21 July 2022
EMA/685994/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Celdoxome pegylated liposomal

International non-proprietary name: doxorubicin

Procedure No. EMEA/H/C/005330/0000

Note

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

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List of abbreviations

AIDS	Acquired immunodeficiency syndrome
AE	Adverse event
%	Percent
(c)GMP	(current)Good Manufacturing Practices
<	Less than
°C	Celsius degree
AIDS(-KS)	Acquired immunodeficiency syndrome (Kaposi's sarcoma)
ANOVA	Analysis of variance
API	Active product ingredient
AR	Assessment report
AUC	Area under the plasma concentration-time curve
AUC_{0-120}	Area under the tissue concentration-time curve from zero to 120 hours
AUC_{0-48}	Area under the plasma concentration versus time curve from time zero to 48 hours post-dose sample
AUC_{0-7}	Area under the plasma concentration-time curve from zero to Day 7
AUC_{0-inf}	Area under the plasma concentration versus time curve from time zero to infinity
AUC_{0-last}	Area under the tissue concentration-time curve from zero to last measurable concentration
AUC_{0-t}	Area under the plasma concentration versus time curve from time zero to the last measurable concentration
$AUC_{48-tlast}$	Area under the plasma concentration versus time curve from time 48 hours post-dose to the last measurable concentration
BALB/c	Immunocompetent mice
BC	Breast cancer
BE	Bioequivalence
CD4	Cluster of differentiation 4
CEP	Certificate of suitability
CFU	Colony Forming Unit
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CL	Clearance
Cmax	Maximum serum concentration
CoA	Certificate of Analysis
CoA	Certificate of analysis
(C)QA	(critical) quality attributes
CRO	Contract research organisation
CSR	Clinical study report
DNA	Deoxyribonucleic acid
DSPE	Distearoylphosphatidylethanolamine
EC	European Commission
EDQM	European Directorate for the Quality of Medicines
EEA	European Economic Area

EMA	European Medicines Agency
EP	European Pharmacopea
EU	European Union
FDA	Food and Drug Administration
Fe(III)	Inorganic iron
g	Gram(s)
GC(FID)	Gas chromatography(flame ionisation detector)
GCP	Good Clinical Practices
G-CSF	Granulocyte-Colony Stimulating Factor
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GMP	Good manufacturing practices
h/hr/hrs	Hour(s)
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
HPLC(-CAD)	High-Performance Liquid Chromatography (charged aerosol detector)
HPLC-MS	high Performance Liquid Chromatography-Mass Spectrometry
HSPC	Hydrogenated soya phosphatidylcholine
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
INN	International non-proprietary name
IP	intraperitoneal(ly)
IV	Intravenous
kg	Kilogram
Km	Correction factor
KS	Kaposi's sarcoma
LC/MS/MS	liquid chromatography with tandem mass spectrometry
LD50	Median lethal dose
LoQ	Limit of quantification/ List of questions
LSmeans	Least squares mean
m2	Square metre
MAA	Marketing authorisation application
MAH	Marketing authorisation holder
MBC	Metastatic breast cancer
mg	Milligram(s)
min	Minute(s)
mL	Milliliter(s)
MM	Multiple myeloma
mm	Millimetre
MO	Major objection
MPEG	Methoxypolyethylene glycol
MPEG-DSPE	N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3phosphoethanolamine sodium salt
MS	Mass spectra/spectrometry
n	Number of observations
N.V.	Naamloze vennootschap (Public limited company)
NADPH	Nicotinamide adenine dinucleotide phosphate

NF	National formulary
ng	Nanogram(s)
NLT	Not lower than
NMT/nmt	Not more than
p	Probability
PD	Pharmacodynamics
PEG	Polyethylene Glycol
pH	Pondus hydrogenii (negative decadal logarithm of the hydrogen ion concentration)
Ph.Eur.	European Pharmacopea
PK	Pharmacokinetic / pharmacokinetics
PLD	Pegylated liposomal doxorubicin
PPE	palmar-plantar erythrodysesthesia
q.s.	Quantity sufficient
QPPV	Qualified person responsible for pharmacovigilance
QWP	Quality working party
RH	Relative humidity
RMP	Risk management plan
RNA	Ribonucleic acid
RRT	Relative retention time
s	Second
SAE	Serious adverse event
SD	Standard deviation
SmPC	Summary of Product Characteristics
t1/2	Elimination half-life
TEM	Transmission electron microscopy
tmax	Time of the maximum measured plasma concentration
UPLC(-FLR)	Ultra Performance Liquid Chromatography (Fluorescence)
USA	United States of America
UV	Ultraviolet
var.	Vvarietas
Vd	Volume of distribution based on terminal phase
V_d	Volume of distribution
vs.	*Versus*

1. Background information on the procedure

1.1. Submission of the dossier

The applicant YES Pharmaceutical Development Services GmbH submitted on 9 January 2020 an application for marketing authorisation to the European Medicines Agency (EMA) for Celdoxome 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 significant technical innovation.

The applicant applied for the following indications:

- 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.

Celdoxome pegylated liposomal 2 mg/ml concentrate for dispersion for infusion 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:

Article 10(3) of Directive 2001/83/EC, as amended – relating to hybrid applications.

The application submitted is composed of administrative information, complete quality data, a clinical bioequivalent study with the reference medicinal product Caelyx and with appropriate own applicant's non-clinical and clinical data.

The chosen reference product is:

Medicinal product which is or has been authorised 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 AsP
- 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 comparability tests and studies have been conducted:

- 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
- Marketing authorisation number(s): EU/1/96/011/001, EU/1/96/011/002, EU/1/96/011/003, EU/1/96/011/004
- Bioavailability study number: 0927-17

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 received the following Scientific advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
4 September 2017	EMEA/H/SA/3682/1/2017/I	Karin Janssen van Doorn, Andrea Laslop

The Scientific Advice pertained to the following clinical aspects:

- the selection of the comparator product for the clinical PK bioequivalence clinical trial in support of the MAA for liposomal doxorubicin.

1.6. Steps taken for the assessment of the product

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

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

The application was received by the EMA on	9 January 2020
The procedure started on	30 January 2020
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	1 May 2020
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	6 May 2020
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	4 May 2020
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	28 May 2020
The applicant submitted the responses to the CHMP consolidated List of Questions on	18 January 2022
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 virtual (IT system), and at a clinical trial site and a bioanalytical site in India between 28-06-2021 and 29-10-2021. The outcome of the inspection carried out was issued on 17-12-2021.	17 December 2021
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	28 February 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	10 March 2022
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	24 March 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	20 May 2022
The CHMP Rapporteurs circulated the CHMP Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	09 June 2022
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	23 June 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	27 June 2022
The CHMP Rapporteurs circulated the CHMP on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	06 July 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 Celdoxome pegylated liposomal on	21 July 2022
The CHMP adopted a report on similarity of Celdoxome pegylated liposomal with Zejula, Darzalex, Farydak, Imnovid, Kyprolis and Ninlaro	21 July 2022

2. Scientific discussion

2.1. Problem statement

Celdoxome pegylated liposomal (in short Celdoxome) contains doxorubicin hydrochloride 2 mg/mL concentrate for dispersion for infusion. The application is a hybrid application which refers to the reference medicinal product Adriamycin 2 mg/mL solution for injection. Adriamycin and Celdoxome pegylated liposomal differ in terms of formulation as Adriamycin contains doxorubicin hydrochloride in a non-liposomal formulation, while Celdoxome contains doxorubicin hydrochloride in a pegylated liposomal formulation. Therefore, Adriamycin could not be used as "reference formulation" in the bioequivalence study and Caelyx was used instead for comparative physico-chemical, non-clinical and bioequivalence studies.

The medicinal product has been designed to have the same indications and posology as Caelyx.

The active substance of Celdoxome pegylated liposomal is doxorubicin hydrochloride, a cytotoxic anthracycline antibiotic obtained from *Streptomyces peucetius var. caesius*. 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.

As a pegylated liposomal, doxorubicin showed reduced cardiotoxicity and an improved pharmacokinetic profile compared to conventional doxorubicin (Gabizon et al, 1997[1], Gabizon et al, 2004[2], Duggan, 2011[3], Tacar et al, 2013[4]). The liposomal form allows doxorubicin to remain in the circulation system for longer periods of time, which facilitates the delivery of a greater amount of the drug to cancerous cells or tumours (Sabeti et al, 2014[5]). It has shown significant clinical benefit for patients with higher risk for anthracycline-induced cardiotoxicity and has moreover been shown to improve efficacy and reduce certain adverse events.

The applicant applied for the same indications as the ones approved for Caelyx.

The recommended indications for Celdoxome pegylated liposomal are, in adults:

- As monotherapy for patients with metastatic breast cancer (MBC), 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 (MM) in patients who have received at least one prior therapy and who have already undergone or are unsuitable for bone marrow transplant.

[1] Gabizon A, Martin F. Polyethylene glycol-coated (pegylated) liposomal doxorubicin. Rationale for use in solid tumours. Drugs. 1997;54 Suppl 4:15-21.

[2] Gabizon AA, Lyass O, Berry GJ, Wildgust M. Cardiac safety of pegylated liposomal doxorubicin (Doxil/Caelyx) demonstrated by endomyocardial biopsy in patients with advanced malignancies. Cancer Invest. 2004;22(5):663-9.

[3] Duggan ST, Keating GM. Pegylated liposomal doxorubicin: a review of its use in metastatic breast cancer, ovarian cancer, multiple myeloma and AIDS-related Kaposi's sarcoma. Drugs. 2011;71(18):2531-58.

[4] Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013;65(2):157-70.

[5] Sabeti B, Noordin MI, Mohd S, Hashim R, Dahlan A, Javar HA. Development and characterization of liposomal doxorubicin hydrochloride with palm oil. Biomed Res Int. 2014;2014:765426.

- For treatment of acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma (KS) in patients with low CD4 counts (<200 CD4 lymphocytes/mm3) and extensive mucocutaneous or visceral disease.

Celdoxome 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) (see SmPC section 4.1).

It is recommended that Celdoxome pegylated liposomal should only be administered under the supervision of a qualified oncologist specialised in the administration of cytotoxic agents.

Celdoxome pegylated liposomal exhibits unique pharmacokinetic properties and must not be used interchangeably with other formulations of doxorubicin hydrochloride (see SmPC section 4.2).

Posology:

Breast cancer/Ovarian cancer

Celdoxome pegylated liposomal 2 mg/ml is administered intravenously at a dose of 50 mg/m^2 once every 4 weeks for as long as the disease does not progress and the patient continues to tolerate treatment.

Multiple myeloma

Celdoxome pegylated liposomal 2 mg/ml is administered at 30 mg/m^2 on day 4 of the bortezomib 3 week regimen as a 1 hour infusion administered immediately after the bortezomib infusion. The bortezomib regimen consists of 1.3 mg/m2 on days 1, 4, 8, and 11 every 3 weeks. The dose should be repeated as long as patients respond satisfactorily and tolerate treatment. Day 4 dosing of both medicinal products may be delayed up to 48 hours as medically necessary. Doses of bortezomib should be at least 72 hours apart.

AIDS-related KS

Celdoxome pegylated liposomal 2 mg/ml is administered intravenously at 20 mg/m^2 every two-to-three weeks. Avoid intervals shorter than 10 days as medicinal product accumulation and increased toxicity cannot be ruled out. Treatment of patients for two-to-three months is recommended to achieve a therapeutic response. Continue treatment as needed to maintain a therapeutic response.

