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INVITED REVIEW

Facilitating Compound Progression of Antiretroviral Agents via Modeling and Simulation

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Abstract Pharmacotherapy in human immunodeficiency virus (HIV)-infected patients and the development of safe and effective antiretroviral dosing regimens has been hindered by numerous issues, including the rapid development of viral resistance to drug therapy, the narrow therapeutic window of the drug compounds, and lack of fundamental knowledge concerning the sources of variation in exposure and response to antiretroviral agents. Sources of variation may include factors such as interpatient differences in genetic expression, immunological response, pathogenesis, epidemiologic and socioeconomic factors, and demographics. Modeling and simulation (M&S) techniques have become valuable tools to identify and quantify variability in exposure and response to antiretroviral agents throughout the drug development process. Before actual entry into human safety and pharmacokinetic (PK) trials, *in vitro* screening and *in vivo* pharmacology studies conducted to assess compound potency and compatibility with agents included in acceptable antiretroviral therapy (ART) regimens can be characterized via quantitative relationships. In addition, physiochemical data is initially used to screen drug candidates based on favorable PK and biopharmaceutic properties. Compound progression can likewise be supported with M&S exercises to ensure the traceability of key assumptions and decisions. The underlying techniques utilize nonlinear mixed effect modeling, Monte Carlo simulation, Neural networks, several regression-based approaches, and less computationally intensive techniques. The application of such an approach promises to be an essential component in the development of new agents to treat HIV-1 and is being implemented in the context of evaluating Nk1r antagonists as potential candidates to treat NeuroAIDS.

Keywords compound progression · ART · modeling · simulation · PK/PD

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Landscape for antiretroviral drug development

The past two decades have offered unique clinical challenges after the recognition of the acquired immunodeficiency syndrome (AIDS) in 1981. The effort to manage viral disease in general was enormous and the clinical tools to diagnose disease, evaluate new pharmacotherapies, investigate treatment resistance patterns, and ultimately individualize patient care have continually evolved. Early drug development strategies were guided by the traditional paradigm of evolving phases during which discrete milestones relating to drug safety, activity, and ultimately clinical outcomes and benefit are evaluated in a predominantly sequential manner based on regulatory hurdles and clinical criteria defined, in part, by the current standard of care. A key milestone in the history of drug development for the treatment of human immunodeficiency virus (HIV)/AIDS was the 1987 decision by the US FDA to create an accelerated process by which antiretroviral agents could be developed and ultimately submitted for regulatory approval. The FDA maintains a chronology of the historical milestones focused on the regulatory involvement in antiretroviral drug development up to the present time

(http://www.fda.gov/oashi/aids/miles.html), which clearly illustrates the impact of accelerated approval on the timelines regarding the ultimate access to new medicines. Before this rule, drugs could be judged only according to their effect on the illness or patients' length of survival. This regulation allowed the FDA to approve drugs based on a reasonable "surrogate endpoint" (i.e., viral RNA suppression). Many early antiretroviral agents, including Epivir (lamivudine, 3TC), Invirase (saquinavir), and Crixivan (indinavir) were approved via the accelerated approval process. Other key historical events include the formation of the National Task Force on AIDS Drug Development in 1994 and the 2002 Guidance on Clinical Trial Design in Developing Treatment for HIV Infection (FDA Guidance 2002). These events highlight the appreciation for a multidisciplinary effort for the development of new antiretroviral agents and the need to conduct more informative clinical trials to support the dynamic environment of the disease progression and mutation and the diversity in the global epidemic.

Four unique drug classes of antiretroviral agents predominate the current targets to combat HIV-1 infection: protease inhibitors (PIs), nucleoside reverse transciptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and entry or fusion inhibitors. Figure 1 shows the HIV-1 cycle and the site of action of the four drug classes (Pomerantz and Horn 2003). Currently, the three most commonly prescribed initial multidrug antiretroviral therapy (ART) are a PI plus two nucleoside analogs, a nonnucleoside reverse transcriptase inhibitor plus two nucleoside analogs, and three nucleoside analogs. As HIV-infected patients usually receive a wide variety of drugs in combination with their antiretroviral drug regimen and because both

