Guidance for Industry

Role of HIV Resistance Testing in Antiretroviral Drug Development

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2007
Clinical Antimicrobial

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Contains Nonbinding Recommendations

Guidance for Industry[1] Role of HIV Resistance Testing in Antiretroviral Drug Development

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance is intended to assist sponsors in the clinical development of drugs for the treatment of human immunodeficiency virus (HIV) infection. Specifically, this guidance addresses the Food and Drug Administration's (FDA's) current thinking regarding the role of HIV resistance testing during antiretroviral drug development and marketing and serves as a focus for continued discussion among the Division of Antiviral Products (DAVP), pharmaceutical sponsors, the academic community, and the public. The goal of this guidance is to stimulate the generation of more complete resistance data and analyses for antiretroviral drug products.

This guidance uses a broad definition of the term *drugs* including, but not limited to, small chemical entities, biologics, monoclonal antibodies, synthetic oligonucleotides, and siRNA, and focuses on resistance to antiretroviral agents as manifested by mutations in the HIV viral genome that result in reduced phenotypic susceptibility to a given drug product. Although mechanisms of cellular resistance to antiretrovirals exist, a discussion of these mechanisms is beyond the scope of this guidance. In addition, loss of susceptibility to drugs is highlighted, rather than hypersusceptibility. However, we acknowledge the potential for results to show increased susceptibility of the virus to one or more antiretroviral drugs and we encourage sponsors to report such observations to the FDA.

Although this guidance focuses on characterization of resistance and cross-resistance during drug development, we recommend application of these principles to currently marketed antiretroviral agents; therefore, we recommend ongoing resistance testing in the postmarketing setting.

[1] This guidance has been prepared by the Division of Antiviral Products in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

This guidance does not imply one type of resistance testing is more useful than another type of resistance testing in the clinical management of HIV infection. This guidance addresses how serial assessments of both genotype and phenotype can be useful in antiretroviral drug development. For characterizing the utility of an antiretroviral drug, both phenotypic and genotypic resistance testing have strengths and limitations as discussed in this guidance.

For information on trial design and endpoints in phase 3 antiretroviral drug development, see the related guidance for industry *Antiretroviral Drugs Using Plasma HIV RNA Measurements — Clinical Considerations for Accelerated and Traditional Approval*.[2]

Because the field of HIV resistance is evolving, we intend to revise this guidance as new information accumulates.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

The primary sources for the recommendations in this guidance are as follows:

- Data analyses and input from the HIV Resistance Collaborative Group (an international, multidisciplinary group consisting of representatives from academic institutions, U.S. and European health regulatory authorities, governmental clinical trial organizations, the pharmaceutical and diagnostics industries, and HIV patient community groups). Data, analyses, and opinions compiled by this group were presented at a 2-day session of the Antiviral Drug Product Advisory Committee, convened November 2-3, 1999, to address issues relating to HIV resistance testing.

- The DAVP's experience with reviewing resistance data for antiretroviral drugs in new drug applications from 1999 to the present, including subsequent analysis and presentation of resistance data to the Antiviral Drug Product Advisory Committee.

- Additional input from pharmaceutical sponsors and the HIV community.

Presentations during the November 1999 advisory committee meeting included the following topics:

- Performance characteristics of genotypic and phenotypic assays
- Prevalence of resistance in antiretroviral naïve patients

[2] We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at http://www.fda.gov/cder/guidance/index.htm.

- The ability of baseline resistance testing to predict subsequent virologic response
- Clinical factors that might influence the results of resistance testing

Summaries of presentations at this meeting have been published in *Antiviral Therapy* (Richman 2000; Hammer and Pedneault 2000; DeGruttola et al. 2000; Laessig et al. 2000), and the transcripts of the FDA advisory committee can be found on the Internet.[3]

III. HIV RESISTANCE TESTING — GENERAL

Because of its high rate of replication (10^9 to 10^{10} virions per person per day) and error-prone polymerase, HIV can easily develop mutations that alter susceptibility to antiretroviral drugs. As a result, the emergence of resistance to one or more antiretroviral drugs is one of the more common reasons for therapeutic failure in the treatment of HIV. In addition, the emergence of resistance to one antiretroviral drug sometimes confers a reduction in or a loss of susceptibility to other or all drugs of the same class.

The application of laboratory technologies, such as gene amplification, automated nucleic acid sequencing, and nucleic acid hybridization, and the availability of recombinant viruses for testing phenotypic susceptibility have permitted advances in HIV resistance testing. Many clinicians and investigators are currently using these technologies in the clinical management of HIV. However, performance characteristics (e.g., sensitivity, specificity, and reproducibility) for many of the assays in investigational use have not been fully established. In addition, the clinical significance of many mutations or mutational patterns has not been defined completely for many antiretroviral drugs. Likewise, the quantitative relationship between reductions of cell culture susceptibility and loss of clinical activity has not been established for most drugs. Consequently, many of the current package inserts are deficient in the amount and type of resistance data describing the utility of a drug in the setting of resistance or reduced susceptibility.

Despite limitations of resistance assays and their interpretation, several randomized controlled studies have demonstrated that virologic outcome, at least over the short term, may be improved when genotypic or phenotypic data are used to guide choice of drug regimens in patients with loss of virologic response to prior regimens (Baxter et al. 2000; Cohen et al. 2002; Durant et al. 1999; Melnick et al. 2000; Meynard et al. 2000; Tural et al. 2002). The FDA recommends that characterization of resistance and cross-resistance be a part of antiretroviral drug development so that clinically relevant information is available at the time of approval. An efficient way to accomplish the goal of having clinically relevant information available at the time of approval is to include resistance testing in all phases of drug development. As discussed below, assessment of resistance should not be delayed until phase 3 or post-approval. We recommend that, before or during phase 1 and phase 2 studies, investigators begin assessing the potential of a drug to select resistant viruses and the drug's activity against HIV isolates resistant to other antiretroviral agents. During early development, a wide range of doses should be evaluated and pharmacokinetic data should be collected, providing information to investigate the relationship between drug exposure and resistance.

[3] See http://www.fda.gov/oashi/aids/advisorycom.html#geno.