Guidelines for Celdoxome pegylated liposomal 2 mg/ml dose modification

To manage adverse events such as palmar-plantar erythrodysesthesia (PPE), stomatitis or haematological toxicity, the dose may be reduced or delayed. Guidelines for Celdoxome pegylated liposomal 2 mg/ml dose modification secondary to these adverse reactions are provided in the tables included in section 4.2 of the SmPC.

2.2. Quality aspects

2.2.1. Introduction

Celdoxome pegylated liposomal is presented as a concentrate for dispersion for infusion containing 2 mg/mL of doxorubicin hydrochloride, as an active substance. There are two dose strengths 20 mg/10 mL and 50 mg/25 mL.

Other ingredients are: N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3 phosphoethanolamine sodium salt (MPEG-DSPE), phosphatidylcholine, hydrogenated (soya bean) (HSPC), cholest-5-en-3β-ol, ammonium sulfate, sucrose, histidine, hydrochloric acid (for pH-adjustment) sodium hydroxide (for pH-adjustment) and water for injection.

The product is available in 10 mL (20 mg/10 mL) clear tubular Type I glass via or 30 mL (50 mg/25 mL) clear molded Type I glass vial, stoppered with a 20 mm grey rubber stopper and sealed with a 20 mm flip-off aluminum seal and polypropylene cap and further stored in individual cardboard containers.

2.2.2. Active Substance

2.2.2.1. General information

The chemical name of doxorubicin hydrochloride is (8S,10S)-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 g/mol and the structure shown in Figure 1.

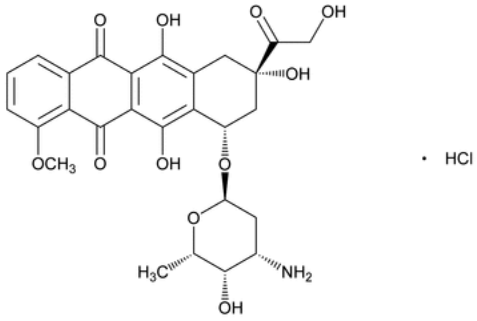

Figure 1: Active substance structure

The active substance (AS) is orange-red, hygroscopic, crystalline powder. It is soluble in water and soluble or slightly soluble in methanol; practically insoluble in other organic solvents. Polymorphism has not been reported in the literature.

As there is a monograph of doxorubicin hydrochloride in the European Pharmacopoeia (01/2008:0714), the manufacturer of the active substance has been granted a Certificate of Suitability of the European Pharmacopoeia (CEP) for doxorubicin hydrochloride which has been provided within the current marketing authorisation application.

2.2.2.2. Manufacture, characterisation and process controls

The active substance is manufactured by one manufacturing site: Olon S.p.A., Torino, Italy.

The relevant information on the manufacture of doxorubicin hydrochloride has been assessed by the EDQM before issuing the CEP (RO-CEP 2017-169-Rev 00).

The CEP specifies that the active substance (AS) is packaged in a glass bottle closed with a polypropylene cap and a polyethylene under-cap, in a polyethylene bag placed in a cardboard box as approved by the European Directorate for the Quality of Medicines (EDQM) in relation to the Certificate of Suitability.

2.2.2.3. Specification

The AS specification is based on the Ph. Eur. monograph and includes tests for description (visual), identification (Ph. Eur.), pH (Ph. Eur.), water content (Ph. Eur.), residual solvents (GC), assay (anhydrous and solvent-free basis: UPLC), assay (as is: UPLC), related substances (UPLC), Total Aerobic Microbial Count (Ph. Eur.), Total Yeast and Mold Count (Ph. Eur.) and Bacterial Endotoxin (Ph. Eur.).The CEP contains a GC method for determination of acetone, ethanol, methanol, chloroform, dichloromethane and 1,4-dioxane. The microbiological quality of the AS will be tested by the finished product manufacturer before using the AS.

Doxorubicin hydrochloride complies with the requirements of monograph for doxorubicin hydrochloride 01/2008:0714 in the current edition of Ph. Eur.

The in-house methods proposed by the finished product manufacturer (assay, related substances, and identification of doxorubicin·HCl) were satisfactorily described and validated. The suitability of the methods used for the control of microbiological quality and bacterial endotoxins of the active substance was demonstrated. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data demonstrating compliance with the specification have been provided for three full-scale batches.

2.2.2.4. Stability

The CEP does not indicate re-test period.

Stability data from nine commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package under long term conditions (25 ± 2 ºC / 60 ± 5% RH) and for up to six months under accelerated conditions (40 ± 2 ºC/75±5% RH) according to the ICH guidelines were provided.

Stability batches were tested for the following stability indicating parameters: description, pH, water content, related substances, assay, Total Aerobic Microbial Count and Total Yeast and Mold Count. The analytical methods used were the same as for release and were stability indicating. The data provided does not show any trend and results for all tested parameters met the specification at all timepoints.

The photostability study as per ICH Q1B Light Option 1 was performed. The results of this study support the claim that the active substance (AS) is not sensitive to light as no decreases in the doxorubicin hydrochloride assay value were detected nor growing of impurities while the Mass Balance was retained.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period in the proposed container.

2.2.3. Finished Medicinal Product

2.2.3.1. Description of the product and pharmaceutical development

The finished product is presented as a translucent red suspension. Each single-dose vial contains 20 mg or 50 mg doxorubicin hydrochloride at a concentration of 2 mg/mL and fill volumes of 10 mL and 25 mL respectively.

The pegylated liposome carriers are composed of cholesterol; fully hydrogenated soy phosphatidylcholine (HSPC); and N-(carbonyl-methoxypolyethylene glycol 2000)-1, 2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE).

Additional excipients used in the formulation are ethanol and nitrogen as processing aids, ammonium sulphate as a gradient material for active loading of doxorubicin, histidine as a buffer, hydrochloric acid and/or sodium hydroxide for pH control, and sucrose to maintain isotonicity. The product also contains water for injections.

This is a generic medicinal product. The European reference medicinal product is Caelyx (EU/1/96/011/001-004 by Baxter Holding B.V.).

The goal of the development studies was to design a generic product that is equivalent to the reference medicinal product. Pharmaceutical formulation development studies have been conducted to evaluate the different unit operations to manufacture doxorubicin-loaded pegylated liposome. In addition, the stability of the generic formulation was compared to the reference medicinal products for assay and related substances data near expiry.

The excipients were chosen based on the excipients listed in the Reference Medicinal Product, Caelyx. All excipients are widely used materials in pharmaceutical formulation, most of the described in Ph. Eur., with a long story of safe use in injectable administration. Stability data demonstrates that the finished product is chemically and physically stable under long term storage; therefore, the stability data supports the compatibility of the AS with the excipients. No novel excipients are used in the production of the finished product.

Comparative characterisation data between the generic medicinal product and reference product has been provided on registration batches and indicate that the physicochemical and biological properties of the generic t and reference products are comparable.

As required in the "Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev02)" various physicochemical properties of Doxorubicin 2 mg/mL concentrate for dispersion for infusion (Pegylated Liposomal Doxorubicin Hydrochloride), 20 mg/10mL and 50 mg/25 mL have been determined. In-vitro liposome characterisation was conducted on the test and reference products.

Analytical results demonstrate that the generic medicinal product can be considered pharmaceutical equivalent to the reference product. The results of Caelyx and Celdoxome CQAs were presented in comparative tables with results of all parameters tested during release. Nonetheless, due to the limited number of available batches in combination with single determination tests the applicant considered that a statistical evaluation was difficult, and therefore, the best available assessment of comparability of critical physico-chemical characteristics was a qualitative evaluation. Moreover, to substantiate this position, the applicant considered that the comparability between test product and reference product is better assessed through *in vivo* comparability gathered from the performed bioequivalence studies between the test product and the reference product, which is the main criteria for assessing pharmaceutical similarity of efficacy of a medicinal product. This was not considered satisfactory and data from comparative studies was requested by the CHMP as a Major Objection (MO). Particularly, the set of Quality Attributes (QAs) subject to analysis, definition/elaboration of the similarity condition, the sampling strategy, the similarity criterion applied per QA should have been predefined in the requested protocol. In addition, sample size representativeness (including impact in conclusions) should have been also addressed along with a pre-specification of the similarity criterion for each (critical) QA selected for comparative data analysis and the submission of the results and

their statistical treatment of all parameters tested during release which were employed in the comparison. Suitable in-process controls or finished product specification acceptance criteria were requested to be set to certify that future batches will also be covered by the variation observed in the reference product.

The applicant provided the comparative data analysis in their response. However, it was considered that although the selected Critical Quality Attributes (CQA) were considered acceptable, the statistical methods employed for the comparison were not acceptable. The CHMP maintained the MO and the applicant was requested to replace the statistical treatment proposed with a statistical treatment recommended by the CHMP. For zeta potential and free doxorubicin specifically the applicant was asked to increase the number of observations in order to show similarity. As requested a revised comparability report was provided following the recommendations from the CHMP. The data gathered from the revised comparability exercise demonstrated that the generic and reference medicinal product should be considered comparable and the previously raised MOs were resolved.

Furthermore, according to the above reflection paper in addition to the characterisation studies conducted under proposed storage conditions, comparative stress test studies of both generic and reference medicinal products, should be conducted in order to compare physical and chemical degradation under stress conditions; this was also raised by CHMP as MO. In response, additional comparative stress test studies were carried out as requested in accordance with the above Reflection Paper. The provided comparative stress test results to support comparable physical and chemical degradation of test and reference product were accepted. Therefore, the MO was considered resolved.

The development of the manufacturing process has been described in sufficient detail. The manufacturing process includes sterile filtration and vial filling under aseptic conditions. As liposomes are temperature-sensitive, terminal sterilisation is not suitable for the proposed finished product. The choice of the sterilisation method has been properly justified based on the Decision Tree for Sterilisation of Aqueous Products in the Guideline (EMA/CHMP/CVMP/QWP/850374/2015) and supported by experimental data and literature information.

The absence of overfill was justified; it has been shown that the extractable volume (Ph. Eur. 2.9.17 test) of can be withdrawn from both the 20 mg or 50 mg vials.

Information on the filters selected during development for manufacturing process and on filter validation was provided. The extractables study performed by sterile filters confirmed that the amount of extractables is very low.

The selection of the in-vitro release method was based on literature which has been provided. The development and optimisation of *in vitro* release of the AS from the finished product (2mg/mL) was provided and found adequate. Method development and validation reports to support the selection of the *in vitro* release method have been provided. The discriminatory power of the method was adequately demonstrated against changes in composition and manufacturing process parameters. For the comparison of dissolution profiles, f2 (and f1) was employed to show similarity of profiles between of test and reference product batches.

Compatibility and stability studies conducted after dilution with 5% dextrose injection were provided for two concentrations. There was no significant change in the parameters tested and the results are found to be well within the specification.

Information sustained by experimental data on the osmolality of the diluted infusion was provided. Osmolality for three of the registration stability batches of finished product diluted in 5% dextrose (sucrose) solution for infusion at different concentrations are presented showing that there is a minimal change to the osmolality of

the infusion solution due to the drug product. As the reference medicinal product has a similar formulation and undiluted osmolality value, a minimal effect on the osmolality of the infusion solution is also expected.

The finished product is provided in Type I glass vial with a bromobutyl rubber stopper and aluminium and PP flip-off cap containing a volume of 10 ml (20 mg) or 25 ml (50 mg). Compliance for primary packaging material with the current EU regulations and Ph. Eur. requirements is confirmed.

