B) was administered at each follow-up visit to measure medication adherence. Extracted data was entered into a SPSS Database (SPSS for Windows, Rel. 12.0.1. 2001. Chicago: SPSS Inc.).

Virologic and immunologic end points

The primary goal of the study was to determine the impact of week 12 pharmacokinetic parameters of DRV/r-based therapy on probability of achieving virologic suppression in treatment-experienced patients, defined as an HIV-RNA was < 50 copies/mL at week 24 of therapy. Participants who did not achieve virologic suppression during the study period were censored at the month of last follow-up (Appendix E). Secondary endpoints included the proportion of patients with a plasma HIV-RNA < 50 copies/mL at week 48 as well as the proportions of patients with plasma HIV-RNA of < 400 copies per mL, the proportion of patients who had a decrease in HIV-RNA of one $\log_{10}$ copies/mL and changes in CD4+ cell count from baseline at weeks 4, 12, 24, and 48 following the initiation of DRV/r-based therapy.

Pharmacokinetic end points

The Cmin value of DRV was collected at weeks 4, 12, 24, and 48. Patients were instructed by study site personnel to take their evening dose of DRV/r with food the night prior to their appointment at the usual time. Subjects were asked to come to the clinic the

next morning, prior to taking their next DRV/r dose. Subjects were instructed to ingest their doses with food. Upon arrival at the clinic, the study nurse obtained 8 mL of venous blood in a heparinized tube. The blood was sent to the McGill University Health Centre – Royal Victoria Hospital Laboratory for analysis (Appendix D). The Cmin was used to calculate the gIQ, vIQ, and NIQ. The gIQ was calculated by dividing the Cmin by the number of DRV related protease mutations. The number of DRV related protease mutations was determined by using all of the patient's available previous genotypes and the IAS – USA mutations(29). The DRV mutations used in the gIQ calculation were protease mutations which occurred at the following positions; V11I, V32I, L33F, I47V, I50V, I54M/L, G73S, I76V, I84V, and L89V. The vIQ was calculated by dividing the Cmin by the wild-type protein adjusted 50% effective concentration (EC_{50}) for DRV (5 ng/L) and multiplying by the darunavir EC_{50} fold-change as per the baseline virtual phenotype. The NIQ was calculated as a ratio between the patient's vIQ and the population IQ. The population IQ was determined by dividing the Cmin from the population curve by the protein-adjusted EC_{50} multiplied by the fold-change for resistance. The fold-change used in the population IQ calculation was the mean of the two clinical cut-off values per Virco™ phenotype.

Adverse Events

At each study visit, subjects were queried by the study nurse about any adverse events that they experienced. All clinical adverse events reported to the study nurses were recorded. The toxicity endpoint of interest was the occurrence of grade 3 (severe) or 4 (life-threatening) clinical adverse events at all study visits.

Statistical analysis

Sample Size Calculation:

The primary objective of the study was to estimate the sensitivity of gIQ at 12 weeks to predict virologic suppression, defined as HIV-RNA < 50 copies/mL at 24 weeks of follow-up. In order to estimate the sensitivity with a 95% confidence interval of +/- 10%, 100 patients were required for gIQ sensitivity at 12 weeks to be 70%, and the 95% confidence interval for sensitivity to be (61%, 79%). A sensitivity of 70% was specifically chosen because it closely resembled the sensitivity (63.6%) used by Bonora et al when determining the gIQ target value associated with virologic response for tipranavir(8).

All statistical analyses were performed using SPSS Version 12.0 (SPSS for Windows, Rel. 12.0.1. 2001. Chicago: SPSS Inc.). Baseline characteristics were summarized using medians and interquartile ranges (IQR) for continuous variables and proportions for categorical variables.

Multivariate logistic regression models was used to determine which of gIQ, vIQ, NIQ and Cmin were most predictive of virologic response at 24 weeks, after controlling for baseline CD4 count and viral load, years of antiretroviral therapy, number of active antiretrovirals in the optimized background regimen, enfuvirtide use, raltegravir use, maraviroc use, etravirine use, number of cumulative darunavir-related mutations and baseline genotypic sensitivity score. For each pharmacokinetic / pharmacodynamic (PK/PD) parameter predictive of response, the best target value to predict virologic response at 24 weeks was determined with receiver operating characteristic curves (ROC). The cohort was stratified into those above the ROC curve-derived target value

and those below the ROC curve-derived target value. Chi-square analysis was employed, using HIV-RNA < 50 copies/mL as the outcome of interest and ROC-derived ?IQ value as the predictor to determine the relationship between the identified target value and virologic suppression.

Additionally, to address secondary objectives, ROC curves were used to determine the best target valuesof GIQ, VIQ, NIQ and Cmin at 4, 12, 24 and 48 weeks to predict virologic response at 12, 24 and 48 weeks if these were related to virologic response following the multivariate analysis. Once the best target values for each PK / PD parameter at each week of follow-up were chosen, the time point most predictive of week 24 virologic response was determined by comparing area under the ROC curve.

Cut-off values for Cmin, gIQ, vIQ, and NIQ were correlated with the presence of grade 3 and 4 adverse events using Chi-square analysis.

Ethical Considerations

Written informed consent was obtained from all patients (Appendix C). Subjects voluntarily participated in the PRIDE study without coercion or compensation. The only variations from the routine standard of care were four pharmacokinetic blood samples and the administration of an adherence questionnaire. The measured plasma concentrations were not used for therapeutic drug monitoring in this study. Therapeutic drug monitoring has not been widely accepted as the standard of care in North America, but is routinely performed as part of clinical practice in other countries. Enrolled subjects did not benefit directly from participation in the study. Potential harm that may have resulted may have been the pain associated with an additional blood draw at the 4 study

visits. Injury that occurred as a result of DRV was the responsibility of the prescribing physician, as it was prescribed as part of the patient's standard of care.

Results

Patient characteristics

Eight patients were screened for entry into the PRIDE study during the study period from Februay 8, 2007 to July 1, 2007. Five patients initiated a DRV/r-based regimen and have completed their week 4 study visit. The PRIDE study is ongoing and actively recruiting patients.

patients did not meet the inclusion criteria and were therefore excluded from the study. Reasons for exclusion included having a baseline HIV-RNA < 50 copies/mL (n = #) and conditions compromising patient safety (n = 1). ## patients were therefore eligible for analysis.

Baseline characteristics of the study population are summarized in Table 3. Patients were highly treatment-experienced, having received a median of ## (IQR ##, ##) antiretroviral agents and treatment for a median of ## (IQR ##, ##) years prior to the initiation of DRV/r-based therapy. Median baseline plasma HIV-RNA and CD4+ cell count were #.# $\log_{10}$ copies/mL (IQR #.#, #.#) and ## cells/mm^3 (IQR ##, ##), respectively.