Optimally, a comprehensive evaluation of a new drug's resistance and cross-resistance profile will promote more rational use of antiretroviral drug combinations in the future.

IV. NONCLINICAL STUDIES

Cell culture studies can provide useful information for the design of in vivo studies and may be predictive of the development of resistant viruses in vivo. This section identifies studies relevant to resistance issues in the development of antiretroviral drugs for the treatment of HIV infection. The FDA also issued a guidance that outlines general nonclinical studies for antiviral drugs (see the guidance for industry *Antiviral Product Development — Conducting and Submitting Virology Studies to the Agency*).

Sponsors should complete nonclinical studies (i.e., mechanism of action, antiviral activity in cell culture, cytotoxicity and therapeutic index, and effects of serum protein binding on antiviral activity) before the initiation of phase 1 clinical studies. Cell culture drug combination activity studies for drugs that are used in clinical trials should be completed before initiation of those trials. Selection of resistant HIV-1 variants in cell culture, the phenotypic and genotypic characterization of resistance viruses, and cross-resistance analyses should be examined before initiation of clinical studies in HIV-infected patients. We recommend sponsors be consistent in the assay used for any particular analysis or measurement in phase 3 studies. Sponsors should provide data supporting the implementation and use of the new or improved assays that become available during drug development.

A. Mechanism of Action

A well-characterized mechanism of action for a new antiretroviral drug can provide insight into the regions of the HIV genome where mutations that confer resistance may develop. These regions are not limited to the site of action (viral-encoded target) of the investigational drug and can include the enzyme substrate(s) (e.g., Gag and Gag-Pol cleavage sites for protease inhibitors) or another viral-encoded protein(s) existing in a quaternary complex with the target protein (e.g., gp120 and gp41). Any metabolite that exerts inhibitory activity should be delineated and its specificity for the target shown. For example, reverse transcriptase (RT) inhibitors should show selectivity for RT over cellular DNA and RNA polymerases.

B. Antiviral Activity in Cell Culture

The antiviral activity in cell culture of a compound indicates that it effectively inhibits replication and forms the basis for defining phenotypic resistance (detected by reductions in susceptibility to the investigational drug (see below)). The concentration of an investigational drug required to inhibit virus replication by 50 percent (EC_{50} for cell-based assays; IC_{50} for biochemical or subcellular assays) should be determined. The use of the EC_{50} value for determining shifts in susceptibility is preferred because it can be determined with greater precision than an EC_{90} or EC_{95} value. A well-characterized wild-type (WT) HIV laboratory strain should serve as a reference standard. Sponsors should obtain some susceptibility data with a limited number of clinical isolates grown in peripheral blood mononuclear cells (PBMCs). The

amount of susceptibility data in PBMCs needed to support an application should be discussed with the division.

The antiviral activity of drugs can vary greatly because of factors such as genetic variation in isolates, host cell type, and multiplicity of infection assay used for measurement of virus replication. Because of genetic variation, determination of antiviral activity against a broad spectrum of viruses of well-characterized HIV laboratory strains and clinical isolates sufficient to assess the breadth of antiviral activity is recommended. The antiviral activity should be assessed in multiple clade B and nonclade B isolates, T-cell tropic HIV-1 and monocyte/macrophage tropic strains, HIV-2, and well-characterized drug-resistant laboratory strains and clinical isolates.

C. Cytotoxicity and Therapeutic Indexes

It is important to establish that an investigational drug has antiviral activity at concentrations that can be achieved in vivo without inducing toxic effects to cells. Furthermore, in a cell culture model, apparent antiviral activity of an investigational drug can be the result of host cell death after exposure to the drug. Cytotoxicity tests use a series of increasing concentrations of the antiviral drug to determine what concentration results in the death of 50 percent of the host cells (CC_{50} or $CCIC_{50}$). The relative effectiveness of the investigational drug in inhibiting viral replication compared to inducing cell death is defined as the therapeutic or selectivity index (i.e., CC_{50} value/EC_{50} value). It is desirable to have a high therapeutic index giving maximum antiviral activity with minimal cell toxicity. We recommend determining CC_{50} values in both stationary and dividing cells from multiple relevant human cell types and tissues to ascertain the potential for cell-cycle, species, or tissue-specific toxicities. Studies determining cytotoxicity and therapeutic indexes should be conducted before the initiation of phase 1 clinical studies.

D. Protein Binding

Protein binding of an antiviral drug to human serum proteins may result in reduced antiviral activity. The effects of 45 percent to 50 percent human serum on the cell culture antiviral activity of the investigational drug should be evaluated for at least one well-characterized laboratory or clinical isolate, and the serum-adjusted EC_{50} value should be determined (see section V.D.2, Data Collection from Dose-Finding Trials). A series of human serum dilutions (e.g., 5 percent, 10 percent, 20 percent, 40 percent) can be used to extrapolate the effect of 100 percent human serum. In addition, an examination of the protein binding effects of α-acid glycoprotein and human serum albumin at physiological concentrations is recommended.

E. Selection of Drug-Resistant HIV-1 Variants in Cell Culture

Selection of resistant viruses in cell culture may indicate whether development of resistance to a drug is likely to involve a few (1 to 2) or several (more than 2) mutations. The ability of an investigational drug to select HIV-1 variants with reduced drug susceptibility (phenotypic resistance) should be determined in cell culture systems. Selection of variants resistant to the investigational drug should be repeated multiple times (e.g., with different strains of WT, with

resistant strains, under high and low selective pressure) to determine if the same or different patterns of resistance mutations develop.

Two basic methods have been developed to identify mutations conferring a reduction in susceptibility to a drug.

1. In the first method, a high initial virus inoculum is propagated for several passages at a fixed drug concentration, using multiple cultures to test different concentrations.

2. In the second method, a low initial inoculum of virus is passaged in the presence of increasing drug concentrations starting near the EC_{50} value for the parental virus.

Virus production is monitored to detect the outgrowth of resistant virus by characterizing intermediate and ending isolates with respect to genotype and phenotype.