The selection of the container closure system was adequately justified based on data obtained from various studies which included compatibility assessment with rubber stoppers and glass vials, stability data and an extractable study. Details on the extraction study have been submitted and the results found to be acceptable. The extractables study performed by bioburden and sterile filters manufacturer confirmed that the amount of extractables is very low. Given the acute dosing nature of the finished product, the extractable profile of the stopper, and the leachable data generated for the finished product, the toxicological risk associated with patient exposure to leachables, which have originated from the container closure system and manufacturing components such as filters and tubing, are negligible and insufficient to warrant the need for further leachable data to be generated on commercial batches which is accepted.

2.2.3.2. Manufacture of the product and process controls

The finished product is manufactured by one manufacturing site: Baxter Oncology GmbH, Westfalen, Germany.

The manufacturing process consists of 9 main steps: preparation of ammonium sulfate buffer, preparation of sucrose buffer, lipid mix, hydration/extrusion, diafiltration, drug loading into liposomes, preparation of and addition of histidine buffer, sterile filtration and filling.

The process is considered to be a non-standard manufacturing process due to the aseptic processing step. The manufacturing process and in-process controls correspond to the normal standards of pharmaceutical technology for liposomal preparations and are suitable to guarantee an appropriate quality of the finished product.

The critical process parameters for the compounding process of the finished product, operating ranges and maximum hold times were provided as were the tests and acceptance criteria performed at each critical parameter of the manufacturing process; these are acceptable.

Confirmation was provided that nitrogen used during manufacture is filtered. The acceptance criteria for bioburden prior to filtration is in accordance with the Note for Guidance on Manufacture of the Finished Dosage Form.

Aspects like washing and sterilisation of equipment, facility and equipment's validation, washing and depyrogenation of glass vials were either briefly or not covered in the dossier since these are covered by GMP; this is acceptable. However, actual results of three media fill simulations, as well as the sterilizing conditions for the primary packaging materials (stoppers and caps) and the corresponding validation report of the sterilisation process of primary packaging material were provided. Sterilisation/depyrogenation validations are conducted at decreased times and/or temperatures, in comparison with the times and temperatures used for production runs.

Information on hold times from the end of pre-filtration up to the sterile final filtration of the product was provided as well as solution compatibility, bacterial filter retention and extractable and leachable reports generated for the final sterilizing filter.

The expiration period is calculated accordingly in line with the regulations described in CPMP/QWP/072/96 Note for Guidance on Start of Shelf-Life of the Finished Dosage Form.

Process validation data on the product has been presented for four full scale batches for the 20 mg fill volume and three full scale batches for the 50 mg fill volume. Overall, it has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

2.2.3.3. Product specification

The finished product release and shelf life specifications include appropriate tests for this kind of dosage form: appearance, (visual), volume in container (Ph. Eur.), identification of AS (UPLC, UV diode array), pH (Ph. Eur.), osmolality (Ph. Eur.), particulate matter (Ph. Eur.), residual ethanol (GC-FID), uniformity of dosage unit (Ph. Eur.),instrumental turbidity (Ph. Eur.), assay (UPLC), related substances (UPLC), free doxorubicin (UPLC-FLR), encapsulation doxorubicin (HPLC), lipid assay (HPLC-CAD), lipid degradants (HPLC-CAD), particle size distribution (dynamic light scattering), ammonium ion concentration (HPLC), sulfate ion concentration (HPLC), zeta potential (particle analyser), bacterial endotoxins (Ph. Eur.), sterility and *in vitro* release (Ph. Eur.).

The specifications were set in line with the requirements of the Ph. Eur. and/or USP monographs, ICH guidelines and batch analysis data from developmental studies and registration stability studies, as well as data from the reference products.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Data for six Celdoxome process validation batches were provided and a maximum daily dose (MDD) of 86.5mg/day was considered. For all elemental impurities tested, the results obtained were found to lower than the limit of quantitation which for all elements is 10% of the specification limit, which obviously were in all cases <30% of the acceptance criteria based on the defined PDEs for the product. Thus it has been concluded that no additional controls are required in the finished product specification.

No risk assessment has been provided for the potential presence of nitrosamines. Considering the advanced cancer indication of Celdoxome this issue has not been raised as a concern.

The analytical methods have been adequately described and non-pharmacopoeia methods have been validated in accordance with the requirements of the relevant ICH guidelines. The methods were found to be suitable for the control of the product and are acceptable. However the CHMP recommended and the applicant committed to consider the following points for the next revalidation of the chromatographic methods: The validity of the conclusions for validation parameters tested based on the SST will be summarised as appropriate; It will be stated clearly whether the concentrations tested for Linearity were obtained by a serial dilution from a mother solution. It will be stated clearly that the accuracy results are obtained testing solutions independent from those used in the study of linearity. This is accepted.

Satisfactory batch analyses, according to the proposed specification and respective criteria, Satisfactory information regarding the reference standards used for assay and impurities testing has been presented. The finished product is released on the market based on the above release specifications through traditional finished product release testing. have been presented for three commercial scale batches of the finished product for both presentations (i.e. the same batches as used in the stability studies), demonstrating compliance with the release specification and showing consistent results.

2.2.3.4. Stability of the product

Stability data from three commercial scale batches of each fill volume stored in upright and inverted orientations for up to 24 months under long term conditions (5 ± 3°C), and for up to six months under accelerated conditions (25 ± 2°C/60 ± 5% RH), according to the ICH guidelines were provided. The stability batches are identical to those proposed for marketing and were packed in the proposed container closure system.

Samples have been tested visual appearance, turbidity, pH, osmolality, particulate matter, ammonium ion, sulfate ion, particle size distribution, doxorubicin assay and related substances, free doxorubicin, encapsulated doxorubicin, drug to lipid ratio, lipids assay and related substances, elemental impurities, bacterial endotoxins and sterility.

The results obtained under long-term conditions comply with specification up until 18 months.

An in-use study was carried out on three batches of each strength by diluting the vial content in 250 mL bags of 5% dextrose injection to a concentration of 0.12 mg/mL (low concentration) or 0.36 mg/mL (high concentration) of doxorubicin. The bags were stored for 24 hours at 5 ± 3°C. At initial time and after 24 hours of storage, the samples were tested for appearance , instrumental colour, turbidity, pH, doxorubicin assay, free doxorubicin, and particle size distribution. Subvisible particulate matter was additionally tested for the 24-hour samples. The results support the recommendation for the diluted product in SmPC section 6.3.

A photostability study was carried out on one batch of each strength (20 mg/10 mL; 50 mg/25 mL) of the finished according to ICH Q1B. Samples have been tested for appearance, instrument colour, turbidity, pH, osmolality, doxorubicin assay and related substances, free doxorubicin, encapsulated doxorubicin, lipid assay and related substances, internal and total ammonium ion, internal and total sulfate ion, particle size distribution, zeta potential, large-diameter droplet and particulate matter. According to the available results, the finished product is susceptible to light. Therefore, the vial is packed in a secondary packaging (such as a carton).

A freeze-thaw cycling study was carried out on one batch of each one of the strengths. Each formulation was subjected to one, two, or three cycles, of two storage conditions: freezing temperature (-20 ± 5°C) and accelerated storage condition (25°C/ 60% RH). Based on the review of the data obtained there were no significant changes in the results during the freeze/thaw study demonstrating that up to three freeze thaw cycles are acceptable. Even so, the product is labelled with "avoid freezing " to align with the reference medicinal product.

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

2.2.3.5. Adventitious agents

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

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and the finished product has been presented in a satisfactory manner. A CEP issued by EDQM has been submitted for the active

substance. An MO that had been raised about the comparison of the finished product quality attributes versus the reference medicinal product has been resolved by provision of additional information and data as requested in line with existing guidance. A second MO was raised with regard to the demonstration of comparable physical and chemical degradation of test and reference products under stress conditions in accordance with the *Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product*; this MO was resolved by provision of respective comparative data as per the above Reflection Paper.

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.

At the time of the CHMP opinion, there was one minor unresolved quality issue having no impact on the benefit-risk balance of the product related to the revalidation of the chromatographic methods. This is proposed as a Recommendation for future quality development (see below).

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable and consistent. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendation 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:

- to summarise as appropriate the validity of the conclusions for validation parameters tested based on the SST for the next revalidation of the chromatographic methods; it should be stated clearly whether the concentrations tested for Linearity were obtained by a serial dilution from a mother solution; it should be stated clearly that the accuracy results are obtained testing solutions independent from those used in the study of linearity.

2.3. Non-clinical aspects

2.3.1. Introduction

A non-clinical overview of the pharmacological, pharmacokinetic and toxicological properties of doxorubicin hydrochloride was submitted in which reference was made to available literature data. To obtain a complete and current update on the published literature, searches in a variety of databases have been performed (up to April 2019) for preclinical pharmacology and toxicology data.

Additionally, study CEL-LD-102/0CLR2 was submitted by the applicant to characterise, from the nonclinical point of view, tissue exposures based on the determination of doxorubicin concentration after administration of Celdoxome pegylated liposomal and Caelyx (7.2 mg/kg, IV, each) in six different rat tissues, i.e. skin, heart, lungs, liver, kidneys, spleen.

2.3.2. Pharmacology

Doxorubicin undergoes enzymatic 1- and 2-electron reduction to the corresponding semiquinone and dihydroquinone. 7-Deoxyalglycones are formed enzymatically by 1-electron reduction, and the resulting semiquinone free radical reacts with oxygen to produce the hydroxyl radical in a cascade of reactions; this radical may lead to cell death by reacting with deoxyribonucleic acid (DNA), ribonucleic acid (RNA), cell membranes, and proteins. The dihydroquinone that results from 2-electron reduction of doxorubicin also can be formed by reaction of two semiquinones. In the presence of oxygen, dihydroquinone reacts to form hydrogen peroxide, and in its absence, loses its sugar and gives rise to the quinone methide, a monofunctional alkylating agent with low affinity for DNA. The contribution of dihydroquinone and the quinone methide to the cytotoxicity of doxorubicin is unclear. Experimental evidence indicated that doxorubicin formed a complex with DNA by intercalation between base pairs, causing inhibition of DNA synthesis and DNA-dependent RNA synthesis by the resulting template disordering and steric obstruction. Doxorubicin also inhibits protein synthesis. Doxorubicin is also reported to be active through the cell cycle including the interphase (Abraham et al, 2005; AHFS, 2015).

Of the cell types tested *in vitro*, cardiac cells were the most sensitive to the effects of doxorubicin, followed by sarcoma and melanoma cells, normal muscle fibroblasts, and normal skin fibroblasts. Normal, rapidly proliferating tissues such as those of bone narrow, gastrointestinal and oral mucosa, and hair follicles are also affected to varying degrees. Doxorubicin hydrochloride was also shown to have immunosuppressive activity (AHFS, 2015).

The long circulation and ability of pegylated liposomes to extravasate through 'leaky' tumour vasculature resulted in localisation of doxorubicin in tumour tissue and reducing cardiotoxicity. In several animal and human tumours, including breast, prostate, pancreatic and ovarian xenografts, pegylated liposomal doxorubicin produced higher intratumoural drug concentrations and better therapeutic responses than equivalent doses of conventional (nonpegylated) liposome encapsulated doxorubicin or free doxorubicin (Gabizon et al., 1997). A number of studies have addressed the mechanism of liposome accumulation in tumours. It was shown that long circulation was critical for liposome accumulation in tumours, as indicated by a correlation between liposome circulation time and tumour uptake. Microscopic observations with colloidal gold-labelled liposomes, and morphological studies with fluorescent liposomes in the skin-fold chamber model, have shown that liposomes extravasate into the tumour extracellular fluid through gaps in tumour microvessels and are found predominantly in the perivascular area with minimal uptake by tumour cells. Studies with ascitic tumours showed a steady extravasation process of long circulating liposomes into the ascitic fluid with gradual release of drug followed by drug diffusion into the ascitic cellular compartment (Gabizon et al, 2003).