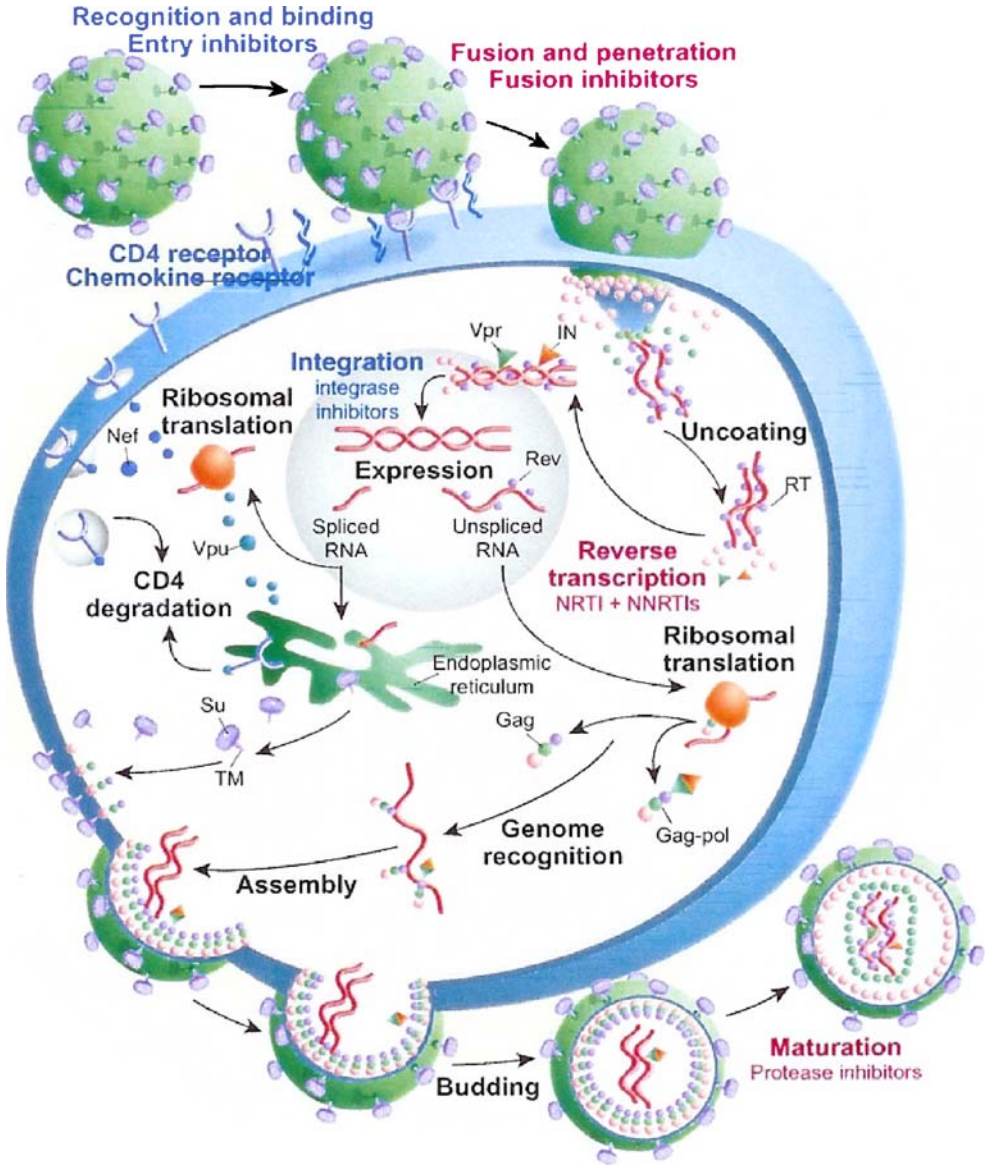


Fig. 1 HIV-1 life cycle and sites of action of antiretroviral drugs from Pomerantz and Horn (2003). Text in red refers to the targets inhibited by the various established drug classes.

PIs and NNRTIs are extensively metabolized by the cytochrome P450 (CYP) system, there is a considerable potential for pharmacokinetic (PK) interactions. The result of such interaction can be either a decrease or increase in drug exposure, which in turn can reduce efficacy or increase toxicity.

The HIV-1 virus itself remains a dynamic target as well. Strains of HIV-1 are classified into three groups (M, O, and N) representing three separate introductions of simian immunodeficiency virus into humans. Whereas more than 90% of HIV-1 infections belong to HIV-1 group, within group M there are known to be at least nine genetically distinct subtypes of HIV-1 (A, B, C, D, F, G, H, J, and K) and occasionally, two viruses of different subtypes can mix together their genetic material to create a new hybrid virus (Wainberg 2004). The HIV-1 subtypes and circulating recombinant forms (CRFs) are very unevenly distributed throughout the world, with the most widespread being subtypes B and C.

The variation in viral distribution globally may affect disease progression (Laeyendecker et al. 2005; Kanki et al. 1999), transmission rates (Bhoopat et al. 2001, Dittmar et al. 1997; Renjifo et al. 2004) and coinfection rates (Centers for Disease Control and Prevention 2001; Phillips et al. 2000). Most current HIV-1 antiretroviral drug regimens were designed for use against subtype B, and so hypothetically might not as effective in Africa or Asia where other strains are more common. At present, there is no compelling evidence that subtypes differ in their sensitivity to antiretroviral drugs. However, some subtypes may occasionally be more likely to develop resistance to certain drugs and in some situations the types of mutations associated with resistance may vary (Wainberg 2004).

A consequence of the effectiveness of ART is that what was once understood as the natural history of HIV infection may no longer hold true. The effectiveness of combination ART that includes a PI in slowing HIV disease progression appears to be altering the disease course significantly (Rachlis et al. 1998). Whereas pharmacotherapy can now reduce HIV RNA to undetectable levels in peripheral blood for long periods, early hopes that HIV might be eradicated in an infected person by therapy have been dampened by the detection of reservoirs of HIV-infected cells outside of the peripheral blood (Lefrere et al. 1997). The possibility of strengthening the initial immune response with early treatment to control infection indefinitely holds out more promise, although it may depend on whether the immune system can control HIV even if therapy is withdrawn at some point. Questions about survival time past an AIDS diagnosis and the impact of the new combination therapies on the incubation period cannot be addressed with any certainty because their effects are observed only in population-based data (e.g., number of annual AIDS cases and AIDS deaths). It is unclear for how many years the benefit of these therapies can be sustained or whether recent encouraging results can be built on with additional treatments to bring HIV infection under control indefinitely.

Hence, the landscape for the development of new agents to combat HIV-1 and related pathophysiologic conditions remains complex. The agents used to treat HIV/AIDS, the virus itself, and the patient populations in whom such agents would be indicated all represent challenges for pharmaceutical researchers seeking to develop new agents. Current challenges to successful pharmacotherapy in treating HIV/AIDS and likewise the development of new antiretroviral compounds will require a development paradigm, which bridges discovery and preclinical and clinical research communities. The accelerated approval process set forth by the FDA is highly dependent on targeted, informative experimentation with emphasis on data-driven decisions about compound selection and development plans.

Compound progression for antiretroviral drug development

Pharmaceutical drug development is a highly regulated process during which numerous discrete experiments are conducted to determine the likelihood that a particular drug candidate possesses the attributes that warrant further evaluation. Successful drug candidates will have had to meet or exceed specified criterion regarding critical attributes at the various development stages for the compound to advance. At early stages of drug discovery attributes will be ranked against potentially thousands of compounds under consideration and other agents identified in the scientific literature or through competitive surveillance. As the development stage progresses, the number of compounds under evaluation declines but the nature and amount of testing increases along with the cost of the evaluation and the risk to the drug sponsor. Historically, the process of compound progression was governed by discrete criterion often disconnected from the preceding or anteceding stage. Likewise, data from a drug candidate was tabulated, summarized, and reported without consideration for how such information could be utilized to learn about drug activity, safety, and the disease process or develop a means to forecast the outcome of future efforts.