1. Genotype

Mutations responsible for reductions in susceptibility to a drug can be identified by DNA sequence analysis of the relevant portions of the virus genome. The complete coding sequence of the gene for the target protein should be determined in the early stages of characterization of mutations associated with reduced drug susceptibility. Once mutations are identified, their ability to confer phenotypic resistance should be evaluated in a recombinant virus system (e.g., by using site-directed mutagenesis or polymerase chain reaction (PCR) amplification of relevant portions of the virus genome to introduce these mutations into a standard laboratory HIV genetic background) or other suitable system, such that the mutations necessary to reproduce the resistant phenotype are identified. If site-directed mutagenesis experiments within the target gene fail to recapitulate the resistance phenotype, then the potential effects of mutations elsewhere in the viral genome should be examined. In the case of studying mutations in the envelope gene, which is highly variable, a possible option is to introduce mutations into the parental envelope to assess their contribution to the resistance phenotype. Recombinant virus should then be tested for drug susceptibility in cell culture. Shifts in drug susceptibility (fold-increases in EC_{50} value) for recombinant virus relative to WT should be determined (see section IV.G., Characterization of Genotypic and Phenotypic Assays).

2. Phenotype

Drug susceptibility (EC_{50} values) for resistant variants and the fold change in EC_{50} values relative to the parent virus should be determined (see section IV.G., Characterization of Phenotypic and Genotypic Assays).

A number of drugs targeting gp120, gp41, and the CCR5 and CXCR4 chemokine co-receptors are in development. A potential concern with CCR5 inhibitors is that loss of effectiveness may develop by the virus switching to the CXCR4 co-receptor. The evolution of HIV to a CXCR4-utilizing virus has been proposed to result in a more virulent virus. Therefore, sponsors should monitor co-receptor usage in cell culture drug selection experiments and in clinical trials evaluating drugs targeting the co-receptors gp120 and gp41.

F. Cross-Resistance

HIV variants resistant to one drug in a class of antiretroviral drugs may be resistant to another drug in the same class. Recombinant viruses containing resistance-associated mutations to an investigational drug should be tested for susceptibility to all approved and any investigational drugs (where possible) of the same class and other classes with the same target protein or protein complex. Conversely, laboratory strains and well-characterized clinical isolates containing resistance-associated mutations for each of the approved and investigational members (where possible) of the same class should be tested for susceptibility to the investigational drug.

Clinical isolates should be representative of the breadth of diverse mutations and combinations of mutations known to confer reduced susceptibility. A standardized panel of virus strains and isolates with diverse resistance-associated mutations and combinations of resistance-associated mutations for the different drug classes can be helpful for profiling investigational drugs and allowing for comparison with approved and other investigational drugs. The panel should be up to date and include mutational changes in current circulating viral populations caused by the introduction of newly approved drugs.

G. Characterization of Genotypic and Phenotypic Assays

Well-characterized genotypic and phenotypic assays can provide the basis for the analysis of the emergence of resistant virus during the development of investigational drugs. Phenotypic and genotypic assays used in clinical practice need more extensive validation than exploratory assays used for the characterization of antiviral activity and/or the resistance profile of the investigational drug. Commercially available assays that are routinely used should be identified, but it may not be necessary to provide the performance characteristics. The amount and nature of validation necessary for an assay and mechanisms of submitting assay performance characteristics should be discussed with the division (also see the guidance for industry *Antiretroviral Drugs Using Plasma HIV RNA Measurements — Clinical Considerations for Accelerated and Traditional Approval*).

1. Genotypic Assays

The performance characteristics of genotypic assays should be described, including elaboration of the following characteristics:

- Minimum plasma viral RNA level with a standard isolate to assess PCR sensitivity
- Purification methodology for viral nucleic acids
- Amplification methodology and primers
- PCR controls
- Clade differences
- Nucleic acid sequencing methodology
- Description of sequencing primers
- Range of mutant and WT ratios detectable
- Interpretation criteria for mutant scoring

The entire coding sequence of the gene for the target protein should be determined in the early stages of resistant variants analysis. Once the mutations leading to resistance are identified, only the relevant portions of the genome need to be sequenced. The pattern of mutations leading to resistance of an investigational drug should be documented and compared with the pattern of mutations of other drugs in the same class.

Reporting the details of methodologies is important. Sponsors should identify sequencing primers and state how many bases from them can be read accurately. Sponsors also should define the sensitivity of the genotypic assay used for detecting minority viral subpopulations.

2. Phenotypic Assays

The performance characteristics (accuracy, precision, limits of detection and quantification, specificity, linearity, range, robustness, stability) of an investigational phenotypic assay should be well documented. The sources of viruses (e.g., blood, plasma), their storage and stability, and cell culture procedures should be described. For definitions on assay validation, refer to the guidance for industry *Bioanalytical Method Validation*. An additional reference is the ICH guideline for industry *Q2A Text on Validation of Analytical Procedures*. Sponsors are encouraged to use a previously characterized and validated assay.

The utility of a phenotypic assay will depend upon its sensitivity (i.e., its ability to measure shifts in susceptibility (fold-changes) in comparison to baseline clinical isolates) and on the drug concentrations achieved. Shifts in susceptibility for a clinical isolate are measured by determining the EC_{50} values for the isolate and a WT standard virus done under the same conditions and at the same time. Simultaneous testing provides for absolute comparisons between assays. Readout of phenotypic assays can be detected with any standard virus assay, such as p24, viral RNA, RT assay, MTT cytotoxic assay, and reporter gene expression.

V. CLINICAL STUDIES: USE OF RESISTANCE TESTING IN CLINICAL PHASES OF DRUG DEVELOPMENT

Before advances in resistance testing technologies, resistance and cross-resistance data were often obtained late in drug development or during postmarketing. However, given the current availability of resistance testing in clinical practice and the need to give health care providers information about an antiretroviral drug's resistance profile, comprehensive resistance testing should be undertaken in all phases of drug development. Crucial decisions in protocol design and drug development hinge on resistance and cross-resistance data.