It was hypothesised that circulating liposomes cross the leaky tumour vasculature, moving from plasma where drug concentration is relatively high into the interstitial fluid of tumour tissue. This was hypothesised to be a slow process, in which long-circulating liposomes possess a distinct advantage because of the repeated passage through the tumour microvascular bed. Cellular delivery of drug is thought to depend on release from liposomes in the interstitial fluid, since pegylated liposomes are seldom taken up by tumour cells. The factors controlling this process and its kinetics are not well understood and may vary among tissues. A gradual loss of the liposome gradient retaining doxorubicin and disruption of the integrity of the liposome bilayer by phospholipases may be involved in the release process. Uptake by tumour-infiltrating macrophages could also contribute to liposomal drug release. In any case, once doxorubicin is released from liposomes, it may diffuse freely through the tumour space and reach deep layers of tumour cells, whereas

most of the liposomes appear to remain in interstitial spaces immediately surrounding the blood vessels (Gabizon et al, 2003).

2.3.3. Pharmacokinetics

Comparative pharmacokinetic studies after IV administration of either PLD or conventional doxorubicin to rats, rabbits, and dogs have shown increased AUC, decreased Vd, and reduced clearance associated with PLD (Table 1). The AUC data best fit a bi-exponential curve, with a short initial t1/2 (1 to 3 hours) followed by a longer second t1/2 of 20 to 30 hours, representing the majority (>95%) of the AUC. After administration of a single 1.5 mg/kg dose of either PLD or conventional doxorubicin to rabbits, plasma doxorubicin levels (and thus plasma AUC) were up to 2,000-fold higher after PLD administration. The plasma level of doxorubicin remained very low following administration of PLD, indicating that most of the doxorubicin in the plasma remained encapsulated in liposomes. With the observed decreased V_d of PLD relative to conventional doxorubicin (Table 1), doxorubicin disposition was shown to be influenced by liposomal encapsulation. Plasma doxorubicin concentration and AUC after PLD administration were dose-dependent, whereas plasma $t_{1/2}$, Vd, and Cl were independent of PLD dose at the dose levels studied (Vail et al., 2004).

Table 1. Pharmacokinetic parameters of PLD (Doxil) and conventional doxorubicin in rats, rabbits, and dogs (Vail et al., 2004)

	$t_{1/2}$ (hr)	AUC_∞ (μg·hr/mL)	Cl (mL/hr)	V_β (mL)
Rat				
PLD 1 mg/kg	λ_1: 1.8 λ_2: 23.6	683 (>95% λ_2)	0.4	13
Conventional doxorubicin 0.9 mg/kg	λ_1: 0.16 λ_2: 29.1	11.1 (>80% λ_1)	24.3	1014
Rabbit				
PLD 1 mg/kg	λ_1: 0.5 λ_2: 21.3	368 (>95% λ_2)	6.0	176
Conventional doxorubicin 1 mg/kg	λ_1: 0.03 λ_2: 4.07	1 (>80% λ_1)	2536	13651
Dog				
PLD 1.5 mg/kg	λ_1: 0.20 λ_2: 25.9	656 (>95% λ_2)	15.5*	596
Conventional doxorubicin	ND	ND	ND	ND

Abbreviations: $t_{1/2}$, half life; AUC_∞, area under the concentration curve; Cl, clearance; V_β, volume of distribution; λ_1, initial half-life; λ_2, second half-life; ND, not determined

*Volume of distribution at steady state.

Absorption

In an *in vivo* study, free doxorubicin or doxorubicin entrapped in cardiolipin liposomes were administered to male Sprague-Dawley rats (300-350 g) at a dose of 6 mg/kg over 2 min via the femoral vein in volumes corresponding to 1% body weight (0.01 mL/g). Plasma, tissues, bile, and urine were analysed for doxorubicin equivalents. The plasma pharmacokinetics of free and liposomal-entrapped doxorubicin in rats at the stated concentration is presented in Figure 2. Plasma pharmacokinetics of doxorubicin in rats following a dose of 6 mg/kg IV of free doxorubicin or doxorubicin entrapped in liposomes (Rahman et al., 1986) Figure 2. Following IV administration of free doxorubicin, the peak plasma concentration achieved immediately following injection was 1.7 μg/mL. This was reduced to 0.3 μg/mL by 1 h. Free drug cleared from plasma very rapidly, with an apparent large volume of distribution. With cardiolipin liposomes, the peak plasma concentration of total doxorubicin achieved was 20.9 μg/mL. The total plasma levels of doxorubicin

decreased gradually and by 1 h the total doxorubicin concentration in plasma was 10 µg/mL (Rahman et al., 1986).

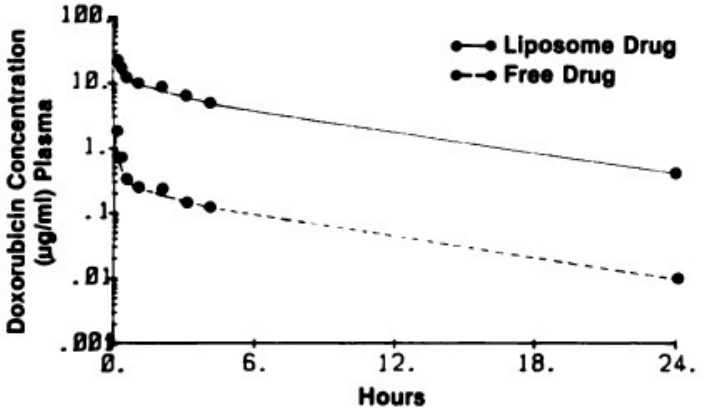

Figure 2. Plasma pharmacokinetics of doxorubicin in rats following a dose of 6 mg/kg IV of free doxorubicin or doxorubicin entrapped in liposomes (Rahman et al., 1986)

The plasma pharmacokinetics of a single dose of Doxil studied in rats and dogs also differed substantially from those of free doxorubicin (Table 2). Free doxorubicin was reported to display biphasic curves with a rapid decline of the initial plasma concentration. The first phase was a rapid distribution phase with a half-life of 5-10 min. The second phase was an elimination and terminal clearance phase with a half-life of 29 h. Clearance was in the order of 121 mL/h/kg, and the volume of distribution was very large (~5 L/kg). In Doxil treated animals, the plasma concentration of total doxorubicin time profile often also displayed a two-phase curve but these two phases actually represented two sections of the distribution phase from the central compartment. In the initial distribution phase, a minor fraction of the injected dose was cleared from circulation with a half-life of about 1 h. During the second extended phase of distribution, accounting for most of the AUC, Doxil was cleared with a half-life ranging between 20 and 35 h. The differences in plasma concentration between the free drug and the pegylated liposomal formulation were considered substantial (Table 2): at least 60-fold increase in AUC for the liposomal drug, with plasma concentrations of doxorubicin several 100-fold greater several hours after injection in liposome-treated animals than in animals treated with free drug. However, most of the drug (95%) found in plasma remained encapsulated in the liposomes and is therefore not yet bioavailable (Gabizon et al., 2003).

Table 2. Pharmacokinetic parameters of doxorubicin administered to animals as the free drug (doxorubicin) or entrapped in pegylated liposomal (Doxil) (Gabizon et al., 2003)

Animal species/ drug form	Dose (mg/kg)	C_{max} (mg/L)	AUC (mg • h/L)	CL (mL/h/kg)[a]	V_β (mL/kg)[a]	$t_{½}$ (h)	Reference
Rat							
Free doxorubicin	0.9	NA	11.1	121	5070	0.16/29.1[b]	20
Doxil®	1	~20	683	2.0	65	1.8/23.6	20
Doxil®	6	~90	3821	1.57	79	35.0	33
Dog							
Doxil®	0.5	7.4	304	1.86	70	27.0	34
Doxil®	0.5	11.8	360	1.39	46	23.1	35
Doxil®	1.5	NA	656	1.03	40	0.2/25.9	20

a For weight normalisation of CL and V_β, average rat and dog weights were estimated at 200g and 15kg, respectively.

b Elimination phase.

AUC = area under the concentration-time curve; **CL** = total plasma body clearance; C_{max} = peak plasma concentration after single dose administration; **NA** = not available; $t_{½}$ = half-life associated with the exponents of distribution phase and, where indicated, of elimination phase; V_β = apparent volume of distribution of β phase.

Considering drugs must be released from liposomes to have activity, a method for measuring levels of released doxorubicin *in vivo* in tumours allowing therapeutic activity to be correlated with bioavailable drug levels was presented. BALB/c mice were orthotopically implanted with 4T1 tumour cells, and 10 days after implantation, the mice were given IV injections via the lateral tail vein (200 µL) with 9 mg/kg of doxorubicin either as free doxorubicin (Doxil) or dioleoylphosphatidylcholine-PLD. To determine if the method scaled with dose, some mice received 16 mg/kg doxorubicin from Doxil. Control mice were injected with 200 µL sterile saline. Mice (n=3-5 per time point) were euthanised, and tumours were excised at various time points up to 7 days after injection or until doxorubicin levels were below detectable levels (0.005 microequivalents doxorubicin/mL). Total and nuclear (free) doxorubicin in 4T1 tumours were determined via the acidified isopropanol extraction method. The concentration of released doxorubicin in tumour tissue was determined from the AUC of nuclear drug relative to the AUC of drug in whole tumours over the time course of the experiment. Results showed that free doxorubicin had high levels of penetration in tumour tissue; 95% of the total doxorubicin in tumours was bound to nuclear DNA by 24 h after injection. Administration of Doxil, a slow-release liposomal formulation of doxorubicin, gave an AUC for total doxorubicin 7 days after injection that was 87-fold higher than that obtained for free doxorubicin, and 49% of the liposomal doxorubicin was released. For liposomes with a more rapid doxorubicin release rate, by 7 days after injection, the AUC_{0-7} days for total doxorubicin was only 14-fold higher than that for free doxorubicin and only 27% of liposomal doxorubicin was released (Laginha et al., 2005).

Distribution

Doxorubicin administered as a conventional injection is widely distributed in the plasma and tissues. As early as 30 seconds after IV administration, doxorubicin is present in liver, lungs, heart, and kidneys. Doxorubicin is absorbed by cells and binds to cellular components, particularly to nucleic acids. The volume of distribution of doxorubicin hydrochloride administered IV as a conventional injection is about 700-1,100 L/m^2. Encapsulations in PEG-stabilised liposomes substantially slowed the rate of distribution of doxorubicin into the extravascular space. Animal studies indicated that liposome encapsulated doxorubicin distributed from blood vessels into tumours, and once distributed into the tissue compartment, the drug was released (AHFS, 2015).

In an *in vivo* study free doxorubicin or doxorubicin entrapped in cardiolipin liposomes were administered to male Sprague-Dawley rats (300-350 g) at a dose of 6 mg/kg over 2 min via the femoral vein in volumes corresponding to 1% body weight (0.01 mL/g). Pharmacologic disposition of free doxorubicin and doxorubicin entrapped in cardiolipin liposomes following IV administration of 6 mg/kg to rats was measured (Table 3) (Rahman et al., 1986).