Compound progression is also driven by the regulatory requirements, which serve as significant hurdles that a successful drug candidate must clear before being considered for entry into the marketplace. These include the demonstration of requirements for a successful investigational new drug application (IND), chemistry and manufacturing controls, an acceptable toxicology program, data to support entry into human phase testing, protocol designs that permit

the assessment of an agents suitability for clinical evaluation, and, of course, the requirements demonstrating safety and efficacy of the compound in support of the NDA (New Drug Application). Given the economic situation of the industry today and the observation of both the FDA (CDER 2004) and the NIH (Zerhouni 2003) that silos in drug development have stagnated the process and prohibited the innovation necessary to develop medicines for the current global healthcare, new mechanisms for compound progression are being implemented by the drug industry and those otherwise involved with the various phases of drug development. These mechanisms are focused on linking the assumptions, data, outcomes, and decisions generated along the drug development path. Specifically, in the case of antiretroviral development, the FDA has created an accelerated review program, which refines the expectation of regulatory milestones to facilitate the review during the development process, thus expediting the review post final NDA submission.

Table 1 lists the critical elements of compound progression from proof of mechanism through health economics and market valuation. Whereas there is an obvious correlation of the various elements with stage of drug development, many of these are at least partly addressed at early development stages and the elements themselves are relevant for any therapeutic area. It is also true that many are revised and refined as a drug candidate is studied. The concept of "druggability" refers to several aspects of feasibility and broadly addresses whether a drug substance has intrinsic properties compatible with the desired systemic exposure, route of administration, and formulation (Keller et al. 2006). The normal industry practice is to create a "target product profile" (TPP) or similar construct to define the minimal, acceptable, and targeted criteria for drug candidates within a specific intended therapeutic area often specific to one or a few primary indications. It provides a starting point from which project management can organize screening tools and sequence the various experiments of various groups to align with project-specific objectives and activities. The TPP represents the perspective of various stakeholders within the project team setting and also serves to prioritize the key features and attributes of the intended drug product—the "to-be-marketed" drug. The next two sections discuss the critical elements for antiretroviral development and how modeling and simulation (M&S) are used to facilitate the critical decisions.

Table 1 Critical elements of compound progression

Elements	Definition
Proof of mechanism	Establish the correlation between drug mechanism of action and the pathophysiology of the intended disease state or condition.
Druggability assessment	Determination of whether the formulation can be made consistent with market image and if the PK properties allow achievement of exposure targets.
Therapeutic window definition	Drug therapy can be administered between an acceptable "window" of drug exposure, which maximizes clinical benefit against any untoward drug action.
Proof of concept	A realization of a method/idea to demonstrate feasibility determines the effectiveness of a drug and confirms safety in patients that the drug may ultimately treat.
Clinical outcome assessment	Assessment of change in a patient's "status" over a period of time to determine if the condition is improving, worsening or remaining unchanged.
Health economics and market valuation	Expected final performance relative to existing medicines used to treat target indications. Analysis of patient costs relative to clinical benefit and health outcomes.

Critical issues in antiretroviral drug development

The critical issues for the development of new antiretroviral agents have evolved over the past two decades. Many of these factors relate to the polypharmacy that exists in this therapeutic setting and the effectiveness of the existing ART therapies. Whereas they represent the various project team disciplines (e.g., pharmacology, drug metabolism, formulation, clinical pharmacology, and biostatistics), most of these have a clinical bias. Table 2 lists the typical criteria for antiretroviral compound progression by development stage, highlighting the critical elements addressed by the various advancement criteria. In some cases (e.g., proof of mechanism) the criteria are somewhat generic as the specific criteria is unique to the mechanism of action and target. Whereas most classes target viral replication, the assays used to compare new molecular entities vary (Adelson et al. 2003) and likewise the criterion is target- and mechanism-specific.

With respect to druggability, particularly in the area of pharmacokinetic/pharmacodynamic (PK/PD)-related attributes, the desired properties for antiretroviral agents (active parent or metabolites) include the following: high potency (viral RNA reduction, CD4+ counts), half life consistent with once-daily dosing, acceptable oral bioavailability, low potential for drug interaction, minimal lifestyle effects (food, time of day administration, etc), blood–brain barrier (BBB) penetration, extensively distributed, and low to moderate protein binding. Each of these attributes of the drug substance are ultimately derived (and confirmed) by experimentation. The critical issue for antiretroviral agents is to be able to achieve sustained drug exposure at the target infec-

Table 2 Criteria for advancement of antiretroviral compounds

Development Stage Decision Elements	Advancement Criteria
Discovery	
Proof of mechanism	*In vitro* and *in vivo* data support the proposed mechanism • Drug actions consistent with mechanism • Sensitivity and specificity demonstrated • Adequate potency relative to agents in class/mechanism • Synergy with other antiretroviral agents demonstrated
Druggability	Structure suggests ADME/Tox properties, which favor development • Absorption → favors acceptable bioavailability • Distribution → crosses BBB; accesses virus "reservoirs" • Metabolism → phase I/II enzyme system involvement; low interaction potential
Therapeutic Window	• Elimination → drug cleared, not sequestered • Toxicology → No evidence of known structure-tox relationships (e.g., vinyl chloride adduct formation)
Preclinical	
Proof of mechanism	Pharmacology model confirms activity within feasible dose range • PK/PD in animals scalable to humans (HIV patients)
Druggability	Low interaction potential • IVIVE identifies several acceptable ART options Acceptable Human Dosage Form Possible • Once daily dosing (bid acceptable) • Acceptable oral formulation (size, taste, number, and delivery) Safety Acceptable
Therapeutic Window	• Parent and/or metabolites nontoxic within acceptable safety margins from expected human dose-exposure.
Phase I	
Therapeutic Window	PK/PD in Humans Favorable • Dose–exposure and exposure–response relationships confirmed and identify regimen options for clinical evaluation
Druggability	Lifestyle Issues Favorable • No or minimal food requirements • Time of day dosing flexibility • Side-effect profile favorable/acceptable (e.g., no issues with dizziness or drowsiness and can operate a vehicle) Drug Interactions Minimal/Acceptable • No or limited impact on concomitant medications
Phase II/III	
Therapeutic Window	PK/PD in Patients Favorable
Proof-of-concept	Clinical Outcomes Suggest Efficacy is Attained
Clinical Outcomes	Compliance Unlikely to Have Impact on Clinical Outcomes
Postmarketing	
Clinical Outcomes	Compliance/Adherence is Manageable Clinical Benefit vs Pricing is Reasonable Utilization in 3rd World Possible
Market Valuation	Health economics