Cell culture resistance and cross-resistance studies of an investigational drug can focus the scope of drug development. For example, drugs that exhibit extensive cross-resistance with approved drugs of the same class are unlikely to be suitable for studies in treatment-experienced patients harboring resistant isolates to that class. Conversely, for drugs that demonstrate a nonoverlapping or unique resistance profile, the division strongly encourages sponsors to develop clinical protocols studying treatment-experienced individuals. Although we encourage

development of new antiretroviral drugs with unique resistance profiles in treatment-experienced patients, we also encourage concurrent clinical development in antiretroviral-naïve patients as appropriate.

Epidemiological data suggest that transmission of drug-resistant HIV is on the rise, meaning one cannot assume treatment-naïve patients harbor WT virus (Little et al. 2002). In this regard, the division strongly recommends the collection and storage of samples for baseline resistance testing (preferably for both genotype and phenotype) from all HIV-infected, multiple-dose study patients, regardless of treatment history. Refer to section V.C., Methods and Types of Analyses, for further information regarding settings where analyses on baseline and follow-up samples are recommended. Knowledge of the genotype and phenotype at baseline can aid in the interpretation of unexpected antiviral responses, particularly in smaller dose-ranging studies.

A. General Considerations

The goals of resistance testing are to:

- Determine the effect of an antiviral drug on the evolution of the virus
- Identify the baseline genotypic and phenotypic determinants of virologic success or failure (or clinical success or failure) in the study

We strongly recommend resistance testing in all phases of development and, in most cases, as soon as the drug is introduced into HIV-infected patients. Data from nonclinical studies and phase 1 and phase 2 clinical trials should provide a preliminary idea of the genotypic mutations that confer reduced drug susceptibility and a lack or loss of virologic response. Phase 3 trial designs should incorporate this information and expand on it, thereby aiming to further characterize drug resistance.

In general, the type of information collected and the types of analyses conducted should be the same for all phases of development. However, the amount of data collected and the types of analyses performed may differ for treatment-naïve versus treatment-experienced patients as described below. Whenever possible, resistance analyses should be prospectively defined. However, since it is not possible to define *a priori* key mutations or susceptibility breakpoints, retrospective analyses can provide important information in characterizing resistance and cross-resistance. The following sections provide recommendations on the type of resistance data that should be collected during drug development and the types of analyses that should be conducted. Appendix A provides guidance for submitting HIV resistance data. Information about the specific assays and mutational algorithms used in protocols also should be provided to the division.

B. Data Collection

To characterize drug resistance during development, sponsors are strongly encouraged to collect samples from both treatment-naïve and treatment-experienced patients. Overall, the extent and type of resistance testing should be discussed and agreed upon with the division throughout drug development. Discussions with the division are particularly important for new classes of

antiretroviral agents as the data and technologies are evolving. The following data should be collected and analyzed:

- Baseline phenotype and genotype samples on all study patients. Samples can be analyzed at a later date as appropriate.

  The reasons for obtaining baseline samples for phenotype and genotype on all clinical trial patients are twofold. First, the prevalence and rate of transmission of drug-resistant virus is increasing (Little et al. 2002), and may continue to increase, as the HIV population becomes more treatment experienced. Second, collection of baseline data provides an opportunity to examine the relationship between genotype and phenotype and virologic response to drug, particularly in treatment-experienced patient populations. Use of resistance testing in study protocols may help in choosing appropriate combination regimens for treatment-experienced patients (see section V.C.4, Genotypic and Phenotypic Correlations: Changes in Susceptibility from Baseline, for further details).

- Post-baseline phenotype and genotype samples on all study patients, regardless of treatment history who demonstrate a lack or loss of virologic response during the trial to determine mutations that may contribute to reduced drug susceptibility.

  Collecting samples for resistance testing when patients are still on study drug, or as soon as possible if study drugs are discontinued, is important. Studies have shown WT virus may outgrow resistant HIV viral strains in the absence of selective drug pressure (Devereux et al. 1999; Halfon et al. 2003). In addition, continuation of resistance monitoring on subsequent regimens can provide useful information regarding cross-resistance and sequencing of therapy. We recognize collection of resistance data on subsequent regimens is not feasible for all clinical trials or patients; however, sponsors are encouraged to consider such studies in their development plans as appropriate (see section V.C.5, Cross-Resistance, for details) and are encouraged to discuss continuation studies with the division.

C. Methods and Types of Analyses

Several types of resistance analyses can be used to characterize a drug's resistance profile. Analyses for treatment-experienced patients are more complicated than for treatment-naïve patients. Some analyses are possible only when larger datasets are available. In phase 3, clinical trial datasets can be sufficiently large to study the effect that mutations confer upon drug susceptibility and outcome. Pooling data from several trials can be appropriate (provided the study populations, endpoints, and assays are similar), but should be discussed in advance with the division. To facilitate pooling data, sponsors should attempt to use similar, if not identical, assays during phase 3. Whenever possible, resistance analyses should be prospectively defined, with the caveat that prospectively defining key mutations or susceptibility breakpoints is not always possible. In some cases, retrospective analyses can provide important information in characterizing resistance and cross-resistance.

Because of a large number of potential comparisons, statistical testing can be problematic for analyses of resistance testing and outcome. Sponsors are encouraged to provide resistance analysis plans to the division in advance.

Analyses of virologic outcome by baseline genotype or phenotype should be based on a censored population to assess the effect of baseline resistance on outcome without confounding factors such as early discontinuation because of adverse events. Therefore, patients who discontinue study treatment while suppressed or who discontinue study treatment before confirmed suppression for adverse event, noncompliance, protocol violation, pregnancy, or withdrawal of consent should be censored. Rules for censoring patients who appear to have a virologic response before discontinuation should be discussed with the division. We encourage sponsors to analyze the baseline resistance data by the primary and secondary endpoints used in the trial. Virologic response parameters recommended for analyses include the following, but are not limited to: proportion of HIV RNA below the limit of quantification (e.g., less than 400 or less than 50 copies/mL), proportion less than 1 $\log_{10}$ decrease from baseline, and mean and/or median change from baseline or time average change from baseline at the protocol-specified time points. Sponsors are encouraged to discuss endpoints with the division in advance. All patients should be included in the dataset until the time of censoring. The datasets should include variables for reasons for censoring patients. Refer to Appendix A for further details.

