
Guidance for Industry

Antiviral Product

Development — Conducting

and Submitting Virology

Studies to the Agency

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

**June 2006
Clinical Antimicrobial**

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Guidance for Industry¹

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I. INTRODUCTION

The purpose of this guidance is to assist sponsors in the development of antiviral drugs and biological products (i.e., therapeutic proteins and monoclonal antibodies) from the initial pre-IND through the new drug application (NDA) and postmarketing stages. This guidance should serve as a starting point for understanding what nonclinical and clinical virology data are important to support the submission of an investigational new drug application (IND), NDA, or biologics license application (BLA) for approval of an antiviral product. This guidance focuses on nonclinical and clinical virology study reports and makes recommendations for collecting and submitting resistance data to the Food and Drug Administration (FDA). Nonclinical and clinical virology study reports, based on collected data, are essential for the FDA's review of antiviral drug investigational and marketing applications. Specific topics discussed in this guidance include:

- Defining the mechanism of action
- Establishing specific antiviral activity of the investigational product
- Assessing the potential for antagonism of other antiviral products that might be used in combination with the investigational product
- Providing data on the development of viral resistance to the investigational product
- Providing data that identify cross-resistance to approved antiviral products having the same target

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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.

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II. BACKGROUND

The recommendations in this guidance are based on the antiviral product review experience of the Division of Antiviral Products and input from pharmaceutical sponsors and the scientific community. Because of the experience, history, and lessons learned with HIV-1 studies, this guidance employs studies commonly used to evaluate HIV-1 products as a paradigm for studies of products to treat other viruses. Although assays and model systems vary with different viruses, many of the principles in this guidance can be applied to antiviral products in development for the treatment of other viral infections (e.g., hepatitis B virus, hepatitis C virus, herpes simplex virus, varicella zoster virus, influenza virus, rhinovirus, cytomegalovirus, and human papillomavirus). Since the field of virology is dynamic and continually evolving, this guidance will be revised as new information accumulates and as circumstances warrant.

Sample formats have been developed to assist sponsors in providing resistance data to the Agency (see the stand-alone documents accompanying this guidance). The sample formats are being provided as stand-alone documents to help sponsors provide the appropriate information to the Agency. These sample formats will be updated as needed, and additional formats for other viruses may be provided.

Guidances on the overall organization of INDs and NDAs can be found at www.fda.gov/cder/regulatory/applications/default.htm. Sponsors are encouraged to contact the division early in the development of an investigational antiviral product to facilitate the review and approval process. To assist prospective sponsors, the FDA accepts submissions in an abbreviated IND format (i.e., pre-INDs) for review and comment. Pre-INDs are especially useful in instances when sponsors are unfamiliar with the process for evaluating investigational products in humans. Information about submitting pre-INDs to the division (“Getting Started with the Pre-IND Process”) can be found at www.fda.gov/cder/ode4/preind/getting.htm. The FDA accepts electronic submissions to expedite the review process. FDA Web sites can be consulted for information on electronic application submission.

Sponsors are advised to consult the division for additional guidance on the development of investigational products against orthopoxviruses, influenza virus, severe acute respiratory syndrome (SARS) virus, or other emerging infections. For antiviral products that treat smallpox and other orthopoxviruses, related information can be found in the draft guidance for industry *Vaccinia Virus — Developing Drugs to Mitigate Complications from Smallpox Vaccination*.² Sponsors should give careful attention to observing all provisions of the Select Agent rule³ and other applicable governmental and institutional biosafety and biosecurity provisions.

² When final, this guidance will represent the FDA’s current thinking on this topic. For the most recent version of a guidance, check the CDER guidance Web page at <http://www.fda.gov/cder/guidance/index.htm>.

³ See 42 CFR part 73 and <http://www.cdc.gov/od/sap/sitemap.htm>.

