A randomized, placebo-controlled, double-blinded trial of the safety and efficacy of tecovirimat for the treatment of adult and pediatric patients with monkeypox virus disease

Statistical Analysis Plan
Appendix 3

Version 1.0

27 June 2024

NCT05559099

Appendix 3 – PCR Data Handling

Background

Prespecified Endpoints, Sample Collection Schedule and History of Changes

The original study design included two key secondary endpoints and a hierarchical plan for analysis of those endpoints (see protocol v1.0). The first of these two key secondary endpoints was time to the first of two consecutive negative results on daily blood PCR tests up to 28 days after randomization; the second was mortality within the first 28 days after randomization. PCR results were obtained daily under this protocol version (until two consecutive negative results separated by 16-32 hours for blood, until day 9 and then every other day until discharge for oropharyngeal swabs, until day 9 and then every other day until lesion resolution for lesion swabs).

Among other changes, protocol v2.0 altered the study schedule of events by reducing the frequency of PCR data collection. For participants enrolled under that protocol version or later, PCR results were anticipated to be obtained for the purposes of establishing eligibility (generally at screening), again at baseline/day 1/day of randomization (ideally within 24 hours prior to randomization, through a specimen collected within 48 hours prior to randomization may be used as baseline if no more recent sample was obtained), and then every other day until consecutive negative results for the specific sample type. Since the transition to protocol v2.0 occurred quite early in the trial, most participants will have PCR results available for each sample type on Days 1, 3, 5, and so on until either achieving sustained PCR negativity (consecutive negative results) or until the data collection sequence ended for other reasons (e.g., for lesion swabs, due to lesion resolution prior to achieving sustained PCR negativity).

Protocol v3.0 dropped the hierarchical testing plan for "key" secondary endpoints and changed the secondary endpoint related to PCR results from time to the first of two consecutive negative results to the proportion of participants with negative PCR results separately by sample type 14 days after randomization (i.e., on Day 15). Versions 1.0 and beyond of the statistical analysis plan describe the planned analysis of this endpoint, which will employ a last-observation-carried-forward approach to handle cases where participants are missing Day 15 PCR data (e.g., if a participant is already negative and hence no longer being tested at Day 15, the analysis will count them as negative on Day 15).

Data Format and Motivation for This Appendix

Available PCR data for a given participant, sample type, and timepoint are generally made up of 4 cycle threshold (CT) values: a clade I mpox CT value, a clade II mpox CT value, a generic orthopox CT value, and a positive control CT value. For certain samples the generic orthopox CT result may not be available—this is a function of the testing supplies available at study sites when results were obtained. For example, the generic orthopox CT value is not available for samples from the Tunda site from October 2022 through February 2023. A CT value less than or equal to 40 indicates a positive result. No cases of clade II disease are expected to enroll in the trial. Therefore, the clade I and generic orthopox CT values are of most interest.

The precise data format was not known during the design phase of the trial since the lab equipment available for running samples could not be determined well in advance of the trial. As the trial evolved,

the precise meaning of “negative” as used in the description of endpoints required clarification. For example, there have been cases where a sample is positive based on the generic orthopox CT value and negative based on the clade I and clade II CT values. The statistical analysis plan (SAP) pointed to the need for exploratory evaluation of study data to understand the best methods of data handling. As described in SAP version 2.0 section 6.5.2:

Little is known about the typical viral load trajectories of mpox patients in this setting. Exploratory work may be required to determine the most appropriate methods of data handling, for example to gain an understanding of how to handle cases where the clade I result is negative but the generic orthopox result is positive. Exploratory analyses will be blinded to study arm and will be described in detail in an appendix to the SAP.

Guiding Principles for Exploratory Analyses

As described above, exploratory analyses will be focused on suggesting a method of handling clade-specific and generic orthopox CT results and possible discrepancies between the two as it relates to the determination of “positive” or “negative” for a given participant, sample type and study day.

Exploratory analyses must be blinded to study arm. Any subject-level presentations of data will use masked subject IDs and will not include any indication of the participant’s study arm. Subject level data presentations may include figures showing CT trajectories over time by target (generic vs clade I) and sample type in order to facilitate an understanding of how strongly the two targets of interest correlate. The unblinded statistics team will be responsible for preparing a report to this effect. To avoid the appearance of any inappropriate unblinding, all materials presented to the study team will be retained. After reviewing the report, the blinded study team will reach consensus on a rule for performing the prespecified analyses as described in the SAP. This rule will be documented in advance of performing or reviewing any comparative analyses.