In general, we recommend analyses of baseline genotype and phenotype for all treatment-experienced patients to evaluate baseline predictors of virologic response. In addition, genotypic and phenotypic analyses of samples obtained at baseline and at the time of virologic failure are recommended for all treatment-experienced patients to characterize resistance and cross-resistance.

The following examples reflect suggested analyses for studies in treatment-experienced patients. Sponsors are strongly encouraged to conduct additional analyses and should discuss these analyses with the division before submission of a new drug application (NDA). In most cases, we do not anticipate baseline genotype and phenotype and virologic outcome analyses, as described below, for all treatment-naïve patients. However, circumstances may arise in treatment-naïve patients where analyses of baseline resistance and virologic outcome data are warranted, particularly in the event of unexpected efficacy results. In addition, in select cases, sponsors can propose to collect samples at baseline and at the time of failure on a subset of treatment-naïve patients, for example, when cell culture data indicate that acquisition of a specific single mutation results in a high degree of phenotypic resistance.

We also acknowledge for various analyses the number of patients in certain subgroups may be limited and as a result definitive conclusions regarding specific baseline factors will not be possible.

1. Baseline Genotype and Virologic Response

Analyses should be conducted to evaluate HIV RNA response according to the presence and absence of baseline mutations. These analyses can help assess the association between a specific mutation or mutational pattern and virologic response rates. For example, for a new nucleoside

analogue, virologic response rates would be determined for patients with and without clinically relevant mutations associated with resistance to other nucleoside analogs. Table 1 shows one example of virologic response rates for a hypothetical nucleoside analog by the presence or absence of zidovudine-associated mutations at baseline.

Table 1. HIV RNA Response by Baseline RTI Mutations at Endpoint

Baseline RTI Mutations*	Virologic Response Rate			
	MUTATION PRESENT		MUTATION ABSENT	
	Drug X (n =)	Control (n =)	Drug X (n =)	Control (n =)
M41L	27%	6%	79%	7%
D67N	65%	3%	63%	2%
K70R	72%	3%	54%	2%
L210W	17%	6%	72%	5%
T215Y/F	38%	3%	80%	7%
K219Q/E/N	60%	9%	58%	7%

* Patients included in these subgroups may have other RTI mutations or mutations in addition to the baseline mutations listed.

Table 1 shows response rates according to the presence or absence of specific mutations. In clinical isolates, however, mutations often occur in patterns, some of which are considered primary and others compensatory or accessory. Exploratory analyses should be conducted to define sets of mutational patterns with the largest effect on subsequent response rates.

For some drugs, defining specific mutational patterns that best correlate with a reduction in treatment response can be difficult. In these cases, another approach can be to investigate the number of baseline mutations that affects overall response. We encourage sponsors to explore how the number of baseline mutations correlate with maximal, reduced, or minimal virologic responses. For example, the response rate (less than 400 copies/mL) in patients with 1 to 2 protease inhibitor- (PI) associated mutations at baseline may be 80 percent, 45 percent if 3 to 4 PI-associated baseline mutations are present, or 10 percent if 5 or more PI-associated baseline mutations are present. For some drugs, the number and types of mutations may be important for overall clinical response. Therefore, we recommend sponsors conduct analyses as suggested in Tables 2A and 2B. Sponsors should discuss in advance with the division the specific mutations to be included in the overall mutation score. Additional exploratory analyses may be recommended to further investigate the effect of certain mutations on virologic response.

Table 2A. HIV RNA Response by Number of Baseline Mutations at Endpoint

Number of Baseline Mutations	Virologic Response Rate	
	Drug X (n =)	Control (n =)
No mutations	78%	80%
1-2 mutations	74%	76%
3-4 mutations	45%	41%
5 or more mutations	10%	3%

Note the number of PI mutations (4) used in Table 2B is for illustrative purposes. The number of mutations that affect virologic response rates vary for each drug; therefore, the number and type

of mutations used for analyses of HIV RNA response should be discussed with the division. We recognize the limitations of this type of analysis in that an interaction between two or more mutations may not be detected. However, this analysis may provide insight into baseline predicators of response and may provide useful information regarding relationships between number and type of mutations for further exploratory analyses.

Table 2B. HIV RNA Response by Number and Type of Mutations at Endpoint

Baseline Primary PI Mutation	Number of Mutations			
	Drug X		Control	
	< 4 PI Mutations %	≥ 4 PI Mutations %	< 4 PI Mutations %	≥ 4 PI Mutations %
46	77%	32%	38%	26%
54	75%	24%	67%	31%
73	67%	34%	75%	50%
82	53%	31%	50%	31%
88	75%	44%	58%	25%
90	70%	40%	56%	9%

2. Development of HIV Mutations

We strongly recommend genotypic testing on all patients who meet the protocol-defined definition of a lack or loss of virologic response, preferably while on study drug or as soon as possible after discontinuation of study drug. Studies have shown WT virus may outgrow resistant HIV strains in the absence of selective drug pressure. For this reason, it can be useful to collect and store samples for resistance testing at the same time points HIV RNA testing are done. These samples can provide important information on the development of resistance, especially for drugs that may have more than one possible resistance pathway.

The proportion of patients who develop any nucleoside analogue reverse transcriptase inhibitor- (NRTI), nonnucleoside reverse transcriptase inhibitor- (NNRTI), or PI-associated mutation and the time to development of these mutations (measured as time to virologic failure) should be presented. Both primary and secondary mutations should be evaluated. For example, for patients receiving a new PI, it is important to evaluate the development of primary and secondary PI mutations along with any other changes in the PR (protease) and RT gene, when applicable. It is also important to assess the genotypic basis of drug susceptibility changes attributable to extragenic sites, such as the protease cleavage sites.

3. Baseline Phenotype and Virologic Response

Analyses also should be conducted to define the decrease in phenotypic susceptibility that adversely affects virologic response (i.e., susceptibility breakpoints). Rather than a single breakpoint, we encourage sponsors to explore incremental subgroups associated with maximal, reduced, or minimal response rates. However, we recognize a single breakpoint may be more appropriate for some drugs. Importantly, the breakpoints determined for a given study are not meant to represent definitive clinical susceptibility breakpoints for all patient populations. Often the data in an initial NDA submission are based on a select patient population. Data displayed in package inserts are provided to give clinicians information on the likelihood of virologic success

based on pretreatment susceptibility to a given agent. Additional data are needed to determine definitive susceptibility breakpoints for a given drug. An example of this analysis is presented in Table 3.