BBB = blood brain barrier, IVIVE = *in vitro–in vivo* extrapolation

tion sites. The exposure should also exceed the IC90 for inhibition of viral replication. With respect to drug distribution, antiretroviral drugs need to be able to suppress the virus throughout body's tissues and blood cells. Key viral reservoirs include long-lived, infected immune cells, lymphatic tissue, including the gastrointestinal tract, tonsils and rectal mucosa, lymph nodes, the central nervous system, the thymus, and the testicles. In many cases ongoing viral replication in these tissues can be detected even after plasma viral load was suppressed below the limit of detection for many months. Another aspect of druggability is, of course, the physiochemical attributes of the drug molecule, which in

part contribute to the observed PK/PD response but also help define the biopharmaceutic properties, which affect the ability to create and manufacture a solid, oral dosage form (Lajiness et al. 2004). Whereas other routes are possible, the oral route is clearly favored from a marketing and compliance standpoint. It is also desirable that the dosage form be of a certain size (not too big or small) and that a single dose unit be administered on each occasion.

The therapeutic window for antiretroviral agents is another key factor for drug candidates under investigation. Specifically, the dose selected should yield the maximum benefit/risk ratio possible for a given agent. This is not always achieved in practice but theoretically it should translate into the desired clinical response outcomes (e.g., viral load reduction) with minimal side effects. As previously discussed, the setting for ART involves multimodal therapy and hence the choice of agents included in the treatment regimen is critical. A key element in this choice is the extent to which the combination therapy (1) ensures compliance/adherence, (2) minimizes the development of resistance, and (3) ensures sustained exposure of all relevant active species. Enzyme inhibition and/or induction are often a reality that must be addressed. It occasionally represents an opportunity to provide dose-sparing gains in target exposures (e.g., ritonavir interaction with CYP 3A4 substrates). Pharmacogenetic differences in treatment populations need to be defined and understood with respect to potential contraindications, warnings, or dose adjustments. The nature of these differences can manifest PK (enzyme polymorphisms) and/or PD (immune pathophysiology) variation in patient response. Likewise, the global nature of the AIDS epidemic presents potential variation in the virus itself and the outcomes of drug therapy from individual studies may not be portable to other study populations.

Postmarketing evaluation presents both a critical stage and often a source of information regarding the potential for emerging (and possibly unforeseen) toxicities to drug therapy, more definitive information on patient adherence/compliance to a given therapy, and pharmacoeconomic information as healthcare providers contend with an ever-complicated insurance and reimbursement landscape. As a drug product's life cycle matures, longitudinal, epidemiologic data may become available to indicate comparative long-term outcomes of various regimen combinations. Such data may likewise affect market performance and possibly alter the choice of agents in early stages of development, particularly if linked to HIV disease progression.

M&S integration to decision making

The benefit of M&S approaches integrated into both a drug development paradigm (Chien et al. 2005; Grasela et al. 2005) and the regulatory review process (Bhattaram et al. 2005; Gobburu and Sekar 2002) were previously discussed and are reasonably well appreciated. Recently, Miller et al. (2005) discussed case studies in which M&S approaches were used in decision making, showing both a preclinical model of behavioral activity to predict potency and time course of response in humans (candidate differentiation) and the planning of a phase IIa dose ranging and proof of concept trial in Alzheimer's disease (criteria for a go/no-go decision) based on disease progression modeling and clinical trial simulation. Both examples illustrate the organization of modeling strategies to address key decision points and the continuity of data and models that become inherited by later development stages. Likewise, the evolution and interrelationships of M&S constructs is a key aspect of deploying the approach broadly and throughout a drug candidate's lifetime.

Models can be physical, conceptual, and/or quantitative. At the discovery stage, molecular modeling is employed extensively to create physical representation of the target site for drug interaction (e.g., enzyme or receptor). Model-based techniques to explore ligand binding are used to prioritize molecular targets by druggability are commonly employed. One such technique uses a three-dimensional protein structure as input and returns the location, volume, and shape of the putative small molecule binding sites by using a physical potential and without any knowledge about a potential ligand molecule (An et al. 2004). Once drug candidates are identified, quantitative structure activity relationships (QSARs) can be constructed to forecast toxicity (Arena et al. 2004), carcinogenicity (Contrera et al. 2005, Richardt and Benigni 2002), and activity (Micheva-Viteva et al. 2005). There are a variety of techniques used to support these approaches, including discriminant analysis, classification and regression tree (CART) analysis, logistic regression, and artificial intelligence/neural networks. In the work of Arena et al. (2004), QSAR models were used to predict the biological activity of potential developmental toxicants whose adverse effects included death, structural abnormalities, altered growth, and functional deficiencies in the developing organism. Physiochemical descriptors of spatial, electronic, and lipophilic properties were used to derive structure activity relationship (SAR) models by logistic regression CART analysis, using a developmental database of 293 chemicals. Both single models and model clusters were derived to predict toxicity. Such models are also employed to forecast ADME (A = absorption, D = distribution, M = metabolism, and E = elimination) properties to judge whether a candidate fulfills expected PK and biopharmaceutic properties identified in the TPP (Votano 2005). Hence, structure alone can provide an early assessment of druggability using model-based techniques and the output of such modeling exercises can be used to rank and subsequently filter potential drug candidates.