The median number of protease and reverse transcriptase associated mutations were ## (IQR #, ##) and # (IQR #, ##) respectively. The median number of DRV-associated mutations was ## (IQR ##,##). ## (# %) patients had a baseline DRV fold change > 40. The median genotypic sensitivity score (GSS) was ## (IQR ##, ##). The

median number of active agents, determined by baseline and previous genotypes, in the regimen was ## (IQR ##, ##). ## patients received enfuvirtide therapy in conjunction with DRV/r based therapy. Enfuvirtide therapy was considered *de novo* in ## patients. The median number of expanded access program (EAP) and/or special access program (SAP) agents used was ## (IQR ##, ##). The most commonly utilized EAP/SAP agents were etravirine (%), raltegravir (%) and maraviroc (%).

Pharmacokinetic Analysis

The calculated pharmacokinetic parameters are shown in Table 4. At week 12, the mean calculated DRV Cmin was #.## mg/L (SD). The median gIQ, vIQ and NIQ values were #.# mg/L/mutation (IQR #.#, #.#), #.# mg/L/mutation (IQR #.#, #.#), and #.# mg/L/mutation (IQR #.#, #.#), respectively. The strongest week 12 pharmacokinetic predictors of 24-week virologic suppression was ?IQ (HR ##, 95% CI ## – ##, p = ##). Based on the ROC curves (Figure 2), the target value of this PK/PD parameter at week 12 associated with virologic response at 24 weeks follow up was #.## mg/mL/mutation (sensitivity ##%, specificity ##%). ## of ## (%) were above the ROC curve derived target value. Subjects with an ?IQ value above the ROC curve derived target value had a higher likelihood of achieving virologic suppression at 24 weeks follow up than those with an ?IQ < than this target value (% vs %, p = 0.###). This is described in Figure 3 and 4. The magnitude of target attainment of ?IQ with change in $\log_{10}$ HIV-RNA is shown in Figure 5. For the other pharmacokinetic values that were predictive, the proposed target values associated with a virologic suppression are summarized in Table 5.

The univariate proportional hazards models identified several predictors of virologic suppression at week 24 (Table 6). When we attempted to fit the proportional hazards model with multiple covariates, XXX (HR ##, 95% CI ## – ##, p = ##), YYY (HR ##, 95% CI ## – ##, p = ##), and ZZZ (HR ##, 95% CI ## – ##, p = ##) remained significantly associated with virologic suppression after adjusting for other covariates.

Virologic Response

of ## (##%) patients included in the analysis attained a plasma HIV-RNA below 50 copies/mL at least once during follow-up. At 24 weeks following the initiation of DRV/r, ## (##%) patients had attained plasma HIV-RNA suppression below 50 copies/mL. HIV-RNA declined by a median of #.## $\log_{10}$ copies/mL (IQR #.##, #.##) following twelve months of DRV/r-based therapy. ## patients (##%) with >## DRV associated resistance mutations at baseline attained virologic suppression during this period. The proportions of patients with a HIV-RNA < 50 copies/mL, < 400 copies/mL and with a 1 $\log_{10}$ copies/mL decrease in viral load during follow-up are summarized in Table 7.

Immunologic Response

CD4+ cell counts increased by a median of ## cells/mm^3 (IQR #, ##) from baseline to week 48 of follow-up (N=##).

Adverse Effects

adverse events were reported in ## subjects. ## of ## adverse events were considered to be grade 3 or higher in nature. XXX (%), YYY (%), ZZZ (%) were the most common grade 3 – 4 adverse events. ## of ## who experienced a grade 3-4 adverse event discontinued therapy. In the regression analysis, these adverse events were independent (or correlated) of DRV Cmin.

Patient Disposition

(## %) patients discontinued DRV/r following a median of ## months (IQR ##, ##). ## of the ## patients discontinued the entire regimen, while ## patients discontinued only DRV/r and remained on their background regimen. ## (%) and ##(%) patients failed to achieve virologic suppression and response, respectively, during the 48 week study duration. The two most common reasons for discontinuing DRV/r were XXX (n = #) and YYY (n = #).

Discussion (*a priori*)

This PRIDE study evaluated the pharmacokinetic predictors of a second generation protease inhibitor and the target values associated with a virologic response to therapy after 24 weeks follow up. Clinically, the results of the PRIDE study will allow for future studies to demonstrate the virologic benefit of measuring DRV plasma concentrations via therapeutic drug monitoring. Attaining plasma concentrations and IQs associated with suppression may preserve future treatments in these patients with already limited treatment options.

Barriers

Several barriers were encountered in the development, initiation and completion of the PRIDE study. The scope of these barriers also extends to the context of a 1-year residency research project.

A general barrier to the completion and execution of the PRIDE study is funding. The purpose of the budget was primarily to cover the costs associated with shipment of samples, raw materials, PK sample analysis and nursing time. Costs associated with access to DRV were not included in the budget as subjects in the PRIDE study were starting DRV as part of their standard of care and obtained the medication independent of the PRIDE study. The PRIDE study budget is displayed in Table 8.

Initially, while drafting the protocol, Tibotec had expressed interest. A funding request application for $140, 875 CAD was then submitted. This application was rejected because of apprehension of results by Tibotec, which may not have fallen in line with their marketing approach. Canadian Foundation for AIDS Research (CANFAR) and Canadian HIV Trials Network (CTN) grant applications were then submitted. The CTN application was approved with suggestions from both the steering committee and the community group. The steering committee suggested increasing the number of CTN sites participating in the study. The community group suggested some changes to the patient informed consent form. The amount of money is yet to be determined. However, this funding does not cover operational funding. We are still awaiting a response from CANFAR.

Barriers to completing PRIDE as a residency research project

The first hurdle was the availability of the medication itself, which significantly delayed enrollment. At the time (November 2006) of submission of the protocol to the McGill University Health Centre (MUHC) and University Health Network (UHN) Research Ethics Boards (REB), DRV was not covered by the Ontario Drug Benefit nor the Régie de l'assurance maladie du Québec (RAMQ) and hence patients were receiving DRV via the Expanded Access Program (EAP) run by the company's manufacturer Tibotec Pharmaceuticals Ltd. Patients in this program were not permitted to enter into other studies that examine the use of DRV.

Tibotec was petitioned to allow EAP patients to participate in the study. Subsequently, the request was also made to Tibotec to release the pharmacokinetic information collected during the POWER studies for reanalysis with our PK predictors of response. Tibotec did acknowledge the scientific merit of the PRIDE study, but due to marketing pressure and scientific apprehension of the results, Tibotec rejected both requests.

As of February 2007, darunavir ethanolate was added to the RAMQ's *medicament d'exception* list of medications that can be obtained for Quebec patients who meet strict predetermined criteria. Ontario followed suit with a similar process known as Section 8 in May. Once patients could access DRV via the aforementioned processes, Tibotec restrictions on participation in the PRIDE study were no longer present. Since then, enrollment quickly began.

A second barrier that existed was the development of an assay to measure DRV plasma concentrations, which impeded the ability to compute interim results for the

residency project. The powder had not been released from Tibotec for assay development. David Colantonio, PhD, biochemist at MUHC – Royal Victoria Hospital, was able to obtain the internal standard from the National Institutes of Health (Bethesda, MD). An assay is currently being developed and validated with other centers assessing plasma concentrations of antiretrovirals.