III. NONCLINICAL VIROLOGY STUDIES

Nonclinical virology studies aid in the evaluation of the safety and efficacy of an investigational product before it is tested in humans. Recommended studies can identify the mechanism of action, help establish specific antiviral activity of the product in a model system, and provide data on the development of viral resistance to the investigational product. Additionally, co-administration of an investigational product with other products approved for the same indication is often necessary to treat viral infections in clinical settings. In these cases, it is desirable to have in vitro combination activity studies designed to identify possible negative interactions on antiviral activity (i.e., antagonism) of the investigational product with other antiviral products.

As more antiviral products are developed to treat particular viral diseases, cross-resistance (i.e., viral resistance to one product causing resistance to more than one product within that drug class) can become a major issue in clinical settings. Therefore, from a scientific perspective, the following information is critical in the development of antiviral products:

- Determining the antiviral activity of an investigational product against relevant viruses resistant to other approved products with the same target molecule or complex
- Determining the antiviral activity of approved products against viruses resistant to an investigational product with the same target molecule or complex

We recommend conducting nonclinical studies (i.e., mechanism of action, antiviral activity in vitro, cytotoxicity and therapeutic indexes, and effects of serum protein binding on antiviral activity) before the initiation of phase 1 clinical studies. When developing products for viruses where in vitro infection systems exist, sponsors should complete in vitro drug combination activity studies of the investigational product with other approved products against the same virus (i.e., all approved and available investigational products that target the same protein and at least one representative product from each of the other existent drug classes) before the initiation of clinical trials that will examine the efficacy of the investigational product in combination with other antiviral products. Furthermore, we recommend examining the in vitro selection of resistant viruses to the investigational product, the phenotypic and genotypic characterization of resistant viruses, and cross-resistance before initiation of clinical studies in patients infected with a particular virus. Complete study reports on nonclinical and clinical virology studies can be submitted to the FDA upon their completion and need not be held until submission of the NDA. Specific details of each of the nonclinical studies recommended by the division are discussed in the following sections.

A. Mechanism of Action Studies

Mechanism of action studies should be conducted before the initiation of phase 1 clinical studies. There are many steps in a virus life cycle that can be targeted by potential antiviral product candidates. Products can act directly to inhibit a virus by targeting a specific viral-encoded function (e.g., an enzyme inhibitor) or act indirectly (e.g., interferon induction of host cell response). We recommend that mechanism of action studies:

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- Demonstrate the investigational product's ability to specifically inhibit viral replication or a virus-specific function
- Establish the site of the product's action (e.g., viral replicase, protease)

Biochemical, structural, cellular, or genetic data can be presented to support the proposed mechanism of action. Data that demonstrate the mechanism of action include, but are not limited to, receptor binding, inhibition of enzymatic activity, X-ray crystallographic structure determination of bound inhibitor complex, and characterization of resistance mutations in the gene encoding the target.

A well-characterized mechanism of action is useful in predicting toxicities and in designing studies to assess the development of resistance. A clear understanding of the mechanism of action for an investigational antiviral product can provide insight into the regions of the viral genome where resistance mutations could develop. These regions are not limited to the site of action (viral-encoded target) of the investigational product but can include the enzyme substrate or another viral-encoded protein existing in a complex with the target protein. Characterization of the resistance mutations can provide *in vivo* validation of the mechanism of action studies.

The specificity of the investigational product should be demonstrated for the viral target over cellular or host proteins, especially in those cases in which a viral enzyme has a cellular counterpart. For example, if the investigational product targets a viral polymerase, we recommend showing the activity of the product against the viral polymerase in comparison with its activity against host DNA polymerases such as DNA polymerase α , β , and γ .

Immunomodulatory products raise additional issues. These products can have the potential for unintended effects on viral replication and other adverse effects resulting from actions on the immune system. For investigational products that act through a general immune stimulatory mechanism, we recommend that sponsors show a reduction in antiviral activity and identify the specific immune system components that are involved. Sponsors should consult the division for specific advice regarding the development of immunomodulatory products for the treatment of viral diseases as well as products with other nonviral host targets.