Drug candidates who survive the initial screening stage will be used in various experimental settings to further refine the selection criteria based on TPP milestones (e.g., preformulation studies, pharmacology and toxicology studies, and ADME studies). Experimental data derived from these studies will then form the basis of the next family of models used to drive preclinical decision making (Chien et al. 2005). These models include the following: physiologic-based pharmacokinetic models to predict drug distribution, *in vitro–in vivo* evaluation (IVIVE) to predict human PK parameters and drug interaction potential, *in vitro–in vivo* correlation to predict the performance of oral dosage forms based on biopharmaceutic data, human allometric models to predict first time in man starting dose, and PK/PD models to inform dose selection for animal and human pharmacology studies (Gobburu and Sekar 2002). In many cases these models utilize data from discrete experiments other than the primary *in vivo* study (i.e., protein binding, receptor affinity/binding, and enzyme inhibition). In either case, the output of these preclinical family of models becomes the input or "priors" for subsequent models developed for clinical phase (phases II–IV) testing. Regression-based techniques are usually employed at this stage given that most of the actual experiments are conducted using rich sampling paradigms.

The clinical evaluation phase is usually focused on only the lead development compound but will occasionally consider a backup compound that has the potential to supplant the lead. In any event, the decision making at this stage is more focused on ensuring that the lead candidate fulfill the benefit/risk target described in the TPP. The critical decisions at this stage also concern the trial designs employed to demonstrate the assumed or theorized response to treatment. Modeling and simulation techniques applied to clinical phase decision making include the following: PK/PD models (and simulation) to confirm/optimize dose regimen (s) for patient phase IIa studies; population PK/PD modeling to examine potential sources of variation in drug exposure in the intended patient population (also used to guide labeling on special populations, drug interactions, and dosing in general); and clinical trial simulation to explore trial, population, and/or conduct scenarios, which ensure the greatest likelihood of clinical success (Michelson et al. 2006). Methods for this phase include linear and nonlinear mixed effect modeling, Bayesian forecasting, and Monte Carlo and Markov Chain Monte Carlo simulation techniques.

Finally, the postmarketing phase will include decisions related to market penetration and performance and pharmacoeconomics in general. It will also address more formally the topics of adherence and compliance and potentially long-term health benefits and/or impact of pharmacotherapy on disease progression. As an example, Weinstein et al. (2001) has examined the role of models as to support recommendations on the cost-effective use of medical technologies and pharmaceuticals with implications for the Food and Drug Administration Modernization Act. Vernon et al. (2005) has explored the feasible range of future product prices when making in-licensing and developmental go/no-go decisions by considering payers' use of the cost-effectiveness method. The approach is theorized, at least in part, to reduce product development and in-licensing risk. Methods at this stage often include regression-based approaches but may also incorporate neural networks, discriminant analysis, CART analysis, and linear and nonlinear mixed effect modeling.

Impact of M&S on antiretroviral drug development

The value of M&S strategies to support the development and registration of antiretroviral agents can be realized at every development stage and for most of the critical decision criteria previously discussed. Table 3 shows M&S applications by development stage with the general compound progression criteria identified. The examples shown involve many of the currently marketed antiretrovirals and likewise span the various classes of agents. The goal of many pharmaceutical sponsors is to incorporate such an approach within the development paradigm of their antiretroviral portfolio, especially for various drug candidates within a target class.

Of the various applications shown in Table 3, several stand out as pivotal with respect to antiretroviral compound progression. The in silico ADME M&S, IVIVE coupled with the FDA guidances (FDA Guidance 2006, 1999) on *Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro* and *Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling* were pivotal events that have reduced the "laundry list" approach of conducting *in vivo* drug interaction studies for every plausible drug interaction to a select few prototypical studies conducted only when enzyme systems involved demonstrate *in vitro* effects suggestive of *in vivo* interaction potential. Population-based PK (pop-PK) M&S were extremely valuable in the investigation of optimal drug combinations, doses, and regimens. Barrett et al. (2005) has recently reviewed the clinical relevance and regulatory impact of such efforts. In addition to the pop-PK effort, the adoption of viral RNA reduction as a surrogate for survival and the modeling of viral dynamics (Bonhoeffer et al. 1997; Huang et al. 2003; Rosario et al. 2005) were instrumental to the design of phase III efficacy trials for new antiretrovirals, the comparison of various ART regimens, and the long-term projection of clinical benefit to therapy.

In the postmarketing area, many studies looking at adherence and compliance of ART were conducted. Modeling

Table 3 Examples of M&S applications in the compound progression of antiretroviral agents at various stages of drug development

Development Stages Subcategory	M&S Application (Reference)
Discovery	
HTS/QSAR	• Screening multiple targets with virus-based assay (Dias et al. 2006)
	• Entry inhibitors targeting gp41 (Renifo et al. 2004)
	• HTS for RTIs (Centers for Disease Control and Prevention 2001)
	• Integration inhibitors (Richardt and Benigni 2002)
	• Latency model (FDA Guidance 2006)
Druggability	• NMR approaches (Shiran et al. 2006)
	• Protein-ligand approach (Evans et al. 1997)
Toxicology Screens	• NRTI-induced mitochondrial Tox model (Tsai et al. 1993)
QSPK	• Human *in vitro* intrinsic CL (Votano 2005)
	• IVIVE and *in vitro* models (Wainberg 2004)
Preclinical	
In vivo Pharmacology	• Macaque/SIV dosing frequency (Riecke et al. 2000)
	• Monocytes and macrophages as physical models (Rosario et al. 2005)
	• Various animal models (Rosario et al. 2006)
In vivo Toxicology	• Developmental Tox of PIs in rats (Swindells et al. 2002)
Clinical	
PK/PD	• Viral dynamics (Hoschele 2006)
	• HIV dynamics relative to drug exposures, adherence and phenotype (Huang et al. 2003)
	• Antiretroviral therapy to predict disease progression (Kanki et al. 1999)
	• PK/PD link to disease progression (Kagay et al. 2004)
Pop-PK	• Clinical and regulatory impact (Ho et al. 2002)
Patient Individualization	• Genetic linkage to biologic phenotype (Vernon et al. 2005)
Clinical Trial Simulation	• Phase IIa trial design (Weinstein et al. 2001)
Postmarketing	
Disease Progression	• ART correlation with multiple risk factors (Rupniak 2002a)
	• Predictive value of viral load (Rupniak 2002b)
	• Impact of antiretrovirals in resource-poor settings (Severini et al. 2002)
Public Policy	• Modeling healthcare and policy decisions (Greco et al. 2004)
Pharmacoeconomics	• In-licensing and go/no-go decision making (Hajduk 2006)
Adherence/compliance	• Motivational interviewing and cognitive behavioral therapy to adherence (Zerhuoni 2003)

