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Draft Guidance on Daunorubicin Citrate

November 2022

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Active Ingredient: Daunorubicin citrate

Dosage Form; Route: Injectable, liposomal; injection

Recommended Studies: One in vivo bioequivalence study with pharmacokinetic endpoints and one in vitro bioequivalence study with supportive characterization studies

To be eligible for the bioequivalence studies recommended in this guidance, the test product should meet the following criteria:

1. Qualitatively (Q1)[1] and quantitatively (Q2)[2] the same as the Reference Listed Drug (RLD).
2. Manufactured by an active liposome loading process with pH gradient.
3. Equivalent liposome characteristics including liposome composition, internal environment of liposome, lamellarity, electrical surface potential or charge, and in vitro drug release/leakage rates comparable to the Reference Standard (RS) product.

One in vivo bioequivalence study with pharmacokinetic endpoints:

1. Type of study: Fasting[3]
   Design: Single-dose, two-way crossover in vivo
   Strength: Eq. 2 mg base/mL
   Dose: 40 mg/m^2
   Subjects: Advanced HIV-associated Kaposi's sarcoma patients

[1] Q1 (Qualitative sameness) means that the test product uses the same inactive ingredient(s) as the reference product.
[2] Q2 (Quantitative sameness) means that concentrations of the inactive ingredient(s) used in the test product are within ±5% of those used in the reference product.
[3] If the health conditions of patients prevent fasting, the sponsor can provide a non-high-fat diet during the proposed study. Alternatively, the treatment can be initiated 2 hours after a standard (non-high-fat) breakfast.

Additional comments:

1. Daunorubicin citrate liposomal injection is a cytotoxic drug. Therefore, a Bio-IND is required for a bioequivalence study of daunorubicin citrate liposomal injection to ensure the safety of human test subjects.
2. Conduct the two arms of the crossover study on two of the days when the patients are scheduled to receive their usual therapy so that the treatment regiment is not altered or delayed.
3. Do not alter the standard of care treatment regimen except to randomize the patients to the Test or Reference therapy on the specified dosing days.
4. Given that the dosage is every two weeks, use two consecutive treatment cycles for the two treatment periods.
5. Any concomitant medications must be the same in both periods of the study.
6. Evaluate cardiac function by means of history and physical examination at Screening Visit and before each dose of study treatment.
7. Determination of left ventricular ejection fraction (LVEF) should be performed at total cumulative doses of daunorubicin citrate liposomal injection 320 mg/m^2, and every 160 mg/m^2 thereafter.
8. Patients who have received prior therapy with anthracyclines (e.g., doxorubicin >300 mg/m^2 or equivalent), have pre-existing cardiac disease, or have received previous radiotherapy encompassing the heart may be less "cardiac" tolerant to treatment with daunorubicin citrate liposomal injection. Therefore, monitoring of LVEF at cumulative daunorubicin citrate liposomal injection doses should occur in these patients prior to therapy and every 160 mg/m^2 of daunorubicin citrate liposomal injection.
9. Obtain Complete Blood Count prior to each dose and withhold dosing if absolute granulocyte count is less than 750 cells/mm^3.
10. Any patient whose weight changes during the study requiring a ± 5% dose adjustment must be discontinued from the study and excluded from the analysis.
11. Study treatment should be administered only under the supervision of a physician who is experienced in the use of chemotherapeutic agents.
12. Inclusion Criteria:
    a. Male of female aged ≥ 18 years ≤ 75 years.
    b. Advanced HIV-associated Kaposi's sarcoma.
    c. Cardiac ejection fraction > 45% by Echo at Screening Visit.
    d. Granulocyte count ≥ 1,500/µL, or WBC ≥ 3,500/µL at Screening Visit.
    e. Platelet count ≥ 75,000 and Hgb ≥ 10 g/dL at Screening Visit.
    f. Liver and renal function testing with no clinically significant abnormality(ies) at Screening Visit.
    g. The sponsor may add additional criteria.
13. Exclusion Criteria:
    a. Female who is pregnant, breastfeeding, or planning a pregnancy.
    b. Female of childbearing potential who does not agree to utilize an adequate form of contraception throughout the study.
    c. Clinically significant or unstable cardiac, liver or kidney disease.
    d. Total cumulative daunorubicin dose approaching 550 mg/m^2.
    e. Patient receiving other myelotoxic drugs.

f. Known allergy or hypersensitivity reaction to daunorubicin, daunorubicin citrate, any reference listed drug excipient or any study treatment excipient.
g. The sponsor may add additional criteria.

Analytes to measure: Free daunorubicin and liposome encapsulated daunorubicin in plasma.

Bioequivalence based on (90% CI): AUC and C_{max} for liposome encapsulated daunorubicin. Submit AUC and C_{max} of free daunorubicin as supportive data.

One in vitro bioequivalence study with supportive characterization studies:

1. Type of study: Liposome size distribution
   Design: In vitro bioequivalence study on at least three batches of both the Test and the RS products

Parameters to measure: D10, D50, D90; or *z*-average diameter and polydispersity index (PDI)

Bioequivalence based on (95% upper confidence bound): Population Bioequivalence (PBE) approach on D50 and SPAN [i.e. (D90-D10)/D50], or alternatively on the harmonic intensity-weighted average particle diameter (*z*-average) and PDI derived from cumulant analysis of the size intensity distribution. Refer to the most recent version of the FDA product-specific guidance on *Budesonide Inhalation Suspension* (NDA 020929)[a] for additional information regarding PBE.

