Attachment to

Guidance on Antiviral Product Development — Conducting and Submitting Virology Studies to the Agency

Guidance for Submitting Influenza Resistance Data

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

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Contains Nonbinding Recommendations

GUIDANCE FOR SUBMITTING INFLUENZA RESISTANCE DATA

Sponsors are encouraged to use the following sample format for submitting influenza resistance data for microbiology review. Sponsors should begin these analyses in the earliest clinical stages of product development so that sample collection, storage, and analyses are well established at the time of the initiation of pivotal studies. If sponsors wish to submit additional information, they are encouraged to discuss these suggestions with the division in advance.

One dataset combines patient data, virologic data, genotypic data, and phenotypic data.[1] Selected clinical outcome data can also be included for exploratory analysis (we recommend discussion with the division to establish the format for including these data in the dataset). There are a number of ways datasets can be subdivided (i.e., by clinical study[1], baseline isolates, or designated later 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.
- For treatment studies, a row should be included for the initial isolate obtained before starting study drug (*baseline isolate*), even if virologic studies (e.g., culture, PCR) are negative. In cases where an assay was negative, insert <LLOQ (lower limit of quantification) or <LOD (limit of detection) where the specific numerical value of the LLOQ or LOD is inserted, as appropriate. Other isolates to be included for treatment studies, and inclusion and designation of isolates for prophylaxis studies, should be discussed with the division.
- Data in columns (with suggested column headings shown below)[2] on all isolates.
- In studies where both treatment and prophylaxis are evaluated, a column should be included identifying the patient as treatment or prophylaxis group.
- In most studies it is anticipated that all isolates for virologic analysis will be obtained using the same methodology (e.g., nasal washing, nasopharyngeal swab, throat swab) that will be described in the protocol and study report. If there is a reason for use of more than one method in the same study, discussion with the division is recommended.
- Genotypic data should be provided for (at a minimum) baseline isolates of all patients and the predetermined time points of isolate collection on the corresponding record for each patient isolate.
- Phenotypic data should be provided for (at a minimum) baseline isolates and the predetermined time points of isolate collection on the corresponding record for each patient isolate. The FDA recognizes the difficulty of phenotypic analysis of influenza virus and the lack of clinical validation of current assays. Sponsors are strongly encouraged to appropriately collect and store isolates for later analysis. If the pathways to resistance as

[1] For datasets containing multiple studies, a column should be added to identify the relevant study in which the subject was enrolled.

[2] 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.

defined by genotypic analysis are straightforward, phenotypic analysis, after consultation with the division, may not be necessary.
- Depending on the timing of serum specimens for antibody determination relative to isolates obtained for viral isolation, the most appropriate incorporation of serologic results into the dataset should be discussed with the division.

Information to Include with Suggested Column Headings[3]

I. Patient Data:

- Patient identification number (ID number should be unique to all clinical studies of the product)
- Isolate (e.g., baseline, day 2 (with day 0 being the start of product administration), discontinuation. Multiple isolates from the same visit should be numbered (e.g., baseline 1, baseline 2)). All isolates should be included even if negative for PCR and cell culture.
- Date of isolate
- Days of study product treatment on day isolate obtained
- Days off study product treatment on day isolate obtained
- Previous therapeutic products, if any
- Treatment group (e.g., specific dose, active control, placebo)
- Indication if data were censored (e.g., discontinuation because of adverse event)

II. Virologic Data (culture and PCR results and quantification as appropriate):

The protocol and study report should include a detailed description of assay methodology and explanation of units of measurement for any qualitative or quantitative assays used to identify viral isolates. Supporting information should be provided to allow review of assay performance; however, use of a specified assay in an antiviral product trial does not constitute FDA review, approval, or endorsement of the assay.

- Baseline measurement (e.g., $\log_{10}$ influenza RNA copies/mL of nasal wash at baseline, $\log_{10}$ $TCID_{50}$/mL). (For therapeutic studies, after onset of illness and before receipt of study product.)
- Measurements using the same units at predefined time points (e.g., day 2), one column for each time point including baseline (i.e., the measurement throughout the course of infection is repeated for each isolate)
- Measurements from additional time points can be included

III. Genotypic Data (for baseline isolates of all patients and predetermined time points):

- Subtype for influenza A isolates (e.g., H1N1, H3N2); insert "B" for influenza B to confirm typing

[3] 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.

- Genotype information for all the relevant coding regions sequenced; one amino acid per column with the wild-type (WT) amino acid as column heading identified using the one amino acid abbreviation. Changes from WT standard sequence indicated (i.e., blanks indicate no change). Known polymorphic amino acid residues should be flagged with an asterisk in the column heading. For neuraminidase inhibitors, genotype information for both the neuraminidase and hemagglutinin proteins would be relevant.

Example (Table 1 highlights how genotype information should be displayed but does not include all column headings previously suggested.)

Table 1. Example of Genotype Information Display

Patient #	Isolate	Subtype/Type	C-291	R292	D-293	N-294
001	BL	H3N2				
001	Day 2	H3N2		K		
001	Day 5	H3N2		K		
002	BL	H1N1				
002	Day 2	H1N1				
002	Day 3	H1N1				

BL = baseline

IV. Phenotypic Data (minimally for baseline isolates and predetermined time points):

1. *Candidate product*
   - Baseline EC_{50} value for candidate product
   - EC_{50} value of reference strain for candidate product (the reference strain should be a widely available standard lab strain)
   - Fold change of baseline EC_{50} value compared to EC_{50} value of reference strain of candidate product
   - EC_{50} value at time of subsequent assessment as appropriate for candidate product
   - Fold change values in EC_{50} value at time of subsequent assessment as appropriate compared to reference strain for candidate product
   - Fold change in EC_{50} value at time of subsequent assessment as appropriate compared to baseline for candidate product

2. *Approved or investigational products in the same class*
   - Fold change in EC_{50} value of baseline compared to reference strain for each of the approved or investigational products in the same class
   - Fold change in EC_{50} value at time of subsequent assessment as appropriate compared to reference strain for each of the approved or investigational products in the same class
   - Fold change in the EC_{50} value at time of subsequent assessment as appropriate compared to baseline for each of the approved or investigational products in the same class

Example (Table 2 highlights how phenotype information should be displayed.)

Table 2. Example of Phenotype Information Display

	Product X				**Other Products in Same Product Class***	
Isolate	EC_{50} value Product X	Ref strain EC_{50} value Product X	Δ resis from ref Product X	Δ resis from BL Product X	Δ resis from ref Product Y	Δ resis from BL Product Y
Baseline						
Day x						

Product X = candidate product
BL = baseline
Day x = predefined time point for subsequent assessment as appropriate (e.g., day 2, day 5)
Δ resis = fold resistance change, e.g.: $\frac{EC_{50} \text{ value of baseline isolate with Product X}}{EC_{50} \text{ value of reference strain with Product X}}$
Ref strain = reference strain (or WT)
*Note: The Δ resis from ref and Δ resis from BL should be included for all approved anti-influenza products.