Scientific and Regulatory Considerations for Doxorubicin HCl Liposome Injection

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Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA’s views or policies.
Objectives

• Review the rationale of liposomal delivery

• Summarize the critical performance attributes of PEGylated liposomal doxorubicin

• Provide the pharmaceutical equivalence recommendations for generic DOXIL

• Discuss common issues and Agency’s expectation in ANDA submissions
Liposomes: Lipid (fat) + Soma (body)

Hydrophilic Head Group

Hydrophobic Tail

100 nm

http://www.avantlipids.com

http://www.fda.gov
## Approved Liposomal Drugs

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Active</th>
<th>Company</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxil</td>
<td>Doxorubicin</td>
<td>ALZA (Jassen)</td>
<td>1995</td>
</tr>
<tr>
<td>Abelcet</td>
<td>Amphotericin B</td>
<td>Enzon</td>
<td>1995</td>
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<tr>
<td>Daunoxxome</td>
<td>Daunorubicin</td>
<td>Gilead</td>
<td>1996</td>
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<tr>
<td>Ambisome</td>
<td>Amphotericin B</td>
<td>Gilead</td>
<td>1997</td>
</tr>
<tr>
<td>Depocyt</td>
<td>Cytarabine</td>
<td>Skye Pharma/Pacira</td>
<td>1999</td>
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<tr>
<td>Visudyne</td>
<td>Verteporfin</td>
<td>QLT/Novartis</td>
<td>2000</td>
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<td>DepoDur</td>
<td>Morphine</td>
<td>Skye Pharma/Endo</td>
<td>2004</td>
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<td>Exparel</td>
<td>Bupivacaine</td>
<td>Pacira</td>
<td>2011</td>
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<tr>
<td>Marqibo</td>
<td>Vincristine</td>
<td>Talon Therapeutics</td>
<td>2012</td>
</tr>
<tr>
<td>Myocet</td>
<td>Doxorubicin</td>
<td>Elan</td>
<td>In EU</td>
</tr>
</tbody>
</table>
Doxorubicin HCl Liposome Injection

- **Drug substance:**
  - Doxorubicin HCl, USP
  - Greater than 90% is encapsulated

- **Drug product:**
  - A sterile, translucent, red liposomal dispersion
  - Single dosage vial for IV infusion, 2 mg/mL, 10 mL and 25 mL
  - Stealth liposome carriers are composed of
    - MPEG-DSPE
    - HSPC
    - Cholesterol
  - Excipients
    - Ammonium sulfate
    - Histidine
    - Sucrose
    - HCl/NaOH
Stealth® Liposomes

- Doxorubicin
- Lipid bilayer
- PEG layer

~100 nm
Rationales

• Clinical Indications:
  – Aids-related Kaposi’s Sarcoma (20 mg/m² every 3 wks)
  – Ovarian cancer (50 mg/m² every 4 wks)
  – Multiple myeloma, with Bortezomib (30 mg/m²)
  – Breast cancer (EU)

• Dosage form design:
  – Injection/Intravenous infusion (liquid suspension of unilamellar vesicles)
  – Weak base API loaded in the aqueous core
  – PEGylated liposomes
Mechanism of Actions

- Delivery barriers
  - blood circulation
  - vascular wall
  - dissemination in tissue
  - cell membrane
  - drug release
  - nucleus

Drummond DC, et al., Pharmacological Reviews, 51 (4); 1994
Critical Performance Attributes

- Sufficient stable drug loading in blood
- Extended circulation time
- Passive targeting of solid tumors via EPR effect
- Release of drug at the site of action

Jiang, et al., Bioanalysis (2011) 3 (3), 342
In vivo PK of free and encapsulated drug

In vitro liposome characterization
- Liposome composition
- Morphology of liposome and number of lamellar
- Surface charge
- Presence of grafted PEG on the surface
- State of encapsulated drug
- Internal environment
- Liposome size distribution
- In vitro leakage under multiple conditions

A manufacturing process using active loading with an ammonium sulfate gradient

Equivalence recommendations

1. Sufficient stable drug loading in blood
2. Extended circulation time
3. Passive targeting to tumor sites
4. Getting active drugs into the tumor cells

Composition/manufacturing characterization → in vivo fate → efficacy and safety
Guidances/References

- Draft Product Specific BE Guidance on Doxorubicin Hydrochloride, OGD, 2010
- Draft Guidance for Industry: Liposome Drug Products, CDER, FDA, 2002
- Advisory committee for Pharmaceutical Science (ACPS) meeting in 2001
Key Considerations for PE

