EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 March 2022
EMA/CHMP/212114/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zolsketil pegylated liposomal

International non-proprietary name: doxorubicin

Procedure No. EMEA/H/C/005320/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

List of abbreviations

AAS	Atomic Absorption Spectrometry
AP	Applicant's Part (or Open Part) of a ASMF
API	Active Pharmaceutical Ingredient
API	Active Pharmaceutical Ingredient
AR	Assessment Report
AS	Active substance
ASM	Active Substance Manufacturer
ASMF	Active Substance Master File = Drug Master File
BCS	Biopharmaceutics Classification System
CCI	container closure integrity
CEP	Certificate of Suitability of the EP
CFU	Colony Forming Units
CHMP	Committee for Medicinal Products for Human use
CMA	Critical Material Attributes
CMS	Concerned Member State
CoA	Certificate of Analysis
CP	Centralised Procedure
CPP	Critical Process Parameters
CQA	Critical Quality Attributes
CRS	Chemical Reference Substance (official standard)
CVMP	Committee for Medicinal Products for Veterinary use
DoE	Design of experiments
DP	Decentralised (Application) Procedure
DPM	Drug Product Manufacturer
DSC	Differential Scanning Calorimetry
EC	European Commission
EDQM	European Directorate for the Quality of Medicines
EP	European Pharmacopoeia
EU	European Union
Eur.Ph.	European Pharmacopoeia
FDA	Food and Drug Administration
FMEA	Failure mode effects analysis
FPM	Finished Product Manufacturer
FT-IR	Fourrier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-MS	Gas chromatography mass spectrometry
GMP	Good Manufacturing Practices
HCT	Hydrochlorothiazide
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
HRMS	High resolution mass spectrometry
HSPC	hydrogenated soy phosphatidylcholine
IC	Ion chromatography

ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma - optical emission spectrometry
IPC	In-process control
IR	Infrared
IU	International Units
IUPAC	International Union of Pure and Applied Chemistry
KF	Karl Fischer titration
LCMS	Liquid chromatography mass spectrometry
LDPE	Low Density Polyethylene
LOA	Letter of Access
LOD	Limit of Detection
LOD	Loss on drying
LOQ	Limit of Quantitation
LoQ	List of Questions
LT	Less than
MA	Marketing Authorisation
MAH	Marketing Authorisation holder
MAV	Marketing Authorisation Variation
MEB	Medicines Evaluation Board
MPEG	2000-DSPE N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl- sn-glycero-3-phosphoethanolamine, sodium salt
MS	Mass Spectrometry
ND	Not detected
NIR	Near Infrared Spectroscopy
NLT	Not less than
NMR	Nuclear Magnetic Resonance
NMT	Not more than
NOR	Normal Operating Range
OOS	Out of Specification
PAR	Proven Acceptable Range
PCTFE	Polychlorotrifluoroethylene
PDE	Permitted Daily Exposure
PE	Polyethylene
Ph.Eur.	European Pharmacopoeia
PIL	Patient Information Leaflet
PIP	Paediatric Investigation Plan
PP	Polypropylene
PVC	Polyvinyl chloride
PVDC	Polyvinylidene chloride
QA	Quality Assessor
QbD	Quality by design
QC	Quality Control
QOS	Quality Overall Summary
QP	Qualified person
QTPP	Quality target product profile

QWP	Quality Working Party
RH	Relative Humidity
RMS	Reference Member State
RP	Restricted Part (or Closed Part) of an ASMF
RRF	Relative Response Factor
RRT	Relative retention time
RSD	Relative standard deviation
Rt	Retention time
SmPC	Summary of Product Characteristics
SST	System Suitability Test (chromatographic methods)
TAMC	Total Aerobic Microbial Count
TGA	Thermo-Gravimetric Analysis
TLC	Thin layer chromatography
tmax	Time to achieve Cmax
TSE	Transmissible Spongiform Encephalopathy
TTC	Threshold of toxicological concern
TYMC	Total Combined Yeasts/Moulds Count
uHPLC	ultra-high performance liquid chromatography
UPLC	Ultra Performance Liquid Chromatography
USP	United States Pharmacopeia
USP/NF	United States Pharmacopoeia/National Formulary
UV	Ultraviolet
XR(P)D	X-Ray (Powder) Diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Accord Healthcare S.L.U. submitted on 3 June 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Zolsketil pegylated liposomal, through the centralised procedure under Article 3 (2) (b) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 April 2019. The eligibility to the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 was based on demonstration of interest of patients at Community level.

The application concerns a hybrid medicinal product as defined in Article 10(3) of Directive 2001/83/EC and refers to a reference product, as defined in Article 10 (2)(a) of Directive 2001/83/EC, for which a marketing authorisation is or has been granted in a Member State on the basis of a complete dossier in accordance with Article 8(3) of Directive 2001/83/EC.

The applicant applied for the following indication:

ZOLSKETIL pegylated liposomal is indicated:

- As monotherapy for patients with metastatic breast cancer, where there is an increased cardiac risk.
- For treatment of advanced ovarian cancer in women who have failed a first-line platinum-based chemotherapy regimen.
- In combination with bortezomib for the treatment of progressive multiple myeloma in patients who have received at least one prior therapy and who have already undergone or are unsuitable for bone marrow transplant.
- For treatment of AIDS-related Kaposi's sarcoma (KS) in patients with low CD4 counts (< 200 CD4 lymphocytes/mm^3) and extensive mucocutaneous or visceral disease.

ZOLSKETIL pegylated liposomal may be used as first-line systemic chemotherapy, or as second line chemotherapy in AIDS-KS patients with disease that has progressed with, or in patients intolerant to, prior combination systemic chemotherapy comprising at least two of the following agents: a vinca alkaloid, bleomycin and standard doxorubicin (or other anthracycline).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Hybrid application (Article 10(3) of Directive No 2001/83/EC).

The application submitted is composed of administrative information, complete quality data and a bioequivalence study with the reference medicinal product Caelyx instead of non-clinical and clinical unless justified otherwise.

The chosen reference product is:

Medicinal product which is or has been authorized in accordance with Union provisions in force for not less than 10 years in the EEA:

- Product name, strength, pharmaceutical form: Adriamycin 2mg/ml solution for injection
- Marketing authorisation holder: Pfizer ApS

- Date of authorisation: (24-10-1979)
- Marketing authorisation granted by:
  - Member State (EEA) : Denmark
    - National procedure
- Marketing authorisation number: 13134
- Difference compared to this medicinal product: change in pharmaceutical form

Medicinal product which is or has been authorised in accordance with Union provisions in force and to which bioequivalence has been demonstrated by appropriate bioavailability studies:

- Product name, strength, pharmaceutical form: Caelyx pegylated liposomal, 2 mg/ml, concentrate for solution for infusion
- Marketing authorisation holder: Baxter Holding B.V.
- Date of authorisation: 21-06-1996
- Marketing authorisation granted by:
  - Union
- Bioavailability study number: 0244-17

1.3. Information on paediatric requirements

Not applicable

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

1.5. Scientific advice

The applicant did not seek scientific advice from the CHMP.

1.6. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Blanca Garcia-Ochoa Co-Rapporteur: Fátima Ventura

The application was received by the EMA on	3 June 2019
The procedure started on	20 June 2019

The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	12 September 2019
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	14 September 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	19 September 2019
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	17 October 2019
The applicant submitted the responses to the CHMP consolidated List of Questions on	24 January 2020
The following GCP inspection were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection at a bioanalytical site and a clinical trial site in India between 21st and 29th October 2021. The outcome of the inspection carried out was issued on.	17 December 2021
The CHMP Rapporteur circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on	6 March 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 March 2020
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	26 March 2020
The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on	22 December 2021
The CHMP Rapporteur circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	18 January 2022
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	27 January 2022
The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on	22 February 2022
The CHMP Rapporteur circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	10 March 2022
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Zolsketil pegylated liposomal on	24 March 2022

The CHMP adopted a report on similarity of Zolsketil pegylated liposomal with Zejula, Kyprolis, Farydak, Ninlaro, Imnovid, Darzalex, Abecma and Blenrep on	24 March 2022

2. Scientific discussion

2.1. Introduction

This centralised application concerns a hybrid application according to article 10(3) of Directive 2001/83/EC for Zolsketil pegylated liposomal 2 mg/ml concentrate for solution for dispersion, which contains doxorubicin hydrochloride as active substance. The reference medicinal product in this application is Adriamycin 2 mg/ml solution for injection (authorised nationally in Denmark on October 24th 1979).

Adriamycin and Zolsketil pegylated liposomal differ in terms of formulation, since Adriamycin contains doxorubicin hydrochloride in a non-liposomal formulation while Zolsketil pegylated liposomal contains doxorubicin hydrochloride in a pegylated liposomal formulation. Hence, due to the differences in formulation, a bioequivalence study was not feasible. Therefore, Caelyx, which contains doxorubicin hydrochloride in a pegylated liposomal formulation was chosen as comparator with regards to quality, non-clinical and clinical comparability. Caelyx 2 mg/ml concentrate for solution for infusion was authorised in the European Union on 20 June 1996 through a centralised procedure.

The indications applied for Zolsketil pegylated liposomal are the same indications authorised for Caelyx:

- As monotherapy for patients with metastatic breast cancer, where there is an increased cardiac risk.
- For treatment of advanced ovarian cancer in women who have failed a first-line platinum-based chemotherapy regimen.
- In combination with Bortezomib for the treatment of progressive multiple myeloma in patients who have received at least one prior therapy and who have already undergone or are unsuitable for bone marrow transplant.

For treatment of AIDS-related Kaposi's sarcoma (KS) in patients with low CD4 counts (< 200 CD4 lymphocytes/mm^3) and extensive mucocutaneous or visceral disease.

Doxorubicin hydrochloride is a cytotoxic anthracycline antibiotic obtained from *Streptomyces peucetius* var. *Caseins*. The exact mechanism of the antitumour activity of doxorubicin is not known. It is generally believed that inhibition of DNA, RNA and protein synthesis is responsible for the majority of the cytotoxic effects. This is probably the result of intercalation of the anthracycline between adjacent base pairs of the DNA double helix thus preventing their unwinding for replication.

Liposomes are microscopic vesicles composed of a phospholipid bilayer that are capable of encapsulating active drugs. The liposomes of Doxorubicin are formulated with surface-bound methoxy polyethylene glycol (MPEG), a process often referred to as pegylation, to protect liposomes from detection by the mononuclear phagocyte system (MPS) and to increase blood circulation time.

Pegylated liposomes have a half-life of approximately 55 hours in humans. They are stable in blood, and direct measurement of liposomal Doxorubicin shows that at least 90% of the drug (the assay used cannot

quantify less than 5-10% unencapsulated Doxorubicin) remains liposome encapsulated during circulation.

It is hypothesized that because of their small size (ca. 100 nm) and persistence in the circulation, the pegylated doxorubicin hydrochloride liposomes are able to penetrate the altered and often compromised vasculature of tumors. This hypothesis is supported by studies using colloidal gold containing pegylated liposomes, which can be visualized microscopically. Evidence of penetration of pegylated liposomes from blood vessels and their entry and accumulation in tumors has been seen in mice with C-26 colon carcinoma tumors and in transgenic mice with Kaposi's sarcoma-like lesions. Once the pegylated liposomes distribute to the tissue compartment, the encapsulated doxorubicin hydrochloride becomes available. The exact mechanism of release is not understood.

Pharmacokinetics

Doxorubicin hydrochloride (Pegylated Liposomal) concentrate for solution for infusion is a long-circulating pegylated liposomal formulation of doxorubicin hydrochloride. Pegylated liposomes contain surface-grafted segments of the hydrophilic polymer methoxy polyethylene glycol (MPEG). These linear MPEG groups extend from the liposome surface creating a protective coating that reduces interactions between the lipid bilayer membrane and the plasma components. This allows the Doxorubicin Hydrochloride Liposomes (Pegylated Liposomal) to circulate for prolonged periods in the blood stream. Pegylated liposomes are small enough (average diameter of approximately 100 nm) to pass intact (extravasate) through defective blood vessels supplying tumours. The pegylated liposomes also have a low permeability lipid matrix and internal aqueous buffer system to keep Doxorubicin hydrochloride encapsulated during liposome residence time in circulation.

The plasma pharmacokinetics of Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion in humans differs significantly from those reported in the literature for standard doxorubicin hydrochloride preparations. Standard Doxorubicin hydrochloride displays extensive tissue distribution (volume of distribution: 700 to 1,100 l/m^2) and a rapid elimination clearance (24 to 73 $l/h/m^2$). In contrast, the pharmacokinetic profile of Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion indicates that Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion is confined mostly to the vascular fluid volume and that the clearance of doxorubicin from the blood is dependent upon the liposomal carrier. Doxorubicin becomes available after the liposomes are extravasated and enter the tissue compartment.

At equivalent doses, the plasma concentration and AUC values of Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion which represent mostly pegylated liposomal Doxorubicin hydrochloride (containing 90 % to 95 % of the measured Doxorubicin) are significantly higher than those achieved with standard Doxorubicin hydrochloride preparations.

Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion should not be used interchangeably with other formulations of Doxorubicin hydrochloride.