A third barrier to completing this study was the need to enroll 100 patients to be adequately powered. For the purposes of a one-year residency project it was difficult to recruit this volume of patients in such a limited period of time. The sample size was required to have a gIQ sensitivity of 70%. The 3 Canadian sites involved are University Health Network, MUHC and Maple Leaf Medical Clinic. Stefano Bonara from Torino, Italy and David Burger from Nijmegen, the Netherlands have agreed to participate. Coordinating with other sites has been challenging due to timing delays in Research Ethics Board (REB) and enrollment of patients, and will remain a barrier while complying with the CTN's request to increase the number of study sites. The EU sites are awaiting approval of DRV before enrolling patients into the PRIDE study.

In addition to these barriers, the REB process has been somewhat limiting. While we were able to obtain REB approval relatively quickly from MUHC (Appendix D) and the Maple Leaf Medical Centre, we are still awaiting a final letter from the UHN REB. On average protocol reviews take approximately 2 months. At UHN, our ethics submission process began with a preliminary presentation to the UHN Community Advisory Research Board in September 2006, with approval being given in October 2006 (Appendix E). The protocol was then submitted to UHN REB in October 2006 and approved for the expedited approval process in November 2006. However, as of August

2007, we are still awaiting a final approval letter. Reasons for a delay in approval were numerous and included delays in obtaining data & material transfer contracts between UHN and MUHC legal departments, clarification requests in the protocol/funding from UHN REB, request from UHN REB to add a list of prohibited natural products, and lack of continuity at UHN REB when our main contact person went on a leave of absence for several months. In retrospect, we could have used better foresight to predict that data & material transfer agreements would be required for transfer of blood samples and case report forms between UHN and MUHC, and these forms could have been sent to the institutional lawyers for approval at an earlier stage. Furthermore, since we did initially prohibit the use of natural products, a list of natural products could have been made *a priori*. However, other factors related to the functioning and workload of the REB department remain out of the control of potential investigators.

Study Limitations

Our tertiary objective was to correlate PK data with toxicity. Our analysis of this endpoint is limited because the instrument used to collect this information is not validated and based on patient query and therefore only includes the clinical toxicities reported to the study nurse at each visit, which can be highly subjective. Because only clinical toxicities were captured, grade 3-4 laboratory changes will go undetected. In a sample of 100 patients, there is a high probability that even if a relationship between toxicity and plasma concentration exists, it will not be adequately powered to truly detect a difference.

Lessons Learned

The PRIDE study represents an early study which examines the PK predictors of response among 2nd-generation protease inhibitors in advanced HIV-1 infected patients. The protocol possesses strong scientific merit, but may have been too ambitious to complete during a 1-year residency. A study of this nature may be more appropriate for candidates pursuing the combined residency/Master's degree program over 2-years.

It was unknown how obstructive a barrier that Tibotec would serve to be with regards to funding and restrictions on patients taking DRV via EAP. Had Tibotec been more cooperative, the study could have moved along much more expeditiously. Future residents should consider choosing research projects that are not dependent upon obtaining new funding during the same academic cycle and using drugs that patients may obtain without manufacturer restrictions.

The REB process at UHN is still in progress. It is impossible to complete a well-designed residency research project when the REB process is greater than 6 months duration for expedited review. The longest delay was due to data & tissue transfer agreements and waiting for legal departments at UHN and MUHC to approve the transfer contracts. Future residents should consider the implications of doing a multi-center research project where transfer of biologic and patient-sensitive material is involved. Prior to submission of an REB protocol at UHN, these transfer contracts should be completed by the legal departments involved. If tissue & material transfer contracts are not necessary, it may be easier and faster to submit the protocol to MUHC, which meets approximately every 2 weeks.

In conclusion, the PRIDE study is an ongoing PK study and will determine the best PK predictors of virologic response in advanced HIV-1 infected patients on DRV based antiretroviral regimens.

Acknowledgements

The PRIDE trial working group wishes to acknowledge the efforts of study site personnel, M. Samia, name #1, name #2, name #3 and name #4; and research nurses, H. Preziosi, nurse #1, nurse #2, nurse #3 and nurse #4. We also wish to acknowledge Stephanie Lam for initial protocol development. We are grateful to all of the referring prescribers who aided in the study. Finally, we thank the study participants for their contribution to the execution of the study.

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Tables and Figures

Table 1: Calculations of Pharmacokinetic / Pharmacodynamic Parameters

gIQ	Cmin ÷ # cumulative DRV-related protease mutations
vIQ	Cmin ÷ DRV wild-type protein adjusted EC_{50} (5 ng/mL) x DRV fold change as per baseline virtual phenotype
NIQ	vIQ of subject ÷ population vIQ†.

gIQ, genotypic inhibitory quotient; Cmin, trough concentration; DRV-related protease mutations: V11I, V32I, L33F, I47V, I50V, I54M/L, G73S, L76V, I84V, and L89V; vIQ, virtual phenotypic inhibitory quotient; NIQ, normalized inihibitory quotient

† Population vIQ calculated by dividing Cmin of the population curve by protein-adjusted EC_{50} multiplied by fold change for resistance. Fold change used to calculate population IQ was the mean of the two reported clinical cut-offs.

Table 2: Summary of IQ Predictors and Virologic Outcomes Among Treatment Experienced Patients(11)

Drug	Sample size (N=)	PK predictor	Actual response	Outcome
Atazanavir(18)	N=92	GIQ	Multivariate analysis: β = -5.4 (95% CI; -10, -1) p = 0.02	HIV-RNA < 50 c/mL at week 24
Fosamprenavir(19, 24, 26)	N=61	PIQ	For virological responses <400 copies /ml population at Week 12 p=0.00065 (n=53), at Week 24 p=0.000002 (n=54) and at Week 48 p=0.000008 (n= 45)	Response defined as HIV-RNA < 400 c/mL and ≥ 1-log decline in HIV-RNA at weeks 12, 24, and 48
	N=49	GIQ	Significant correlation between HIV-RNA decline from day 0 and week 12 and the GIQ ($R = 0.49$; $P = 0.001$)	Viral load endpoint used was ≥ 1-log decline in HIV-RNA
	N=121	GIQ	GIQ independently associated with failure in logistic regression OR = 30.8 and $P < 10^{-4}$	Virologic success defined as <2.3 log in HIV-RNA or 1-log decline
Lopinavir/r(9, 20, 21, 23)	N= 59	NIQ	Logistic regression OR = 1.433 (95% CI; 1.141, 1.997) p = 0.009	NIQ predictive of HIV-RNA < 80 c/mL at week 48