- Q1 and Q2 equivalent composition
- Active loading with an ammonium sulfate gradient
- Liposome characterizations
- *In vitro* release test in multiple conditions
Critical Material Attributes

• API
  – Specify impurities
  – Endotoxin, bioburden

• Lipid excipients
  – HSPC, MPEG-DSPE (Na), and cholesterol
  – Obtain lipids from the same category of synthesis route (natural, synthetic)
  – Specify impurities, e.g., lyso-PC
  – Conduct comparative characterization of lipids beyond spec (M.W., molecular species)
Comparative Characterization Tests

• Liposome composition
  – Lipid quantities
  – Free and encapsulated drug
  – Internal and total sulfate conc.
  – Histidine conc.
  – Sucrose conc.
  – Lipid-to-drug ratio

• Liposome size distribution
  – Dynamic light scattering (DLS)
  – Static light scattering and field flow fractionation (FFF)
  – Size-exclusion chromatography (SEC)
  – Electron microscopy (EM)
  – Flow cytometry
Comparative Characterization Tests, cont’d

• Morphology of liposome and number of lamellar
  – Transmission electron microscopy (TEM)
  – Atomic force microscopy (AFM)
  – Cryo-TEM
• State of encapsulated drug
  – Fluorescence studies
  – X-ray diffraction (XRD)
  – Cryo-TEM
• Presence of grafted PEG on the surface
  – Fixed aqueous layer thickness (FALT)
  – NMR spectroscopy
• Lipid bilayer phase transition
  – Differential scanning calorimetry (DSC)
Comparative Characterization Tests, cont’d

• Internal environment
  – pH
  – sulfate conc.
  – volume

• Surface charge
  – Zeta potential
  – Electrophoretic mobility distribution

• In vitro leakage under multiple conditions
  – A range of physiological conditions
    • 37°C in 50% human plasma for 24 hr
    • 37°C with pH 5.5, 6.5 and 7.5 for 24 hr
  – Evaluate the lipid integrity
    • 43, 47, 52 and 57 °C in pH 6.5 for up to 12 hr
  – Evaluate the state of encapsulated drug
    • 37°C under low-frequency (20 kHz) ultrasound for 2 h
Critical Process Parameters

• To be identified based on different manufacturing process

• Particle formation
  – Thin layer evaporation
  – Ethanol injection
  – Freeze thaw
  – Detergent dialysis

• Size reduction
  – Extrusion
  – High pressure homogenization
  – Microfluidization
  – Ultrasonication
Critical Process Parameters

• Purification
  – Tangential flow diafiltration
  – Dialysis
  – Centrifugation

• Drug loading
  – Active drug loading with ammonium sulfate gradient

• Sterile filtration
  – Membrane selection
  – Filtration time
  – Flow rate
Specifications

• Liposome specific quality attributes
  – Particle size
  – Zeta potential
  – Assay (API and lipids)
  – Impurities
  – Lipid degradants
  – Drug encapsulation (free and entrapped)
  – In vitro release

• Standard quality attributes
  – Description
  – Identification
  – pH
  – Osmolality
  – Residual solvent
  – Particulate matter
  – Sterility/Bacterial endotoxin
  – Volume in container
Stability

• Three batches
  – Commercial scale is encouraged
  – Biobatch has to be at commercial scale
• Accelerated stability data
• Long-term stability data
• In-use stability
  – Compatibility with IV sets using the diluent (D5W)
Common Issues

• Composition
  – Qualitatively (Q1) and quantitatively (Q2) not the same as the RLD
  – Tonicity adjuster is not an exception excipient
  – Sulfate conc. not determined
  – Lipid quality issues
Common Issues, cont’d

• Characterization
  • Particle size distribution
    • DLS not sufficient
    • PDI and/or 3-tier
  • In vitro leakage
    • Should be at different conditions
      – Temperature
      – pH
      – Plasma
    • Lack of negative controls

• Sulfate conc.
  • Inside liposome
Common Issues, cont’d

• Manufacturing
  – Insufficient IPCs
    • Lipid conc.
    • Particle size
    • Lamellarity
    • Bioburden
  – Scale up
    • Any increase in batch size requires PAS
    • Product characterization and stability data (3 batches at minimum)
Common Issues, cont’d

• Stability
  – In use dilution stability
    • Dilution at highest and lowest conc.
    • % encapsulation
    • Particle size AND particulate matter
  – Degradants
    • API
    • Lipids
Summary

• Demonstrate the understanding of critical attributes that impact in vivo performance – QbD approach encouraged

• Develop adequate characterizations and IPCs of the nanoparticle properties – specification is only part of QC strategy. BE testing alone is insufficient.

• Avoid common quality issues
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