Contains Nonbinding Recommendations

Draft Guidance on Doxorubicin Hydrochloride

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the Office of Generic Drugs.

Active Ingredient: Doxorubicin hydrochloride

Dosage Form; Route: Injectable, liposomal

Recommended Studies: Two studies: in vivo and in vitro

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

- Qualitatively (Q1)[1] and quantitatively (Q2)[2] the same as the Reference Listed Drug (RLD)
- Manufactured by an active liposome loading process with an ammonium sulfate gradient
- At least one batch of the Test product should be produced by the commercial scale process and be used in the in vivo bioequivalence study
- Equivalent liposome characteristics including liposome composition, state of encapsulated drug, internal environment of liposome, liposome size distribution, number of lamellar, grafted PEG at the liposome surface, electrical surface potential or charge, and in vitro leakage rates comparable to the Reference Standard (RS).

In Vivo Study:

Type of study: Fasting*
Design: Single-dose, two-way crossover in vivo
Strength: 50 mg/vial or 20 mg/vial
Dose: 50 mg/m2
Subjects: Ovarian cancer patients whose disease has progressed or recurred after platinum-based chemotherapy and who are already receiving or scheduled to start therapy on doxorubicin hydrochloride (liposomal).
Additional comments:

- The pivotal bioequivalence study should be conducted using test product produced by the proposed commercial scale manufacturing process
- Doxorubicin is a cytotoxic drug. Therefore, a Bio-IND is required for bioequivalence studies of a doxorubicin HCl liposomal injection to ensure the safety of human test subjects

[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.

- The two arms of the crossover study must not alter or delay the treatment regimen; the study is to be conducted on two of the days that the patients are scheduled to receive their usual therapy
- The standard of care treatment regimen should not be altered, except to randomize patients to the test or reference therapy on the specified dosing days
- Given that the dosage is every 4 weeks, two consecutive treatment cycles should be used for the two treatment periods
- Any concomitant medications must be identical in both periods of the study.
- Due to concerns about cardiac toxicity, cardiac status should be documented at baseline
- Any patient with weight changes during the study requiring a ±5% dose adjustment must be discontinued from the study and excluded from the analysis.

Exclusion Criteria:

- Patients with prior doxorubicin exposure that would result in a total lifetime exposure of 550mg/m^2 or more after four cycles of treatment
- Patients with significantly impaired hepatic function
- Patients who have a history of hypersensitivity reactions to a conventional formulation of doxorubicin HCl or the components of the RLD should not be in the study
- Females patients should not be pregnant or lactating
- Patients are < 18 years of age or > 75 years of age
- Patients with active opportunistic infection with mycobacteria, cytomegalovirus, toxoplasma, *P. carinii* or other microorganism if under treatment with myelotoxic drugs
- Patients with clinically significant cardiac, liver or kidney disease.

* 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.

Analytes to measure (in appropriate biological fluid): Free doxorubicin and liposome encapsulated doxorubicin.

Bioequivalence based on (90% CI): AUC and Cmax for free doxorubicin and liposome encapsulated doxorubicin.

In Vitro Study:

1. Type of study: Liposome Size Distribution

   Design: In vitro bioequivalence study on at least three lots of both Test and RS product. At least one lot of the Test product should be produced by the proposed commercial scale manufacturing process.

Parameters to measure: D10, D50, D90

Bioequivalence based on (95% upper confidence bound): D50 and SPAN [(i.e. (D90-D10)/D50] using the Population Bioequivalence (PBE) approach. Please refer to the *Guidance on Budesonide* inhalation suspension for additional information regarding PBE.

Dissolution test method and sampling times: The dissolution information for this drug product can be found on the FDA-Recommended Dissolution Methods Web site, 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 all strengths of the test and reference products. Specifications will be determined upon review of the abbreviated new drug application (ANDA).

Additional information:

Same drug product composition

Being a parenteral drug product, a generic doxorubicin HCl liposomal injection product must be Q1/Q2 the same as the RLD, except for differences in buffers, preservatives and antioxidants; under the condition 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 the impact of using different buffers, preservatives or antioxidants on the safety/efficacy profile of the drug product.