	N= 109	GIQ	Multivariate regression analysis: ANRS LPV- GIQ, OR= 1.21; 95% CI = 1.02–1.43; *P* = 0.02; major- Stanford-LPV-GIQ, OR= 1.17; 95% CI = 1–1.35; *P* = 0.04	Several different GIQ calculations exist for LPV. In this analysis, ANRS LPV GIQ and Standford LPV GIQ were predictive of HIV-RNA < 400 c/mL at week 24
	N=67	GIQ	Univariate analysis: LPV GIQ predictive of change in HIV-RNA from baseline to month 6 R^2 = 0.099; p= 0.001	Median HIV-RNA decline from baseline to month 6 was –1.50 (+0.73, –4.64) log_{10} copies/ml
	N=95	GIQ	Logistic regression demonstrated that cumulative GIQ were significantly stronger associated with the virologic response than the GIQ based on a single resistance test. Cumulative GIQ values associated with response vs non-response were: GIQ calculated using protease inhibitor associate mutations 1.4 (0.8 – 2.4) vs 0.7 (0.4 – 0.8) p =	Virologic response defined as HIV-RNA < 500 copies/mL at 12 months

			0.005; GIQ calculated using lopinavir associated mutations 2.3 (1.0 – 4.2) vs 0.8 (0.4 – 1.0) p = 0.002; GIQ calculated lopinavir mutation score 2.4 (1.0 – 4.4) vs 0.9 (0.6 – 1.1) p = 0.005.	
Saquinavir(16, 22)	N=53	vIQ	Virologic response achieved in 30 (66.67%) of patients. Predictors of virologic response at week 16 were vIQ < 0.5 (p = 0.006); baseline viral load > 50000 copies/mL (p < 0.05); mutations > 5 (PI or SQV) (p < 0.05)	vIQ <0.5 predictive of response (HIV-RNA < 200 copies/mL or > 1-log decline)
	N=139	GIQ	Virologic response (%) in subjects with GIQ < 0.04 vs > 0.04 was: Week 12: 50 vs 88.4 (p = 0.003) Week 24: 46.1 vs 76.7 (p = 0.07) Week 48: 18.2 vs 77.1 (p = 0.001)	GIQ predictive of >1-log decline in HIV-RNA at wk 12, 2, and 48
Tipranavir(8, 25)	N= 27	GIQ	Of the patients who had virologic suppression, 7/9 had a GIQ > 13000 vs 4/18 with a GIQ < 13000 (X^2=7.6, p=0.011)	GIQ > 13000 predictive of HIV-RNA < 50 c/mL at week 12
	N=513	> 5 baseline PI mutations	Overall response rates for all patients, patients without T20 in their regimen, and patients with T20 in regimen were 47% (241/513), 40% (148/369), and 65% (93/144), respectively. In patients with 1-2 baseline PI mutations, response rates were 70% (30/43)	Response rates were defined as ≥ 1-log decline in HIV-RNA by week 24

			overall; 67% (27/39) without T20; and 75% (3/4) with T20. Patients with 3-4 baseline PI mutations, response rates were 50% (117/236) overall; 44% (78/176) without T20; and 65% (39/60) with T20. Patients with >5 baseline PI mutations, response rates were 41% (94/231) overall; 28% (43/151) without T20; and 64% (51/80) with T20.	

Table 3: Characteristics of PRIDE Study Participants at Screening

Characteristic	N =
Age*	Years
Male Gender (%)	
Ethnicity	
• Caucasian (%)	
• African (%)	
• Hispanic (%)	
• Other (%)	
Years on ARVs *	Years
Previous number of ARVs*	
Previous number of PIs*	
Previous NNRTI*	
Number of protease mutations*	
Number of reverse transcriptase mutations*	
Baseline Darunavir IC_{50*}	
Baseline CD4+ count* (cells/mm^3)	
Baseline viral load (log_{10} copies/mL)*	
Enfuvirtide used (%)	
• De Novo (%)	
Genotypic Sensitivity Score*	
Number of active drugs*	

*Median (interquartile range); ARVs, antiretrovirals; PIs, protease inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitor; IC50, 50% inhibitory concentration

Table 4: Median Calculated Pharmacokinetic Values with Interquartile Ranges

	Week 4	Week 12	Week 24	Week 48
Cmin				
gIQ				
vIQ				
NIQ				

Cmin, trough concentration; gIQ, genotypic inhibitory quotient; vIQ, virtual phenotypic inhibitory quotient; NIQ, normalized inihibitory quotient

Table 5: Receiver Operating Characteristic curve (ROC)-derived PK Target Values Associated with Virologic Suppression

	Week 4	Week 12	Week 24	Week 48
Cmin				
gIQ				
vIQ				
NIQ				

Cmin, trough concentration; gIQ, genotypic inhibitory quotient; vIQ, virtual phenotypic inhibitory quotient; NIQ, normalized inihibitory quotient

Table 6: Univariate Cox Proportional Hazard model with virologic suppression as the outcome

Covariate	Hazard Ratio	95% CI	p-value
Years on ARVs			
Number of previous ARV regimens			
Number of previous PIs			
Number of cumulative PI mutations			
Number of cumulative RT mutations			
Baseline viral load (log_{10} copies/mL)			
Baseline $CD4^+$ (cell/mm^3)			
GSS			
Number of ARVs in current regimen			
Number of NRTIs in current regimen			
NNRTI use in current regimen			
Enfuvirtide in current regimen			
Etravirine use			
Raltegravir use			
Maraviroc use			
Number of Active Drugs in current regimen			
Number of DRV associated mutations			

DRV Cmin*

DRV GIQ*

DRV NIQ*

DRV vIQ*

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* = week 12 values; ARV, antiretroviral; PI, protease inhibitor; RT, reverse transcriptase; GSS, genotypic sensitivity score; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; DRV, darunavir; Cmin, trough concentration; gIQ, genotypic inhibitory quotient; vIQ, virtual phenotypic inhibitory quotient; NIQ, normalized inihibitory quotient

Table 7: Proportion of Patients Achieving Virologic Suppression and Response

	VL < 50 copies/mL	**VL < 400 copies/mL**	**One log_{10} decline copies/mL**
4 weeks			
12 weeks			
24 weeks			
48 weeks			

VL, viral load (HIV-RNA)

Table 8: Detailed Budget

DETAILED BUDGET - Year 1 of 2

Personnel (list paid and unpaid personnel) **(Year 1 of 2)**

Name	Position	Time allocated to project (hours)	Salary (weeks)
To be determined at each participating site	Research nurse	600 (6h/patient)	40$/hour (24 000$)
To be determined	Data collection	100 (1h/patient)	25$/hour (2500$)
Data management for database design for data entry	Data management	80	55$/hour (4400$)

Subtotal	$	**30 900**
Benefits	$	6180 (20%)
Salaries Total	**$**	**37 080**

Equipment **(Year 1 of 2)**

Description	Quantity	Unit Cost	Totals
Shipping boxes	~ 12	75	900

Equipment Total	**$**	**900**

Supplies / Services **(Year 1 of 2)**

Description	Quantity	Unit Cost	Totals

Pharmacokinetic sampling of darunavir	200	28.75	5750
Genotypic / virtual phenotypic analysis (Virco)	50	325	16 250
Shipping of PK samples in bulk to Montréal (3/year/site)	12	~ 120 (may vary depending on distance)	1440
	Supplies Total	**$**	**23 440**

