Notes on the Design of Bioequivalence Study: Zanamivir

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. In particular, 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 pre-dispensed inhalation powder containing zanamivir.

Pharmacokinetics of zanamivir

The absolute oral bioavailability of zanamivir is low, averaging 2% (range 1 to 5 %). After oral inhaled administration, a median of 10 to 20% of the dose was systemically absorbed, with maximum serum concentrations (47 mcg/ml) generally reached after 0.75 h (0.08 – 2 h). The mean serum elimination half-life of 3.56 – 5.05 h ranges between 2.23 and 9.49 h, suggesting that the elimination rate is limited by absorption. Approximately 90% of zanamivir was excreted unchanged in the urine. The kinetics of inhaled zanamivir were linear, as suggested by the dose proportionality.

Guidance for the design of bioequivalence studies

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

Design: Two single-dose cross-over designs are recommended. A single dose study without active charcoal blockade to compare systemic exposure as measurement of systemic safety, and a single dose study with active charcoal blockade to assess pulmonary deposition as measurement of efficacy. It should be demonstrated previously that oral absorption of the swallowed fraction is blocked by the administration protocol of active charcoal.

Dose: As the EoI includes only the 5 mg/dose, a therapeutic dose of 2 x 5 mg should be administered in the bioequivalence studies to obtain measurable levels.

Fasted/fed: As this product is administered by pulmonary route, bioequivalence should be investigated in fasted state.

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

Parent or metabolite data for assessment of bioequivalence: The parent drug is considered to best reflect the biopharmaceutical quality of the product. In addition, zanamivir is not metabolised. Therefore, bioequivalence should be based on the determination of zanamivir.

Sample size: There is limited pharmacokinetic data available on inhaled Zanamivir to estimate the intra-subject variability of C_{max} and AUC but, the available data suggests an intra-subject CV around 45% for C_{max} (Cass et al. Pharmacoscintigraphic evaluation of lung deposition of inhaled zanamivir in healthy volunteers. Clin Pharmacokinet. 1999;36 Suppl 1:21-31). Therefore, pilot studies might be conducted to design the pivotal studies. The intra-subject variability is highly dependent on the consistency and correctness of the inhalation maneuvers. Therefore, the bioequivalence studies should be conducted in centers experienced with inhalation products to ensure that participants are trained adequately and the subjects with inadequate inhalation maneuvers are excluded.

Washout: Taking into account the elimination half-life of zanamivir of up to 9.5 h a washout period of approximately 7 days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be very frequent for the first minutes after administration to properly characterize the absorption from the lungs. For example: pre-dose, 0.03, 0.08, 0.17, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, 12.00, 18.00 and 24.00 h after drug administration.

Analytical considerations: Information currently available indicates that it is possible to measure zanamivir in human serum 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).

Statistical considerations: The data for zanamivir should meet the following bioequivalence standards in both single-dose crossover design studies:

- 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%.

Information currently available to the PQT/MED suggests that inhalation products are frequently highly variable drug products due to the variability in the inhalation maneuvers. Therefore, if the Applicant suspects that the variability of C_{max} or AUC_{0-t} is high (CV>30%), the applicant may prefer to employ a full replicate design study in order to widen the acceptance range of C_{max} and/or AUC_{0-t}. For more information on replicate study designs and widening the limits of average bioequivalence, refer to Section 7.9.3 of Annex 8, TRS 1052 and WHO guidance document "*Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED*".