Table 3. HIV RNA Response to Drug X by Baseline Susceptibility

Baseline Drug X Susceptibility	Drug X (n =) HIV RNA < 400 copies/mL
0-5	56%
>5-10	33%
>10	10%

4. Genotypic and Phenotypic Correlations: Changes in Susceptibility from Baseline

Assessing changes in susceptibility over time on treatment is an important factor in the characterization of a drug's resistance profile. For patients who meet the protocol-specified definition of a lack or loss of virologic response, evaluation of mean and median-fold changes in susceptibility from baseline for the investigational drug and other approved drugs from both inside and outside the same class is important. In addition, analyses should be conducted on patients who develop a particular new mutation during treatment, and the median-fold change in susceptibility from baseline should be presented. Table 4 shows an example of how data from this analysis can be displayed. Efforts also should be made to define relationships between genotype and phenotype as shown in Table 5.

Table 4. Development of Mutations and Median Change in Susceptibility from Baseline

Patients Developing New Entry Inhibitor Mutations	N	Median-Fold Change in Susceptibility from Baseline	
		Drug X	Control
Mutation A			
Absent through week 24		2.1	3.2
Present by week 24		10.5	5.5
Mutation B			
Absent through week 24		1.6	2.6
Present by week 24		5.2	2.2
All patients analyzed		1.9	3.2

Table 5: Relationship between Genotype and Phenotype and Response

Number of Baseline Mutations	Drug X	Control	Baseline Phenotype	Drug X Susceptibility
1-2	78%	75%	0-5	Susceptible
3-4	43%	45%	>5-10	Decreased susceptibility
5 or more	10%	3%	>10	Resistant

5. Cross-Resistance

The evaluation of cross-resistance with other drugs in the same class is important. Characterization of cross-resistance of a drug can provide health care providers and patients with information on how to choose and sequence antiretroviral drugs. Evaluation of the effect of the investigational drug on subsequent use of other drugs and how previous treatment with other drugs may affect the response to the investigational drug is essential in drug development. The former can be accomplished by designing rollover studies evaluating virologic response rates in patients discontinuing study drug in clinical trials.

We encourage sponsors to incorporate prospective rollover designs to provide for assessment of virologic responses in study patients administered subsequent antiretroviral regimens. When possible, the design of a rollover study should include a randomized control. Every effort should be made to capture as much information as possible from the original studies. Resistance testing can be used to assess the genotype and phenotype of antiretroviral-experienced patients that predict success or failure after exposure to previous therapies. This testing can involve longer follow-up of study patients, perhaps continuing into the postmarketing period.

6. Additional Analyses

In addition to the analyses previously suggested, sponsors should consider conducting the following investigations:

- Genotypic sensitivity scores (GSS) and phenotypic sensitivity scores (PSS) and virologic outcome analyses can be investigated in all phases of development. Sponsors should discuss with the division in advance methods to calculate GSS and PSS for the optimized background regimens.

- Sponsors are encouraged to conduct exposure-response analyses throughout the drug development process, beginning with phase 2a studies in HIV-infected patients. One goal of exposure-response evaluations is to aid dose selection for phase 2b and phase 3 studies. Results from exposure-response evaluations also can help determine whether dose adjustments are warranted for special populations and whether therapeutic drug monitoring may be helpful in some patients.

  Exposure-response evaluations require the collection of exposure data (drug concentrations) and response data (efficacy and safety). The sponsor should discuss

exposure-response evaluation plans with the division and the Office of Clinical Pharmacology before study initiation. Exposure-response evaluations should indicate which drug exposure measures (e.g., area under the curve, C_{trough}) are relevant to a given virologic outcome. In addition, sponsors are encouraged to evaluate the proportion of responders to a given drug regimen by inhibitory quotient (IQ), steady-state C_{min} values, and other methods, as appropriate. An IQ value is the ratio of C_{min}/EC_{50} (protein-binding corrected).

- Pharmacogenetic analyses can be conducted to determine genetic factors that may be involved in virologic response (e.g., for co-receptor inhibitors targeting a host receptor, genetic differences in the receptor may affect response).

D. Other Considerations

1. Role for Supporting Initial Activity and Dose-Finding Studies

Primary objectives of initial studies in HIV-infected patients should be to establish that the new investigational agent has anti-HIV activity, to determine the magnitude of that effect, and to determine the most active dose that can be taken forward in larger studies. Often, studies that incorporate short periods of monotherapy (e.g., less than or equal to 2 weeks) or functional monotherapy (when a drug is added to a failing but stable regimen) have been helpful in accomplishing these objectives. Compared to combination studies, such protocols can more clearly delineate the effect of the drug of interest. However, resistant viruses sometimes emerge rapidly for certain drugs, such that periods of monotherapy can jeopardize a patient's future therapeutic options. Thus, some drugs are not candidates for use in monotherapy trials, including trials of short duration. Drugs for which a single mutation is easily selected and able to confer large reductions in susceptibility to the new drug and other drugs of the same class should not be studied as monotherapy, particularly in treatment-naïve individuals. Data from cell culture resistance studies, along with pharmacokinetic and safety data, should be used to determine whether a new drug could be safely administered as a single agent for limited periods of time.

2. Data Collection from Dose-Finding Trials

Sponsors should collect baseline genotype and phenotype information in HIV-infected patients who participate in pharmacokinetic and dose-finding studies. As stated previously, we encourage sponsors to analyze baseline resistance information and outcome in treatment-experienced patients. Analysis of baseline resistance and virologic outcome data in treatment-naïve patients may not be routinely needed; however, analysis of this information may be helpful to interpret unexpected virologic results. Current evidence indicates virologic response is better when drug levels can be maintained some increment above the serum-adjusted EC_{50} value (see section III, HIV Resistance Testing — General). Study patients with baseline resistance mutations may require higher drug concentrations of the antiretroviral drug to achieve an antiviral response that is comparable to that in patients with WT virus. Patients with particular genotypes and/or phenotypes of interest should be prospectively identified for inclusion in dose-ranging studies.