Other **(Year 1 of 2)**

Description	Totals
Research ethics board submission (2000$ / site, 4 sites have expected costs related to REB submission)	8 000

Other Total	**$**	**8 000**

Total Funds Requested $ 69 420

** Maximum request $80,000*

DETAILED BUDGET - Year 2 of 2)

Personnel (list paid and unpaid personnel) **(Year 2 of 2)**

Name	Position	Time allocated to project (hours)	Salary (weeks)
To be determined at each participating site	Research nurse	700 (7h/patient)	40$/hour (28 000$)
To be determined	Data collection	200 (2h/patient)	25$/hour (5000$)

To be determined	Statistical analysis	320	55$/hour (17 600$)

Subtotal	**$**	50 600
Benefits	$	10 120 (20%)
Salaries Total	**$**	**60 720**

Equipment **(Year 2 of 2)**

Description	Quantity	Unit Cost	Totals
Shipping boxes	~ 12	75	900
Dry ice bags	4	25	100

Equipment Total	**$**	1000

Supplies / Services **(Year 2 of 2)**

Description	Quantity	Unit Cost	Totals
Pharmacokinetic sampling of darunavir	200	28.75	5750
Shipping of PK samples in bulk to Montréal (3/year/site)	12	~ 120 (may vary depending on distance)	1440

Supplies Total	**$**	7190

	Travel **(please note: a maximum of $3,000 will be awarded for travel, but only in the second year of a two year grant) (Year 2 of 2)**			
	Description			Totals
	Travel to present data in HIV related international conference (ie: International HIV Pharmacology Workshop or CROI): flight, registration, accomodations			3000
		Travel Total	**$**	3000
	Other **(Year 2 of 2)**			
		Other Total	**$**	0

Total Funds Requested **$ 71 910**

** Maximum request $80,000*

Total Funds Requested (Year 1 & 2 Combined) $ 141 330

Figure 1. Change in Log10 Viral Load from Baseline at Week 24 by DRV Exposure and by Baseline Fold-change(27)

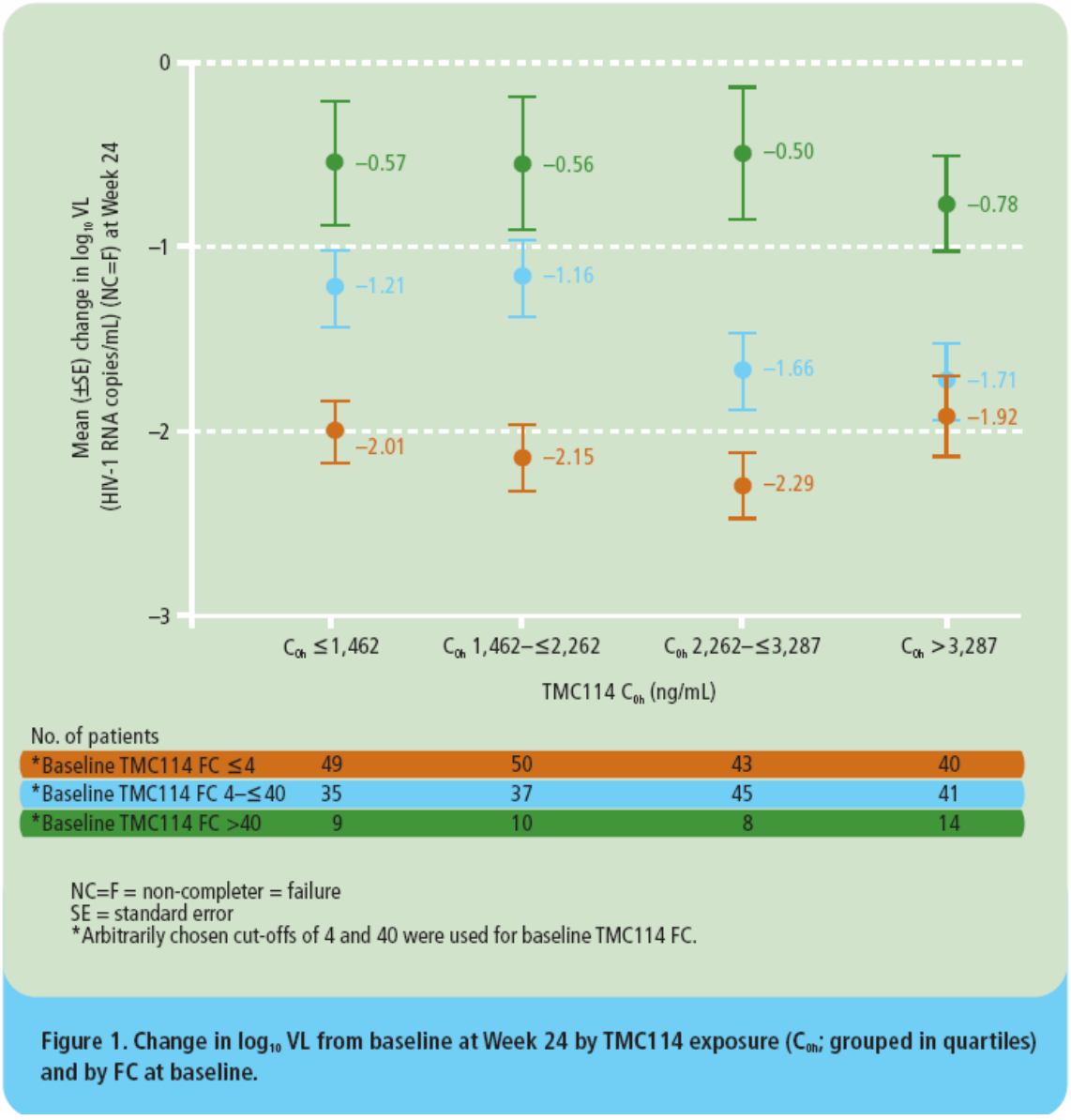

Figure 1. Change in log_{10} VL from baseline at Week 24 by TMC114 exposure (C_{0h}; grouped in quartiles) and by FC at baseline.