B. Antiviral Activity

1. Antiviral Activity in Vitro

For many human viruses, there are cell culture systems or animal hosts in which the infectious agent can undergo a complete virus life cycle. In these cases, we recommend that the sponsor document that the investigational product and/or its metabolites show specific, quantifiable antiviral activity *in vitro* before initiating tests in humans (i.e., before initiation of phase 1 studies). It is important that these data support clinical testing in humans by providing clear evidence of antiviral effects at drug concentrations that can be achieved *in vivo* with acceptable risk-benefit. Additionally, *in vitro* antiviral activity and cytotoxicity assessments (see Section III.C., Cytotoxicity and Therapeutic Indexes) using relevant cell types and virus isolates can be

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used to guide the selection of appropriate dose ranges in early clinical trials. Sponsors are encouraged to obtain antiviral activity data using primary human target cells, if possible. Because of viral genetic variation, the antiviral activity of the investigational product should be examined for multiple clinical isolates and viral isolates representative of the virus population in clinical trials. Antiviral activity evaluations that are recommended to support the development of the investigational product include:

- Assessing specific antiviral activity of the investigational product against a broad range of clinical and laboratory viral isolates including different clades, subtypes, or genotypes
- Evaluating the antiviral activity of the investigational product against mutant viruses that are resistant to products with the same target molecule or complex as the investigational product as well as a representative sample of viruses resistant to other approved products for the same indication

We recommend determining specific antiviral activity using a quantitative assay to measure virus replication in the presence of increasing concentrations of the product compared to replication in the absence of the product. The effective concentration is the concentration of product at which virus replication is inhibited by 50 percent (e.g., EC₅₀ for cell-based assays; IC₅₀ for biochemical or subcellular assays). Assays that evaluate antiviral activity and cytotoxicity include, but are not limited to, virus inactivation assays, plaque reduction assays, cytopathic effect inhibition assays, peripheral blood mononuclear cell (PBMC) assays, and binding and fusion assays. Other factors that can be assessed in these assays include the effect of an increasing multiplicity of infection and the effect of pretreatment of virus or cells before infection versus treatment post-infection. We suggest that host cell lines be low in passage number for reproducible results.

It is important that the effective concentration be consistent with data supporting the mechanism of action. An investigational product that inhibits virus replication at concentrations lower than biochemical data for the proposed mechanism indicates that another target or mechanism of inhibition may be affected. Resistance analyses can provide *in vivo* validation of the proposed mechanism of action when biochemical data are not consistent with antiviral activity data. For nucleoside or nucleotide analogs, we recommend that sponsors determine the intracellular half-life ($t_{1/2}$) of the triphosphate form of the active drug moiety in stationary and dividing cells from the target tissue.

For some human viruses (e.g., hepatitis B and hepatitis C viruses), no satisfactory cell culture or animal model exists, and in these cases, inhibition of an essential viral function or activity against related viruses can be used to indicate potential activity. When no satisfactory cell culture or animal model exists for the target human virus, it is particularly important to know whether or not the active moiety of an antiviral product enters cells, if it has a proposed intracellular site of action, and if the intracellular concentration correlates with biochemical studies. Cell-based assays and host cell lines for studying viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV) replication have advanced, but at the present time are limited. Currently, assays that examine HBV replication include, but are not limited to:

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- Measuring HBV DNA polymerase activity in biochemical assays
- Cell culture assays with baculovirus-mediated cell transfer or transfection of HBV genomes into human hepatoma cell lines followed by quantitative Southern blot analysis with HBV DNA probes
- Cell culture assays with stably-transfected cell lines containing the HBV genome
- Quantitative PCR of extracellular HBV DNA

For analyses of HCV replication, replicon systems have been developed that permit studies of viral replication and can be used to assess antiviral activity of some anti-HCV products.^{4,5} In addition, recent studies indicate that cell-based systems for the replication of HCV in vitro are in development and might be available to assess antiviral activity.⁶ Currently, assays that examine HCV replication using the HCV replicon system include, but are not limited to:

- Measuring the level of HCV RNA by RT-PCR in HCV replicon cells
- Measuring HCV RNA polymerase, HCV serine protease, or reporter enzyme (e.g., luciferase) activity in biochemical assays