Patients with impaired hepatic function

Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion pharmacokinetics determined in a small number of patients with elevated total bilirubin levels do not differ from patients with normal total bilirubin; however, until further experience is gained, the Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion dosage in patients with impaired hepatic function should be reduced based on the experience from the breast and ovarian clinical trial programs as follows: at initiation of therapy, if the bilirubin is between 1.2-3.0 mg/dl, the first dose is reduced by 25%. If the bilirubin is > 3.0 mg/dl, the first dose is reduced by 50%. If the patient tolerates the first dose without an increase in serum bilirubin or liver enzymes, the dose for cycle 2 can be increased to the next dose level, i.e., if reduced by 25% for the first dose, increase to full dose for cycle 2; if reduced

by 50% for the first dose, increase to 75% of full dose for cycle 2. The dosage can be increased to full dose for subsequent cycles if tolerated.

Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion can be administered to patients with liver metastases with concurrent elevation of bilirubin and liver enzymes up to 4 x the upper limit of the normal range. Prior to Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion administration, evaluate hepatic function using conventional clinical laboratory tests such as ALT/AST, alkaline phosphatase, and bilirubin.

Patients with impaired renal function

As doxorubicin is metabolized by the liver and excreted in the bile, dose modification should not be required. Population pharmacokinetic data (in the range of creatinine clearance tested of 30-156 ml/min) demonstrate that Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion clearance is not influenced by renal function. No pharmacokinetic data are available in patients with creatinine clearance of less than 30 ml/min.

Population pharmacokinetics

The pharmacokinetics of Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion was evaluated in 120 patients from 10 different clinical trials using the population pharmacokinetic approach. The mean intrinsic clearance of Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion was 0.030 l/h/m^2 (range 0.008 to 0.152 l/h/m^2) and the mean central volume of distribution was 1.93 l/m^2 (range 0.96 – 3.85 l/m^2) approximating the plasma volume. The apparent half-life ranged from 24 – 231 hrs with a mean of 73.9 hrs.

Breast cancer patients

The pharmacokinetics of Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion determined in 18 patients with breast carcinoma were similar to the pharmacokinetics determined in the larger population of 120 patients with various cancers. The mean intrinsic clearance was 0.016 l/h/m^2 (range 0.008 - 0.027 l/h/m^2), the mean central volume of distribution was 1.46 l/m^2 (range 1.10 - 1.64 l/m^2). The mean apparent half-life was 71.5 hrs (range 45.2 - 98.5 hrs).

Ovarian cancer patients

The pharmacokinetics of Doxorubicin Hydrochloride (Pegylated Liposomal) concentrate for solution for infusion determined in 11 patients with ovarian carcinoma were similar to the pharmacokinetics determined in the larger population of 120 patients with various cancers. The mean intrinsic clearance was 0.021 l/h/m^2 (range 0.009 – 0.041 l/h/m^2), the mean central volume of distribution was 1.95 l/m^2 (range 1.67 – 2.40 l/m^2). The mean apparent half-life was 75.0 hrs (range 36.1 – 125 hrs).

Relevant for the assessment are the Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02), the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98) as well as the Guideline on Bioanalytical method validation (EMEA/CHMP/EWP/192217/09), Question number 4 of the Questions & Answers: Generic Applications" (CMDh/272/2009/Rev. 03, December 2017) and Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance (EMA/CHMP/800775/2017).

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as a concentrate for dispersion for infusion containing 2 mg/mL of doxorubicin hydrochloride in a pegylated liposomal formulation.

Other ingredients are: N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl- sn-glycero-3-phosphoethanolamine, sodium salt (MPEG 2000-DSPE), hydrogenated soy phosphatidylcholine (HSPC), cholesterol, ammonium sulphate (E 517), sucrose (E 473), histidine, hydrochloric acid concentrated (E 507) (for pH adjustment), sodium Hydroxide (E-524) (for pH adjustment) and water for injections.

The product is available in type I glass vials with a siliconised grey bromobutyl stopper, and an aluminium seal containing a deliverable volume of 10 mL (20 mg) or 25 mL (50 mg) as described in section 6.5 of the SmPC.

2.2.2. Active substance

2.2.2.1. General Information

The chemical name of doxorubicin hydrochloride is (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride, corresponding to the molecular formula $C_{27}H_{29}NO_{11}$.HCl. It has a relative molecular mass of 580 and the following structure:

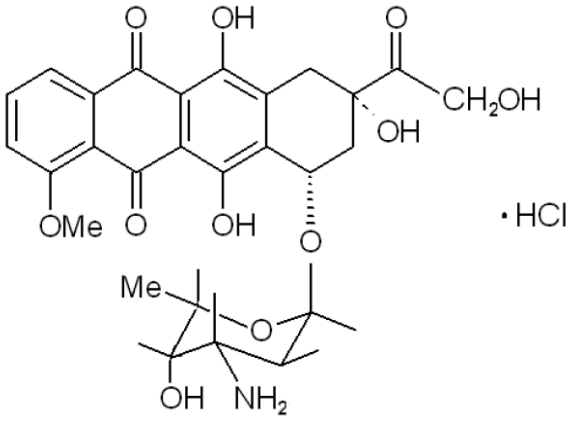

Figure 1: doxorubicin hydrochloride structure

Doxorubicin hydrochloride is subject of Ph. Eur. monograph 01/2008:0714. According to Ph. Eur., the active substance is an orange-red, hygroscopic, crystalline powder: It is soluble in water and slightly soluble in methanol.

The active substance does not show polymorphism.

The Certificate of Suitability of the European Pharmacopoeia (CEP) has been provided within the current marketing authorisation application (MAA).

2.2.2.2. Manufacture, characterisation and process controls

The relevant information has been assessed by the EDQM before issuing the CEP.

The CEP-holder is Transo-Pharm Handels GMBH.

The active substance is packaged in a brown glass container with a polypropylene cap and a polyethylene cap insert. Bottles comply with all applicable requirements for EP Type (III) glass as defined by the current Ph. Eur. 3.2.1. The container material is suitable for the storage of a powder for parenteral use.

2.2.2.3. Specification

As indicated above, the active substance manufacturer holds a CEP for doxorubicin hydrochloride certifying that the quality of the active substance is suitably controlled as per current version of the Ph. Eur. monograph 0714.

The active substance specification from the finished product manufacturer includes tests for description, identification (IR, HPLC, chloride), pH (Ph.Eur.), bacterial endotoxins (Ph.Eur.), water (Ph.Eur.), related substances (HPLC), assay (HPLC), residual solvents (GC) and microbial limit test (TAMC, TYMC, *E. coli*) (Ph. Eur.). The active substance specification is in line with Ph. Eur. and CEP tests and limits.

The in-house methods proposed by the finished product manufacturer (assay of doxorubicin·HCl by HPLC, related substances by HPLC and residual solvents by GC) have been validated. However, the applicant is recommended (REC1) to improve the analytical procedures description as defined in the section 2.2.6.

Details of the reference standards used for assay and impurities have been provided.

Certificates of analysis for three batches of active substance issued by the active substance manufacturer as well by the finished product manufacturer were provided. The results are within the specifications and consistent from batch to batch.

2.2.2.4. Stability

The CEP indicates that the re-test period of the substance is 36 months when stored in a brown glass container with a polypropylene cap and a polyethylene cap insert.

The finished product manufacturer will test every batch of active substance upon receipt as per the specification proposed.

2.2.3. Finished medicinal product

2.2.3.1. Description of the product and Pharmaceutical development

The finished product is a concentrate for dispersion for infusion. It consists of a pegylated liposomal formulation of doxorubicin hydrochloride 2 mg/mL. Two fill volumes are proposed: 10 mL and 25 mL.

The finished product is a translucent red coloured dispersion filled in a clear glass vial.

The excipients used in the formulation are hydrogenated soy phosphatidylcholine, N-(carbonylmethoxypoly-ethylene glycol-2000)-1,2- distearoyl- sn-glycero- 3-phosphoethanolamine,

sodium salt (MPEG 2000-DSPE), cholesterol, ammonium sulfate, histidine, sucrose, ethanol anhydrous, hydrochloric acid, concentrated, sodium hydroxide, water for injection, and nitrogen gas.

The aim of the pharmaceutical development was to formulate a robust and stable product, with essentially similar qualitative and quantitative composition and being a pharmaceutical equivalent generic formulation of Caelyx® 2mg/ml (MA Holder: Janssen-Cilag International NV).

The development of the formulation and manufacturing process has been described. The development of the formulation and manufacturing process was done using Quality by Design (QbD) approach. The quality target product profile (QTTP) was defined based on the European reference product; Caelyx. Major QTTP elements were appearance/description, route of administration, dosage form, strength, therapeutic indication, pharmacokinetic properties, storage, shelf life, packaging and drug product quality. The critical quality attributes were identified for the drug substance and drug product. The CHMP raised a MO asking the applicant to determine the pH of the internal compartment of the liposomes, and liposomes aggregation in 5% glucose. This was addressed and the MO resolved, concluding that the quality characterization of the product was performed in line with the CHMP reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02). The comparison for the test and reference product was performed for specification parameters.

The identified CQAs of the active substance were description/appearance, assay, related substances, pH, bacterial endotoxins and microbial tests.

The identified CQAs of the finished product intermediate (bulk stage) were appearance, pH, assay, bioburden and weight per mL.

The identified CQAs of the finished product were appearance, identification of doxorubicin, identification of lipids, pH, osmolality, particulate matter, particle size distribution, zeta potential, assay, related substances, lipid content, lipid degradants, encapsulation efficiency, free drug, drug to lipid ratio, sulfate and ammonium ion concentration, colour, sterility and bacterial endotoxin.

The applicant determined the qualitative and quantitative composition of the reference product based on literature survey, SmPC/Labelling and analytical evaluation of reference product, and adopted an equivalent composition for the formulation development of his product. The excipients selected are same as in the reference product. These are:

- Hydrogenated soy phosphatidylcholine (HSPC). This is the main lipid component, which forms the lipid bilayer along with other lipids during the manufacturing of the liposome formulation.

- MPEG-DSPE. This is also a lipid component which forms the lipid bilayer along with other lipids during the manufacturing of the liposome formulation as well as the pegylated layer over the surface of the liposome.

- cholesterol is the lipid component of the liposomes which provides the stability to the lipid bilayer of the liposomes.

- histidine which acts as a buffering agent.

- sucrose, used to maintain the isotonicity of the formulation.

- ammonium sulfate, used for the active substance precipitation and active drug loading in the liposomes.
- hydrochloric acid, to adjust the pH of the formulation.
- sodium hydroxide, to adjust the pH of the formulation
- ethanol, used for lipid solubilization
- water for injections, as the vehicle
- nitrogen gas, as the inert gas used for sparging and flushing

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph.Eur., US/NF or in-house specifications. As requested during the MA evaluation, the applicant presented a detailed justification for the proposed specifications for the non-compendial excipients, discussing the relevance of the parameters included and their limits and justifying the omission of some tests. It was also confirmed that cholesterol complies with Ph. Eur. monograph 2397 on "cholesterol for parenteral use". There are no novel excipients used in the finished product formulation.

To support this MAA, the applicant conducted an extensive comparative characterisation study on multiple batches of the reference and the test product. The quality attributes evaluated as part of this comparability exercise were the same as those tested in the reference product, listed above. The original submission included only tables with analytical results, and was missing a scientific discussion and a statistical evaluation of the results, in line with the CHMP reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development (EMA/CHMP/138502/2017). This was unacceptable and resulted in a major objection (MO) for which the applicant provided acceptable responses.

In response, the applicant provided a statistical comparison protocol and report of CQAs in line with the CMHP Reflection paper. According to the information provided, for each set quality attributes the similarity criteria, as well as the sample strategy, have been predefined in a protocol. Based on the analysed results the applicant confirmed that both products test and reference products are similar. The analyses provided are acceptable. These data demonstrated that the test product can be considered pharmaceutical equivalent to the reference product.

The comparability results on the general physicochemical properties of the test product were found to be equivalent to Caelyx. There were no significant differences between both products.

Upon request from the CHMP, and in line with the CHMP reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02), this comparative physicochemical characterization was also conducted on samples of the reference and test products stored for up to 3 months at accelerated condition (25°C/60%RH in inverted vials as the worst case condition). Comparable degradation behaviour was observed between the two products.

In addition, the comparability exercise also included an evaluation of the phase transition temperature, the composition and morphology [lamellarity, shape of liposomes, location of drug substance, size distribution, drug encapsulation and grafted PEG layer thickness] of the liposomes, in vitro leakage on multiple conditions, determination of the internal pH of the liposomes and determination of aggregates. There results showed no notable differences between the applicant's product and the reference product Caelyx.

Finally, in terms of drug release as measured by in vitro methods, both products were found to be equivalent in the various multiple conditions tested and the final *in vitro* release method proposed for quality control (QC) release testing. Comparability of the drug release profiles between the test and reference product under different conditions (e.g. temperature, pH) was also investigated. The results indicated that both products are highly comparable regardless of the *in vitro* release conditions.

During initial assessment, the CHMP raised an MO on the choice of QC dissolution method and its discriminatory nature. In response, the choice of the QC method conditions and its discriminatory nature towards changes to product composition and manufacturing process parameters has been further discussed and found to be satisfactory.

