ADME Studies in Support of Development of Liposomal Formulations of Marketed Chemotherapeutics

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Presentation Outline

- Review of Liposomal Drug Delivery
- Regulatory Requirements for ADME/PK Data
- Case Study: CPX-351
  - Introduction to CPX-351 Development
  - Non-clinical ADME Study Results
- Summary
Liposomal Drug Delivery
A liposome is an artificially-prepared spherical vesicle composed of a lipid bilayer. Liposomes are often composed of phosphatidylcholine-enriched phospholipids and may also contain mixed lipid chains with surfactant properties such as egg phosphatidylethanolamine. The major types of liposomes are the multilamellar vesicle (MLV), the small unilamellar vesicle (SUV), the large unilamellar vesicle (LUV), and the cochleate vesicle. A liposome encapsulates a region of aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer.
By encapsulating a drug(s) in a liposome, the PK/PD profile may be improved compared to the non-liposomal form.

Liposomal drug delivery systems may be designed to shape the drug disposition profile to:

- increase the circulation time within the body (e.g. via protective PEGylation) avoiding detection “stealth”, thus increasing the exposure profile to disease cells/tissues,
- target (active (peptide or mab) or passive) and release within specific disease tissue types, and
- minimize the harmful effects on healthy tissues (e.g. cardio or liver toxicity).
Pharmacokinetics of Radioactivity in Plasma

Passive targeting

Hydrophilic polymer modified liposomes

Hydrophobic moiety

Antibody-anchored liposomes

Active targeting to antigen expressed over cancer cell

Ligand-anchored liposomes

Active targeting to receptor expressed over cancer cell

Cell-penetrating peptide-anchored liposomes

Cytosolic delivery

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<table>
<thead>
<tr>
<th>Name</th>
<th>Trade name</th>
<th>Company</th>
<th>Indication</th>
<th>Liposomal Excipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomal amphotericin B</td>
<td>Abelcet</td>
<td>Enzon</td>
<td>Fungal infections</td>
<td>DMPC, DMPG</td>
</tr>
<tr>
<td>Liposomal amphotericin B</td>
<td>Ambisome</td>
<td>Gilead Sciences</td>
<td>Fungal and protozoal infections</td>
<td>HSPC, Cholesterol, DSPG</td>
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<tr>
<td>Liposomal cytarabine</td>
<td>Depocyt</td>
<td>Pacira (formerly SkyePharma)</td>
<td>Malignant lymphomatous meningitis</td>
<td>DOPC, Cholesterol, DPPG</td>
</tr>
<tr>
<td>Liposomal daunorubicin</td>
<td>DaunoXome</td>
<td>Gilead</td>
<td>HIV-related Kaposi’s sarcoma</td>
<td>DSPC, Cholesterol</td>
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<tr>
<td>Liposomal doxorubicin</td>
<td>Myocet</td>
<td>Zeneus</td>
<td>Combination therapy with cyclophosphamide in metastatic breast cancer</td>
<td>LIPOVA-E120, Cholesterol</td>
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<td>Liposomal IRIV vaccine</td>
<td>Epaxal</td>
<td>Crucell</td>
<td>Hepatitis A</td>
<td>LECIVA-S70</td>
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<tr>
<td>Liposomal IRIV vaccine</td>
<td>Inflexal V</td>
<td>Berna Biotech</td>
<td>Influenza</td>
<td>LECIVA-S90</td>
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<td>Liposomal morphine</td>
<td>DepoDur</td>
<td>SkyePharma, Endo</td>
<td>Postsurgical analgesia</td>
<td>DOPC, Cholesterol, DPPG</td>
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<td>Liposomal verteporfin</td>
<td>Visudyne</td>
<td>QLT, Novartis</td>
<td>Age-related macular degeneration pathologic myopia ocular histoplasmosis</td>
<td>Egg PG, DMPC</td>
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<tr>
<td>Liposome-proteins SP-B and SP-C</td>
<td>Curosurf</td>
<td>Chiesi Farmaceutici, S.p.A.</td>
<td>pulmonary surfactant for Respiratory Distress Syndrome (RDS)</td>
<td>Leciva-S90</td>
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<tr>
<td>Liposome-PEG doxorubicin</td>
<td>Doxil/Caelyx</td>
<td>Ortho Biotech, Merck</td>
<td>HIV-related Kaposi’s sarcoma, metastatic breast cancer, metastatic ovarian cancer</td>
<td>MPEG-DSPE, HSPC, Cholesterol</td>
</tr>
<tr>
<td>Liposomal vincristine</td>
<td>Marqibo</td>
<td>Spectrum Pharmaceuticals</td>
<td>Acute Lymphoblastic Leukemia (ALL) and Melanoma</td>
<td>Cholesterol and egg sphingomyelin</td>
</tr>
</tbody>
</table>
Lipid Soluble Drug in the Bilayer
Hydrophilic Drug Contained within the Liposome

Doxorubicin

Polyethylene glycol (PEG)

~ 100 nm
Liposomal Drug Development

Regulatory Requirements for ADME/PK Data
Introduction

“A drug substance in a liposome formulation is intended to exhibit a different pharmacokinetic and/or tissue distribution (PK/TD) profile from the same drug substance (or active moiety) in a nonliposomal formulation given by the same route of administration. The complete characterization of the PK/TD profile of a new liposome drug product is essential to establish the safe and effective dosing regimen of the product”…

This guidance does not cover clinical efficacy and safety studies or bioequivalence studies of those to document sameness.

III. Human Pharmacokinetics and Bioavailability

A. Bioanalytical Methods… for liposomal drug products the bioanalytical method should also be capable of measuring encapsulated and unencapsulated drug substance.