2. Antiviral Activity in Vitro in the Presence of Serum Proteins

Serum proteins can bind to and sequester many products and interfere with a product's antiviral activity. We recommend that sponsors ascertain if the investigational product is significantly bound by serum proteins. Common methods for determining protein binding include equilibrium dialysis, ultrafiltration methods, and fluorescence-based high throughput human serum albumin and α -acidic glycoprotein protein binding. If the investigational product is highly protein bound, sponsors are encouraged to examine the in vitro antiviral activity of the investigational product in the presence of a series of dilutions of human serum up to 40 percent (e.g., 5 percent, 10 percent, 20 percent, 40 percent). An EC₅₀ value for 100 percent human serum can be extrapolated from these data and the serum-adjusted EC₅₀ values reported. In addition, sponsors are encouraged to determine EC₅₀ values in the presence of physiological concentrations of α -acidic glycoprotein and human serum albumin.

⁴ Lohmann V, F Korner, J-O Koch, U Herian, L Theilmann, and R Bartenschlager, 1999, Replication of Subgenomic Hepatitis C Virus RNAs in a Hepatoma Cell Line, *Science* 285:110-113.

⁵ Blight K, A Kolykhalov, and C Rice, 2000, Efficient Initiation of HCV RNA Replication in Cell Culture, *Science* 290:1972-1974.

⁶ Wakita T, T Pietsschmann, T Kato, T Date, M Miyamoto, A Zhao et al., 2005, Production of Infectious Hepatitis C Virus in Tissue Culture from a Cloned Viral Genome, *Nat Med.* 11:791-6.

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3. *Inhibitory Quotient*

Information on plasma and intracellular product concentrations is important in assessing the dose-response of antiviral therapy and evaluating the potential for resistance development; therefore, it is useful to determine an inhibitory quotient (IQ). An IQ is the C_{\min} /serum-adjusted EC_{50} value. (For more information on determining EC_{50} values, see Section III.B.1., Antiviral Activity in Vitro.) We view IQ values as a useful tool integrating in vivo product concentrations and antiviral activity for an individual product. It is a measure that characterizes the relationship between product exposure and the susceptibility of a virus to a product. A high IQ indicates that an effective product concentration can be achieved in a patient to inhibit the virus and minimize the development of resistance. Since one dose may not be adequate for all patient populations, IQ values can be used to aid in the selection of doses to further evaluate in phase 3 and phase 4 clinical studies.

4. *Antiviral Activity in Vivo*

In cases where no in vitro cell culture or replicon system has been shown to be predictive of antiviral activity in humans, measurement of viral titers after treatment in animal model systems can be used to assess antiviral activity of the investigational product. Analyses from animal models include the morbidity and mortality of animals following documented infection, histological examination of tissues, quantification of viral titers over time, isolation and characterization of resistant isolates in animals that experience viral rebound, quantification of viral antigens and antibodies, pharmacokinetics of the investigational product, and a description of symptoms (e.g., neurological, weight loss).

C. Cytotoxicity and Therapeutic Indexes

It is important to establish that an investigational product 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 product can be the result of host cell death after exposure to the product. Cytotoxicity tests use a series of increasing concentrations of the antiviral product to determine what concentration results in the death of 50 percent of the host cells (see also Section III.B.1., Antiviral Activity in Vitro). This value is referred to as the median cellular cytotoxicity concentration and is identified by CC_{50} or $CCIC_{50}$. The relative effectiveness of the investigational product 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.

Because of the myelosuppressive effects of some antiviral products, we recommend assessing the potential effects of certain investigational products (e.g., nucleoside analogs) on the growth of human bone marrow progenitor cells in colony formation assays. Additionally, some investigational antiviral products are potential inhibitors of cellular DNA polymerases, which are

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responsible for normal nuclear and mitochondrial DNA synthesis and repair. Sponsors should determine IC₅₀ values for investigational antiviral products against cellular polymerases and show specificity for the viral target over cellular polymerases. The inhibition of human pol γ , the enzyme responsible for mitochondrial DNA synthesis, has been linked to defects in mitochondrial function that can lead to adverse events in humans. Therefore, it is important to examine the effects of certain investigational products (e.g., nucleoside analogs) on human pol γ activity and on mitochondrial toxicity (e.g., lactic acid production, mitochondrial DNA content, mitochondrial morphology, glucose utilization).