Overall, the applicant has discussed the physicochemical properties of the proposed product versus the reference product (Caelyx) in view of the required quality characterisation data as detailed in the CHMP reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02).

The liposomes are prepared using an ammonium sulfate gradient to load doxorubicin into the liposomes aqueous phase. Pre-formulation and development studies were described in sufficient detail. Based on the presented data, it is acceptable the use of overages proposed for the active substance and the lipids.

The recommended hold time of 24 hours is acceptable for manufacturing / filtration / filling of doxorubicin hydrochloride pegylated liposomal 2 mg/mL concentrate for dispersion for infusion. The filter selection has been justified for the filtration of bulk solution. Further, four types of stoppers MOC were observed to be compatible with the finished product, but the rubber stopper was selected as similar to the innovator stopper MOC. The product is oxygen sensitive and should be stored under nitrogen headspace or atmosphere; the product is stable throughout the studied pH range.

The manufacturing process includes sterile filtration and vial filling under aseptic conditions. As liposomes are temperature-sensitive, terminal sterilisation is not suitable for proposed finished product. The choice of the sterilisation method (sterile filtration) is justified.

Process optimisation studies were conducted at laboratory and large batch sizes, with identification of scale dependent and independent process parameters. The process parameters considered of high risk were identified in the lipid extrusion and filtration steps.

An overage of doxorubicin active substance to compensate for manufacturing process loss during filtration stage is justified by the results obtained during the validation of the manufacturing process. An excess lipid is employed to compensate for manufacturing losses observed during manufacturing of the lipid suspension prior to doxorubicin addition.

The primary packaging material consists of Type I glass vials closed with a siliconised grey bromobutyl stopper, and an aluminium seal containing a deliverable volume of 10 mL (20 mg) or 25 mL (50 mg). The flip off seals for each fill volume are of different colour (blue for the 10 mL, red for the 25 mL). The glass container and rubber stoppers comply with the requirements of Ph. Eur. The finished product glass vial is further wrapped with PharmaShield®. PharmaShield® is a system consisting of a superficial plastic sheathing around the vial, going from the reinforced non-PVC base to the vial seal.

The container closure was chosen based on the results from compatibility studies with the rubber stoppers and the glass vials, a 6-month stability study in inverted condition, a container closure integrity (CCI) study and an extractable and leachable study. The choice of the container closure system has been validated by stability data and is concluded to be adequate for the intended use of the product.

Some extractable impurities were observed at different concentrations; the impurities/extractables above AET level were considered as potential leachables and will be monitored during the leachable study in finished product (REC2).

2.2.3.2. Manufacture of the product and process controls

The manufacturing process consists of following main steps: preparation of buffer solution, preparation of lipid solution, preparation of lipid suspension and hydration, extrusion, diafiltration, drug solution preparation and loading, pre-filtration, aseptic filtration, filling and sealing. The CHMP raised a MO about the level of detail of the manufacturing process description provided in the original submission which was insufficient. This was addressed satisfactorily by the provision of further details. The process is considered to be a non-standard manufacturing process.

The finished product is manufactured through a validated manufacturing process.

Following a request from the CHMP, the applicant confirmed the maximum batch manufacturing time from the start of product manufacturing (doxorubicin addition) to the completion of packaging into the final primary container.

The manufacturing process and in-process controls correspond to the actual standards of pharmaceutical technology for liposomal preparations and are suitable to guarantee an appropriate quality of the final finished product. Although the applicant proposed to delete the IPC for "phosphorous content" for commercial production, a recommendation is made to keep this test in future process validation exercises (REC3).

The provided validation data on three batches of each fill volume (10 mL & 25 mL) show a good reproducibility with all presented data matching the specifications and in compliance with results obtained from finished product release testing. These imply that the manufacturing process is adequately controlled, reproducible and robust.

2.2.3.3. Product specification

The finished product release and shelf-life specifications include appropriate tests for this kind of dosage form: description, identification for doxorubicin hydrochloride (HPLC), identification for lipids (HPLC for hydrogenated soy phosphatidylcholine, HPLC for MPEG-DSPE, HPLC for cholesterol), pH, extractable volume, uniformity of dosage units, degradation products (HPLC-UV), assay of doxorubicin hydrochloride (HPLC-UV/DAD), in-vitro release, content of cholesterol (HPLC-UV), content of hydrogenated soy phosphatidylcholine (HPLC-UV), content of MPEG-DSPE (HPLC-RI), lipid related impurities, encapsulation efficacy (HPLC-UV), free drug, drug to lipid ratio, histidine content (HPLC-UV), sucrose content (HPLC-RI), sulfate ion concentration (IC), ammonium sulfate (IC), ammonium ion concentration (IC), ethanol content (GC), particle size distribution, particulate contamination, sterility, bacterial endotoxins, zeta potential, phosphorous content (ICPS-OES), osmolality (Ph.Eur.), turbidity (Ph.Eur.), and colour test (Ph.Eur.).

Although some of the limits proposed by the applicant in the original submission were wider than the batch analysis data provided, following a MO raised by the CHMP, the applicant revised and tightened them. The revised release and shelf-life tests and limits are acceptable. Nonetheless, the applicant is recommended to continue the characterisation studies of impurities and submit the data as soon as available (REC4). The applicant is also recommended to revise the limit of impurity at RRT 0.95 to further guarantee that the limit is not exceeded during the shelf-life (REC5).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. However, additional recommendations related to analytical methods (REC 6 and REC 7) are proposed to the applicant, as specified under the section 2.2.6.

Batch analysis results on three production scale finished product batches of 10 mL and three batches of 25 mL (i.e. the same batches as used in the stability studies) have been provided.

Satisfactory details and certificates of analysis for standards used for the analysis of the finished product have been provided.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on three process validation batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

Following a request from the CHMP (other concern) the applicant performed a risk assessment on the potential presence of nitrosamine impurities in the product concluding that the presence of nitrosamines is negligible. The information on the potential presence of nitrosamine impurities was considered satisfactory.

2.2.3.4. Stability of the product

Stability data from three production scale batches of finished product of each presentation stored for up to 24 months under long term conditions (5°C ± 3°C) and for up to six months under accelerated conditions (25°C ± 2°C/60% ± 5%RH) according to the ICH guidelines were provided. The batches were stored in inverted position in clear tubular glass vial (type I), with 20 mm grey bromobutyl rubber stopper, and a coloured flip off seal. The batches of Zolsketil liposomal pegylated concentrate for dispersion for infusion used in the stability studies are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for description, pH, related substance, assay of doxorubicin hydrochloride, cholesterol content, hydrogenated soy phosphatidylcholine content, MPEG-DSPE content, lysophophatidyl choline, lyso PEPEG 2000, free fatty acids, encapsulation efficiency, free drug, drug to lipid ratio, sulfate ion concentration, ammonium sulfate, ammonium ion concentration, particle size, particle size distribution, zeta potential, phosphorous content, osmolality, particulate contamination, sterility, bacterial endotoxins, turbidity and colour test. The analytical procedures used have been demonstrated to be stability indicating.

At long term conditions, there was an increase in the level of related substances. No trends are observed in the assay of the lipids, or in-vitro release. With regards to the lipid related impurities, there is no change in the level of lysophosphatidyl choline. However, the levels of lyso PEPEG 2000 and free fatty acid increase over time. There are no trends in any of the other parameters observed.

To note, although at day 120 list of questions (LoQ) a MO was raised on the proposed shelf-life for the 10 mL vials given that the observed level of the impurity at RRT 1.15 was not justified as being qualified, this was addressed by the applicant justifying the qualification level by use and the issue was resolved. At accelerated conditions, out of specification results were observed for related substances, in-vitro release, and MPEG-DSPE content.

A forced degradation study (covering acid, base, oxidative, heat, humidity, photolytic and metal ions degradation) was carried out as part of the analytical method validation in order to prove the specificity of the HPLC method for assay and related substances in the finished product, and therefore the stability indicating capability of those methods.

In addition, one batch of Zolsketil pegylated liposomal concentrate for dispersion for infusion was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Samples were tested for: description, pH, related substance, assay of doxorubicin hydrochloride, cholesterol content, hydrogenated soy phosphatidylcholine content, MPEG-DSPE content, lysophophatidyl choline, lyso PEPEG 2000, free fatty acids, encapsulation efficiency, free drug, drug to lipid ratio, particle size mean diameter and particle size distribution. The results demonstrate that the product is photostable.

A freeze thaw study was conducted. The purpose was to generate data to support possible temperature excursions during transit. The results showed that the product physical and chemical parameters remain unaffected after three cycles of freeze thaw, concluding that there is no risk for short-term storage at freezing conditions. Nonetheless, the applicant proposed to maintain the stringent storage condition "do not freeze" in line with reference product. The inclusion of this additional condition is acceptable. A dilution study was performed to evaluate the stability of doxorubicin hydrochloride pegylated liposomal 2 mg/mL concentrate for dispersion for infusion after dilution with 5% dextrose solution in non PVC infusion container for a period of 24 hrs at 2 - 8°C. The study is in accordance with the instructions in the reference product's SmPC. One test batch and one reference batch were included in the study. Samples were analysed for description, pH of diluted sample, assay of doxorubicin hydrochloride, related substances, particle size (mean), particle size distribution, encapsulation efficiency, free drug particulate matter and osmolality. The study results revealed that the applicant's product after diluted with 5% dextrose injection in the studies conditions meets the predetermined acceptance criteria for a period of 24 hours at 2 - 8°C. The results on the test and reference products were comparable and support the administration instructions included in section 4.2 of the SmPC.

Based on the available stability data, the proposed shelf-life of 18 months and storage conditions "Store in a refrigerator (2°C - 8°C); do not freeze" as stated in the SmPC (section 6.3 and 6.4) are acceptable.

2.2.3.5. Post approval change management protocols

n/a

2.2.3.6. Adventitious agents

There is no risk of Transmissible Spongiform Encephalopathy (TSE) / Bovine Spongiform Encephalopathy (BSE) from the raw materials used in manufacturing of Zolsketil pegylated.

Declarations by the suppliers for each excipient and the active substance used in this formulation regarding human or animal origin (TSE/BSE free declarations) have been submitted.

A copy of the current CEP for cholesterol is provided, as well as an updated confirmation that the cholesterol material complies with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via medicinal products

There are no other substances of ruminant animal origin present in the product or used in the manufacturing of this product.

2.2.4. Discussion on chemical, and pharmaceutical aspects

The active substance of the product, doxorubicin hydrochloride, is subject of a Ph. Eur. monograph (01/2008:0714). A CEP is used to support this application.

The finished product has been developed in essence as a "generic" of Caelyx (pegylated liposomes containing doxorubicin) and has been compared with it. The comparison of physicochemical attributes has been conducted in line with the CHMP reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02) and the CHMP reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development (EMA/CHMP/138502/2017). Although five MO were raised during the evaluation, which pertained to comparability exercise (attributes included and statistical evaluation) of the test and reference products, quality characterisation of the product (e.g. pH of internal compartment of the liposomes, liposomes aggregation), level of detail of the manufacturing process description, description of the dissolution method, finished product specification and the proposed shelf-life, all have been resolved by provision of the relevant additional information.

There are a few recommendations to the applicant for further development which do not impact the benefit/risk of the product.

Overall, the information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on TSE safety.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The applicant is recommended to improve the following analytical procedures description in terms of:

   In–house Related substances by HPLC:

   - The SST should include the symmetry factor. The acceptance criterion for the symmetry factor should meet the requirements on Eur. Ph. 2.2.46.
   - Calculation formula: Note that Ph. Eur. indicates: any impurity*: not more than the area of the peak due to doxorubicin in the chromatogram obtained with reference solution (b) (0.5 per cent).* Therefore, the calculation formula for individual impurities (as % of total area) should be amended in order to guarantee that the individual content of each impurity is expressed relative to main component.

In–house Assay by HPLC:

- SST:
  - The acceptance criterion set for RSD should meet the corresponding requirements on Eur. Ph. 2.2.46.
  - Apart from resolution and RSD, the SST for assay should include the symmetry factor. The acceptance criterion for the symmetry factor should be set in accordance with the corresponding requirements on Eur. Ph. 2.2.46.

The validation parameters should be revised in accordance with the System Suitability Test (SST) amended as requested above.

2. The applicant is recommended to monitor as part of the leachable study in finished product the potential leachables identified.

3. The applicant is recommended to keep the IPC for "phosphorous content" in future process validation exercises.

4. The applicant is recommended to continue the characterization studies of impurities and submit the data as soon as available.

5. The applicant is recommended to revise the limit of impurity at RRT 0.95, to further guarantee that the limit is not exceeded during the shelf-life.

6. For the method validation results for Zeta potential, the applicant is recommended to set tighter limits for RSD (repeatability/intermediate precision) in subsequent (re)validation exercise(s).

7. With regards to the content determinations by HPLC-UV and also for HPLC-RI, the applicant is recommended to update RSD in system suitability test (SST).