Table 3. Pharmacologic disposition of free doxorubicin and doxorubicin entrapped in cardiolipin liposomes following IV administration of 6 mg/kg (Rahman et al., 1986)

	Heart		Liver		Spleen	
Time	Free drug	Liposome-entrapped drug	Free drug	Liposome-entrapped drug	Free drug	Liposome-entrapped drug
30 min	15.1 ± 1.3[a]	8.8 ± 0.2	26.4 ± 0.2	54.3 ± 3.2	26.4 ± 4.5	71.9 ± 16.1
2 h	11.5 ± 0.9	8.7 ± 1.3	16.5 ± 3.7	36.1 ± 3.7	21.8 ± 2.1	60.7 ± 13.4
4 h	9.8 ± 1.1	5.8 ± 0.8	15.2 ± 4.3	33.9 ± 4.3	16.4 ± 4.9	46.0 ± 2.9
24 h	4.7 ± 0.5	2.3 ± 1	4.1 ± 0.6	31.8 ± 0.3	11.5 ± 2.8	42.5 ± 14.2
	Lungs		Kidney		Small intestine	
	Free drug	Liposome-entrapped drug	Free drug	Liposome-entrapped drug	Free drug	Liposome-entrapped drug
30 min	16.31 ± 2.4	12.9 ± 2.05	37.2 ± 5.8	23 ± 2.8	12.1 ± 4.3	8.0 ± 0.7
2 h	14.5 ± 6.2	10.1 ± 2.4	20.6 ± 3.1	21.6 ± 0.2	11.3 ± 0.0	7.2 ± 1.2
4 h	12.2 ± 3.5	11.7 ± 1.4	18.1 ± 2.2	18.6 ± 2.7	7.9 ± 0.16	3.7 ± 0.31
24 h	5.01 ± 2.0	6.48 ± 1.0	5.18 ± 0.2	5.28 ± 0.9	5.94 ± 0.6	3.59 ± 0.4

[a] Mean ± SD.

According to results obtained by Gabizon et al. (Table 3), the volume of distribution of Doxil was very small and approximated the blood volume in each species, whereas that of free doxorubicin was very large and indicative of rapid distribution/dispersion into the tissues. Altogether, Doxil treatment resulted in an increased AUC of doxorubicin equivalents and a longer mean residence time, whereas clearance and volume of distribution of total drug were significantly decreased compared with free doxorubicin treatment. Studies with drug-free liposomes indicated linear, dose-independent pharmacokinetics for pegylated liposomes. However, studies with tumour-bearing mice in which the dose of Doxil was escalated from 2.5 to 20 mg/kg bodyweight (that is much higher than the maximum recommended human dose) indicated that dose escalation resulted in saturation of clearance and a disproportionate increase of plasma concentrations (Gabizon et al., 2003).

- *Study CEL-LD-102/0CLR2*

CEL-LD-102/0CLR2 study was conducted to characterise tissue exposures based on the determination of doxorubicin concentration after administration of Celdoxome pegylated liposomal and Caelyx (7.2 mg/kg, IV, each) in six different rat tissues, i.e. skin, heart, lungs, liver, kidneys, spleen.

Material and methods

Sixty (60) naïve female Sprague-Dawley rats (n=6/time point/compound) were administered a single IV bolus injection of Celdoxome pegylated liposomal or Caelyx 2 mg/mL at a dose of 7.2 mg/kg with an administration volume of 3.6 mL/kg body weight.

Groups	Number of animals per groups	Test items	Dose	Organ collection
1	6	Celerity's Doxorubicin HCl Liposome 2 mg/mL concentrate for solution for infusion (Celdoxome pegylated liposomal)	7.2 mg/kg	8 h
2				24 h
3				48 h
4				72 h
5				120 h
6		Caelyx		8 h
7				24 h
8				48 h
9				72 h

10				120 h

After administration, the organs (skin, heart, lungs (left and right), liver, kidneys (left and right), and spleen) were collected and concentration of total doxorubicin determined at the following timepoints: 8 h, 24 h, 48 h, 72 h and 120 h by validated LC/MS/MS method.

Statistical analysis

A non-compartmental pharmacokinetic analysis was performed using Phoenix WinNonlin (version 8.1) on total doxorubicin concentrations (ng/mL of homogenate) in each tissue (heart, lungs, spleen, liver, kidneys and skin). Six (6) different animals were used per time point and per compound, the different areas under the concentration-time curve (AUC) were calculated using the linear trapezoidal summation (both ascending and descending phase) using the mean tissue concentrations at each time point for each compound: $AUC_{0\text{-last}}$ and $AUC_{0\text{-}120}$.

Results

Following administration of a single intravenous dose of Celdoxome pegylated liposomal, total doxorubicin was detected in skin, heart, lung, kidney, spleen and liver tissues from the first sampling time-point (8 h) up to the last sampling time point (120 h). The mean maximum (±SEM) concentration of total doxorubicin was 516±54 ng/mL in skin tissue at 72 h, 579±55 ng/mL in heart tissue at 48 h, 927±58 ng/mL in lung tissue at 72 h, 744±39 ng/mL in kidney tissue at 72 h, 3123±293 ng/mL in spleen tissue at 24 h and 707±57 ng/mL in liver tissue at 24 h. Following administration of Caelyx, very similar concentration-time profiles were observed for each tissue. The mean maximum concentration of total doxorubicin was 476±30 ng/mL in skin tissue at 48 h, 662±53 ng/mL in heart tissue at 72 h, 911±53 ng/mL in lung tissue at 72 h, 653±34 ng/mL in kidney tissue at 72 h, 3337±304 ng/mL in spleen tissue at 24 h, and 817±33 ng/mL in liver tissue at 24 h.

However, it was noted that the liver concentration of total doxorubicin peaked higher and dropped faster in animals treated with Caelyx than in animals treated with Celerity's doxorubicin, before reaching similar concentrations over the rest of the studied period, i.e. liver concentration of total doxorubicin at 24 h was significantly higher in animals treated with Caelyx compared to Celdoxome (817±33 ng/mL vs. 707±57 ng/mL, p=0.0145) and at 48 h liver concentration of total doxorubicin was significantly lower in animals treated with Caelyx compared to Celdoxome (571±22 ng/mL vs. 668±47 ng/mL, p=0.0301). For the rest of the studied period administration of both preparations resulted in similar total doxorubicin concentrations in the liver, i.e. at 72 h: 470±25 ng/mL vs. 434±27 ng/mL (NS); and at 120 h: 222±17 ng/mL vs. 246±14 ng/mL (NS).

Based on the statistical analysis, no difference in skin, heart, lung, kidney, spleen and liver tissue concentrations between the two formulations were shown (p-values: 0.5142, 0.9802, 0.8338, 0.8786, 0.6669, 0.7226, respectively). Additionally, no interaction between formulations and time were reported to be observed in all tissues except for liver tissue in which a statistically significant interaction between formulations and time (p=0.0239) were noted. However, the ratio of the means for $AUC_{0\text{-last}}$ and $AUC_{0\text{-}120}$ was inside the boundaries of 80-125% for all tested tissues (98.6%, 97.7%, 102.8%, 104.6%, 98.7%, 98.8%, respectively) and it was stipulated that the 2 formulations led to overall similar tissues exposure. Thus, single administrations of 7.2 mg/kg of Celdoxome pegylated liposonal or Caelyx were reported to lead to similar exposures ($AUC_{0\text{-last}}$ and $AUC_{0\text{-}120}$) in skin, heart, lung, kidney, spleen and liver tissues, with similar concentration-time profiles.

Table 4. Pharmacokinetics: Organ Distribution

Species:	Rat, Sprague-Dawley
Gender (M/F)/Number of Animals:	60 F/6 per time-point/compound
Feeding Condition:	Fed
Vehicle/Formulation:	Saline/Liposomal parenteral formulation
Method of Administration:	IV bolus
Dose (mg/kg):	7.2
Radionuclide:	Non-radiolabeled
Specific Activity:	–
Analyte/Assay (unit):	Total doxorubicin/LC/MS/MS
Sampling Time:	8, 24, 48, 72 and 120 hours

Tissues/Organs	**Total Doxorubicin Concentration (ng/mL)**										**$AUC_{0\text{-last}}$ (ng·h/mL)**		**Ratio[1,2]**
	Celerity's Doxorubicin HCl Liposome 2 mg/mL concentrate for solution for infusion					**Caelyx® 2 mg/mL**					**Celerity's Doxorubicin HCl Liposome 2 mg/mL concentrate for solution for infusion**	**Caelyx® 2 mg/mL**	
	8 h	**24 h**	**48 h**	**72 h**	**120 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**			
Skin	172±70	317±124	369±119	516±132	328±76	227±114	310±122	476±73	453±137	321±37	43718	44326	0.9863
Heart	291±48	527±168	579±136	559±80	363±39	289±88	490±72	511±112	662±130	363±73	56742	58083	0.9769
Lungs	429±204	499±155	696±243	927±142	579±164	487±286	510±165	625±286	911±131	547±133	79082.8	76955.6	1.0276
Kidney	203±39	519±224	594±109	744±94	492±123	337±212	386±55	621±281	653±83	525±165	65631.2	62776	1.0455
Spleen	2872±459	3123±717	2723±417	2248±317	1722±224	3022±418	3337±744	2618±378	2322±290	1622±247	284545.2	288335.6	0.9869
Liver	588±101	707±139	668±114	434±65	246±34	599±80	817±80	571±55	470±60	222±42	58758	59454.8	0.9883

Additional Information:
Values are expressed mean±SD.
LC/MS/MS=liquid chromatography with tandem mass spectrometry.
1 Celerity's Doxorubicin HCl Liposome 2 mg/mL concentrate for solution for infusion /Caelyx® 2 mg/mL.
2 The formulations were considered as equivalent if the ratio is between 0.8-1.25.

In addition to the biodistribution study, results from a bibliographic data search were discussed to further understand different aspects of pharmacokinetics. Considering the differences between studies identified and CEL-LD-102/0CLR2, including differences in liposomes, doses, weight of rats, study duration (672 hours in Burade et al. 2017, 24 hours in Rahman et al. 1986 and 120 hours in the applicant's study CEL-LD-102/0CLR2) and between study variability, the tissue exposures seen across the mentioned studies, and especially compared for Cmax values, were similar and comparable.

Metabolism

Non-encapsulated doxorubicin is metabolised by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent aldoketoreductases to the hydrophilic 13-hydroxyl metabolite doxorubicinol, which exhibits antineoplastic activity and is the major metabolite. These reductases are present in most if not all cells, but particularly in erythrocytes, liver, and kidney. Although not clearly established, doxorubicinol also appears to be the moiety responsible for the cardiotoxic effects of the drug. Undetectable or low plasma concentrations (i.e., 0.826.2 ng/mL) of doxorubicinol have been reported following IV administration of a single 10- to 50 mg/m^2 dose of doxorubicin hydrochloride as a PEG-stabilised liposomal injection. Substantially reduced or absent plasma concentrations of the usual major metabolite of doxorubicin observed with the PEG-stabilised liposomal injection suggests that either the drug is not released appreciably from the liposomes as they circulate or that some doxorubicin may be released but that the rate of doxorubicinol elimination greatly exceeds the release rate; doxorubicin hydrochloride encapsulated in liposomes that have not been PEG-stabilised is metabolised to doxorubicinol. Other metabolites, which are therapeutically inactive, include the poorly water-soluble aglycones, doxorubicinone (adriamycinone) and 7-deoxydoxorubicinone (17-deoxyadriamycinone), and conjugates. The aglycones are formed in microsomes by NADPH-dependent, cytochrome reductase-mediated cleavage of the amino sugar moiety. The enzymatic reduction of doxorubicin to 7-deoxyaglycones is important to the cytotoxic effect of the drug since it is reported to result in hydroxyl

radicals that cause extensive cell damage and death. With non-encapsulated doxorubicin, more than 20% of the total drug in plasma is reported to be present as metabolites as soon as 5 minutes after a dose, 70% in 30 minutes, 75% in 4 hours, and 90% in 24 h (AHFS, 2015).