D. In Vitro Combination Activity Analysis

Within an infected individual, viruses can exist as a heterogeneous population of variant viruses, some of which may show reduced susceptibility to one or more antiviral products. Therefore, for some viruses, administration of multiple antiviral products (e.g., three-drug combination antiretroviral therapy against HIV-1) can be more effective than a single product in establishing and maintaining inhibition of virus replication. However, the interactions of products are complex and can result in antagonistic, additive, or synergistic effects with respect to antiviral activity. For this reason, sponsors should evaluate the in vitro antiviral activity of investigational products in two-drug combinations with other products approved for the same indication. Specifically, combinations that should be tested include the investigational product with all approved products that target the same protein and at least two appropriate products from each class of products approved for the same indication. We recommend completing the in vitro drug combination activity studies of the investigational product with approved products before initiation of clinical trials that will evaluate the efficacy of the investigational product in combination with other antiviral products. Often patients are infected with two or more viral diseases (e.g., HIV and HBV or HCV); therefore, we also recommend that the in vitro antiviral activity of antiviral products used in co-infected patients for different indications be assessed in in vitro combination activity studies.

E. Resistance

1. Selection of Resistant Virus in Vitro

This guidance focuses on resistance to antiviral products caused by mutations in viral genomes that result in reduced phenotypic susceptibility to a given antiviral product. Resistance, as it is used here, is not an absolute term, but relative. We recommend that the in vitro selection of resistant viruses to the investigational product, the phenotypic and genotypic characterization of resistant viruses, and cross-resistance analyses be examined before initiation of clinical studies in patients infected with the particular virus. We understand that the resistance data generated in vitro are not necessarily predictive of clinical resistance. However, in vitro resistance selection studies are recommended to assess the potential barrier of a target virus to develop reduced

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susceptibility (i.e., resistance) to the investigational product and to aid in designing clinical studies.⁷

Selection in cell culture of virus resistant to the investigational product can provide insight into whether the genetic threshold for resistance development is high or low. A product with a low genetic threshold may select for resistance with only one or two mutations. In contrast, a product with a high genetic threshold may require multiple mutations to select for resistance. Several factors specific to the investigational product and the target virus affect the development of resistance (e.g., product concentration). The rate of appearance of mutant viruses depends on the rate of replication of the virus, the number of virus genomes produced, the fidelity of the replicative machinery, and host factors. Consideration of these factors can help in designing tests to detect resistant virus in vitro. For example, when multiple mutations are required to develop resistance to high concentrations of the investigational product, many cell culture systems do not produce sufficient virus titers to select resistant virus. In these instances, serial passage of the virus in cell culture under conditions of increasing concentrations of the investigational product can lead to the isolation of resistant virus. Sponsors are encouraged to assess the development of resistance in vitro over the concentration range spanning the anticipated in vivo concentration. Selection of variants resistant to the investigational product should be repeated more than once (e.g., with different strains of wild-type, with resistant strains, under high and low selective pressure) to determine if the same or different patterns of resistance mutations develop, and to assess the relationship of product concentration to the genetic barrier to resistance.

If the targeted virus replicates in a cell culture system, two basic methods can be employed to isolate viruses that have reduced susceptibility to the investigational product:

- A high initial virus inoculum is propagated for several passages at a fixed product concentration, using multiple cultures to test different concentrations.
- A low initial virus inoculum is passaged in the presence of increasing product concentrations starting near the EC₅₀ value for the parental virus.

Virus production is monitored by determining the genotype and phenotype of isolates throughout the selection process to detect the outgrowth of resistant viruses.

HCV resistance to investigational products can be examined using HCV replicon systems. Methods that select HCV resistance using HCV replicon cells include the following:

- HCV replicon cells are cultured at low density in the presence of neomycin and a fixed concentration of the investigational product using multiple cultures to test different

⁷ For an in-depth review of resistance issues relating to HIV-1, see the draft guidance for industry *Role of HIV Drug Resistance Testing in Antiretroviral Drug Development*. When final, the guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the CDER guidance Web page at <http://www.fda.gov/cder/guidance/index.htm>.