Figure 2. Receiver Operating Curves for PK Parameters Most Predictive of Week 24 VL < 50 copies/mL

Figure 3. Proportions of Subjects Achieving Virologic Suppression with ROC-derived ?IQ Breakpoint (Bar Graph)

Figure 4. Median Likelihood of Achieving Virologic Suppression (HIV-RNA < 50 copies/mL) and Response (HIV-RNA < 400 copies/mL or >1-log decline) with ROC-derived ?IQ Breakpoint (Box and Whisker Plots with Medians)

Figure 5. Magnitude of Target Attainment ?IQ with log10 change in HIV-RNA

Appendix A: Study Timeline

Procedures	Screening/Baseline	Week 4	Week 12	Week 24	Week 48
Informed Consent	X				
Medical Assessment* (History, Physical Exam, Adverse Events)	X	X	X	X	X
Genotype History*	X				
VL*	X	X	X	X	X
CD4+ count*	X	X	X	X	X
PK sample (Cmin)		X	X	X	X
Genotype Virtual phenotype	X				
Adherence (SMAQ)		X	X	X	X

*Denotes standard of care procedures

Appendix B: Data Collection Forms and Case Report Forms

Eligibility Screening Form:

Inclusion Criteria	Circle one choice per statement below
a) Has the patient signed informed consent?	Yes No
b) Does the patient have documented HIV-1 infection?	Yes No
c) Is the patient older than 18 years?	Yes No
d) Does the patient have limited treatment options due to virologic failure or intolerance to multiple regimens?	Yes No
e) Has the patient tried at least 3 different classes of antiretroviral medications?	Yes No
f) Has the patient received 2 different protease inhibitor-based regimens?	Yes No
g) The subject is not achieving adequate virologic suppression on his/her current regimen and at risk of clinical or immunologic progression?	Yes No
h) The patient is starting darunavir/ritonavir by the treating physician as a standard of care	Yes No
Exclusion criteria	Circle one choice per statement below
a) Is this a primary infection?†	Yes No
b) Does the patient exhibit documented resistance to all currently approved PIs?*	Yes No
c) Does the patient have a condition, including but not limited to alcohol or drug use, whereby in the opinion of the investigator, could compromise the subject's safety or	Yes No

adherence to the study protocol?	
d) Has the patient used tipranavir within the past 15 days?	Yes No
e) Does the patient have any active or clinically significant disease (eg. Cardiac dysfunction, pancreatitis, acute viral infection) or finding during the screening of medical history or physical examination that is not either resolved or stabilized for at least 30 days prior to the screening phase of the trial?	Yes No
f) Has the patient demonstrated clinically significant allergy or hypersensitivity to any of the excipients of darunavir or ritonavir?**	Yes No
g) Will the patient be adherent to darunavir therapy?	Yes No

*If response to this statement is "Yes", the patient does not need to fulfill inclusion criteria d, e, f or g.

**Darunavir is a sulfonamide derivative. Subjects who previously experienced a sulfonamide allergy will be allowed to enter the trial. To date, no potential for cross-sensitivity between drugs in the sulfonamide class and darunavir have been identified in subjects participating in Phase II trials.

† If yes, must also have answered yes to exclusion criteria b.

DEMOGRAPHICS

BIRTH DATE dd/mmm/yyyy	SEX	RACE	CURENTLY SMOKING
	☐ Male ☐ Female	☐ White ☐ Black ☐ Asian ☐ Hispanic ☐ Other (specify) _____	☐ No ☐ Yes, _____pk/day

MEDICAL HISTORY

Indicate whether or not there is a medical history for each of the following:

SITE	NO	YES	IF "YES", GIVE PERTINENT DETAILS (Include Dates)
Eyes-Ears-Nose-Throat			
Respiratory			
Cardiovascular			

Gastrointestinal			
Genito-Urinary			
Central Nervous System			
Endocrine-Metabolic			
Dermatological			
Musculoskeletal			
Psychiatric			
Drug/Alcohol Abuse			
Drug Allergy			
Non-Drug Allergy			

Surgical History			
Other			

SCREENING

PHYSICAL EXAMINATION

DATE OF EXAMINATION dd/mmm/yyyy	HEIGHT (cm)	WEIGHT (kg)	ORAL TEMPERATURE (°C)	SITTING BLOOD PRESSURE	SITTING PULSE RATE	SITTING RESPIRATORY RATE

Check (√) Normal/Abnormal for each site/system; describe abnormalities.

SITE/SYSTEM	NORMAL	ABNORMAL	NOT DONE	DESCRIBE ANY ABNORMALITIES
Skin				
Eyes (Fundoscopy)				
Ears-Nose-Throat				

Neck				
Heart				
Lungs				
Abdomen				
Neurological				
Extremities				
Genital/Urinary				
Other : Specify _____				

Antiretroviral Regimen History

Date regimen started yy/mm/dd	Antiretrovirals in regimen§	Date regimen discontinued	Reason regimen discontinued†

§ AZT = zidovudine, 3TC = lamivudine; d4T = stavudine; ddI = didanosine; ddC = zalcitabine; ABC = abacavir; TNF = ténofovir; FTC = emtricitabine; HU = hydroxyurea; NVP = nevirapine; DLV = delavirdine; EFV = efavirenz; TMC125 = etravirine; AMP = amprenavir; TAZ = atazanavir; DRV = darunavir; fAMP = fosamprenavir; IDV = indinavir; LPV = lopinavir; NLF = nelfinavir; RTV = ritonavir; SQV = saquinavir; TPV = tipranavir; T-20 = enfuvirtide; O = other (please specify)

† VF = virologic failure; IF = immunologic failure; TX = toxicity; PC = patient's choice; 0 = other (please specify)

SCREENING

LABORATORY TESTS

Sample date: _____

☐ **CD4**

☐ **Viral load (bDNA)**

☐ **Genotype / Virtual phenotype (if not done during the previous 8 weeks)**

Past Genotypic / Virtual Phenotypic Data

Date of genotype and/or virtual phenotype	**Antiretrovirals at time of genotype and / or virtual phenotype**	**Company doing Genotype / Virtual Phenotype**	**Protease mutations (including amino acid substitution)**	**Reverse transcriptase mutations (including amino acid substitution)**	**Darunavir fold change in IC50 (virtual phenotype), if available**

Week – 8 to day 0 result

Date of genotype and/or virtual phenotype	Antiretrovirals at time of genotype and / or virtual phenotype	Company doing Genotype / Virtual Phenotype	Protease mutations (including amino acid substitution)	Reverse transcriptase mutations (including amino acid substitution)	Darunavir fold change in IC50 (virtual phenotype), if available

- ☐ **Verify if medication changes since last visit**
- ☐ **Adverse event report**
- ☐ **SMAQ adherence questionnaire**

LABORATORY TESTS

Sample date: _____

- ☐ **CD4**
- ☐ **Viral load (bDNA)**
- ☐ **Darunavir PK sample [1 heparin tube (green top)]; must be done, as much as possible, just before the next dose (12 hours post-dose)**

Pharmacokinetic Test Request

Medication Information			
Drug	**Last dose taken** **Date** **Time**		**Quantity taken**
Darunavir 300mg			**_____# Tablets**
Ritonavir 100mg			**_____# Capsule**
Last dose taken with food? Yes No			
Sample			
Date of Sample:		**Time of Sample:**	

Procedure to obtain sample:

About 8ml of blood should be colleted for drug level analysis using heparinized tubes (green top). Mix tube immediately after collection by inverting 10 times. Plasma should be isolated within 4 hours after collection by centrifugation at room temperature (5 minutes at 3000 G), and stored at –70C (or lower) until shipment. ONLY PLASMA should be shipped for analysis, in a TIGHTLY CLOSED PLASTIC tube, safely packed and shipped at ambient temperature according to the regulations for the shipment of infectious material. If shipping is expected to take more than 24 hours, ship the plasma samples frozen with dry ice. Please prepare the box as per the procedure for packing with dry ice to ensure safety. Samples should be sent to:

McGill University Health Centre (Royal Victoria Hospital)

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Montréal, Québec

H3A 1A1

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Samples are to be shipped in bulk. Each site will receive a notice when samples are to be shipped to the laboratory.