2.3. Non-clinical aspects

2.3.1. Introduction

Doxorubicin hydrochloride, a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius var. caesius*. Doxorubicin interferes with DNA synthesis by interacting strongly with the phosphate backbone of nucleic acids, presumably by specific intercalation of the planar anthracycline nucleus with the DNA double helix. Doxorubicin is an approved antineoplastic agent. Human tumors shown to be responsive to doxorubicin include acute leukemia, resistant Hodgkin's and non-Hodgkin's lymphomas, sarcoma, neuroblastoma, ovarian and endometrial carcinoma, breast carcinoma, bronchogenic carcinoma, lung cancer and thyroid and bladder carcinoma. AIDS-related Kaposi's sarcoma (KS) is somewhat responsive to doxorubicin as a single agent and in combination regimens. Dose dependent toxicities, including stomatitis/mucositis, nausea/vomiting, bone marrow suppression and cardiomyopathy, limit the amount of doxorubicin patients are able to tolerate. Conventional

liposomal formulations of doxorubicin have been proposed as a means to reduce doxorubicin-related toxicities and thereby improve the drug's therapeutic index.

2.3.2. Pharmacology

Zolsetil Pegylated Liposomal is a long-circulating liposomal formulation of doxorubicin. This type of liposome contains surface-grafted segments of the hydrophilic polymer methoxypolyethylene glycol (MPEG). These linear MPEG groups extend from the liposome surface creating a protective coating that reduces interactions between the lipid bilayer membrane and plasma components.

The critical design features of the Stealth Liposome include:

- Polyethylene glycol ("Stealth" polymer) coating: reduces MPS uptake and provides long plasma residence times.
- Average diameter of approximately 85 nm: balances drug carrying capacity and circulation time, and allows extravasation through endothelial defects/gaps in microvasculature of tumors.
- Low permeability lipid matrix and internal aqueous buffer system: provide high drug loading and stable encapsulation, i.e., drug retention during residence in plasma. The "steric stabilization" effect provided by MPEG is believed to be responsible for the remarkable stability of Accord Doxorubicin in plasma. The MPEG coating also inhibits the interaction (close approach) of liposomes with macrophage cells, thus reducing hepatic uptake and prolonging liposome residence time in the circulation.

A study evaluated whether multiple sessions of Lipo-DOX administered after FUS-induced blood-brain barrier (BBB) disruption (FUS-BBBD) induces severe adverse events in the normal brain tissues. While delivery of Lipo-DOX to the rat brain might result in minor damage, the severe neurotoxicity seen in earlier works does not appear to occur with delivery via FUS-BBB disruption. The damage may be related to capillary damage produced by inertial cavitation, which might have resulted in excessive doxorubicin concentrations in some areas.

Comparative *in vitro* cytotoxicity of doxorubicin liposome injection and Caelyx in cancer cell lines

In vitro cytotoxicity of Doxorubicin Liposome injection (Test) and Caelyx injection (Reference) were tested with WST-1 cell viability assay in two cancer cell lines, T47D, a human breast cancer line and P388, a murine leukemia cell line. The T47D cells were incubated with either Test or Reference over a concentration range of 0.01 and 100 µM for 96 hrs, while P388 cells were incubated over a concentration range of 0.001 and 10 µM for 72 hrs. Cell viability was quantified at the end of the incubation. The study showed comparable *in vitro* cytotoxicity between Test and Reference formulation in both T47D and P388 cells.

The P388 cell viability vs. concentration of doxorubicin of both formulations is showed in Figure 2. The 50% inhibition concentration (IC50) for both formulations estimated from the curves was around 0.4 µM.

The T47D cell viability vs. concentration of doxorubicin of both formulations is showed in Figure 3. The 50% inhibition concentration (IC50) for both formulations estimated from the curves was around 0.9 µM. The cytotoxicity studies showed equivalent inhibition of cancer cell lines by both formulations.

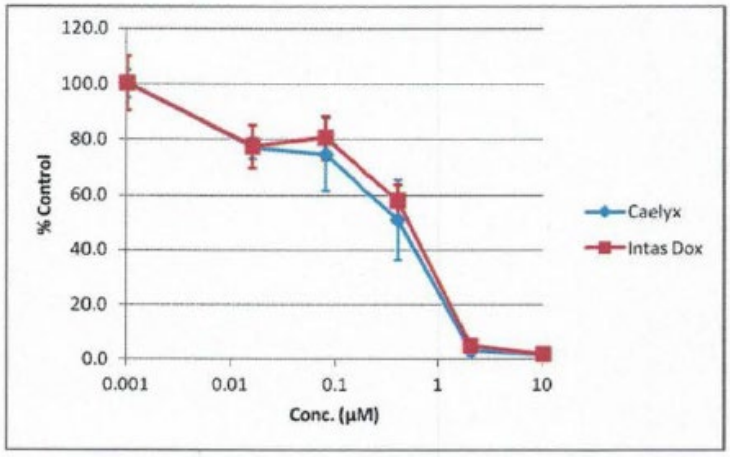

Figure 2: Viability of P388 Murine Leukemia Cells Treated with Doxorubicin
Cells were incubated with doxorubicin for 72 hrs.

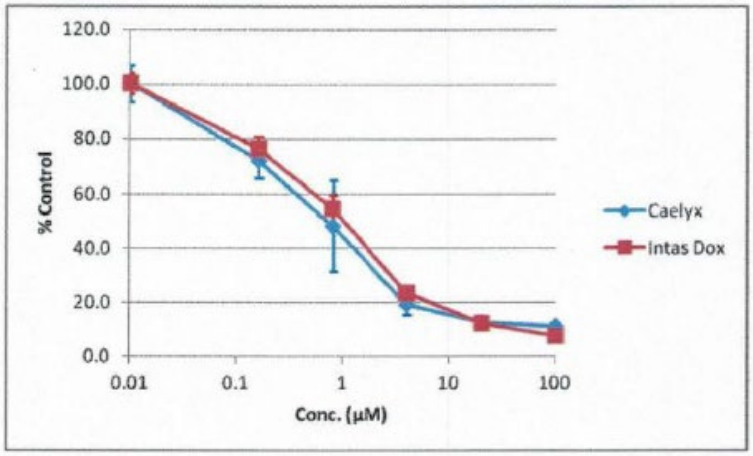

Figure 3: Viability of T47D Human Breast Cancer Cells Treated with Doxorubicin
Cells were incubated with doxorubicin for 96 hrs.

2.3.3. Pharmacokinetics

Daunorubicin, doxorubicin, epirubicin, and idarubicin usually are administered intravenously and are cleared by a complex pattern of hepatic metabolism and biliary excretion. The plasma disappearance curve for doxorubicin is multiphasic, with elimination half-lives of 3 hours and about 30 hours. All anthracyclines are converted to an active alcohol intermediate that plays a variable role in their therapeutic activity. Idarubicin has a half-life of about 15 hours, and its active metabolite, idarubicinol, has a half-life of about 40 hours. There is rapid uptake of the drugs in the heart, kidneys, lungs, liver, and spleen. They do not cross the blood-brain barrier.

Doxorubicin is eliminated by metabolic conversion to a variety of aglycones and other inactive products. Doxorubicin is metabolized predominantly by the liver to the major metabolite, doxorubicinol, and several cytotoxic aglycone metabolites. Doxorubicinol is up to 10 times more potent than doxorubicin at inhibiting isometric contraction of the papillary muscle isolated from the right ventricle of rabbit heart.

Pegylated liposomal doxorubicin (doxorubicin HCl liposome injection) is a liposomal formulation of doxorubicin, reducing uptake by the reticulo-endothelial system due to the attachment of polyethylene glycol polymers to a lipid anchor and stably retaining drug as a result of liposomal entrapment via an ammonium sulfate chemical gradient. These features result in a pharmacokinetic profile characterized

by an extended circulation time and a reduced volume of distribution, thereby promoting tumor uptake. Preclinical studies demonstrated one- or two-phase plasma concentration-time profiles. Most of the drug is cleared with an elimination half-life of 20–30 hours. The volume of distribution is close to the blood volume, and the area under the concentration-time curve (AUC) is increased at least 60-fold compared with free doxorubicin. Studies of tissue distribution indicated preferential accumulation into various implanted tumors and human tumor xenografts, with an enhancement of drug concentrations in the tumor when compared with free drug.

Comparative pharmacokinetics of doxorubicin following an intravenous administration of doxorubicin liposome injection (Intas) and Caelyx in female ICR (CD-1) mice.

The pharmacokinetics (PK) of doxorubicin in reference formulation, Caelyx (Janssen) and a test formulation, Doxorubiocin Liposome Injection (Intas) following a single intravenous (IV) dose administration was determined in female ICR (CD-1) mice (Study No. JIN026). Blood samples were collected at 1, 24, 48 and 72 hrs following each IV administration. Plasma was prepared and the concentrations of free (unencapsulated) and encapsulated doxorubicin in plasma sample were analyzed by HPLC. The major PK and relative bioavailability of free and encapsulated doxorubicin were calculated.

Both free and encapsulated doxorubicin profiles were comparable between Test and Reference formulations. The 90% confidence interval (CI) of relative bioavailability (T/R) of both free and encapsulated doxorubicin was well within 80.00-125.00%, indicating the two formulations are bioequivalent.

Relative Bioavailability of Free Doxorubicin:

Parameter	Units	Ref GeoLSM	Test GeoLSM	Ratio_%Ref	90%CI
$Ln(C_{max})$	ng/mL	506.9	538.5	106.2	101.65- 111.04
$Ln(AUC_{1ast})$	h*ng/mL	20187.4	19564.2	96.9	91.99- 102.10
$Ln(AUC_{INF\_obs})$	h*ng/mL	21801.1	20942.1	96.1	92.70- 99.55

Relative Bioavailability of Encapsulated Doxorubicin:

Parameter	Units	Ref GeoLSM	Test GeoLSM	Ratio_%Ref	90%CI
$Ln(C_{max})$	ng/mL	157884.1	157622.1	99.8	96.33- 103.47
$Ln(AUC_{1ast})$	h*ng/mL	3857830.8	3814019.4	98.9	96.08- 101.73
$Ln(AUC_{INF\_obs})$	h*ng/mL	4061759.2	4253806.5	104.7	101.34- 108.22

GeoLSM: geometric least square mean

The time course of mean plasma concentrations of free doxorubicin for both formulations are shown in Figure 4 and encapsulated for both formulations are presented in Figure 5.

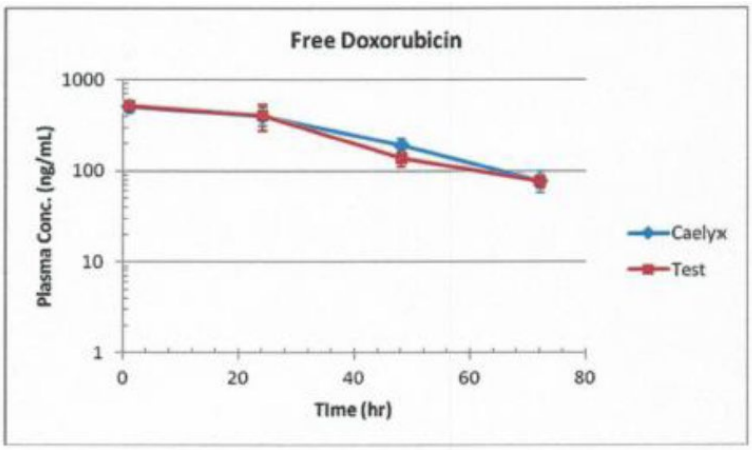

Figure 4: Plasma Concentration - Time Profiles of Free Doxorubicin

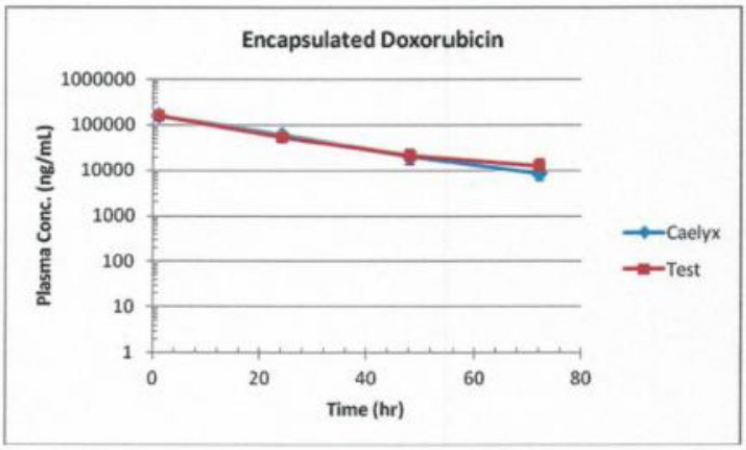

Figure 5: Plasma Concentration - Time Profiles of Encapsulated Doxorubicin

The animal pharmacokinetics study (JIN026) as described above in female ICR (CD-1) mice, also compared the volume of distribution (Vd) between the reference (R) product Caelyx (Janssen) and the test (T) product Doxorubicin Liposome Injection (Intas) following a single intravenous (IV) dose administration was determined in female ICR (CD-1) mice. The comparative data obtained is as shown below:

Table 1: Volume of distribution (Vd), clearance (Cl) and elimination (t1/2 el) data for free and encapsulated doxorubicin in female ICR (CD-1) mice from study number JIN026.