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concentrations. The cells containing resistant replicons will form colonies that are then expanded for genotypic and phenotypic characterization.

- HCV replicon cells are passaged in the presence of a fixed product concentration, but in the absence of neomycin, using multiple cultures to test different concentrations. The HCV replicon cells from each passage are harvested and stored for phenotypic and genotypic characterization.

2. Genotypic Analysis

Genotypic analysis of resistant viruses selected in vitro determines the mutations that might contribute to reduced susceptibility to the investigational product. Identifying resistance mutations by DNA sequence analysis of the relevant portions of the virus genome can be useful in predicting clinical outcomes and supporting the proposed mechanism of action of the investigational product. Sponsors should determine the entire coding sequence of the gene for the target protein and compare the pattern of mutations leading to resistance of the investigational product with the pattern of mutations of other products in the same class. For larger viruses (e.g., herpesviruses, poxviruses), the relevant portions of the viral gene targeted by the investigational product should be sequenced and analyzed for mutations that could contribute to product resistance (e.g., marker rescue). We recommend that resistance pathways be characterized in several genetic backgrounds (i.e., strains, subtypes, genotypes) and that isolates be obtained throughout the selection process to identify the order in which multiple mutations appear.

For genotypic assays, sponsors are encouraged to identify sequencing primers, state how many bases can be read from the primer accurately, and define the sensitivity of the genotypic assay used for detecting minority viral subpopulations. It is important that sponsors define what percentage of the population a mutation has to represent to be detected in their genotypic assay.

3. Phenotypic Analysis

Phenotypic analysis determines if mutant viruses have reduced susceptibility to the investigational product. When mutations that may be associated with resistance are identified by genotypic analysis, the ability of each of these mutations to confer phenotypic resistance should be evaluated in a recombinant virus system if possible (e.g., by using site-directed mutagenesis, PCR amplification of relevant portions of virus genome to introduce these mutations into a standard laboratory genetic background, or other suitable system). Recombinant viruses can then be tested in vitro for susceptibility to the product to determine an EC₅₀ value. The fold resistant change should be calculated as the EC₅₀ value of the isolate/EC₅₀ value of the reference or parental strain. Phenotypic results can be determined with any standard virus assay (e.g., protein assay, viral RNA assay, polymerase assay, MTT cytotoxic assay, reporter gene expression). The shift in susceptibility (or fold resistant change) for a viral isolate should be measured by determining the EC₅₀ values for the isolate and comparing it to the EC₅₀ value of a reference (well-characterized wild-type laboratory strain) or parental virus done under the same conditions and at the same time. The use of the EC₅₀ value for determining shifts in susceptibility is preferred because it can be determined with greater precision than an EC₉₀ or

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EC₉₅ value. The utility of a phenotypic assay depends on its sensitivity (i.e., its ability to measure shifts in susceptibility (fold resistance change) in comparison to reference, parental strains, or baseline clinical isolates). Calculating the fold resistant change (EC₅₀ value of isolate/EC₅₀ value of reference strain) allows for comparisons among phenotypic assays.

4. Cross-Resistance

Antiviral products targeting the same protein (typically products of the same drug class) may develop mutations that lead to reduced susceptibility to one antiviral product and can result in decreased or loss of susceptibility to other antiviral products in the same drug class. This observation is referred to as cross-resistance. Cross-resistance is not necessarily reciprocal, so it is important to evaluate both possibilities. For example, if virus X is resistant to drug A and drug B, and virus Y is also resistant to drug A, virus Y may still be sensitive to drug B. We recommend that the effectiveness of the investigational product against viruses resistant to other approved products in the same drug class and the effectiveness of approved products against viruses resistant to the investigational product be evaluated by phenotypic analyses. Additionally, we recommend that cross-resistance be analyzed between drug classes in instances where more than one drug class targets a single protein or protein complex (e.g., nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), which both target the HIV-encoded reverse transcriptase). We suggest that multiple recombinant and clinical isolates representative of the breadth of diverse mutations and combinations of mutations known to confer reduced susceptibility to products in the same drug class be tested for phenotypic susceptibility to the investigational product. If phenotyping is performed in cell lines with recombinant viruses, the EC₅₀ value should be validated with clinical isolates.