Excretion

The rate at which liposome encapsulated drug can be cleared from the systemic circulation is reported to be affected by three main mechanisms (Gabizon et al., 1997):

- Uptake and destruction of circulating liposomes by cells of the RES, with active drug then being metabolised and excreted,
- Leakage of drug from liposomes in circulation, followed by rapid and extensive tissue distribution and elimination of free drug,
- Accumulation of liposome encapsulated drug in non-reticuloendothelial system tissue (including solid tumour).

Plasma clearance of non-encapsulated doxorubicin (when administered as a conventional injection) was reported to range from 8-20 mL/minute per kg (or 324-809 mL/min/m^2). Plasma clearance of doxorubicin encapsulated in PEG-stabilised liposomes appears to be substantially slower than that of non-encapsulated doxorubicin, and this results in a substantial increase in the AUC (AHFS, 2015).

Non-encapsulated doxorubicin and its metabolites are excreted predominantly in bile; about 10-20% of a single dose in excreted in feces in 24 h, and 40-50% of a dose is excreted in bile or feces within 7 days. About 50% of the drug in bile is unchanged drug, 23% is doxorubicinol, and the remainder is other metabolites including aglycones and conjugates, about 4-5% of the administered dose is excreted in urine after 5 days, principally as unchanged doxorubicin (AHFS, 2015).

2.3.4. Toxicology

Single dose toxicity

Single dose IV injection studies in mice showed the encapsulated form of doxorubicin to be less toxic (LD50 of 32 mg/kg, being two times the 50 mg/m^2 human dose on a mg/m^2 basis) than free doxorubicin (LD50 of 17 mg/kg, being the same as the 50 mg/m^2 human dose on a mg/m^2 basis). Histologic examination of cardiac sections taken from DBA/2J mice 7 days after a single IV injection of free or liposomal doxorubicin (25 mg/kg) revealed that the liposomal preparation was much less cardiotoxic. Myelosuppression was seen in a study in dogs being less severe after intraperitoneal dosage than after IV dosage of liposomal doxorubicin (Caelyx Product Monograph, 2019).

Cynomolgus monkeys (3/sex) were administered a single IV dose of Caelyx of 10 mg/kg (120 mg/m^2; approximately two times the clinical dose) and followed for 28 days as a comparator arm in an acute toxicity study with an investigational doxorubicin formulation. Three male and one female monkey were sacrificed on Day 11 or 15 in poor condition attributable to renal toxicity. 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 neutrophil infiltration, and/or necrosis of renal adipose tissue. Renal toxicity has been observed with even lower single doses of doxorubicin HCl in rats and rabbits. However, since an evaluation of the postmarketing safety database for Caelyx in patients has not suggested a significant nephrotoxicity liability of Caelyx, these findings in monkeys may not have relevance to patient risk assessment (Caelyx Product Monograph, 2019).

Repeat-dose toxicity

A preclinical toxicology study evaluating liposome encapsulated doxorubicin and free doxorubicin carried out in beagle dogs was presented. Dogs received single intraperitoneal (IP) infusions of 1.5 mg free or 1.5, 2.25 or 3.37 mg liposomal doxorubicin/kg. One group of four dogs received 1.5 mg liposomal doxorubicin/kg every three weeks for 4 cycles. The dose limiting toxicity of free or liposomal doxorubicin given by the IP route was a dose-related chemical peritonitis. This toxicity was more severe in dogs that received by the IP route the previously determined maximally tolerated IV dose of liposomal doxorubicin (2.25 mg/kg). The abdominal toxicity was characterised by capsular fibrosis and ascites formation. Abdominal toxicity was life-threatening after single doses of 3.37 mg liposomal doxorubicin/kg, or after multiple (4) doses of 1.5 mg liposomal doxorubicin/kg. Thoracic toxicity (increased fluid, mediastinal oedema, thickening of the parietal pleura) was seen after multiple (every 3 weeks for 4 cycles) IP doses of 1.5 mg or single doses of 3.37 mg liposomal doxorubicin/kg. Myelosuppression was seen in all groups but was less severe after intraperitoneal dosage than after IV dosage of liposomal doxorubicin (Kanter et al., 1994).

In a rabbit multidose study using well-established histopathological parameters, the cardiac toxicity of Doxil was reported to be significantly less compared to doxorubicin (Gabizon et al., 2003).

The toxicity of Caelyx following repeated administration was reported to be similar in rats and dogs and an extension of the findings in the acute studies. Treatment-related effects included dermatologic toxicity, body weight and food consumption changes, alopecia, myelotoxicity (bone marrow cellularity changes), and haematologic effects (leukopenia and lower erythron mass). Dogs also showed gastrointestinal toxicity but no pathologic signs of toxicity (Caelyx Product Monograph, 2019).

In studies performed after the repeated administration of liposomal doxorubicin to rats and dogs, serious dermal inflammations and ulcer formations were observed at clinically relevant doses. In the study in dogs, the occurrence and severity of these lesions was reduced by lowering the dose or prolonging the intervals between doses. Similar dermal lesions, which are described as palmar-plantar erythrodysesthesia were also observed in patients after long-term intravenous infusion (Caelyx Summary of Product Characteristics,2021).

Genotoxicity

The mutagenicity of doxorubicin has been documented using the Salmonella/microsome mutagenicity assay. Doxorubicin has been shown to induce His+ revertants in the *Salmonella typhimurium (S. typhimurium)* strains TA98, TA 1538, TA100, TA2637 and TA102. In several studies, the addition of S9-mix for metabolic activation did not modify the mutagenic response of tester strains TA98 and TA1538 to doxorubicin; instead, addition of microsomes decreased doxorubicin -induced mutant yields, which were linked to the formation of inactive aglycones. This and the fact that doxorubicin binds and intercalates DNA, are said to provide support that intercalation processes explain the mechanism of mutagenicity of doxorubicin (Kostoryz, 2001).

Doxorubicin has a high affinity for inorganic iron, Fe(III), and has potential to form doxorubicin-Fe(III) complexes in biological systems. Indirect involvement of iron has been substantiated in the oxidative mutagenicity of doxorubicin. However, direct involvement of Fe(III) was evaluated in mutagenicity studies with the doxorubicin-Fe(III) complex. The Salmonella mutagenicity assay with strain TA102 was used with a pre-incubation step. The highest mutagenicity of doxorubicin-Fe(III) complex was reported to be observed at the dose of 2.5 nmol/plate of the complex. The S9-mix decreased this highest mutagenicity but increased the number of revertants at a higher dose of 10 nmol/plate of the complex. On the other hand, the mutagenicity of the doxorubicin-Fe(III) complex at the doses of 0.25, 0.5, 1 and 2 nmol/plate was said to be enhanced about twice by the addition of glutathione plus H_2O_2. This enhanced mutagenicity as well as of the complex

itself, the complex plus glutathione, and the complex plus H_2O_2 were reduced by the addition of ADR-529, an Fe(III) chelator, and potassium iodide, a hydroxyl radical scavenger. These results indicate, according to the authors, that doxorubicin-Fe(III) complex exert the mutagenicity through oxidative DNA damage and that Fe(III) is a required element in the mutagenesis of doxorubicin (Kostoryz, 2001).

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* (AHFS, 2015; Caelyx Product Monograph, 2019).

Reproductive and developmental toxicity

Doxorubicin was shown to be embryotoxic and teratogenic in rats and embryotoxic and abortifacient in rabbits, and trace amounts of drug have been found in mouse foetuses and in one aborted human foetus following administration of conventional doxorubicin. Liposomal doxorubicin was embryotoxic at doses of 1 mg/kg daily (corresponding to 6 mg/m^2 being 6 the correction factor (K_m) for rats; about 1/8 of the 50 mg/m^2 human dose) in rats and is embryotoxic and abortifacient at doses of 0.5 mg/kg daily (corresponding to 6 mg/m^2 being 12 the K_m for rabbits; about 1/8 of the 50 mg/m^2 human dose) in rabbits. Embryotoxicity consisted of increased embryofoetal deaths and reduced live litter sizes (Caelyx Product Monograph, 2019).

The potential developmental toxicity of Caelyx was evaluated in rats and rabbits. In the first study, IV bolus injections of Caelyx 0.1, 0.5, or 1.0 mg/kg were administered on gestation days 6, 9, 12, and 15; or STEALTH placebo liposomes or saline on the same treatment schedule. An additional group received doxorubicin 0.2 or 0.4 mg/kg daily between gestation days 6 and 15. Equivalent maternal toxicity occurred in the Caelyx 0.5 and 1.0 mg/kg groups and in the doxorubicin groups. Caelyx 1.0 mg/kg induced decreased fetal weights, increases in fetal resorptions, and retarded ossification of caudal vertebrae and xiphoid canters in the fetuses. No adverse effects were seen in dams or fetuses in the placebo liposome or Caelyx 0.1 mg/kg groups (Caelyx Product Monograph, 2019).

The embryotoxicity of Caelyx was confirmed in the study in pregnant New Zealand White rabbits administered intravenous injections of Caelyx 0.5, 1.5, or 2.5 mg/kg on gestation days 6, 9, 12, 15, and 18. All doses were maternally toxic. Four females that died (3 and 1 in the high- and mid-dose groups, respectively), surviving females in the mid- and high-dose groups (4 and 2, respectively), and 4 females (low-dose group) who aborted prior to the end of the study all had 100% resorbed conceptuses. The uterine of another female (low-dose group) who aborted prior to the end of the study consisted of 3 normal conceptuses, 4 late resorptions, and 5 early resorptions. Caelyx is both embryotoxic and an abortifacient in rabbits (Caelyx Product Monograph, 2019).

Liposomal doxorubicin hydrochloride has been associated with mild to moderate ovarian and testicular atrophy in mice after a single 36 mg/kg dose (corresponding to 98 mg/m^2 being 3 times the K_m for mice; about two times the 50 mg/m^2 human dose), decreased testicular weight and hypospermia in rats after repeated dosages of 0.25 mg/kg or more daily (corresponding to 1.5 mg/m^2 being 6 the K_m for rats; about 1/30 of the 50 mg/m^2 human dose), and diffuse degeneration of the seminiferous tubules and marked decreases in spermatogenesis in dogs after repeated dosages of 1 mg/kg daily (corresponding to 20 mg/m^2 being 20 the K_m for rabbits; about 1/2 of the 50 mg/m^2 human dose) (AHFS, 2015).

Local tolerance

A study in rabbits showed liposomal doxorubicin was well tolerated with no gross or microscopic evidence of irritation at injection site. In contrast, histopathological evaluation of the subcutaneous injection sites showed reversible mild to moderate dose-related inflammation and necrosis (Caelyx Product Monograph, 2019). In animal experiments, the injection of granulocyte–macrophage colonystimulating factor (GM-CSF) 6 µg at the injection site of doxorubicin had a beneficial effect on the doxorubicin-induced tissue necrosis (Schrijvers, 2003).