B. In Vivo Integrity (Stability) Considerations… if the bioanalytical method can distinguish between encapsulated and unencapsulated drug substance, the in vivo stability of the liposome should be determined.

The liposome is considered stable in vivo, if over time (from PK study), the:
- Drug substance, when in circulation, remains substantially in the encapsulated form.
- Ratio of unencapsulated to encapsulated drug substance remains constant

When the liposome is stable in vivo, the total drug substance can be measured to determine the PK and Bioavailability.

C. Protein Binding

D. In Vitro Stability

FDA Draft Guidance – Liposomal Drug Products, 2002
III. Human Pharmacokinetics and Bioavailability

E. Pharmacokinetics and Bioavailability

- ADME/PK parameters may be different between the liposome and nonliposome drug products.

- Conduct comparative MB/PK studies between the liposome and nonliposome drug products when (1) the two products have the same active moiety, (2) the two products are given by the same route of administration, and (3) one of the products is already approved for marketing.

  1. Mass Balance (MB) Study
     - a crossover or non-crossover design
     - drug substance tagged with a radioactive label (e.g. $^{14}$C, $^{3}$H)

  2. PK Studies
     - Single Dose, Multi-dose, dose proportionality (*measure encapsulated and unencapsulated drug substance if needed*)

  3. Additional PK Studies
     - Food-Effect
     - Drug Interaction and/or Special Populations
     - Exposure-response

FDA Draft Guidance – Liposomal Drug Products, 2002
Introduction

“...liposomal medicinal products have formulation and manufacturing-specific
distribution characteristics after intravenous administration and similar plasma
concentrations may not correlate with therapeutic performance... the complete
characterisation of the stability, pharmacokinetics (including tissue distribution)
of a new liposomal product is critical to establish safe and effective use. This is
because differences between the applicant’s product and innovator product with
regard to manufacturing process steps and formulation may substantially modify
efficacy/safety due to changes in specific liposome-cell interactions and liposome
distribution characteristics which are not detectable by conventional bioequivalence
testing alone”.

2013/03/WC500140351.pdf
3.2 Non-Clinical and Clinical Requirements

- Significant changes in pharmacokinetic characteristics are evident when an active substance is administered in a liposomal formulation, i.e. volume of distribution and clearance may be reduced and half-life prolonged. The clearance of the liposomal active substance is dependent on:
  1. the clearance of the liposomal carrier itself,
  2. the rate of release of entrapped drug from the liposomal carrier, and
  3. the clearance and metabolism of unencapsulated drug upon its release.

- The rate and location of in vivo drug release is a crucial parameter which can affect toxicity and efficacy.

- Therefore, the pharmacokinetics of the developed liposomal product should always be compared with the innovator’s product. Only certain aspects of the conventional bioequivalence approach are applicable and in some cases additional requirements should be set on a case-by-case basis.

- Comparative human pharmacokinetic investigations should demonstrate not only the similarity of exposure of the total, unencapsulated and liposome encapsulated drug, but they should also demonstrate similar distribution and elimination characteristics.

EMA Reflection Paper – 2013
3.2.3 Non-clinical Studies *(ADME requirements!)*

**Non-clinical pharmacokinetic studies**

- Some pharmacokinetic aspects of liposomal products with regard to their performance in humans can be predicted by animal and, where applicable, cell-based models. However, the choice of appropriate species and models to investigate the in-vivo release of the drug from liposomes should be justified with special emphasis on areas such as accumulation and retention in target organs, pharmacokinetics and distribution. In addition to the systemic exposure, similarities in the distribution and elimination should be demonstrated. These studies provide pivotal evidence of the comparability of disposition of liposomal drug products, as it is not possible to have a full picture of the distribution in man from blood/plasma data alone.

- Sampling time points and sampling duration should be carefully selected so as to accurately quantify the time course of unencapsulated and total drug and metabolite in tissues balancing the need to quantify early drug release from liposomes (e.g. over first 15 min) and persistence of drug in particular tissues. If due to analytical reasons free concentrations cannot be measured then attempts should be made to compare the metabolite concentrations in the target organs.

- *Analytes to be measured*
  - The kinetics (including tissue distribution and excretion) of both the unencapsulated drug and the encapsulated drug should be investigated if feasible.

EMA Reflection Paper – 2013
Combination Chemotherapy

✔️ For most patients with cancer, standard of care usually involves the use of combinations of individual drugs. For example, the use of cytarabine in combination with an anthracycline (such as daunorubicin) is the standard of care for the treatment of patients diagnosed with acute myeloid leukemia (AML).

✔️ Individual drugs are combined at their “maximum tolerated dose” (MTD), the dose at which a drug has been shown to deliver maximum benefit balanced by an acceptable level of toxicity.

✔️ Complementary mechanism of actions
Understanding the Impact of Drug Ratios

✓ Dosing individual drugs at MTD does not always produce combination drug regimens that deliver maximum efficacy.

✓ The same drugs combined at different ratios can result in distinctly varied efficacy and safety profiles. Depending on the ratio of the combined drugs, the outcome can be:

**Additive** – the anti-cancer effect of the drugs is equal to the sum of the individual drugs.

**Synergistic** – the anti-cancer effect of the drugs is greater than the sum of the individual drugs.

**Antagonistic** – the anti-cancer effect of the drugs is less than the sum of the individual drugs.
Summary

Non-clinical ADME Studies to support Liposomal Drug Development

• Comparative studies of free vs. encapsulated forms of radiolabel drug(s) provide key information required by agencies for approval.

• May help to reduced the number and scope of additional clinical trials required for regulatory approval.
Thank you