Analyte	Formulation	Dose	$t_{1/2}$ el	V_d	Cl
		mg/kg	h	mL/kg	mL/h/kg
Free	R	6	20.2	7450.7	256.2
	T	6	20.0	7763.9	268.8
Encapsulated	R	6	16.3	33.7	1.4
	T	6	19.0	38.6	1.4

2.3.4. Toxicology

The toxicity profile of pegylated liposomal doxorubicin is characterized by dose-limiting mucosal and cutaneous toxicities, mild myelosuppression, decreased cardiotoxicity compared with free doxorubicin

and minimal alopecia. The mucocutaneous toxicities are dose-limiting per injection; however, the reduced cardiotoxicity allows a larger cumulative dose than that acceptable for free doxorubicin (Gabizon A et al. 2003).

2.3.4.1. Single dose toxicity

In single dose studies, the acute toxicity of liposomal doxorubicin injection was similar for mice, rats, and dogs. In the rat, the incidence and severity of clinical observations were dose- related and included tail and footpad lesions, swelling and inflammation of the penis and scrotum, rough haircoat, alopecia, hypoactivity, hunched posture, respiratory distress, and reduced body weight gain. Reversible myelotoxicity was noted based on decreased RBC, WBC, hemoglobin, and hematocrit. Increases occurred in BUN and cholesterol levels (Product monograph, Caelyx, 2018). Dogs were the most sensitive species. Treatment-related toxicity included dermal toxicity, reversible myelotoxicity, hematologic changes, increased BUN, gastrointestinal toxicity, body weight loss, reversible cutaneous lesions, and alopecia. Myelotoxicity was less severe compared with the doxorubicin hydrochloride group. In a single dose study, MPEG-DSPE micelles, a component of the Doxorubicin liposome formulation, had no acute toxic effects in mice when administered at a lipid dose approximately 30-fold that found in the dose of 20 mg/m^2 recommended for humans. The acute toxicity of doxorubicin in Swiss mice varies greatly according to the route of administration. The LD50 is 8.5 mg/kg by the intra-peritoneal route, 21.1 mg/kg by the intravenous route, and greater than 750 mg/kg by the oral route.

2.3.4.2. Repeat dose toxicity

The toxicities of free doxorubicin (F-DOX) and liposome-associated doxorubicin (L-DOX) were investigated in inbred BALB/c and outbred Sabra mice treated iv with 5, 7.5, and 10 mg doxorubicin (DOX)/kg body weight every 2 weeks up to 8 injections and observed for 6 months. Two distinct patterns of death were observed: 1) an acute phase type occurring early after injection of high doses of DOX and apparently related to gastrointestinal toxicity and 2) a delayed phase type requiring a long latency after initial drug exposure and characterized by a complex pattern of abnormalities. Delivery of DOX by liposomes effectively protected against both types of lethal effects. Reduced toxicity of L- DOX resulted in reduced body and organ weight losses, reduced severity of pathologic changes, and fewer blood biochemical alterations. The pathological damage to the heart muscle found in mice treated with L-DOX was less severe than with F-DOX, and in some cases, it was reversible. Nephrotoxicity was extremely frequent and severe among F-DOX- treated mice, while it was totally insignificant among L-DOX-treated mice. Hyperlipidemia, hypoglycemia, and glycogen-depleted hepatocytes were characteristic findings in mice treated with F-DOX. Altogether, the data obtained in this study indicate that liposomes significantly diminish the toxicity of DOX with the use of an intermittent schedule of chemotherapy.

Repeated dose toxicity of a generic version of Doxil (Pegylated Doxorubicin Hydrochloride Liposome), and Doxil were also conducted in male and female mice and rats, respectively. Mice (5 males and 5 females in each group) received 3 i.v. injections (via tail vein) at a dose of 6 mg/kg on days 1, 8 and 15. Rats (6 males and 6 females in each group) received an i.v. injection at dose levels of 0.5, 1.0 and 1.5 mg/kg on days 1 and 8. The toxicity profiles of Pegylated Doxorubicin Hydrochloride Liposome in both mice and rats were comparable to those of Doxil. The body weight changes from Day 1 to Day 29 were comparable between those treated with Pegylated Doxorubicin Hydrochloride Liposome and Doxil in both mice and rats. Necropsy results showed that all organ weights were within the normal limits for animals treated with Pegylated Doxorubicin Hydrochloride Liposome and Doxil. There were no abnormal changes in Complete Blood Count and Blood Chemistry values for both male and female

animals treated with Doxil or Pegylated Doxorubicin Hydrochloride Liposome. There were no histopathological changes or generic toxicity in kidneys, liver, heart, spleen and lungs in mice treated with either Pegylated Doxorubicin Hydrochloride Liposome or Doxil. In rats, mild toxic effects were observed only in cardiac myocytes of some of the animals treated with Doxil or Pegylated Doxorubicin Hydrochloride Liposome. No other toxic effects in kidneys, liver, spleen and lungs were observed in any of the animals. It was thus concluded that Pegylated Doxorubicin Hydrochloride Liposome have comparable toxicity profile to that of Doxil in both mice and rats (Ali SM et al. 2016).

2.3.4.3. Carcinogenicity and mutagenesis

Doxorubicin, is both mutagenic and carcinogenic so conducting carcinogenicity and mutagenicity studies was not deemed necessary. Four studies were carried out with Stealth placebo liposome to confirm their lack of mutagenicity and genotoxicity. Negative results were obtained in the Ames, the L5178Y mouse lymphoma, and chromosomal aberration assays in vitro, and the mouse bone marrow micronucleus assay in vivo.

Formal long-term carcinogenicity studies have not been conducted with doxorubicin. Doxorubicin and related compounds have been shown to have mutagenic and carcinogenic properties when tested in experimental models (including bacterial systems, mammalian cells in culture, and female Sprague-Dawley rats) (Report on carcinogens, 1985; PDR, 2002; SPC, 2008; HSDB: Hazardous Substance Data Bank: National Library of Medicine). Doxorubicin is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. There is inadequate evidence for the carcinogenicity of doxorubicin in humans (Report on carcinogens, 1985). No epidemiological study of doxorubicin alone was available for review. However, in one study of cancer patients receiving doxorubicin in combination with alkylating agents and radiotherapy, the patients developed leukemia and bone cancer.

2.3.4.4. Local tolerance

Histopathological evaluation of the intravenous injection sites in Rabbits revealed that liposomal doxorubicin injection, doxorubicin hydrochloride, and placebo liposomes were well tolerated with no gross or microscopic evidence of irritation. In contrast, histopathological evaluation of the subcutaneous injection sites showed reversible mild to moderate dose-related inflammation at liposomal doxorubicin injection injections sites compared to moderate to severe inflammation and necrosis at doxorubicin hydrochloride injection sites that showed no signs of resolution during a 4-week recovery period.

2.3.4.5. Other toxicity studies

Cardiotoxicity: Cardiotoxicity is a risk of anthracycline treatment that may be manifested by early (i.e., acute) or late (i.e., delayed) events (Doroshow et al. 1981; Shan et al., 1996; Andersson et al. 1999; Wang et al. 1999; Forrest et al. 2000; Olson et al. 2003; Minotti et al. 2004; Kim et al. 2006). Results in two species (dogs and rabbits) demonstrate that the cardiotoxicity of doxorubicin is significantly decreased when administered as pegylated liposomal doxorubicin. Significantly more pegylated liposomal doxorubicin can be given without incurring an increased risk of cardiomyopathy (Working PK et al. 1999).

Anionic liposomes containing doxorubicin were evaluated in mice for therapeutic potential in reducing the risks of chronic cardiotoxicity characteristic of long-term high-dose anthracycline therapy. It was concluded that anionic liposomes can function as efficacious carriers of doxorubicin. These vesicles

possess improved therapeutic action as reflected by their ability to reduce cardiac toxicity, overcome growth inhibition, and increase antileukemic activity (Forssen EA *et al*. 1981).

Nephrotoxicity: Renal toxicity reflected in increased serum creatinine and blood urea nitrogen levels included tubular and/or glomerular changes and presented as renal hemorrhage and/or edema (cortex, pelvis or papilla), distal tubular dilatation, tubular protein casts, hypertrophy of the Bowman's capsular epithelial cells, interstitial

Administration of lip^- DXR resulted in lower DXR levels in renal tissue compared to administration of free DXR. After administration of lip^+ DXR, very low tissue and tumor DXR levels were found. The authors conclude that treatment with lip^- DXR or lip^+ DXR resulted in a prolonged survival, less albuminuria, and higher serum albumin levels. Also, fewer lesions in kidney was found, correlating with lower DXR levels in the kidney (vanHoesel QGCM et al. 1984).

Hemolytic Potential: The hemolytic potential of liposomal doxorubicin injection and Stealth placebo liposomes in human blood was assessed in vitro, as well as their compatibility with human serum and plasma. Neither liposomal doxorubicin injection 1.0 mg/mL nor empty Stealth liposomes induced hemolysis of human erythrocytes, nor did either cause coagulation or precipitation of human serum or plasma. Lysophosphatidylcholine (LPC) is a degradation product of the phosphatidylcholine component of the liposomes. An additional hemolytic potential study using liposomal doxorubicin injection formulations prepared with 0 mg/mL, 0.5 mg/mL, or 0.88 mg/mL LPC caused no hemolysis of rat blood cells.

Dermal Lesion Development: The effect of peak dosage and dose frequency on dermal lesion development and myelosuppression was studied in dogs. Liposomal doxorubicin injection 0.5, 1.0, 1.5 mg/kg was administered q7d, q14d, or q28d by intravenous (cephalic) catheter for 6-12 weeks. The higher dose intensities with lower dose frequency (1.0 mg/kg q14d and 1.5 mg/kg q28d) produced minimal evidence of cyclic depression of hemoglobin and hematocrit. In both groups, the hemoglobin and hematocrit values recovered to prestudy values at the end of the study. The onset of lesions occurred within 1 to 2 weeks after initiation of treatment and began to heal at rates that varied depending on lesion severity and dose frequency. Myelosuppression was mild with all treatment regimens and no evidence of treatment-related leukopenia was observed. Dosages of 0.5 mg/kg given every 2 or 4 weeks were tolerated much better than the weekly doses at 0.5 mg/kg. Comparison of groups that received 0.5 mg/kg/treatment showed clear dose frequency-related effects on lesion development, lesion severity, and general toxicity. Integration of current results with previous studies showed a similar frequency-dependent effect with 1.0 mg/kg; weekly and every 2-week regimens produced severe toxicity while a 3-week dose cycle was better tolerated.

Stealth Liposome Placebo: In addition to the mutagenicity and developmental studies, and the acute and long-term studies in which placebo liposomes were used as controls, Stealth Liposome Placebo was evaluated for its potential to induce cardiovascular changes in dogs and neurobehavioral changes in rats. In the cardiovascular study, dogs showed a significant decrease in blood pressure (19-70%) immediately after the start of dosing followed by a rapid partial recovery after the end of dosing, and a return to normal values within 4-6 hours post-dose. Compensating acceleration in heart rate was not seen. The dose rate did not affect the extent of hypotension, but inversely affected the duration. In the rat study, placebo liposomes did not induce any adverse neurobehavioral effects or evidence of neurotoxicity.

Other toxicity Studies: The excipients in the Doxorubicin Hydrochloride concentrate for solution for infusion are: Fully hydrogenated soy phosphatidylcholine (HSPC), α-(2-[1,2-distearoyl-sn glycero(3)phosphooxy] ethylcarbamoyl) -ω-methoxypoly(oxyethylen)-40 sodium salt (MPEG-DSPE),

Cholesterol, Ammonium sulphate, Sucrose, Histidine, sodium hydroxide, hydrochloric acid and water for injection. Suitability and safety of excipients in the formulation and the toxicity of inactive ingredients were discussed. The excipients presented in Doxorubicin Hydrochloride concentrate for solution for infusion 2 mg/ml are essentially similar to that of reference product. These excipients are widely used in pharmaceutical products and are well characterized. In line with EC directive 92/27 all excipients should be declared on the leaflet. There is no safety or clinical concerns regarding the presence of any of these excipients in this formulation.

Several new studies were added to non-clinical dossier, in line with the EMA´s Reflection Paper on the data requirements for intravenous liposomal products developed regarding an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02). In the light of the studies provided by the Applicant, the product is comparable and has similar exposure as with the reference. Consequently, it is concluded that all the toxicological data generated for the reference medicinal product, can be extrapolated to Doxorubicin Liposome Injection (Intas), and the outcome to the comparability studies support equivalent toxicity.

2.3.5. Ecotoxicity/environmental risk assessment

No dedicated environmental risk assessment (ERA) has been provided. The introduction of Zolsketil pegylated liposomal is considered unlikely to result in any significant increase in the combined sales volumes for all Doxorubicin hydrochloride, liposomal, containing products and the exposure of the environment to the active substance.

2.3.6. Discussion on non-clinical aspects

As this is a "Hybrid" application claiming essential similarity to an existing product, no specific non-clinical studies have been undertaken; however, an analysis of data pertaining to related substances and formulation supported the authorization of this product under article 10(3) of European Directive 2001/83/EC. The non-clinical part of the submission provided considered the pharmacological and toxicological literature.