HTS = high-throughput screening

approaches have focused on understanding patterns of noncompliance (Huang et al. 2003) and characterizing the long-term dynamics of viral load, including resurgence of the HIV virus quantifying the effect of adherence in the dynamic of HIV1–RNA (Kagay et al. 2004). Such approaches were extended to look at HIV1 disease progression (Kagay et al. 2004) and prediction of the potential impact that low to moderate usage rates of antiretrovirals might have in developing countries (Birbeck 2005). In the latter example, models are developed to predict the relationship between the specific usage rate of antiretrovirals (in terms of the percentage of those infected with HIV who receive such treatment) and (1) the prevalence of drug-resistant HIV that will arise, (2) the future transmission rate of drug-resistant strains of HIV, and (3) the cumulative number of HIV infections that will be prevented through more widespread use of antiretrovirals. Thus, the framework for M&S integration into the progression of antiretrovirals through the development process is well established and should be a valuable tool despite the recent revelations regarding HIV disease progression and the various disease etiologies resultant from long-term ART administration.

Academic medical research example: retrofitting compound progression for NeuroAIDS indication

In June 2003, the National Institutes of Allergy and Infectious Disease (NIAID) and the National Institutes of Mental Health (NIMH) of the National Institutes of Health issued a program announcement (PAR-03-138) inviting applications from groups capable of establishing a multidisciplinary research and development program targeted toward the discovery, development, and evaluation of innovative therapies for HIV infection (http://grants.nih.gov/grants/guide/pa-files/PAR-03-138.html). The overall goal of this NIAID/NIMH program is to support and accelerate scientific and technical progress in nontraditional

and traditional drug-based therapies that exploit novel viral and cellular targets of importance in HIV infection. A benchmark for a successful program is the development of a new treatment concept that can be introduced to clinical practice.

One such program focuses on the ability of neurokinin-1 receptor (NK-1R) antagonists to target the substance P (SP) receptor demonstrating antiviral and immunomodulatory effects. The NK-1R antagonists represent a new therapeutic target with the potential to interrupt a pathway critical to HIV replication. The goal of this specific Integrated Preclinical/Clinical Program (IPCP) is to identify an NK-1R antagonist that is: (1) active as an anti-HIV agent through interaction with chemokine/cytokine receptors (*project 1*); (2) is specific for chemokine and G-protein-coupled receptors (*project 2*); (3) is safe for use in SIV-infected nonhuman primates and provides proof of concept related to antiviral, immunomodulatory, and neurobehavioral effects (*project 3*); and (4) is safe in humans and has positive immunomodulatory effects (*project 4*). All projects contribute to understanding the basic virologic, molecular, and cellular immunologic mechanisms of SP, NK-1R antagonists, and HIV/SIV infection.

There are several physiologic and immunologic effects of the release of SP (Greco et al. 2004; Maggi 1997). These diverse biologic effects include immune stimulation (Maggi 1997), secretion stimulation (gastrointestinal or pulmonary), smooth muscle contraction (pulmonary airways, urinary, and gastrointestinal tract and vascular system), and unique effects on the central nervous system. The NK-1R receptors are expressed in a number of important sites in the nervous system (Caberlotto et al. 2003) and the immune system (Lai et al. 2001, 2002) as well. Substance P and its preferring receptor, NK-1R, are central mediators in the interaction between the immune system and the nervous system (Maggi 1997, Severini et al. 2002; Hartung et al. 1986). Moreover, SP and SP-preferring receptors (NK-1R) have important associations with depression, anxiety, and psychologic and physiologic stress, in general, particularly in HIV-infected individuals (Michaels et al. 1998). It was demonstrated that HIV-infected men and women have elevated levels of circulating plasma SP in comparison to uninfected subjects (Douglas et al. 2001). The addition of SP *in vitro* enhances HIV replication in blood-isolated mononuclear phagocytes (Ho et al. 1996). It was demonstrated that the nonpeptide SP antagonist (CP-96,345) inhibits HIV replication in human mononuclear phagocytes, at least in part, through downregulation of CCR5 chemokine receptor, a principal coreceptor for HIV entry into macrophages, and also through inhibition of endogenous SP production (Lai et al. 2001, 2002). The SP receptor, NK-1R, facilitates HIV entry into macrophages through downregulation of CCR5 (Lai et al. 2001). The effects of SP on HIV infection are most likely mediated through several interrelated pathways: by enhancing HIV entry into immune cells, by facilitating HIV replication directly within immune cells, and/or by indirectly affecting virus proliferation through replication through effects on HIV coreceptors (Lai et al. 2001; Ho et al. 2002). Our measurement of circulating SP level demonstrates higher levels in HIV-infected men (Douglas et al. 2001) and women (unpublished) than in healthy individuals. Furthermore, plasma SP levels exhibit diurnal variation (AM higher than PM) and gender differences (men higher than women) (Douglas et al. 2001). Neurokinin-1 receptor antagonists downregulate SP production by macrophages (Lai et al. 2002). There is evidence that men with HIV/AIDS have increased life-event stress (Evans et al. 1995, 1997) and women have increased incidence of depression (Evans et al. 2002). We demonstrated that impairment in innate immunity, in particular, natural killer cell function, is associated with stress/depression in HIV. An improved understanding of the effects of interrupting the interaction between SP and NK-1R will provide insights into the regulatory mechanisms of NK-1R in HIV/AIDS. Manipulation of NK-1R through antagonists has direct potential therapeutic application in NeuroAIDS.