IV. PROPOSAL FOR MONITORING RESISTANCE DEVELOPMENT

It is important to disseminate information about an antiviral product's resistance profile to health care professionals and patients so that they can make optimal treatment decisions. Furthermore, crucial decisions in protocol design and product development plans often hinge on resistance and cross-resistance data. Therefore, it is strongly recommended that comprehensive resistance testing be undertaken during all phases of product development consistent with the way the product will be used in clinical practice.

For some viruses, measurements of changes in viral concentrations are accepted endpoints to determine clinical effectiveness of antiviral products. In these cases, assays that measure and monitor viral load can be used. Well-characterized genotypic and phenotypic assays provide the basis for examination of the emergence of resistant virus to investigational products and attempt to show a relationship between viral resistance and clinical virologic failure. In addition, phenotypic and genotypic results are used to define treatment options and predict the utility of treating an individual with an investigational product. Genotypic analysis of viral isolates from patients failing to respond to therapy or undergoing viral rebound can help identify mutations that contribute to reduced susceptibility to the investigational product. In addition, we recommend genotypic and phenotypic analyses of baseline isolates be used to determine

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response to treatment outcomes based on baseline mutations and polymorphisms and baseline phenotypic drug susceptibilities. In cases where measurements of viral concentrations have not been established as principal endpoints, development of assays and monitoring of resistance emergence will be important for exploratory analyses of relationships between virologic measurements and clinical outcomes and can assist in refining designs of future studies.

We recommend that sponsors develop and submit a plan for monitoring the development of resistant viruses in clinical studies before the initiation of clinical studies in virus-infected individuals. A resistance monitoring plan should include, but not be limited to:

- A description of the assays that will be used to monitor viral loads
- Viral load assay protocols and performance characteristics (if required)
- The genotypic and phenotypic assays that will be used
- Genotypic and phenotypic assay protocols and performance characteristics (if required)
- The methods for sample collection and storage
- The methods for sample handling and shipping (frozen or ambient)
- A description of additional resistance analyses
- Time points when samples for viral loads, genotypic and phenotypic assays, and other resistance analyses will be collected (i.e., baseline, week 24, week 48, following regimen failure or discontinuation)

The resistance monitoring plan should be included with the overall clinical development plan in the IND.

We also recommend developing and submitting with the IND plans for genotypic and phenotypic baseline studies and resistance substudies early in product development. We suggest that genotypic and phenotypic analyses of baseline and post-treatment isolates be completed in a timely manner to characterize the resistance profile of the investigational product and its cross-resistance potential with other antiviral products. For viruses such as HBV and HCV, baseline and post-treatment genotypic analyses are key to monitoring the development of genotypic resistance. Comparing genotypic analyses of samples from patients exhibiting virologic breakthrough (or rebound) with baseline samples can identify mutations associated with resistance. The extent and type of resistance monitoring and analysis should be discussed and agreed to with the division in advance.

Generally, in studies of treatment-experienced patients, sponsors are strongly encouraged to collect phenotypic and genotypic data for baseline isolates from all patients and endpoint isolates from all virologic failures and discontinuations (not suppressed). Virologic failure and discontinuation samples should be collected when the patient is still on the study product. In studies of treatment-naïve patients, phenotypic and genotypic data for baseline and endpoint isolates from all virologic failures and discontinuations (not suppressed) should be obtained. Therefore, in treatment-naïve studies, a baseline sample should be collected and stored from all patients for potential phenotypic and genotypic analysis of virologic failures. Additional genotypic and phenotypic assessments and subset analyses may be appropriate depending on the clinical study protocol or population, thus re-emphasizing the need to collect and store baseline

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samples and treatment samples throughout studies. In select cases, sponsors may propose to collect samples at baseline and at the time of failure on a subset of treatment-naïve patients when in vitro data indicate that acquisition of a single mutation results in a high degree of phenotypic resistance. However, any such proposal should be discussed with the division in advance.