Lipid excipients are critical in the liposome formulation. ANDA sponsors 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 in FDA's guidance for industry: *Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation.* ANDA sponsors should have specification on lipid excipients that are similar to the lipid excipients used to produce the RLD. Additional comparative characterization (beyond meeting specifications) of lipid excipients, including the distribution of the molecular species should be provided.

Active liposome loading process with an ammonium sulfate gradient

To meet the compositional equivalence and other equivalence tests, an ANDA sponsor would be expected to use an active loading process with an ammonium sulfate gradient. The major steps include: 1) formation of liposomes containing ammonium sulfate, 2) liposome size reduction, 3) creation of ammonium sulfate gradient, and 4) active drug loading. An active loading process uses an ammonium sulfate concentration gradient between the liposome interior and the exterior environment to drive the diffusion of doxorubicin into the liposomes.[3]

[3] A. Gabizon, H. Sheemda, Y. Barenholz. Pharmacokinetics of pegylated liposome doxorubicin: review of animal and human studies. Clin Pharmcokinet 42(5): 419-436 (2003)

Sponsors should use an appropriate approach to identify critical material attributes and critical process parameters, and guide process optimization. It is recommended to identify the critical process parameters and critical material attributes by evaluating the sensitivity of liposome characteristics to changes in process parameters and attributes. The optimal values of critical process parameters should be selected based on comparison of resulting liposome characteristics to those of the RS product.

Equivalent liposome characteristics

Comparative physicochemical characterization studies should be performed on at least three batches of both the Test and RS products, at least one Test batch should be produced by the commercial scale process and be used in the in vivo bioequivalence study, and should include:

- Liposome composition: Liposome composition including lipid content, free and encapsulated drug, internal and total sulfate and ammonium concentration, histidine concentration, and sucrose concentration should be measured. The drug-to-lipid ratio and the percentage of drug encapsulation can be calculated from liposome composition values.

- State of encapsulated drug: Doxorubicin is largely in the form of a doxorubicin sulfate crystal inside the liposome. The proposed Test product must contain a comparable doxorubicin sulfate crystal inside the liposome, as the RS product.

- Internal environment (volume, pH, sulfate, and ammonium ion concentration): The internal environment of the liposome Test product should be comparable to the RS, including its volume, pH, sulfate, and ammonium concentration maintains the doxorubicin sulfate crystal.

- Liposome morphology and number of lamellae: Liposome morphology and lamellarity should be comparable to the RS as drug loading, drug retention, and the rate of drug release from the liposomes are likely influenced by the degree of lamellarity.

- 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.

- Liposome size distribution: The ANDA sponsor should select the most appropriate particle size analysis method to determine the particle size distributions of both Test and RS products. The number of liposome product vials to be studied should not be fewer than 30 for each of the Test and RS products (i.e., no fewer than 10 from each of three batches). See recommended study 2 (above) for details of the recommended statistical equivalence tests.

- Grafted PEG at the liposome surface: The surface-bound methoxypolyethylene glycol (MPEG) polymer coating protects liposomes from clearance by the mononuclear phagocyte system (MPS) and increases blood circulation time. The PEG layer thickness is known to be thermodynamically limited and estimated to be in the order of several nanometers. The PEG layer thickness should be comparable to the RS.

- Electrical surface potential or charge: Surface charge on liposomes can affect the clearance, tissue distribution, and cellular uptake. Liposome surface charge should be comparable to the RS.

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

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

In Vitro Drug Leakage Condition	**Purpose**	**Rationale**
At 37ºC in 50% human plasma for 24 hours	Evaluate liposome stability in blood circulation.	Plasma mostly mimics blood conditions.
At 37ºC 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 Insider cancer cells (endosomes and lysosomes): pH 5-6 (Endosome and lysosomes of cancer cells may be involved in liposome uptake and induce drug release).
At a range of temperatures (43ºC, 47ºC, 52ºC, 57ºC) in pH 6.5 buffer for up to 12 hours or until complete release	Evaluate the lipid bilayer integrity	The phase transition temperature (Tm) of lipids is determined by lipid bilayer properties such as rigidity, stiffness and chemical composition. Differences in release as a function of temperature (below or above Tm) will reflect small differences in lipid properties
At 37ºC 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 (20 kHz) disrupts the lipid bilayer via a transient introduction of pore-like defects and will render the release of doxorubicin controlled by the dissolution of the gel inside the liposome.