A key element in the IPCP is the integration of M&S strategies to support the various projects and inform the Administrative Core. Specifically, computational techniques are employed to challenge the "druggability" of the candidate agents. In silico ADME techniques are used as part of the ranking criteria by which we will prioritize the advancement of selected agents. This issue is not trivial as more than 50% of the candidates presumably fail because of ADME/Tox deficiencies during development. The approach enables the calculation of molecular descriptors and prediction of drug likeness and drug absorption data (Caco-2, MDCK, BBB, etc) using 2D molecular structures, and without the need for special computational chemistry knowledge as described above. As pharmacology and biology/mechanism data are generated for the various agents (projects 1 and 2), we will explore the generalizability of quantitative structure/pharmacokinetic (QSPK) relationships for this compound class.

The licensed drug, aprepitant (Emend®) is a NK-1R (SP receptor) antagonist that augments the antiemetic activity of the 5-HT_3-receptor antagonist, ondansetron, and the corticosteroid dexamethasone, thus inhibiting the acute and delayed phases of cisplatin-induced emesis. Aprepitant (Emend®) was approved by the FDA for this indication. This drug was not approved for use in the treatment of depression, although a number of recent published studies have reported activity of this family of compounds in depression, in particular, beneficial effects on stress, mood, and sleep (Kramer 2000; Kramer et al. 2004; Rupniak 2002a, b). Neurokinin-1 receptor occupancy by aprepitant was predictable

Table 4 M&S-facilitated decision points in the advancement of compound progression for the investigation of NK1r antagonists in the treatment of HIV-1-infected patients

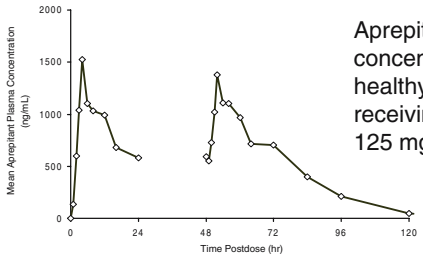
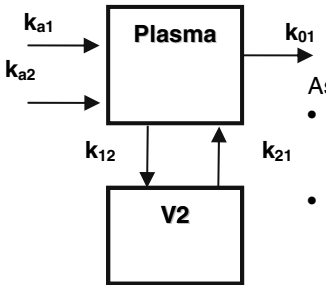
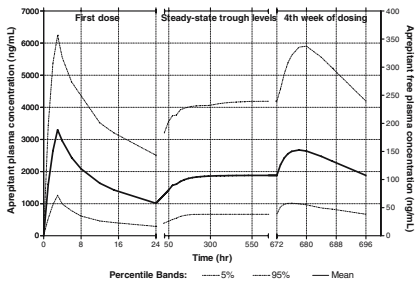
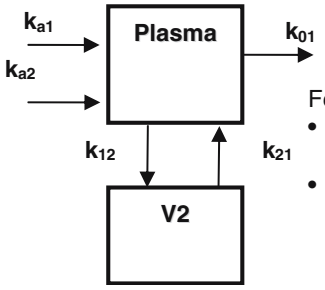
Phase/Activity	Key questions
Compound screening/ranking	
Create mol file for chemical structures under consideration	Does the structure suggest ADME properties that are consistent with "druggable" agents?
Define and model NK1r mechanistic, virologic and immunomodulatory activity	Is the compound "active" in the range of concentrations, which would be pharmacologically achievable?
Project criteria for advancement based on "druggability"	Is there evidence of synergy with the various experimental agents and marketed antiretrovirals?
Examine IVIVE-drug interaction potential	
In silico Tox evaluation	
Pharmacology studies on viable candidates	
PK/PD in SIV	
Define target profile and ITW in the cynomolgus monkey	Is the monkey a reasonable model for the presumed NK-1R mechanism(s) of action?
Exposure-response models in monkeys for antiretroviral and neurologic effects	Can the SIV/monkey model be extrapolated to the human PK/PD behavior (neurocognitive, antiretroviral, etc.)?
Scale doses to obtain human equivalent exposures	Can the SIV/monkey model be extrapolated to the human HIV disease?
PK/PD in HIV	
Project exposure-response profile in HIV-1 infected patients (use human PK for aprepitant and SIV/monkey for PD)	Can activity be demonstrated at the dose and duration of therapy negotiated with FDA?
Simulate Phase IB exposure-response for various dose/regimen scenarios	Does the variation in response suggestive of responder/nonresponder partition given the small sample size?
Conduct trial pop-PK/PD in patients	Is the PK/PD adequately defined such that dose extrapolation can be made reliably?
Simulate phase IIb Proof of concept trial outcomes	
Knowledge transfer to preclinical screening	
Derive relationship between clinical response from Phase IB trial and preclinical ranking metrics	Is there sufficient correlation between SARs and the ranking criteria to suggest a backup compound to aprepitant?
Reconsider ranking based on clinical response metrics	Is there a therapeutic response for NeuroAIDS based on the aprepitant phase IB trial that can be targeted from earlier (preclinical) data?

from plasma concentration using a Hill equation model (Bergstrom et al. 2004). Positron emission tomography results indicate that brain NK-1R receptor occupancy occurs with daily oral doses of aprepitant (Bergstrom et al. 2004). Aprepitant was not administered to HIV-infected subjects. It is known to be a moderate CYP3A4 inhibitor. In the approved product labeling, a number of drugs are contraindicated for use with Emend®. In particular, inhibition of the CYP isoenzyme 3A4 (CYP3A4) may result in elevated plasma concentrations of certain drugs (Merck & Co., Inc. 2003).