The reference product (Adriamycin) contains doxorubicin hydrochloride in a different form (not enclosed in pegylated liposomes), whereas in the applicant's product the active substance doxorubicin hydrochloride is enclosed in tiny fatty spheres called liposomes (pegylated liposomal formulation). Therefore, Caelyx 2 mg/ml concentrate for solution for infusion, which contains doxorubicin in pegylated liposomal form, was used for comparisons with Zolsketil pegylated liposomal.

The cytotoxic effect of doxorubicin on malignant cells and its toxic effects on various organs are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities of doxorubicin. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of doxorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of doxorubicin cytocidal activity.

Doxorubicin and related compounds have been shown to have mutagenic and carcinogenic properties when tested in experimental models (including bacterial systems, mammalian cells in culture, and female Sprague-Dawley rats).

Animal studies have shown activity in a spectrum of experimental tumors, immunosuppression, induction of a variety of toxic effects, including delayed and progressive cardiac toxicity, myelosuppression in all species and atrophy to testes in rats and dogs.

Zolsketil pegylated liposomal is a long-circulating liposomal formulation of doxorubicin. This type of liposome contains surface-grafted segments of the hydrophilic polymer methoxypolyethylene glycol (MPEG). These linear MPEG groups extend from the liposome surface creating a protective coating that reduces interactions between the lipid bilayer membrane and plasma components. The "steric stabilization" effect provided by MPEG is believed to be responsible for the stability of Accord Doxorubicin in plasma. The MPEG coating also inhibits the interaction (close approach) of liposomes with macrophage cells, thus reducing hepatic uptake and prolonging liposome residence time in the circulation. Decreased systemic elimination, increased penetration into the tumor, and long liposome presence with slow drug release into the tumor probably accounted for the enhanced therapeutic effect of doxorubicin in sterically stabilized liposomes.

Besides, as requested, the Applicant performed several new non-clinical studies to follow the EMA recommendations stated in the "Reflection paper on the data requirements for intravenous liposomal products developed with reference an innovator liposomal product" (EMA/CHMP/806058/2009/Rev.02). The studies addressed specific questions of Pharmacology and, pharmacokinetics. The studies display similar outcome between the free and encapsulated in both products (reference and test formulations). Therefore, no new studies in animals are required to support the present marketing authorisation application.

The Applicant conducted an in-vitro cytotoxicity study to demonstrate the similarity in pharmacodynamic response. To perform the mentioned test, two cancer cell line were tested T47D, a human breast cancer line, and P388, a murine leukaemia cell line. The cytotoxicity studies showed equivalent inhibition of cancer cell lines by both formulations i.e. test product and reference product.

The Applicant conducted two in vivo tests to address the pharmacokinetic studies question inquiry by EMA´s reflection paper "Reflection paper on the data requirements for intravenous liposomal products developed regarding an innovator liposomal product" (EMA/CHMP/806058/2009/Rev. 02), a comparative pharmacokinetic study in mice; and a distribution study in mice. Both studies displayed a similar outcome between the free and encapsulated doxorubicin in both products (reference vs test formulation). After one single intravenous dose administration in female ICR mice, the plasma concentration for encapsulated and unencapsulated remains at comparable levels. Secondly, a distribution study was conducted in mice and compared to human data (reference and a test formulation). The result displays similar data between the free and encapsulated in both products (reference vs test formulation) either in the volume of distribution and clearance.

Some impurities are present at higher than the qualification threshold, this may be considered acceptable, based on levels found in the EU and US originator product, which are considered as qualified.

Environmental Risk Assessment

The applicant has submitted a review of the doxorubicin active-product-ingredient (API) consumption from year 2017 to year 2019 (data not shown). According to the EMA/CHMP/SWP/44609/2010 Rev. 1 guideline, the introduction of Zolsketil pegylated liposomal is considered unlikely to result in any significant increase in the combined sales volumes for all Doxorubicin hydrochloride, liposomal, containing products and the exposure of the environment to the active substance, and not further studies on environmental risk assessment are necessary.

2.3.7. Conclusion on the non-clinical aspects

The Applicant presented new studies to confirm the similarity between the reference product and the test product. The studies display similar outcomes in cytotoxicity, volume of distribution and clearance

between the reference and the test product. Considering all the results provided (bibliographic and new studies) and acknowledging that the Applicant follows the EMA´s reflection paper on the data requirements for intravenous liposomal products developed regarding an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02), and, takes into account the principles of the EMEA´s 3R Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches, no further animal studies are required to support the present marketing authorisation application.

The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.

2.4. Clinical aspects

2.4.1. Introduction

The reference medicinal product (Adriamycin) contains doxorubicin hydrochloride (not enclosed in pegylated liposomes), whereas in the Applicant's product the active substance doxorubicin hydrochloride is enclosed in tiny fatty spheres called liposomes (pegylated liposomal formulation). Therefore, Caelyx 2 mg/ml concentrate for solution for infusion, which contains doxorubicin in pegylated liposomal form was used for comparisons with the Applicant's product.

The applicant provided a clinical overview outlining the pharmacokinetics and pharmacodynamics as well as efficacy and safety of doxorubicin based on published literature. The SmPC is in line with the SmPC of the reference product.

No CHMP scientific advice pertinent to the clinical development was given for this medicinal product.

For the clinical assessment the Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02), the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1) as well as the Guideline on Bioanalytical method validation (EMEA/CHMP/EWP/192217/09), <Question number 4 of the Questions & Answers: Generic Applications" (CMDh/272/2009/Rev. 03, December 2017) and Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance (EMA/CHMP/800775/2017) are of particular relevance.

GCP aspect

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Tabular overview of clinical studies

To support the application, the Applicant has submitted one bioequivalence study, "a multicentre, open label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, cross-over, bioequivalence study of Doxorubicin hydrochloride (Pegylated Liposomal) in comparison with Caelyx [Doxorubicin Hydrochloride (Pegylated Liposomal)] in patients with ovarian cancer".

Table 2. Tabular overview of the clinical studies

Type of study	Study Identifier	Location of study report	Study title	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of treatment	Study Status; Type of Report
Pivotal	Project No. 0244-17	Module 5, Section 5.3.1.2	A Multicenter, Open Label, Balanced, Randomized, Two-Treatment, Two-Period, Two-Sequence, Single Dose, Cross-Over, Bioequivalence Study of Doxorubicin Hydrochloride (Pegylated Liposomal) in comparison with Caelyx® [Doxorubicin Hydrochloride (Pegylated Liposomal)] in Patients with Ovarian Cancer	Test Product: Doxorubicin Hydrochloride (Pegylated Liposomal) 2 mg/mL (20 mg/10 mL) concentrate for solution for infusion Ref product: Caelyx® [Doxorubicin Hydrochloride (Pegylated Liposomal)] 2 mg/ml (20 mg/10 mL) concentrate for solution for infusion Single dose: 50 mg/m^2 Intravenous infusion	Total subjects included in pharmacokinetic and statistical analysis, N = 50	Patients with Ovarian Cancer	Single dose	Completed, Full

2.4.2. Clinical pharmacology

2.4.2.1. Pharmacokinetics

Study Project No. 0244-17: A multicentre, open label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, cross-over, bioequivalence study of Doxorubicin hydrochloride (Pegylated Liposomal) in comparison with Caelyx [Doxorubicin Hydrochloride (Pegylated Liposomal)] in patients with ovarian cancer.

Methods

- **Study design**

This was a multicenter, open label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, cross-over, bioequivalence study. There was at least 28 days of washout period between first dosing of IMP administration in the two periods. Window period of +14 days for adverse event management was allowed between two periods of the study.

As per the protocol, a total of fifty (50) samples (25 samples in each period), each of 5 mL were collected from each patient during the study.

The venous blood samples were withdrawn at pre-dose (0.000 hour) and at 0.333, 0.667, 1.000*, 1.250, 1.500, 1.750, 2.000, 2.500, 3.000, 4.000, 5.000, 6.000, 8.000, 12.000, 16.000, 24.000, 48.000, 72.000, 120.000, 168.000, 216.000, 264.000, 312.000 and 360.000 hours after first-dose administration in each period. Blood sample at 72.000, 120.000, 168.000, 216.000, 264.000, 312.000 and 360.000 hours were collected on an ambulatory basis.

*That was the adjustable time-point: The sample was taken immediately at the end of infusion (1 hour ± 5 minutes).

Pre-dose blood sample were collected within 5 minutes prior to dosing. A deviation of ± 1 minute was allowed for samples to be collected during infusion up to the end of infusion. A deviation of ± 2 minutes was allowed for post-dose samples up to 48 hours. The ambulatory samples were collected at an allowable deviation of ± 2 hours. All deviations outside the range allowed as above were

documented as protocol deviations.

The actual time of sample collection was reported in eCRF.

The blood samples were withdrawn using pre-labelled syringe and transferred into pre-chilled (maintained at temperature of 2 to 8°C), vacutainers containing K_3EDTA as the anticoagulant, kept in ice cold water bath (maintained at temperature of 2 to 8°C).

Treatments administered and method of administration

After an overnight fast of at least 8 hours, the patients were served with a non-high fat breakfast, which they consumed completely within 30 minutes. The breakfast derived approximately 15-20%, 60-65%, and 20-25% calories from protein, carbohydrate, and fat respectively. The IMP was administered at two hours (± 10 minutes) after serving of breakfast. They were administered 50 mg/m^2 dose (based on BSA) of either the test or reference product as per the randomisation schedule on the first day of the chemotherapy cycle under fasting condition (Day 1) in Period-I. Patients were crossed over to the other arm in Period-II (Day 29).

The IMP was administered by intravenous infusion after dilution over 1 hour (+ 5 minutes window period).

The sampling schedule is considered adequate. It is necessary to take sample beyond 72 h because this is not an immediate release product, but a prolonged release product. Samples up to 360 h are considered sufficient.

The washout period of at least 28 days between the dosing days is considered sufficient for encapsulated, unencapsulated and total doxorubicin taking into account the half-life described for encapsulated doxorubicin of 73 h. However, pre-dose values were observed for subjects [redacted] (169.980 ng/mL) and [redacted] (168.189 ng/mL) for encapsulated doxorubicin and subjects [redacted] (8.033 ng/mL) and [redacted] (37.637 ng/mL) for unencapsulated doxorubicin. Only in the last case the pre-dose level is higher than 5% of the corresponding Cmax. This profile was excluded from statistical calculations.

Further, the dosing interval is in line with the recommended posology of one dose once every 4 weeks as this BE study is conducted in ovarian cancer patients.

Conducting the bioequivalence study with standardized light meals due to patient's needs is appropriate and in line with the product-specific bioequivalence guidance. Moreover, the relative caloric content (approximately 15-20%, 60-65%, and 20-25% calories from protein, carbohydrate, and fat respectively) is considered also acceptable. The detailed composition of the meal is not described, but it is not considered critical.

The administration of the meal two hours before drug product administration is considered acceptable since this product is not an orally administered product.

- **Test and reference products**

Test and reference product information is presented below:

Product Characteristics	Test Product-T	Reference Product-R
Name	Doxorubicin Hydrochloride (Pegylated Liposomal) 2 mg/mL (20 mg/10 mL) concentrate for solution for infusion	Caelyx® [Doxorubicin Hydrochloride (Pegylated Liposomal)] 2 mg/ml (20 mg/10 mL) concentrate for solution for infusion
Strength	2 mg/mL (20 mg/10 mL)	2 mg/mL (20 mg/10 mL)
Dosage form	Concentrate solution for infusion	Concentrate solution for infusion
Manufacturer	Intas Pharmaceuticals Limited, India.	-
Marketing Authorization Holder	Accord	Janssen-Cilag International NV, Turnhoutseweg 30, B-2340 Beerse, Belgium.
Measured content(s) (% of label claim)	97.0%	98.9%
Commercial Batch Size	-	
Expiry date (Retest date)	04/2019	03/2019
Location of Certificate of Analysis	Appendix No. 16.3.2-Other CRFs Submitted Page # 70-71	Appendix No. 16.3.2-Other CRFs Submitted Page # 72
Member State where the reference product is purchased from:		European Union
This Product was used in the following trials:	0244-17	0244-17

- **Population(s) studied**

The sample size computation was determined considering the following assumptions:

- T/R ratio = 95.0-105.0%
- Intra-patient CV (%) ~ 35%
- Significance Level = 5%
- Power ≥ 80%
- Bioequivalence Limits = 80.00-125.00%

Based on the above estimates, 52 completers were required to establish bioequivalence between formulations with adequate power. Considering approximately 20% dropout and/or withdrawn, 66 patients were sufficient to establish bioequivalence between formulations with adequate power for this study.

Patient with confirmed ovarian cancer whose disease has progressed or recurred after platinum-based Chemotherapy who are already receiving or scheduled to start the therapy with Doxorubicin HCl (Pegylated Liposomal) 2 mg/mL (20 mg/10 mL) concentrate for solution for infusion. Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2. Cardiac function (left ventricular ejection fraction [LVEF] ≥ 50%.

A total of 66 patients were dosed in the trial as per the randomization schedule. Out of these, a total of 15 patients discontinued/ were withdrawn.

In all, a total of 50 patients completed the clinical phase of the study successfully and were used in the pharmacokinetics and statistical analysis with the exception of one patient (Period-I) whose pre-dose concentration was > 5% of C_{max} for unencapsulated doxorubicin.