2.3.5. Ecotoxicity/environmental risk assessment

A justification for not providing ERA studies was provided (see discussion on non-clinical aspects). The introduction of Celdoxome pegylated liposomal is considered unlikely to result in any significant increase in the combined sales volumes for all doxorubicin hydrochloride containing products and the exposure of the environment to the active substance.

2.3.6. Discussion on non-clinical aspects

Pegylated liposomes cause doxorubicin to be slowly released into tissues over several days. Thus, the proportion of doxorubicin that is available at the tumour site at any one time is thought to be several times higher than it is in normal tissues. Long-circulating pegylated liposomal formulations of doxorubicin have been shown to result in increased accumulation of drug in solid tumours and reduced dose-limiting toxicities. Comparative pharmacokinetic studies after IV administration of either PLD or conventional doxorubicin to rats, rabbits, and dogs have confirmed increased AUC, decreased distribution volume, and reduced clearance of total drug associated with PLD. Doxorubicin is metabolised by NADPH-dependent aldoketoreductases to the hydrophilic 13-hydroxyl metabolite doxorubicinol, which exhibits antineoplastic activity and is the major metabolite. These reductases are present in most if not all cells, but particularly in erythrocytes, liver, and kidney. Doxorubicin and its metabolites are excreted predominantly in bile.

Results from a biodistribution study (CEL-LD-102/0CLR2) in which the level of test formulation was quantified in level in skin, heart, lungs, liver, kidneys and spleen, were presented. The distribution equivalence criteria for each tissue was based on AUC which represents the overall exposure to the tissue. Similar exposures were obtained for both liposomal formulations in skin, heart, lung, kidney, and spleen, with comparable concentration-time profiles, but significant differences were found in the liver concentrations at 24 h and 48 h time points. Although the Test/Reference C_{max} ratios for each tissue were not part of the analysis plan and the study was not powered to demonstrate equivalence on C_{max}, the C_{max} T/R ratio in liver was 86.5% and falls between the BE boundaries of 80-125%, which is considered acceptable.

During the procedure, the applicant conducted a bibliographic data search, and the results were discussed in conjunction with the biodistribution study. The distribution results from the applicant's study are in line with those previously reported for liposomal doxorubicin and Caelyx. The conclusion supports the equivalence between test and reference formulations regarding biodistribution.

The toxicity profile of capsuled and free doxorubicin HCl has been well characterised based on available literature data on conventional liposomes and free drug as well as on studies performed specifically on Caelyx or Doxil. Single dose IV injection studies in mice showed the encapsulated form of doxorubicin to be less toxic than free doxorubicin. Histologic examination of cardiac sections after a single IV injection of free or liposomal doxorubicin revealed that the liposomal preparation was much less cardiotoxic.

Myelosuppression was seen in a study in dogs being less severe after intraperitoneal dosage than after IV dosage of liposomal doxorubicin.

Doxorubicin has been shown to be carcinogenic in experimental models (AHFS, 2015).

Liposomal doxorubicin was shown to be embryotoxic in rats and embryotoxic and abortifacient in rabbits. Liposomal doxorubicin hydrochloride has been associated with mild to moderate ovarian and testicular atrophy in mice after a single dose, decreased testicular weight and hypospermia in rats, and diffuse degeneration of the seminiferous tubules and marked decreases in spermatogenesis in dogs, after repeated dosages.

Histopathological evaluation of the IV injection sites revealed that Caelyx, doxorubicin hydrochloride, and placebo liposomes were well tolerated with no gross or microscopic evidence of irritation (Caelyx Product monograph, 2019).

The applicant has not presented new genotoxicity studies on Celdoxome pegylated liposomal. The mutagenicity of doxorubicin has been well documented using the Salmonella/microsome mutagenicity assay.

Studies for qualification of impurities were presented, during the procedure. Although Doxorubicin Dimer Impurity 1, was predicted to be plausible for *in vitro* mutagenicity by Derek and positive by LSMA, it is agreed that API, which is a cancer therapeutic, and is not expected or predicted to be more toxic than the API, formation of this impurity in the drug product will not increase the concentration of this specific chemical class. This impurity is considered qualified at the proposed qualification limit of 1.2%, which is considered acceptable.

Environmental Risk Assessment

Doxorubicin is already used in existing marketed products and no significant increase in environmental exposure is anticipated. This medicinal product should be used according to the precautions stated in the SmPC (section 6.6) in order to minimise any potential risks to the environment.

2.3.7. Conclusion on the non-clinical aspects

The non-clinical studies and literature review provided are considered adequate to support a hybrid application. The presented data indicated comparability of biodistribution, pharmacokinetics and toxicological properties of Celdoxome pegylated liposomal and Caelyx.

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

In support of this application, results from a bioequivalence study comparing Celdoxome pegylated liposomal to Caelyx have been provided. Scientific literature was also presented to support the clinical pharmacokinetics and pharmacodynamics.

GCP aspects

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**

Table 5. Listing of clinical studies

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	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
Reports of biopharmaceutic studies									
Comparative BA and BE study reports									
BE	CT Report Project No. 0927-17; Sponsor Protocol No. CEL-LD-202	Mod. 5.3.1.2	Primary objective: Bioequivalence of the test product to the reference medicinal product; Secondary objective: Safety and tolerability of test and reference medicinal product	Multi-centre, open label, randomised, two-treatment, two-period, two-sequence, single-dose, cross-over BE study	1. Test product: Doxorubicin hydrochloride (pegylated liposomal) injection 20 mg/ 10 mL (2 mg/mL) 2. Reference product: Caelyx® [doxorubicin hydrochloride (pegylated liposomal) injection] 20 mg/ 10 mL (2 mg/mL) Both: 50 mg/m^2 of BSA i.v. infused within 1 h (at least 28 days of washout period)	68	Patients with metastatic breast cancer and who were scheduled to start therapy with doxorubicin hydrochloride (pegylated liposomal)	Single dose	Complete, final study report

2.4.2. Clinical pharmacology

2.4.2.1. Pharmacokinetics

Study 0927-17: "A Multicenter, Open label, Randomized, Two-treatment, Two-period, Two-sequence, Single dose, Crossover Study to Test for Bioequivalence between Celerity's Doxorubicin hydrochloride (Pegylated Liposomal) Injection 20 mg/10 mL (2 mg/mL) and the Reference Product Caelyx [Doxorubicin hydrochloride (Pegylated Liposomal) Injection 20 mg/10 mL (2 mg/mL)] in patients with Metastatic Breast cancer".

Study design

The study consisted of a 14-day screening period prior to Day 1 and treatment phase with 50 mg/m^2 dose [based on body surface area (BSA)] of doxorubicin hydrochloride in each study period. As the recommended dosage interval of doxorubicin hydrochloride is every 4 weeks, two consecutive treatment cycles were used for the two treatment periods. The washout period was therefore 28 days (dosing days were specifically Day 1 and Day 29 per protocol - with allowed windows when needed). Following a screening visit, there were 18 other visits (a total of 19 visits) during the study. The total duration of the study for an individual patient was approximately 71 days (including the screening phase).

Drug products were to be administered as intravenous infusion via infusion pump over 60 minutes (± 5 minutes) period. Drug products were administered as intravenous infusion via infusion pump over 60 minutes (± 5 minutes) period. After a supervised overnight fasting of at least 8 hours, patients were served a non-high fat breakfast, which they have 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.

The dose of doxorubicin hydrochloride liposome injection for individual subject was period specific and was calculated according to body surface area using appropriate formula (Du Bois method):

Body Surface Area (m^2) = 0.007184 x Weight $(kg)^{0.425}$ x Height $(cm)^{0.725}$

Pre-medication with dexamethasone and granisetron was used for all subjects. Prophylactic anti-allergy treatment and Granulocyte-Colony Stimulating Factor (G-CSF) were permitted at the discretion of the investigator until 2 hours prior or after study drug administration. Chronic medication was permitted also until 2 hours prior or after study drug administration Prophylactic or chronic medications were accepted for ethical reasons and considering the target population, although evaluated for any potential PK interactions with the test product impacting efficacy and safety. Patients receiving non-permissible concomitant medications were documented as protocol deviations and the PK data excluded and considered for the bioequivalence assessment.

For doxorubicin, 27 blood samples were to be drawn into blood collection tubes (5 mL each) containing pre-cooled k_3EDTA as anticoagulant. Samples were withdrawn at pre-dose (within 5 min prior to dosing) and post dose at 0.333, 0.667, 1.00, 1.08, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00, 24.00, 36.00, 48.00, 72.00, 120.00, 168.00, 216.00, 264.00, 312.00 and 360.00 hours. Blood samples collected at 01.00 h and 1.08 h were carried out immediately before and immediately after the completion of infusion, respectively. Blood samples at 72.00, 120.00, 168.00, 216.00, 264.00, 312.00 and 360.00 hours blood draw were collected as ambulatory blood draw. Adverse events and vital signs have been monitored at the specified time-points during the study.

The plasma samples of patients were analysed using three separate validated liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods for doxorubicinol (single method), encapsulated doxorubicin and total doxorubicin (single method) and free doxorubicin (single method).

Test and reference products

Test and reference product information is presented below:

Test Product-T		
Name of IMP	:	Doxorubicin hydrochloride (Pegylated liposomal) Injection 20 mg/10 mL (2 mg/mL)
Strength	:	20 mg/10 mL (2 mg/mL)
Manufacturer	:	Celerity Pharmaceuticals, LLC, Rosemont, Illinois, USA.
Lot No.	:	7H001
Manufacturing Date	:	18 August 2017
Retest date	:	May 2019
Storage Condition	:	Store at 2°C-8°C.

Reference Product-R		
Name of IMP	:	Caelyx® [Doxorubicin hydrochloride (Pegylated liposomal) Injection] 20 mg/10 mL (2 mg/mL)
Strength	:	20 mg/10 mL (2 mg/mL)
Marketing Authorization Holder	:	Janssen-Cilag International NV, Turnhoutseweg 30, 2340 Beerse, Belgium.
Batch No.	:	HKZSL00
Expiry Date	:	06/2019
Storage Condition	:	Store at 2°C-8°C.

Population(s) studied

Sixty-eight (68) female patients with confirmed metastatic breast cancer ($T_xN_xM_1$ (any T, any N M1) stage based on TNM Classification of Malignant Tumours), who were scheduled to start therapy with doxorubicin hydrochloride (pegylated liposomal) injection, 20 mg/10 mL (2 mg/mL) were considered eligible and were initially included in this study after meeting the criteria of inclusion/exclusion described on study protocol/final report. Fifty-nine patients (aged 32 to 71 years) completed the two study periods.

A total of 8 patients discontinued/ were withdrawn after dosing of Period I and one patient was withdrawn after dosing of Period II.

From the 59 subjects that have concluded both study periods, 2 subjects were excluded from bioequivalence statistical analysis due pre-dose concentrations above 5% of subject's respective C_{max} and one subject was excluded from PK and statistical analysis due to non-reportable concentration values before actual t_{max}. Exclusion of these subjects from PK and statistical analysis is in agreement with the Guideline CPMP/EWP/QWP/1401/98 Rev1 and study Protocol.

A total of 100 patients were screened and, of these, 68 received at least one dose of the study medication. A list of screen failures and motives was provided. Overall 59 (87%) patients completed the treatment for the two treatment periods.