The key questions that this IPCP must answer along with the M&S effort to support their inquiry are shown in Table 4. The NK-1R IPCP project is dependent on two primary progression pathways: (1) examine the extent to which the NK1r antagonist aprepitant is a suitable agent for ART therapy in NeuroAIDS patients based on exposure–response criteria constructed from its presumed actions and (2) examine the correlation between aprepitant preclinical pharmacology, druggability criteria, and PK/PD relationships to generalize and ultimately rank suitable backup compounds. Within the first pathway there is a progression of experiments, predefined criteria for stage advancement and decision trees, which will guide the overall progression. These are facilitated by M&S exercises, which permit scenario testing about subsequent experiments and testing of assumptions, which are fundamental to key assumptions about the overall program. One of the key bridges in this IPCP is the suitability of an SIV pharmacology/disease model to predict success outcomes in HIV. Likewise, much of the initial effort was to establish a colony of SIV-infected rhesus macaque, develop methods and procedures to detect the exposure of aprepitant and metabolites in various biologic fluids (e.g., plasma, urine, and CSF), and assess response to chronic administration of aprepitant related to psychological and immunologic indices. In the series of experiments that has ensued, the pharmacokinetics and pharmacodynamics of aprepitant in SIV-infected animals were characterized and used in conjunction with relevant human data in chemotherapy-induced nausea and vomiting (CINV) patients to build simulation models that project expected response in HIV-infected patients.

We have illustrated on such approach in Table 5. Based on our initial findings, the inhibition of HIV Bal strain in MDM by aprepitant (10–6 M) is 79.5% (preliminary data);

Table 5 A model-based approach illustrating the progression of exposure–response relationships with aprepitant from *in vitro* data, *in vivo* data in animals, and *in vivo* data in CINV patients to ultimately predict HIV-infected patient response

Step	M&S task	Data/Models/Assumptions
1	Assemble prior information with respect to aprepitant activity and ADME properties	80% Inhibition of HIV Bal strain in MDM (10^{-6} M) Assume exposure target similar to *in vitro* activity → target trough-free concentration ~ 100–500 ng/ml. Metabolized primarily by CYP3A4; minor metabolism by CYP1A2 and CYP2C19. Enzyme induction reduces the exposure after chronic administration. Protein binding ~95%; F~60–65%; half-life 9–13 h Elimination by metabolism; no renal
2	Use aprepitant ADME properties from step 1 with actual PK data (Majumdar et al. 2006) in CINV patients to derive mechanistic PK model	 Aprepitant concentrations in 12 healthy volunteers receiving 80 then 125 mg QD
3	Use PK model to simulate concentration-time profiles in HIV-infected patients for regimen scenarios under consideration for Phase IB trial	 Assumptions / Features: • Induction reduces exposure by 50% at SS (↑ CL by 2-fold) • Moderate variability in CL and V
4	Evaluate model predictions (simulations) to identify doses with greatest likelihood of achieving clinical activity targets	
5	Refine model upon completion of Phase IB trial incorporating relevant covariates and simulate phase IIb trial designs based on PK/PD models	 Features: • Patient demographics, drug interactions, etc • Links to PD response

the inhibition by CP-96,345 is 54% (Lai et al. 2001). Converting molar to mass units of concentration and assuming that the human exposure target is similar to the *in vitro* activity yields a trough-free drug concentration of approximately 500 ng/ml. Projections of dose requirements to achieve this exposure target were derived by modeling data recently published (Majumdar et al. 2006) on the exposure of aprepitant after oral administration of the dose indicated

for CINV (120 mg for the first dose followed by 80 mg QD for 3 days; see Table 5). Aprepitant is metabolized primarily by CYP3A4 with minor metabolism by CYP1A2 and CYP2C19. Seven metabolites of aprepitant, which are only weakly active, were identified in human plasma. It was described in preclinical reports that enzyme induction reduces the exposure of aprepitant after chronic administration although this data was not published. Likewise, a conservative simulation model might assume a doubling in systemic clearance, effectively reducing trough exposure by 50% because of induction. Table 5 also shows the expected aprepitant exposure after 120 mg once daily dosing over the 4-week study duration. These projections also assume that HIV patients behave similarly to the healthy volunteers described by Majumdar et al. (2006). Based on the results of the simulation model, more than 50% of the patients will achieve trough levels in excess of the 500-ng/ml of trough target exposure. When correcting for protein binding (95% in the case of aprepitant), target exposures are well below the projected target exposure at the 120-mg QD dosing regimen.

Thus, higher doses of aprepitant are warranted to treat HIV although the intended utilization in NeuroAIDS is clearly beyond its antiretroviral potency. Our initial trial will explore higher doses deemed suitable to explore the safety of chronic aprepitant administration in an HIV-infected population and permit the exploration of exposure–response relationships to guide future studies in which coadministration with effective ART therapeutic regimens will be studied.

Future challenges

The Pharmaceutical Research and Manufacturing Association of America reports that only 1 in 5,000 compounds screened is approved as a new medicine. This occurs despite a heavy investment in discovery stage technology, including high throughput screening techniques. Recent findings from FDA also report a phase III failure rate of 50%. The standard drug development paradigm for discovery, screening, preclinical (pharmacology and toxicology), and clinical (human safety, tolerability, and efficacy) evaluation is predicated on sequential experimentation in which knowledge hurdles (drug activity /toxicity → PK → safety → PD → efficacy) are overcome to pass to the next phase of development. This approach has come under criticism from the FDA. In a recent communication entitled, *Innovation and stagnation: Challenge and opportunity on the critical path to new medicinal products*, FDA has implored pharmaceutical sponsors to develop new tools to "identify successful products and eliminate impending failures more efficiently and earlier in the development process." The plea from FDA in this area is based on a request that sponsors identify ways to bridge between the laboratory and the whole organism and correlate early markers of safety and benefit with actual outcomes in patients.

A key development component for many pharmaceutical sponsors is the linkage between the translational science and clinical outcomes coupled with M&S techniques to aid in (1) the ranking of various preclinical candidates; (2) the criteria for advancement to animal pharmacologic testing (proof of principle/proof of mechanism); (3) the evaluation of drug properties, which constitute suitable criteria for advancement to human testing; and (4) the specific experimental and study design features, which will permit specific, hypothesis-driven evaluation of the clinical utility of a particular mechanism as a treatment modality in the given target population. This is especially true in the area of antiretroviral drug development and specifically in evolving clinical conditions such as the NeuroAIDS setting. The application of M&S approaches to this arena will not only have to facilitate decision making but be a measuring stick by which this approach ultimately delivers improved healthcare.

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