Nancy Sheehan should be notified via email when the samples are shipped nancy.sheehan@umontreal.ca.

- ☐ **Verify if medication changes since last visit**
- ☐ **Adverse event report**
- ☐ **SMAQ adherence questionnaire**

LABORATORY TESTS

Sample date: _____

- ☐ **CD4**
- ☐ **Viral load (bDNA)**
- ☐ **Darunavir PK sample [1 heparin tube (green top)]; must be done, as much as possible, just before the next dose (12 hours post-dose)**

Pharmacokinetic Test Request

Medication Information			
Drug	**Last dose taken** **Date** **Time**		**Quantity taken**
Darunavir 300mg			**_____# Tablets**
Ritonavir 100mg			**_____# Capsule**
Last dose taken with food? Yes No			
Sample			
Date of Sample:		**Time of Sample:**	

Procedure to obtain sample:

About 8ml of blood should be colleted for drug level analysis using heparinized tubes (green top). Mix tube immediately after collection by inverting 10 times. Plasma should be isolated within 4 hours after collection by centrifugation at room temperature (5 minutes at 3000 G), and stored at –70C (or lower) until shipment. ONLY PLASMA should be shipped for analysis, in a TIGHTLY CLOSED PLASTIC tube, safely packed and shipped at ambient temperature according to the regulations for the shipment of infectious material. If shipping is expected to take more than 24 hours, ship the plasma samples frozen with dry ice. Please prepare the box as per the procedure for packing with dry ice to ensure safety. Samples should be sent to:

McGill University Health Centre (Royal Victoria Hospital)

LABORATOIRE DE BIOCHIMIE – Pièce C6.31

Centre Universitaire de Santé McGill - Hôpital Royal Victoria

687, AVENUE DES PINS Ouest

Montréal, Québec

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Laboratory results ($CD4^+$ / Viral Loads)

Study Visit	Baseline – Day 0	Week 4	Week 12	Week 24	Week 48
Viral Load bDNA (copies/mL)					
Viral load bDNA (log_{10})					
$CD4^+$ ($cell/mm^3$)					
$CD4^+$ %					
$CD8^+$ ($cell/mm^3$)					
$CD8^+$ %					
CD4:CD8 ratio					

MEDICATIONS / HERBAL MEDICINE / SUPPLEMENTS / OVER THE COUNTER MEDICATIONS / RECREATIONAL DRUGS

(Record all medications taken from screening to End of Study.)

MEDICATION NAME dose route and frequency	START DATE			STOP DATE			REASON FOR USE
	dd/mmm/yyyy	OR	pre trial	dd/mmm/yyyy	OR	post trial	

Study week: 4 ☐ 24 ☐

12 ☐ 48 ☐

Simplified Medication Adherence Questionnaire (SMAQ)(30) Questionnaire d'adhésion médicamenteuse simplifié (version française non validée)
Est-ce que cela vous est déjà arrivé d'oublier de prendre vos médicaments? **a) Oui** **b) Non**
Est-ce que vous êtes à l'occasion négligeant avec la prise de vos médicaments? **a) Oui** **b) Non**
Parfois, si vous vous sentez moins bien, est-ce que vous cessez de prendre vos médicaments? **a) Oui** **b) Non**
En réfléchissant sur la dernière semaine. Combien de fois n'avez-vous pas pris vos médicaments? **a) Jamais** **b) 1-2 fois** **c) 3-5 fois** **d) 6-10 fois** **e) >10 fois**
Est-ce que vous n'avez pas pris un de vos médicaments lors de la dernière fin de

semaine? **a) Oui** **b) Non**
Au cours des 3 derniers mois, combien de jours avez-vous pris aucun de vos médicaments? **a) ≤ 2 jours** **b) > 2 jours**

GEEMA Study Group. Validation of a simplified medication adherence questionnaire in a large cohort of HIV-infected patients: the GEEMA Study. AIDS. 2002 Mar 8; 16(4): 605-13.

Study week: 4 ☐ 24 ☐

12 ☐ 48 ☐

Simplified Medication Adherence Questionnaire (SMAQ)(30)
Do you ever forget to take your medicine? **c) Yes** **d) No**
Are you careless at times about taking your medicine? **c) Yes** **d) No**
Sometimes if you feel worse, do you stop taking your medicines? **c) Yes** **d) No**
Thinking about the last week. How often have you not taken your medicine? **f) Never** **g) 1-2 times** **h) 3-5 times** **i) 6-10 times** **j) >10 times**
Did you not take any of your medicine over the past weekend? **c) Yes** **d) No**

Over the past 3 months, how many days have you not taken your medicine at all?

c) ≤ 2 days

d) > 2 days

GEEMA Study Group. Validation of a simplified medication adherence questionnaire in a large cohort of HIV-infected patients: the GEEMA Study. AIDS. 2002 Mar 8; 16(4): 605-13.

ADVERSE EVENT REPORT

Adverse Event	Date of Onset	Maximum Intensity	Date of resolution	Outcome	Action taken with antiretroviral	Causal relationship to study drug	Other action taken?
	dd/mmm/yyyy	1= mild 2=moderate 3= severe 4= Serious	dd/mmm/yyyy	R= resolved S= resolved with Sequelae O= Ongoing	0 = none 1 = reduced 2 = increased 3 = discontinued and reintroduced 4 = discontinued	0 = unrelated 1 = remote 2 = possible 3 = probable	1 = concomitant meds (specify) 2 = other (specify) 3 = none

STUDY COMPLETION

Did the subject complete the study?

_____ Yes

_____ No, date of last contact _____

Specify below,

_____ adverse event

_____ intercurrent illness

_____ failure to return

_____ death

_____ refused treatment/withdrew consent

_____ protocol violation

_____ Other, specify_____

Additional observations ☐ No ☐ Yes, specify below

_____

_____

_____

I have reviewed the case report form for this subject. To the best of my knowledge the entries are complete and accurate.

_____ _____

Investigator Signature Date

Appendix C: PARTICIPANT INFORMATION AND CONSENT FORM

Evaluating the *P*ha*R*macok*I*netic Profile of *D*arunavir *E*thanolate/Ritonavir to Determine the Most Appropriate Predictor of Virologic Response in Advanced HIV-1 Infected Patients (The PRIDE Study)

Principal Investigators: **Nancy Sheehan, B.Pharm, M.Sc**

Richard Lalonde, MD

Study Site: **Montréal Chest Institute**

Introduction

You are being asked to participate in a study because your doctor has prescribed you the anti-HIV drug named darunavir (Prezista™), a new drug in the class of anti-HIV drugs known as protease inhibitors.

Before deciding to participate in the study, you should clearly understand its requirements, risks, and benefits. This document provides information about the study, and it may contain words you do not fully understand. Please read it carefully and ask the study staff any questions you may have. They will discuss the study with you in detail. If you decide to participate, you will be asked to sign this form and a copy will be given to you.