3. Use of Resistance Data to Establish an Indication

The amount of evidence sufficient to characterize a drug's general resistance profile and the amount of data to support a specific efficacy claim against a particular resistant strain of HIV are not always the same. Well-controlled, randomized, prospective trials are preferred when attempting to develop a drug product to obtain a specific indication for use in a select group of patients with a particular resistance profile at baseline or antiretroviral treatment history. The amount of evidence sufficient to establish an efficacy claim in a specific patient population will be substantial, and studies of small numbers of patients are unlikely to accomplish this goal. Sponsors are encouraged to discuss their development plans with the division in advance. In addition, resistance data also can be considered for usage statements in a package insert.

4. Use of Resistance Data for Study Enrollment Criteria, Background Regimen Selection, and Stratification Factors

As mentioned earlier, resistance testing at baseline can be helpful in selecting antiretroviral-experienced study patients with particular resistance profiles. For example, to evaluate the efficacy of a new NNRTI in patients who have failed previous NNRTI regimens, sponsors can elect to enroll patients with confirmed genotypic and/or phenotypic NNRTI resistance. We strongly encourage sponsors to use all available phase 1 and phase 2 and nonclinical data to define which mutation or mutations adversely affect response rate. Early determination of the effect of baseline genotype and phenotype for new investigational agents can be important for patient selection into phase 3 clinical trials, thereby restricting specific mutational patterns or limiting the number of mutations to ensure all patients, regardless of randomized treatment, have a reasonable chance of virologic response.

Some studies evaluate a new drug combined with an *optimized background*, meaning that the concomitantly administered antiretrovirals were chosen based on data from resistance testing. For trials that include an optimized background regimen, genotypic and/or phenotypic resistance testing is used to guide the selection of the background antiretroviral regimen. In addition, some studies have used genotypic and/or phenotypic susceptibility scores to quantify the number of drugs to which a patient may still be susceptible. Sponsors also can consider using an external expert committee to aid in the selection of background regimen, especially for patients with limited therapeutic options.

Baseline resistance testing also can be used to stratify patients. Rationale for specific stratification factors should be discussed with the division in advance.

5. Nonclade B Subtypes

Sponsors are also encouraged to evaluate baseline resistance data and response and the development of resistance in patients with nonclade B viruses versus clade B viruses. Since many sponsors are conducting global development plans and it is unknown if different HIV subtypes develop resistance via different pathways, these data will add to the overall characterization of an investigational drug's resistance profile (Hirsch et al. 2003).

E. Monitoring during Phase 4

Resistance should continue to be monitored and further described during postmarketing of an antiretroviral drug (phase 4). As more drugs enter the market, additional cross-resistance studies can help to further characterize the cross-resistance profile between new and existing antiretroviral agents.

VI. SUMMARY

Resistance analyses provide information regarding which mutations may have an effect on the therapeutic success of a given antiretroviral drug product. Such information potentially can be included in drug labeling to facilitate appropriate prescribing of products and to maximize the chance for therapeutic success.

The preceding sections of this guidance include recommendations for how and when to obtain HIV drug resistance information. Appendix A provides guidance for submitting HIV resistance data. The decision that any given mutation is clinically relevant and deserves inclusion in product labeling will be determined during the course of an NDA review.

GLOSSARY

Accessory or compensatory mutation: A mutation that by itself does not confer a decrease in susceptibility to antiretroviral agents. Accessory or compensatory mutations can augment key mutations and, perhaps, fitness mutations.

Fitness mutation: An amino acid change that compensates for the reduced virus growth resulting from a drug resistance-conferring mutation.

Key mutation: A treatment-selected amino acid change that can cause a decrease in susceptibility to one or more antiretroviral agents of the same class.

Polymorphism: Natural variation in the HIV-1 genome.

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APPENDIX A:
GUIDANCE FOR SUBMITTING HIV RESISTANCE DATA

Sponsors are encouraged to use the following sample format for submitting HIV resistance data.

One dataset combines patient data, endpoint data, genotypic data, and phenotypic data. There are a number of ways datasets can be subdivided (i.e., by clinical study, baseline isolates, or virologic failure isolates) and this should be discussed with the division before submission.

For each study, we recommend constructing datasets as SAS transport files containing the following information:

- One record (row) per patient per isolate (e.g., baseline, failure, and other time points).
- Data in columns (with suggested column headings shown below)[4] on all isolates.
- Genotypic data should be provided on the corresponding record for each patient isolate for baseline isolates of all patients in treatment-experienced studies and the endpoint isolates of virologic failures and discontinuations in all studies.[5] In treatment-naïve studies, a baseline sample should be collected and stored from all patients for future phenotypic and genotypic analysis of virologic failures.
- Phenotypic data should be provided on the corresponding record for each patient isolate for baseline isolates and the endpoint isolates of virologic failures and discontinuations.[5] In treatment-experienced studies, it is recommended that baseline phenotypic data be obtained for all patients.

The specific criteria for defining virologic failures should be discussed with the division and may include multiple primary and secondary protocol endpoints. The endpoints for clinical virologic and resistance outcome analyses should be consistent.

Information to Include with Suggested Column Headings[4]

I. Patient Data:

- Patient identification number (ID number should be unique for all studies)
- Isolate (e.g., baseline, week 24, week 48, discontinuation. Multiple isolates should be numbered.)
- Date of isolate
- Study day (number of days since the patient started the study product)
- Previous therapeutic products where available
- Treatment group
- Censored for analysis (yes or no)

[4] In the SAS transport files, column headings can be given abbreviated column names to fit the SAS format; however, it is suggested that a description of column names be provided to the reviewer in the submission.

[5] Treatment and endpoint samples should be collected when the patient is still on the study product.

