Notes on the Design of Bioequivalence Study: Lamivudine/Tenofovir Alafenamide

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Unit – Medicines Assessment Team (PQT/MED) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.

The current notes should be read and followed in line with the general guidelines of submission of documentation for WHO prequalification. For guidance on issues related to bioequivalence (BE) studies for immediate-release, solid oral dosage forms, see the ICH Harmonised Guideline M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms (2024). For BE issues outside the scope of the ICH M13A guideline, e.g., for additional strength biowaivers, please consult the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability" in: *Fifty-seventh report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations,* Geneva, World Health Organization, 2024. WHO Technical Report Series, No. 1052, Annex 8.

Below, additional specific guidance is provided on the invited immediate release products containing lamivudine and tenofovir alafenamide (TAF).

Pharmacokinetics of lamivdudine and tenofovir alafenamide

Maximum lamivudine concentrations are observed in serum within 0.5 to 3.0 hours of dosing in the fasted state (median T_{max} of 1 hour). TAF peak plasma concentrations are observed after about 1 hour after dosing in fed state.

The elimination half-lives of lamivudine and TAF are 5-7 and 0.51 hours, respectively, following single doses.

Co-administration of lamivudine with food results in a delay of Tmax and a lower C_{max} (decreased by 47%). However, the extent (based on the AUC) of lamivudine absorbed is not influenced. Therefore, lamivudine can be taken with or without food.

Administration of TAF with a high-fat meal increased the systemic exposure of tenofovir alafenamide by 65%; therefore, TAF should be administered with food. However, the emtricitabine/TAF fixed dose combination (FDC) comparator product is labeled to be taken with or without food.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of lamivudine and TAF, the following guidance with regard to the study design should be taken into account:

Design: A single-dose cross-over design is recommended.

Dose: As the EoI includes lamivudine/tenofovir alafenamide 300 mg/25 mg tablets, the bioequivalence study should be conducted with this strength versus a combination of the following comparator products: Epivir (ViiV Healthcare Co.) and Vemlidy (Gilead Sciences Int. Inc.).

Fasted/fed: As the APIs involved may be taken without regard to food for HIV indications, the bioequivalence study should be conducted in the fasted state.

Subjects: Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence study.

Parent or metabolite data for assessment of bioequivalence: The parent drug is considered to best reflect the biopharmaceutical quality of the product. The data for the parent compound should be used to assess bioequivalence of lamivudine and TAF.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for lamivudine is around 20%, while the intra-subject variability for TAF C_{max} is reported to be approximately 36%. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: Taking into account the elimination half-life of lamivudine (5 – 7 hours) and TAF (0.51 hours) in healthy volunteers, a washout period of seven days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive for the first three hours after administration to properly characterize the C_{max} of lamivudine and TAF. For example, blood samples should be taken at pre-dose, 0.083, 0.17, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00, 24.00, and 36.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure lamivudine and TAF in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the C_{max} in most profiles of each formulation (test or comparator). The bioanalytical method for each analyte should be validated in the presence of the other analyte (see Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for lamivudine and TAF should meet the following bioequivalence standards in a single-dose cross-over design study:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to reference product should be within 80.00–125.00%

- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80.00– 125.00%.