Purpose of the Study

Now that darunavir is available in Canada, many patients who have tried several other anti-HIV drugs may consider starting this therapy. However, because darunavir is a new medication, information still needs to be collected about how various drug levels in the blood relate to response to therapy.

The purpose of this study is to collect blood samples to evaluate which pharmacokinetic parameter is the most appropriate predictor of virologic response in advanced HIV-1 infected patients at week 12 of treatment.

Approximately 100 subjects from multiple treatment sites will be enrolled in the study, including 20 at the Montreal Chest Institute.

Study Procedures

If you agree to participate in this study, you will be asked to come to the clinic for 5 visits. During these visits, four to five blood samples will be taken for a total of 32 to 40 ml (approximately 3 tablespoons) of blood throughout a period of 48 weeks.

Screening Visit (Visit 1)

- You will be examined by a physician.
- You will be asked to provide information about your medical and medication history.
- Your medical chart will be reviewed and information about your current level of resistance to anti-HIV medications will be obtained.

- A genotype/virtual phenotype will be performed to measure the degree of resistance to medications that your virus has developed (only if not done in the last 8 weeks). In this case, one blood sample (approximately 8 ml) will be taken.

Medications

To be eligible to participate in the study, you must be taking a new anti-HIV regimen containing darunavir (Prezista™) in combination with ritonavir (Norvir™), which includes two 300 mg tablets of darunavir with one 100mg capsule of ritonavir twice daily. These medications should be taken within 30 minutes of eating a meal.

Study Weeks 4, 12, 24, and 48 (Visits 2 to 5)

You will be asked to come to the clinic early in the morning, 12 hours after your last dose of darunavir / ritonavir medications from the evening before. A small plastic needle (intravenous catheter) will be placed in a vein in one of your arms to draw an 8 ml blood sample. This blood sample is in addition to the one that you would regularly have drawn during your normal clinic visits, which measure how well your anti-HIV drugs are working by measuring CD4 and viral load. You may take your morning doses of darunavir/ritonavir after your blood sample has been obtained. A short questionnaire will be given to you to fill out, which asks questions about how you take your medications.

Use of Blood Samples

Blood samples will be used to measure the blood levels of darunavir and ritonavir. The blood samples will be kept and analyzed at the Royal Victoria Hospital. Blood collected for this study will be

destroyed once the results are analysed and published. The blood samples will not be used for other studies, including any genetic studies.

Benefits

You should not expect any direct benefits from participating in this study. However, the information collected from this study may benefit future patients.

Risks and Inconveniences

There are no risks associated with your participation in this study.

Blood Draws

There is minimal risk from routine blood drawing. Side effects include mild pain, discomfort, swelling, bleeding, or bruising at the needle entry site, and in rare cases, fainting or infection.

Voluntary Participation and Termination of Participation

Your participation in this research study is strictly voluntary. You can refuse to participate or you may discontinue your participation at any time without explanation, and without penalty or loss of benefits to which you are otherwise entitled. If you decide not to participate, or if you discontinue your participation, you will suffer no prejudice regarding your medical care or your participation in any other research studies.

The study doctor may choose to withdraw you from the study if you experience a serious reaction to any of the drugs you receive or if you fail to keep appointments. It is important to follow study directions.

In the Case of a Research-Related Injury

In the event that you become ill or injured as a result of participating in this study necessary medical treatment will be made available to you.

The McGill University Health Centre, the MUHC Research Institute, and the investigator would not be able to offer compensation in the unlikely event of an injury resulting from your participation in this research study. However, you are not giving up any of your legal rights by signing this consent and agreeing to participate in this study.

Costs and Compensation

You will not be paid for your participation in this research study.

Confidentiality

The research team will consult your medical file to collect information relating to your medical history, and issues that you do not remember precisely.

All information obtained during the course of the study will be coded and kept strictly confidential. The code list will be locked in the filing cabinet in the Investigator's office with limited access. The results from this study may be published, however, your name will not be used in any publication. The

research data will be available only to the research team and to persons taking part in managing and analyzing the research information. In order to verify the research study data, Allergy Therapeutics, the Canadian Therapeutic Products Programme (TPP), the United States Food and Drug Administration (FDA), or the Quality Assurance Officer of the MUHC-Research Ethics Boards (REBs) may review these records.

By signing this consent form, you give us permission to release information regarding your participation in this study to these entities, and to inform your treating physician of any significant findings that may occur during the study. Your confidentiality will otherwise be protected to the extent permitted by applicable laws and regulations.

Contact Information

For answers to questions relating to this research study, or for information about study procedures you may contact: **Nancy Sheehan at (514) 934-1934, extension 32304.**

If you have questions about your rights as a research participant and wish to discuss this with someone not associated with the study, you may contact the **McGill University Health Centre Ombudsman at (514) 934-1934, extension 35655.** In case of a research-related injury, you should call **Nancy Sheehan at (514) 934-1934, extension 32304** during work hours and after hours, you may call **(514) 934-1934, extension 33333** and ask for **the physician-on-call for the Immunodeficiency Service.**

Title: Evaluating the *P*ha*R*macok*I*netic Profile of *D*arunavir *E*thanolate/Ritonavir to Determine the Most Appropriate Predictor of Virologic Response in Advanced HIV-1 Infected Patients (The PRIDE Study)

Principal Investigators: Nancy Sheehan, B.Pharm, M.Sc

Richard Lalonde, M.D.

DECLARATION OF CONSENT

I have read the contents of this consent form, and I agree to participate in this research study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I understand that I will be given a signed copy of this consent form. By signing this consent form, I am not giving up any of my legal rights.

Participant Signature	Date	Participant Printed Name

Signature of Investigator/Delegate Obtaining Consent	**Date**	**Name of Investigator/Delegate Obtaining Consent**

Appendix E – Community Advisory Board Approval Letter

Evan J. Collins
#304 – 833 King Street West
Toronto, Ontario Canada
M5V 1N9
416-603-6027 ● ecollins@interlog.com

October 23, 2006

Dr. Nimesh Patel
Re: "Evaluating the Pharmacokinetic Profile of Darunavir ethanolate/ritonavir to Determine the Most Appropriate Predictor of Virologic Response in Advanced HIV-1 Infected Patients"

Dear Dr. Patel,

I am writing you on behalf of the Community Advisory Board (CAB), Immunodeficiency Clinic, University Health Network. Thank you for attending the CAB meeting on September 18, 2006 to present your protocol and allow our members to discuss it with you.

Four members of the CAB were in attendance and after full discussion we saw your research study as being important to people living with HIV/AIDS and seemed to pose little risk or time commitment to the research subjects. As such we fully endorse your study as it goes forward for Research and Ethics review.

Please contact me if you have any questions.

Best of luck with your research,

Evan Collins,
Chair, Community Advisory Board, Immunodeficiency Clinic, UHN

Cc - Dr. Sharon Walmsley
- CAB members