Demographics and baseline characteristics are presented in the following table:

	Statistics	TR (N=32)	RT (N=34)	Total (N=66)	Completed patients (N=50)
Age (years)	n	32	34	66	50
	Mean (SD)	50 (10.5)	51 (9.9)	51 (10.1)	50 (9.4)
	Median	48	52	51	49
	Min, Max	27, 74	27, 67	27, 74	27, 70
Gender					
Female	n (%)	32 (100.00%)	34 (100.00%)	66 (100.00%)	50 (100.00%)
Race					
Asian	n (%)	32 (100.00%)	34 (100.00%)	66 (100.00%)	50 (100.00%)
Height (cm)	n	32	34	66	50
	Mean (SD)	152 (6.5)	153 (5.4)	153 (5.9)	153 (6.0)
	Median	152	152	152	152
	Min, Max	142, 171	142, 163	142, 171	142, 171
Weight (kg)	n	32	34	66	50
	Mean (SD)	53.89 (11.241)	58.31 (12.431)	56.16 (11.986)	58.03 (11.961)
	Median	53.20	59.20	56.25	57.50
	Min, Max	32.50, 80.00	34.00, 92.00	32.50, 92.00	32.50, 92.00
BMI (kg/m^2)	n	32	34	66	50
	Mean (SD)	23.3 (4.86)	24.9 (5.32)	24.1 (5.12)	24.8 (5.16)
	Median	22.5	25.2	23.2	24.8
	Min, Max	14.1, 33.0	16.6, 37.8	14.1, 37.8	14.1, 37.8

Prophylactic Inj. Granisetron (Antiemetic) 2 mg IV (30 minutes prior) and Inj. Dexamethasone 8 mg (45 minutes prior) (to avoid hypersensitivity reaction) were given to all the patients before IMP administration in both the periods. Prophylactic anti-allergy treatment and G-CSF are permitted at the discretion of the investigator.

- **Analytical methods**

Pre-study validation

The concentrations of unencapsulated doxorubicin (non-liposomal, free) and encapsulated doxorubicin in human plasma were determined using two separate LC-MS/MS methods. Apparently, the pre-study validation of both methods are satisfactory. Both methods met the acceptance criteria for all the validation parameters evaluated, demonstrating acceptable performance. In addition, the analyte stability was demonstrated. The analytical range for unencapsulated doxorubicin was modified to 4.063 ng /mL - 750.770 ng/ mL] in the partial validation (MV(I)-205-16 (A-IV). This new calibration range met also the acceptance criteria or all the validation parameters evaluated. The long-term stability data in frozen human plasma stored at -65 ºC was demonstrated in K_3EDTA for 280 days for unencapsulated doxorubicin and at least 250 days for encapsulated doxorubicin and it covers the maximum storage samples at -65 ºC [i.e., 232 days at -65±10°C for unencapsulated doxorubicin (June 06th, 2018 to January 23th, 2019 for Patient No. 10202 and 243 days at -65±10°C for encapsulated doxorubicin (May 28th, 2018 to January 25th, 2019 for Patient No. 10802)].

In-study validation:

The calibration standards of the in-study validation were acceptable, although, for encapsulated doxorubicin the processing of calibrators, QC samples and study samples should be identical. However, in the determination of encapsulated doxorubicin the calibrators and QC samples prepared with

working solutions were extracted by protein precipitation, whereas the QC samples prepared with the formulation and the study samples were extracted by solid phase extraction. In the submitted response the Applicant has clarified these concerns.

Encapsulated doxorubicin

The reasons for reanalysis of the individual samples for unencapsulated doxorubicin (significant analyte concentration in pre-dose sample, concentration above highest standard, poor chromatography and significant variation in response of internal) and for encapsulated doxorubicin (significant analyte concentration in pre-dose sample of patient, and laboratory accident) are acceptable. In addition, one run for unencapsulated doxorubicin and four runs for encapsulated doxorubicin were re-analysed due to the runs did not meet the acceptance criteria. In addition, fifty samples (50) were reanalysed due to the run did not meet the analytical run acceptance.

Dilution of some samples for unencapsulated doxorubicin was necessary (a dilution factor x5 was validated; the maximum C_{max} was 1466.705 ng/mL).

Dilution of some samples for encapsulated doxorubicin was not necessary (the maximum C_{max} was 54,163.493 ng/mL).

Neither a sample re-integration nor a sample re-injection was performed for encapsulated doxorubicin. For unencapsulated doxorubicin, the re-injection and the re-integration of the samples were carried out according to the internal SOPs. The applicant submitted a table concerning the re-integration of chromatograms for free doxorubicin and a table concerning the reanalysis of samples runs containing subjects No. [redacted] and [redacted], including the original and the repeated concentration values. The ISR was performed in a total of 125 samples for each analyte, which is in accordance with section 6 "Incurred Samples reanalysis" of the Guideline on bioanalytical method validation that require at least 194 samples out of 2876 (10% of the samples should be reanalysed in case the number of samples is less than 1000 samples and 5% of the number of samples exceeding 1000 samples). For the samples reanalysed, the ISR was acceptable as 90.7% (195 samples out of 215 samples) and 76.7% (165 samples out of 215 samples) of the samples reanalysed for encapsulated and unencapsulated doxorubicin, respectively, were within the acceptance range (± 20%).

- **Pharmacokinetic variables**

The following pharmacokinetic parameters were calculated:

Primary pharmacokinetic parameters

C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for unencapsulated and encapsulated doxorubicin.

Secondary pharmacokinetic parameters

T_{max}, AUC_{0-48}, AUC_{48-t}, λ_z, $t_{½}$, AUC_%Extrap_obs, Cl and V_d for unencapsulated and encapsulated doxorubicin.

These parameters were derived individually for each analysed patient from the concentration vs. time profiles of Encapsulated and unencapsulated Doxorubicin in plasma using non-compartmental of Phoenix WinNonlin.

The selected primary pharmacokinetics variables are not completely in line with the product-specific guidance for liposomal doxorubicin, because the partial AUC should have been considered as primary PK parameters for the encapsulated doxorubicin to ensure profile comparability. In any case, the Applicant has submitted these partial AUC and we have considered them as primary.

Pharmacokinetic software is considered acceptable and the method of C_{max} and AUC estimation is correct since the linear trapezoidal method was employed as described in the protocol.

According to the product-specific guidance for liposomal doxorubicin, only encapsulated drug and unencapsulated drug need to be analysed, but both have to be quantified separately.

For unencapsulated doxorubicin the results of two samples have been excluded for the analysis because of suspected mishandling of the samples at the clinical site, which cannot be accepted. The Applicant has conducted new pharmacokinetic calculations again with the original concentrations of the samples from patients [redacted] and [redacted] (please refer to the results section).

- **Statistical methods**

The statistical software and method is considered the correct method to be employed.

The calculation of the 90% CI of the test / reference ratio of the pharmacokinetic parameters of interest is based on an ANOVA analysis containing the centre factor because it was defined so in the protocol. In addition, the centre effect was significant in several PK parameters (Encapsulated doxorubicin Cmax, AUC_t, AUC_{inf}, AUC_{0-48} and AUC_{48-t} and unencapsulated doxorubicin Cmax, AUCt, AUCinf, AUC40-t), except for unencapsulated doxorubicin $AUC_{0-48\ h}$ with P value = 0.0512), according to the ANOVAs reported by the Applicant. In the assessor opinion the existence of a significant centre effect is not relevant since patients in the difference centers may exhibit different demographic characteristics, which are likely responsible for the different exposure in the centers. In addition, the number of patients per centre is very limited and some of the centers were pooled.

The Applicant has also submitted an additional pharmacokinetic and statistical analysis that was performed using the original concentration values to calculate pharmacokinetic parameters of unencapsulated Doxorubicin and these results show that the 90% CI of all the primary PK parameters remains within the acceptance criteria of 80.00-125.00% (see results section).

Results

The pharmacokinetic parameters of Unencapsulated Doxorubicin for Test Product-T and Reference Product-R are summarized in the following table (Period-I):

[Excluding Patient No. [redacted] (Period-I) whose pre-dose concentration was >5% of C_{max}]

Parameters (Units)	Mean ± SD (untransformed data)	
	Test Product-T (N=50)	Reference Product-R (N=49)
T_{max} (h)[#]	16.000 (0.667 - 168.000)	16.000 (1.000 - 120.000)
C_{max} (ng/mL)	125.785 ± 39.1102	144.967 ± 196.1236
AUC_{0-t} (ng.h/mL)	14208.298 ± 4068.4027	15707.305 ± 13073.7238
$AUC_{0-\infty}$ (ng.h/mL)	15555.246 ± 4876.9338	16777.882 ± 13331.1367
AUC_{0-48} (ng.h/mL)	4126.787 ± 914.6318	4485.950 ± 3448.3333
AUC_{48-t} (ng.h/mL)	10081.510 ± 3688.8756	11221.355 ± 9761.5076
λ_z (1/h)	0.009 ± 0.0038	0.009 ± 0.0032
$t_{½}$ (h)	85.374 ± 31.2855	84.124 ± 29.8037
AUC_%Extrap_obs (%)	7.634 ± 8.1586	6.789 ± 4.4463
Cl (L/h)	5.368 ± 1.6020	5.534 ± 2.0452
V_d (L)	631.294 ± 214.2492	633.881 ± 232.5733

[#]T_{max} is represented as median (min-max) value.

The pharmacokinetic parameters of Encapsulated Doxorubicin for Test Product-T and Reference Product-R are summarized in the following table:

Descriptive Statistics of Formulation Means for Encapsulated Doxorubicin (N = 50)

Parameters (Units)	Mean ± SD (untransformed data)	
	Test Product-T	Reference Product-R
T_{max} (h)[#]	2.500 (1.000 - 120.000)	2.500 (1.017 - 6.000)
C_{max} (ng/mL)	37118.891 ± 4968.7401	39210.181 ± 5439.0007
AUC_{0-t} (ng.h/mL)	3241512.135 ± 725000.4072	3300540.378 ± 863650.9482
$AUC_{0-\infty}$ (ng.h/mL)	3351310.408 ± 775389.3182	3410411.509 ± 915120.5940
AUC_{0-48} (ng.h/mL)	1237931.778 ± 168209.1830	1293412.150 ± 190549.9190
AUC_{48-t} (ng.h/mL)	2003580.357 ± 621483.7402	2007128.228 ± 725681.8307
λ_z (1/h)	0.012 ± 0.0063	0.012 ± 0.0054
$t_{½}$ (h)	65.950 ± 18.0723	62.293 ± 17.8972
AUC_%Extrap_obs (%)	3.078 ± 2.4119	3.007 ± 3.7775
Cl (L/h)	0.024 ± 0.0061	0.024 ± 0.0086
V_d (L)	2.210 ± 0.6326	2.064 ± 0.5260

[#]T_{max} is represented as median (min-max) value.

Bioequivalence Confidence Intervals and intra-subject CV% of doxorubicin

Encapsulated Doxorubicin (N = 50)

Parameters	Geometric Least Squares Means			90% Confidence Interval	Intra Patient CV (%)	Power (%)
	Test Product-T	Reference Product-R	Ratio (T/R) %			
lnC_{max}	36902.723	39021.710	94.6	91.94 - 97.28	8.4	100.0
$lnAUC_{0-t}$	3139300.162	3160177.808	99.3	95.19 - 103.67	12.7	100.0
$lnAUC_{0-\infty}$	3237807.262	3260433.151	99.3	95.13 - 103.66	12.7	100.0
$lnAUC_{0-48}$	1225501.545	1276933.864	96.0	93.70 - 98.30	7.1	100.0
$lnAUC_{48-t}$	1880421.634	1843416.490	102.0	95.66 - 108.78	19.2	100.0

(Refer Table No. 14.2.1.1)

Unencapsulated Doxorubicin (N=49)

[Excluding patient No. [redacted] (Period-I) whose pre-dose concentration was >5% of Cmax]

Parameters	Geometric Least Squares Means			90% Confidence Interval	Intra Patient CV (%)	Power (%)
	Test Product-T (N=50)	Reference Product-R (N=49)	Ratio (T/R) %			
lnC_{max}	119.743	115.635	103.6	92.08 - 116.46	35.3	93.1
$lnAUC_{0-t}$	13529.238	13562.056	99.8	91.91 - 108.28	24.3	99.7
$lnAUC_{0-\infty}$	14726.369	14521.978	101.4	93.45 - 110.05	24.2	99.7
$lnAUC_{0-48}$	4002.014	3988.060	100.3	91.66 - 109.87	26.9	99.1
$lnAUC_{48-t}$	9372.159	9418.035	99.5	91.00 - 108.82	26.5	99.2

New pharmacokinetic and statistical calculations were done with the original concentrations of the samples from patients [redacted] and [redacted] as shown below:

PK Parameters	Geometric Least Square Mean Ratio (T/R)%	90% Confidence Interval
C_{max}	96.6%	83.23%-112.19%
AUC_{0-t}	98.5%	90.29%-107.41%
AUC_{0-inf}	102.0%	93.88%-110.84%

Linear and log-linear plots have been submitted for both analytes.