Additional information:

Same drug product composition:
Per 21 CFR § 314.94 (a)(9)(iii), as a parenteral drug product, a generic daunorubicin citrate liposomal injection must be Q1 and Q2 the same as the RLD, except differences in buffers, preservatives, and antioxidants provided that the applicant identifies and characterizes these differences and demonstrates that the differences do not impact the safety/efficacy profile of the drug product. Currently, FDA has no recommendations for the type of studies that would be needed to demonstrate that differences in buffers, preservatives, and antioxidants do not affect the safety/efficacy profile of the drug product.

Lipid excipients are critical in the liposome formulation. Abbreviated New Drug Application (ANDA) applicants should obtain lipids from the same category of synthesis route (natural or synthetic) as found in the RLD. Information concerning the chemistry, manufacturing and control of the lipid components should be provided as per the recommendations the most recent version of the FDA guidance for industry on *Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation.*[b] ANDA applicants should have specifications on lipid excipients that are comparable to the lipid excipients used to produce the RLD. Provide additional comparative characterization (beyond meeting specifications) of lipid excipients, including the distribution of the molecular species.

Active liposome loading process with a pH gradient:
To meet the compositional equivalence and other equivalence tests, an ANDA sponsor would be expected to use an active loading process with a pH gradient. The major steps include (1) formation of liposomes containing citric acid and sucrose, (2) liposome size reduction, (3) creation of a pH gradient, and (4) active drug loading. The size reduction and drug loading steps should be conducted at a temperature over the phase transition temperature of lipids. An active loading process uses a pH gradient between the internal and external aqueous phases of the liposomes to drive diffusion of daunorubicin into liposomes.

Equivalent liposome characteristics:
Additional in vitro characterizations are recommended to demonstrate the Test product is comparable to the Reference product. The comparative physicochemical characterization studies should be conducted on at least three batches of the Test and the RS products, and at least one Test batch should be produced by the commercial scale process and used in the in vivo bioequivalence study. Attributes that should be characterized include:

1. Liposome composition: lipid content, free and encapsulated drug content, internal and total citric acid concentration, and sucrose concentration should be measured. The drug-to-lipid ratio and the percentage of drug encapsulation can be calculated from liposome composition measurements.

2. Internal environment: internal volume, pH, and citrate concentration should be measured. Different from doxorubicin salt in Doxil®, daunorubicin is unlikely to form precipitate in liposomes,[4] and the physical state of daunorubicin in the Test product should be equivalent to that in the Reference product.

3. Liposome morphology and number of lamellae: drug loading, drug retention, and the rate of drug release from liposomes are likely influenced by lamellarity. Morphological assessment should be conducted on an appropriate number of liposomes using suitable imaging techniques to demonstrate equivalent morphology and lamellarity between Test and Reference products.

4. Lipid bilayer phase transitions: equivalence in lipid bilayer phase transitions will contribute to demonstrating equivalence in bilayer fluidity and uniformity. The phase transition profile of the liposomal Test product should be comparable to the RS product.

5. Liposome size distribution: critical to ensuring equivalent passive targeting of tumors. Sponsors should select the most appropriate particle size analysis method to determine the particle size distributions of both Test and Reference products. The number of liposomal product vials to be studied should be no fewer than 30 vials for each of the Test and Reference products (i.e., no fewer than 10 vials for each batch, minimum of three batches). Equivalency should be established based on an appropriate statistical equivalence test.

[4] Dicko A, Kwak S, Frazier A, Mayer L, Liboiron B, Biophysical characterization of a liposomal formulation of cytarabine and daunorubicin. International Journal of Pharmaceutics 2010, 391: 248-259.

6. Electrical surface potential or charge: surface charge on liposome can affect clearance, tissue distribution and cellular uptake. Sponsors should demonstrate comparable electrical surface potential or charge between Test and Reference products.

7. In vitro leakage under multiple conditions: in vitro drug leakage testing to characterize the physical state of the lipid bilayer and encapsulated daunorubicin should be conducted to support a lack of uncontrolled leakage under a range of physiological conditions and equivalent drug delivery conditions to the tumor cells. Below are some examples of proposed conditions:

Table 1. Examples of in vitro leakage conditions of daunorubicin liposomes

In Vitro Drug Leakage Condition	**Purpose**	**Rationale**
At 37°C in 50% human plasma for 24 hours	Evaluate liposome stability in blood circulation	Plasma mostly mimics blood conditions
At 37°C with pH values 5.5, 6.5, and 7.5 for 24 hours in buffer	Mimic drug release in normal tissues, around cancer cells or inside cancer cells	Normal Tissues: pH 7.3 Cancer Tissues: pH 6.6 Endosomal/Lysosomal environment: pH 5-6
At a range of temperatures (43, 47, 53, 60°C) in pH 6.5 buffer for up to 12 hours or until complete release	Evaluate the lipid bilayer integrity	The phase transition temperature (T_m) of lipids is determined by the bilayer properties such as rigidity, stiffness, and chemical composition. Differences in release as a function of temperature (below and above T_m) may reflect differences in lipid properties
At 37°C under low-frequency (20 kHz) ultrasound for 2 hours or until complete release	Evaluate the state of encapsulated drug in the liposome	Low-frequency ultrasound disrupts the lipid bilayer via transient introduction of pore-like defects, and it will render the release of daunorubicin controlled by the dissolution of the gel inside the liposomal structure.

Waiver request of in vivo testing: Not applicable

Dissolution test method and sampling times: The dissolution information for this drug product can be found on the FDA-Recommended Dissolution Methods website available to the public at the following location: http://www.accessdata.fda.gov/scripts/cder/dissolution/. Conduct comparative dissolution testing on 12 dosage units each of the Test and Reference products. Specifications will be determined upon review of the ANDA.

Revision History: Recommended July 2014; Revised November 2022

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[a] For the most recent version of a product-specific guidance, check the FDA product-specific guidance web page at https://www.accessdata.fda.gov/scripts/cder/psg/index.cfm.
[b] For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.