II. Endpoint Data:

- HIV RNA (copies/mL) at baseline (viral loads less than LLOQ should be listed as "<LLOQ value" and values greater than ULOQ should be listed as "<LLOQ value" and not left blank)
- HIV RNA (copies/mL) at predefined time points (e.g., week 24 and week 48), one column for each time point including baseline (i.e., the viral load throughout the course of the study is provided for each sample)
- HIV RNA (copies/mL) at other times (e.g., loss of virologic response or discontinuation because of adverse event)
- Endpoint assessment (e.g., mean log change in viral load from baseline)
- Other endpoint assessments (e.g., DAVG)
- Indication of data were censored for reasons other than virologic failure (e.g., discontinuation because of adverse event)
- Outcome (i.e., responder, virologic failure, discontinuation while suppressed, discontinuation before achieving viral suppression)
- Reason for discontinuation (i.e., adverse event, pregnancy) or failure (i.e., never suppressed, rebound)
- HIV RNA (copies/ml) from additional time points can be included

III. Genotypic Data:[6]

- Clade
- Genotype for the RT, protease, and gp160 (for products targeting entry only), one amino acid per column with the WT amino acid as column heading. Changes from WT standard sequence indicated (i.e., blanks indicate no change).
- Column with total number of PI mutations in patient isolate (for baseline and endpoint isolates). The specific mutations to include should be discussed with the division in advance.
- Column with total number of NRTI mutations in patient isolate (for baseline and endpoint isolates). The specific mutations to include should be discussed with the division in advance.
- Column with total number of NNRTI mutations in patient isolate (for baseline and endpoint isolates). The specific mutations to include should be discussed with the division in advance.

Example (Table A highlights how genotype information can be displayed, but does not include all column headings previously suggested.)

[6] Genotypic data should be provided for baseline isolates of all patients in treatment-experienced studies and the endpoint isolates of virologic failures and discontinuations in all studies. In treatment-naïve studies, a baseline sample should be collected and stored from all patients for future phenotypic and genotypic analysis of virologic failures.

Table A. Example of Genotype Information Display

Patient Number	Isolate	V-82	N-83	I-84	I-85	G-86	R-87	N-88	L-89	L-90	Number of PI Mutations
001	BL							S		M/L	2
001	WK48			V				S		M	3
002	BL	A/T		V				D		M	4
002	WK48	T		V						M	3
003	BL	T		V							2
004	BL			V						M	2

BL = baseline
WK48 = week 48 for investigational product

IV. Protease Cleavage Sites (for protease inhibitors only):

- NC/p1 Gag cleavage sites: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant
- p1/p6 Gag cleavage sites: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant

V. Phenotypic Data:[7]

1. *Information on the investigational product*
   - Baseline EC_{50} value for investigational product
   - Baseline EC_{50} value of reference strain for investigational product
   - Fold resistant change of baseline EC_{50} value compared to EC_{50} value of reference strain of investigational product
   - EC_{50} value at time of endpoint assessment or failure for investigational product
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to reference strain for investigational product
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to baseline for investigational product
   - Replication capacity (if available)

2. *Information on approved and other investigational anti-HIV products (if available) in the same class*
   - Fold change in EC_{50} value of baseline compared to reference strain for each of the approved and other investigational anti-HIV products (if available)
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to reference strain for each of the approved and other investigational anti-HIV products (if available)
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to baseline for each of the approved and other investigational anti-HIV products (if available)

[7] Phenotypic data should be provided for baseline isolates and the endpoint isolates of virologic failures and discontinuations. In treatment-experienced studies, it is recommended that baseline phenotypic data be obtained for all patients.

3. *Information on approved and other investigational products (if available) outside the investigational product's class with same target protein (e.g., NRTIs and NNRTIs)*
   - Fold change in EC_{50} value of baseline compared to reference strain for approved and other investigational products (if available) outside the investigational product's class
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to reference strain for each of the approved and other investigational products (if available) outside the investigational product's class
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to baseline for each of the approved and other investigational products (if available) outside the investigational product's class

4. *Information on other antiretroviral products in the regimen*
   - Fold change in EC_{50} value of baseline compared to reference strain for other antiretroviral products in the regimen, one column per product
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to reference strain for other antiretroviral products in the regimen, one column per product
   - Fold change in EC_{50} value at time of endpoint assessment or failure compared to baseline for other antiretroviral products in the regimen, one column per product

Example (Table B highlights how phenotype information can be displayed, but does not include all column headings previously suggested.)

Table B. Example of Phenotype Information Display

	Agent X				Other Agents in the Same Class*		Other Agents Outside Agent Class*	
Sample	EC_{50} value Agent X	Ref strain EC_{50} value Agent X	Δ resis from ref Agent X	Δ resis from BL Agent X	Δ resis from ref Agent Y	Δ resis from BL Agent Y	Δ resis from ref Agent A	Δ resis from BL Agent A
Baseline								
Endpoint								

Agent X = candidate agent

Δ resis = fold resistance change, e.g.: $\frac{EC_{50} \text{ value of sample with Agent X}}{EC_{50} \text{ value of reference (or baseline) strain with Agent X}}$

Ref strain = reference strain (or WT)

Endpoint = predefined time point for endpoint assessment (e.g., week 24, week 48, failure or discontinuation)

*Note: The Δ resis from ref and Δ resis from BL should be included for all approved anti-HIV products

VI. Co-Receptor Usage (for all agents targeting co-receptors):

- Co-receptor usage of baseline isolates. Indicate R5, X4, D for dual-tropic, M for mixed-tropic, or D/M if the assay cannot distinguish between dual or mixed, in a column.
- Baseline R5 tropism assay value (e.g., RLUs).
- Baseline X4 tropism assay value (e.g., RLUs).

- Co-receptor usage of virologic failures and end-of-study isolates (on therapy). Indicate R5, X4, D for dual-tropic, M for mixed-tropic, or D/M if the assay cannot distinguish between dual or mixed, in a column.
- R5 tropism assay value at failure or end of study (e.g., RLUs).
- X4 tropism assay value at failure or end of study (e.g., RLUs).

VII. Therapeutic Drug Monitoring Data (when available):

- Patient's C_{min}
- Serum adjusted IQ (inhibitory quotient = C_{min}/serum adjusted EC_{50} value)