A pre-dose concentration levels were detected for subjects [redacted] (8.033 ng/mL) and 10104 (37.637 ng/mL) for unencapsulated doxorubicin and subjects [redacted] (169.980 ng/mL) and [redacted] (167.189 ng/mL) for encapsulated doxorubicin. For subject [redacted], the pre-dose value was > 5% of C_{max} (95.063 ng/mL), hence this subject was excluded from the statistical analysis. This is considered acceptable in accordance with the Guideline on the investigation of bioequivalence. For the rest of the subjects the pre-dose values were < 5% of their respective C_{max} and were included in the statistical analysis.

No t_{max} was observed at the first sample time point for both analytes.

The LLOQ was 4.133 ng/mL for unencapsulated doxorubicin, therefore, it was not sensitive enough to detect levels of 5% of the minimum C_{max} (3.2884 ng/mL is the 5% of the minimum C_{max}= 65.768 ng/mL), but it was sensitive enough to detect levels of the 5% of the mean C_{max} (7.248 ng/mL is the 5% of the mean C_{max}= 144.967 ng/mL).

The LLOQ was 151.002 ng/mL for encapsulated doxorubicin, therefore, it was sensitive enough to detect levels of 5% of the minimum C_{max} (1379.458 ng/mL is the 5% of the minimum C_{max}= 27,589.161 ng/mL).

The AUC estimated is considered representative enough of the extent of absorption/exposure since the extrapolation is lower than 20% in all individual profiles for both analytes with the exception of subject [redacted] (25.823%) for encapsulated doxorubicin of the test product and subjects [redacted] (48.928%), [redacted] (34.915%) and [redacted] (22.294%) for unencapsulated doxorubicin of the test product and subject [redacted] (23.589%) for reference product. This is considered acceptable in accordance with the Guideline on the investigation of bioequivalence.

For encapsulated doxorubicin and unencapsulated doxorubicin it can be concluded that the 90% CI for AUC_{0-t}, AUC_{0-inf}, C_{max} and partial AUC (AUC_{0-48h} and AUC_{48-t}) were within the 80.00% to 125.00% acceptance criterion. Therefore, bioequivalence has been demonstrated for these two types of doxorubicin (encapsulated and unencapsulated). The Applicant has submitted additional pharmacokinetic and statistical analyses that were performed using the original concentration values to calculate pharmacokinetic parameters of unencapsulated Doxorubicin and these results show that the 90% CI of all the primary PK parameters remains within the acceptance criteria of 80.00-125.00%.

- **Safety data**

A total of 66 patients were dosed at least once and were included in safety evaluation. A total of two hundred and thirty-one (231) adverse events (AEs) were reported by forty (40) patients during the conduct of study. As per CTCAE, of the two hundred and thirty-one (231) adverse events, one hundred and forty (140) AEs were classified under Grade 1 (mild), fifty-nine (59) AEs were classified under Grade 2 (moderate), twenty-six (26) AEs were classified under Grade 3 (severe) and six (6) AEs were classified under Grade 5 (death related to AE).

The causality assessment was judged as unlikely for one hundred and thirty-four (134) AEs, as probable/likely for sixty (60) AEs, as possible for thirty-one (31) AEs and as certain for six (6) AEs to the study drug administered.

One hundred and twenty-five (125) AEs were reported after administration of Test Product-T and one hundred and six (106) AEs were reported after administration of Reference Product-R.

The outcome of the adverse event was "recovered without sequelae" for two hundred and nine (209) AEs, "unknown" for nine (9) AEs, "death" for six (6) AEs, "change in severity" for five (5) AEs, "not yet recovered " for one (1) AE and "stable" for one (1) AE.

There were ten (10) SAEs reported during the study. Out of which, one (1) SAE was sudden death and from the remaining nine (9) SAEs, five (5) SAEs resulted in death of 2 patients. Hence, a total of three (3) patients died during the study.

The outcome of the SAEs was "recovered without sequelae" for two (2) SAEs, "unknown" for one (1) SAE, "death" for six (6) SAEs and "change in severity" for one (1) SAE.

The causality assessment was judged as unlikely for nine (9) SAEs (including 3 deaths) and possible for one (1) SAE.

2.4.2.2. Pharmacodynamics

No new pharmacodynamic studies were presented and no such studies are required for this application.

2.4.3. Discussion on clinical aspects

The application is for a hybrid medicinal product of the reference product Adriamycin 2 mg/mL Solution for Injection, which has been authorized nationally in Denmark on 24th October 1979. The proposed indications for the developed product are the same indications of the centrally authorised product Caelyx 2 mg/mL Concentrate for Solution for Infusion of Janssen-Cilag International NV.

To support the application, the Applicant has submitted one bioequivalence study, "a multicentre, open label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, cross-over, bioequivalence study of Doxorubicin hydrochloride (Pegylated Liposomal) in comparison with Caelyx [Doxorubicin Hydrochloride (Pegylated Liposomal)] in patients with ovarian cancer".

The Applicant has stated that the trial has been conducted in compliance with GCP and GLP requirements, and the monitoring reports of the bioequivalence study have been submitted. Both, clinical and analytical sites were inspected by the European Authorities.

The test and reference (from the Romanian market) products and the mode of administration are adequate in accordance with Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance. Further, the dosing interval is in line with the recommended posology of one dose once every 4 weeks as this BE study is conducted in ovarian cancer patients.

Both batches were tested before expiry date and the CoA shows a similar content. Therefore, content correction is not necessary.

The provided comparative bioavailability study is appropriate in accordance with pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/mL product-specific bioequivalence guidance, that require a single dose study with standardized light meals rather than in the fasting state due to patient's needs (please refer to mode of administration). In addition, the study was carried out in patients with ovarian cancer, which is also in accordance with the product-specific bioequivalence guidance.

Conducting the bioequivalence study with standardized light meals due to patient's needs is appropriate and in line with the product-specific bioequivalence guidance.

A total of 66 patients were dosed in the trial as per the randomization schedule. The number of subjects is adequate to show equivalence based on the intra-subject variability obtained from in house data. Out of these, a total of 15 patients discontinued/ were withdrawn.

The subject withdrawals due to adverse events (subjects [redacted] and [redacted]), protocol non-compliance (subject [redacted]), due to disease progression (subject [redacted]), died during the study (subjects [redacted] and [redacted], lost to follow-up ([redacted]) and dropout (freely of consent; [redacted], [redacted]) are considered to be acceptable. The exclusion of patient [redacted] from the statistical analysis due to pre-dose concentration > 5% of Cmax for unencapsulated doxorubicin is considered acceptable in accordance with the Guideline on investigation of bioequivalence. This pre-dose level occurred in period 1, which may be understood as he/she was a patient.

The study population is considered acceptable with regards to demographic characteristics and the inclusion and exclusion criteria are considered to be acceptable.

The study patient population is in line with the recommendation of the product-specific bioequivalence guidance (stable ovarian/breast cancer patients) and with the therapeutic indications of the reference product used in the BE study.

The concentrations of unencapsulated doxorubicin (non-liposomal, free) and encapsulated doxorubicin in human plasma were determined using two separate LC-MS/MS methods.

The selected primary pharmacokinetics variables (i.e., C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were not completely adequate for this study, since partial AUCs are also required in the product-specific guidance for liposomal doxorubicin. The Applicant has submitted them and these were considered as secondary PK parameters.

The applied statistical methodology is adequate and in accordance with the Guideline requirements. A Statistical Analysis Plan is presented in appendix 16.1.9.4 of the Clinical Study Report, with details on the statistical analysis. ANOVA model includes clinical site as a factor. The center effect was assessed by the applicant with MS error as denominator at it should be the MSsubject(sequence*center). With the correct analysis it has not been detected a centre effect. Therefore, the differences between centers are not significant compared with the existing variability.

It could be considered more relevant to conduct the ANOVA including additionally the centre-by-formulation interaction in order to assess if the ratio T/R is consistent in all centers. This exploratory analysis is not proposed to estimate the 90% CI of the ratio T/R of the primary PK parameters, since the estimation would be biased when the number of subjects per center is disbalanced, but it is considered valuable to give consistency to the study results. These analyses have been conducted by the assessment teams and no center-by-formulation interaction has been detected. The centre effect is not considered relevant, the conventional ANOVA ignoring the centre, i.e. including only formulation, period, sequence and subject (sequence) has been calculated and the corresponding 90% confidence interval of the ratio test / reference of the PK parameters of interest are similar to those reported by the MAH based on the ANOVA including the centre without centre-by-formulation interaction. Therefore, this sensitivity analysis confirms the bioequivalence of the product.

For encapsulated doxorubicin and unencapsulated doxorubicin it was concluded that the 90% CI for AUC_{0-t}, AUC_{0-inf}, C_{max} and partial AUC (AUC_{0-48h} and AUC_{48-t}) were within the 80.00% to 125.00% acceptance criterion. Therefore, bioequivalence has been considered as appropriately demonstrated for these two substances. However, for unencapsulated doxorubicin this conclusion was only achieved after the presentation of the outcome of new calculations that were repeated with the original values of two samples that were previously excluded (not reportable) due to pharmacokinetic reasons.

The safety profile of both products seems to be comparable, although it is obvious that the design was not powered to compare the safety profile. No difference in the safety profile can be anticipated.

2.4.4. Conclusions on clinical aspects

Based on the presented bioequivalence study Zolsketil pegylated liposomal is considered bioequivalent with Caelyx.

2.5. Risk Management Plan

2.5.1. Safety concerns

Table SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	None

2.5.1. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.5.2. Risk minimisation measures

None.

2.5.3. Conclusion

The CHMP and PRAC considered that the risk management plan version 1.4 is acceptable.

2.6. Pharmacovigilance

2.6.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.6.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.7. Product information

2.7.1. User consultation

No full user consultation with target patient groups on the package leaflet has been performed on the basis of a bridging report making reference to Caelyx. The bridging report submitted by the applicant has been found acceptable.

3. Benefit-risk balance

This centralised procedure concerns a hybrid application for Zolsketil pegylated liposomal 2 mg/ml concentrate for dispersion for infusion (pegylated liposomal formulation) with Adriamycin 2 mg/ml solution for injection (non-pegylated liposomal formulation) as reference medicinal product. However, since Caelyx contains doxorubicin hydrochloride in a pegylated liposomal formulation, it was considered an appropriate comparator. Therefore, the indications applied for this new medicinal product are the same as those authorised for Caelyx: *"As monotherapy for patients with metastatic breast cancer, where there is an increased cardiac risk. For treatment of advanced ovarian cancer in women who have failed a first-line platinum-based chemotherapy regimen. In combination with bortezomib for the treatment of progressive multiple myeloma in patients who have received at least one prior therapy and who have already undergone or are unsuitable for bone marrow transplant. For treatment of AIDS-related Kaposi's sarcoma (KS) in patients with low CD4 counts (<200 CD4 lymphocytes/mm^3) and extensive mucocutaneous or visceral disease"*.

Nonclinical studies have been provided for this application and considered sufficient. From a clinical perspective, this application does not contain new data on the pharmacokinetics and pharmacodynamics as well as the efficacy and safety of the active substance; the applicant's clinical overview on these clinical aspects based on information from published literature was considered sufficient.

The bioavailability study in patients with ovarian cancer forms the pivotal basis with an open label, randomized, two-treatment, two-period, two-sequence, single dose, cross-over, design. The study design was considered adequate to evaluate the bioavailability of this formulation and was in line with the respective European requirements. Choice of dose, sampling points, overall sampling time as well as wash-out period were adequate. The analytical method was validated. Pharmacokinetic and statistical methods applied were adequate.

The test formulation of Zolsketil pegylated liposomal met the protocol-defined criteria for bioequivalence when compared with Caelyx. The point estimates and their 90% confidence intervals for the parameters AUC0-t, AUC0-72h, and Cmax were all contained within the protocol-defined acceptance range. Bioequivalence of the two formulations was demonstrated.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus decision is of the opinion that Zolsketil pegylated liposomal is not similar to Zejula, Kyprolis, Farydak, Ninlaro, Imnovid, Darzalex, Abecma and Blenrep within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. (See appendix)

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Zolsketil pegylated liposomal is favourable in the following indication:

ZOLSKETIL pegylated liposomal is indicated:

- As monotherapy for patients with metastatic breast cancer, where there is an increased cardiac risk.
- For treatment of advanced ovarian cancer in women who have failed a first-line platinum-based chemotherapy regimen.

- In combination with bortezomib for the treatment of progressive multiple myeloma in patients who have received at least one prior therapy and who have already undergone or are unsuitable for bone marrow transplant.
- For treatment of AIDS-related Kaposi's sarcoma (KS) in patients with low CD4 counts (< 200 CD4 lymphocytes/mm^3) and extensive mucocutaneous or visceral disease.

ZOLSKETIL pegylated liposomal may be used as first-line systemic chemotherapy, or as second line chemotherapy in AIDS-KS patients with disease that has progressed with, or in patients intolerant to, prior combination systemic chemotherapy comprising at least two of the following agents: a vinca alkaloid, bleomycin and standard doxorubicin (or other anthracycline).

ZOLSKETIL pegylated liposomal is indicated in adults.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription.

Other conditions and requirements of the marketing authorisation

- ***Periodic Safety Update Reports***

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

These conditions fully reflect the advice received from the PRAC.