Virologic failures and discontinuation definitions are protocol defined. Sponsors with investigational products can solicit advice from the division early in product development on definitions of clinical response-failure and plans to monitor resistance in clinical studies. We recommend that sponsors consult with the division for detailed descriptions and examples of how to submit clinical viral resistance data. In conjunction with this guidance, we are providing stand-alone documents to aid sponsors in submitting resistance data for HIV, HBV, HCV, and influenza studies. These sample formats may be updated periodically and additional formats for other viruses added as needed.

Sponsors can choose to quantify viral loads and conduct phenotypic and genotypic analyses themselves or send samples to companies that have been certified by Clinical Laboratory Improvement Amendments (CLIA). Proper handling procedures should be followed for laboratory samples. If an assay is not performed according to the manufacturer's specifications, the assay results might not be acceptable. Sponsors are encouraged to use approved assays (if possible) that are characterized and validated. If the assay is investigational, we recommend that sponsors provide the performance characteristics of the assay (e.g., accuracy, precision, limits of detection and quantification, specificity, linearity, range, robustness, stability), as well as sources of viruses (e.g., blood, plasma), their storage and stability, and cell culture procedures. For definitions on assay validation, refer to the guidance for industry *Bioanalytical Method Validation*, the guidance for industry *Antiretroviral Drugs Using Plasma HIV RNA Measurements — Clinical Considerations for Accelerated and Traditional Approval*, and the ICH guideline for industry *Q2A Text on Validation of Analytical Procedures*. Assays that will be used in clinical practice should have more extensive validation than exploratory assays that are used for the characterization of antiviral activity and resistance of investigational products. 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 (e.g., Data Master File) should be discussed with the division. We recommend that sponsors consistently use the same assay for any particular analysis or measurement in phase 3 studies and that the same assay be used for a particular patient throughout the study. Sponsors should provide data supporting the implementation and use of any new assays that become available during product development.

V. VIROLOGY STUDY REPORTS

Complete virology study reports can be extensive and should include the primary data and derived data, the procedures used to obtain the data, and information necessary to evaluate the data. Virology study reports should contain information on nonclinical studies, clinical antiviral activity of the investigational product, resistance development to the investigational product in treated patients, cross-resistance with other products in the same drug class, and baseline

Contains Nonbinding Recommendations

genotypic and phenotypic virologic response analyses (if applicable). The formats of virology study reports should be similar to that of a scientific publication and typically should include the following sections: summary, introduction, materials and methods, results, and discussion. The methods section should describe all the protocols employed and include a description of the statistical analyses used. Virology study reports on nonclinical and clinical studies can be submitted upon their completion and need not be held until submission of the NDA. If the NDA is submitted in the common technical document (CTD) format, sponsors should submit virology study reports and datasets in Module 5, Section 5.3.5.4, Other Studies, under the specific heading, Antiviral Reports.

VI. SUMMARY

This guidance identifies virology studies relevant to the development and application review of antiviral products for the treatment of viral infections. The goal of this guidance is to stimulate the generation of more complete analyses for antiviral products. Such analyses help provide data that support the introduction of an investigational product into humans and provide the data necessary for determining dose-response relationships, designing clinical trials, and selecting appropriate patient populations. Thus, the data collected during these studies can affect the therapeutic success of a given product.

Because nonclinical in vitro virology studies can provide useful information for the design of in vivo studies and can help predict the development of resistant viruses in vivo, we recommend conducting nonclinical studies before the initiation of phase 1 clinical studies. In vitro selected resistant viruses should be analyzed carefully before initiation of studies in patients infected with a particular virus. This guidance includes recommendations for how and when to perform virology studies. Such information could be included in product labeling to facilitate appropriate prescribing of antiviral products and maximize the chance for therapeutic success. To assist sponsors in providing data from clinical resistance studies, we have developed stand-alone documents that accompany this guidance and provide a format for submitting resistance data to the Agency.