Demographics and baseline characteristics for the safety population are presented in the following table:

Table 6. Demographics and baseline characteristics (Safety set)

	Statistics	TR (N=32)	RT (N=36)	Total (N=68)	Completed patients (N=59)
Age (years)	n	32	36	68	59
	Mean (SD)	50 (9.9)	53 (9.7)	52 (9.8)	52 (9.5)
	Median	53	53	53	53
	Min, Max	32, 68	33, 71	32, 71	32, 71
Gender					
Female	n (%)	32 (100.00%)	36 (100.00%)	68 (115.25%)	59 (100.00%)
Race					
Asian	n (%)	32 (100.00%)	36 (100.00%)	68 (115.25%)	59 (100.00%)
Ethnicity					
Not Hispanic Or Latino	n (%)	32 (100.00%)	36 (100.00%)	68 (115.25%)	59 (100.00%)
Height (cm)	n	32	36	68	59
	Mean (SD)	152.7 (6.20)	152.0 (7.37)	152.3 (6.80)	152.1 (6.97)
	Median	152.0	152.5	152.0	152.0
	Min, Max	140.0, 166.0	130.0, 169.0	130.0, 169.0	130.0, 169.0
Weight (kg)	n	32	36	68	59
	Mean (SD)	56.8 (11.32)	57.1 (10.12)	57.0 (10.62)	57.6 (10.87)
	Median	55.2	55.5	55.2	56.4
	Min, Max	39.3, 77.0	40.0, 80.0	39.3, 80.0	39.3, 80.0
BMI (kg/m²)	n	32	36	68	59
	Mean (SD)	24.3 (4.02)	24.7 (3.86)	24.5 (3.91)	24.8 (4.00)
	Median	24.5	23.8	24.1	24.7
	Min, Max	18.3, 30.9	18.5, 30.8	18.3, 30.9	18.3, 30.9

Analytical methods

A liquid chromatography tandem mass spectrometry (LC/MS/MS) method has been developed and validated for the determination of free doxorubicin and encapsulated doxorubicin in human plasma. Unencapsulated drug concentrations were therefore achieved by means of appropriate bioanalytical methods rather than by subtracting encapsulated from total drug, in accordance with the product-specific bioequivalence Guidance.

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.

Pharmacokinetic data analysis

Pharmacokinetic (PK) parameters were calculated by non-compartmental methods for free doxorubicin (uncapsulated) and liposome encapsulated doxorubicin, using Phoenix WinNonlin v 6.4. PK parameters AUC_{0-t}, AUC_{0-48h}, $AUC_{0-\infty}$ were calculated by the linear trapezoidal method. C_{max} and t_{max} were based on the actual data. The apparent first-order terminal elimination constant (k_e) was calculated from the semi-log plot of the plasma concentration versus time curve for each analyte. The parameter was calculated by linear least-

squares regression analysis using the maximum number of points in the terminal log-linear phase (three at least or more non-zero plasma concentrations). Parameter $AUC_{0\text{-}inf}$ was calculated as the sum of $AUC_{0\text{-}t}$ plus the ratio of the last measurable plasma concentration to the k_e. The elimination $t_{1/2}$ was calculated as (ln 2)/k_e.

Actual sampling time points were used for the calculation of pharmacokinetic parameters.

Statistical methods

For sample size, calculations were based on CRO data. An intra-subject C.V. of 35% for the bioequivalence metrics of free doxorubicin, and an expected Test to Reference ratio of geometric LS means within 95% and 105% were assumed.

Continuous variables were summarised with the following descriptive statistics: n (number of observations), arithmetic mean, geometric mean, standard deviation (SD), coefficient of variation (CV%), minimum, median and maximum. Categorical data are summarised with frequencies and percentages.

Demographic parameters were summarised descriptively. Treatment-emergent adverse events were summarised descriptively by treatment for all subjects who were dosed (Safety population).

For the required analytes, individual (per subject) and mean plasma concentration versus time curves were presented for both linear and semi-log scales. Descriptive statistics (arithmetic mean, SD, CV%, minimum, median and maximum value) of the plasma concentrations were presented for each timepoint.

C_{max}, $AUC_{0\text{-}t}$ $AUC_{0\text{-}inf}$ for free (uncapsulated) and liposomal (encapsulated) doxorubicin, as well as partial AUCs ($AUC_{0\text{-}48h}$ and $AUC_{48\text{-}tlast}$) for encapsulated doxorubicin, were the primary pharmacokinetic parameters for bioequivalence evaluation. The other PK parameters were considered for information purposes only. The analysis of bioequivalence was conducted in the pharmacokinetic analysis population. Statistical calculations were performed by SAS v.9.4 software.

Using a general linear model, an analysis of variance (ANOVA) was performed on the primary pharmacokinetic parameters. The terms included in the ANOVA model were Centre, Sequence, Sequence by Centre, Subject nested within Sequence by Centre, Period and Formulation. These terms were used as fixed effects and were assessed at the 5% two-sided level. As an exploratory analysis, the MAA performed a new ANOVA with terms Centre, Sequence, Sequence by Centre, Patient (Sequence by Centre), Formulation, Period (Centre) and Formulation by Centre interaction.

For the pairwise comparisons of interest, the 90% confidence intervals for the ratios of the treatment means (Test/Reference) were calculated. Bioequivalence between Test and Reference products was declared when the 90% confidence interval of the ratio of the means (Test/Reference) for the primary pharmacokinetic parameters was within 80.00-125.00% range.

Results

Sixty-eight (68) female patients were included in this study and dosed in period I. From these, fifty-nine (59) patients completed both treatment periods. Each subject received a single dose of doxorubicin hydrochloride liposome concentrate for dispersion for infusion (2 mg/mL) at a dose of 50mg/m^2, obtained from 20 mg/10 mL vials of both Test and Reference products, according to the randomisation scheme. Based on pre-specified analysis rules, 58 patients were included the PK and statistical analysis for encapsulated doxorubicin while fifty-six were included in the PK and statistical analysis for unencapsulated doxorubicin.

For the majority of subjects, dosing differences were between [-0.5 to 0.5 mg], with 24 subjects presenting no dosing differences between the two periods.

Clinical phase of the study was initiated on 27th August 2018 and was completed on 27th May 2019.

Major protocol deviations were visit schedule related and safety reporting.

Free doxorubicin

The pharmacokinetic variables obtained for free doxorubicin for Test and Reference products are shown in table 7, the ANOVA results in Table 8 and the 90% confidence intervals (90% CI) for the bioequivalence metrics in Table 9.

Table 7. Pharmacokinetic variables obtained for free doxorubicin (mean; coefficient of variation (%)

Free doxorubicin N=56		**Test**		**Reference**	
		Mean	C.V.%	Mean	C.V.%
C_{max}	(ng/mL)	151.732	34.7	146.138	40.8
t_{max}*	(h)	24.0	--	16.0	--
AUC_{0-t}	(ng.h/mL)	18480.589	32.9	17754.006	28.3
AUC_{0-inf}	(ng.h/mL)	20156.521	34.4	19315.623	31.2
$t_{1/2}$	(h)	85.374	36.6	84.124	35.4

*median

Table 8. ANOVA results for free doxorubicin

Free doxorubicin	**Period (Centre)**	**Sequence**	**Centre**	**$CV_{intra-indiv}$**
N=56	(p-value)	(p-value)	(p-value)	(%)
C_{max}	0.6287	0.5189	0.0010	26.5
AUC_{0-t}	0.4652	0.1777	<0.0001	19.4
AUC_{0-inf}	0.3048	0.2433	<0.0001	19.3

Table 9. 90% CI's for free doxorubicin

Free doxorubicin	**μ_T/μ_R**	**90% Confidence Interval**
N=56	%	%
C_{max}	105.8	[97.13 – 115.18]
AUC_{0-t}	104.6	[98.21 – 111.35]
AUC_{0-inf}	105.1	[98.76 – 111.87]

Encapsulated doxorubicin

The pharmacokinetic variables obtained for encapsulated doxorubicin for Test and Reference products are shown in Table 10, the ANOVA results in Table 11 and the 90% confidence intervals (90% CI) for the bioequivalence metrics in

A pre-dose concentration of encapsulated doxorubicin above the LLOQ was seen for patients no. a and b before Test product administration and in patient no. c before Reference product administration. For these cases, pre-dose concentration corresponded to < 5% of patient's C_{max} and patient's data were not excluded from the analysis.

Table 10. Pharmacokinetic variables obtained for encapsulated doxorubicin (mean; coefficient of variation (%))

Encapsulated doxorubicin		**Test**		**Reference**	
N=58		Mean	C.V.%	Mean	C.V.%
C_{max}	(ng/mL)	44512.409	12.7	44281.534	12.9
t_{max}*	(h)	2.50	--	2.50	--
AUC_{0-t}	(ng.h/mL)	3995945.358	22.3	4028859.501	24.7
AUC_{0-48}	(ng.h/mL)	1495037.581	13.6	1488493.890	13.0
AUC_{48-360}	(ng.h/mL)	2500907.776	30.2	2540365.611	33.7
AUC_{0-inf}	(ng.h/mL)	4160708.033	23.7	4216400.824	26.0
$t_{1/2}$	(h)	65.95	27.4	62.29	28.7

*median

Table 11. ANOVA results for encapsulated doxorubicin

Encapsulated doxorubicin	**Period (Centre)**	**Sequence**	**Centre**	**$CV_{intra-indiv}$**
N=50	(p-value)	(p-value)	(p-value)	(%)
C_{max}	0.2062	0.8879	<0.0001	6.1
AUC_{0-t}	0.0013	0.0050	<0.0001	11.2
AUC_{0-48}	0.3595	0.1106	<0.0001	5.9
AUC_{48-360}	0.0016	0.0052	<0.0001	17.5
AUC_{0-inf}	0.0005	0.0191	<0.0001	11.9

Table 12. 90% CI's for encapsulated doxorubicin (n=50)

Encapsulated doxorubicin	**μ_T/μ_R**	**90% Confidence Interval**
	%	%
C_{max}	100.0	[98.11 – 102.02]
AUC_{0-t}	100.6	[97.08 – 104.28]
AUC_{0-48}	100.2	[98.38 – 102.15]
AUC_{48-360}	101.2	[95.70 – 106.97]
AUC_{0-inf}	100.1	[96.35 – 103.95]

- **Safety data**

The 68 patients in the bioequivalence study Study 0927-17 were included in safety evaluation. Patients were administered doxorubicin hydrochloride injection 2 mg/mL (50 mg/m2 dose) by IV infusion after dilution over 1 hour (+ 5 minutes window period). The washout between administration of Period-I and Period-II was 28 days, with a window period of + 14 days for adverse event management. A total of 63 subjects received one dose of Doxorubicin Celdoxome and 65 subjects received one dose of Caelyx.

The MAA assessment of causality was "Related" (Certain, Probable /Likely and Possible) and "not related" (Unlikely).

68 patients were dosed in the study (98.53%). According to severity (per CTCAE), out of the 454 AEs, 276 were mild, 144 moderate, 30 severe, 1 life-threatening and 3 not classified. The CSR refers data by Test Product-T and Reference Product-R.

Test Product:

214 AEs were reported by 88.89% (n=56) of the 63 patients; 124 were mild, 73 moderate, 16 severe, and 1 life-threatening. 112 were considered related to test product and 102 unrelated. 117 were "recovered without sequelae", 59 "not yet recovered", 19 "change in severity", 18 "stable" and 1 "converted to SAE".

Reference Product:

240 AEs were reported by 90.77% (n=59) of 65 patients; 152 were mild, 71 moderate, 14 severe, and 3 not classified (Thrombocytosis, Leukocytosis and Neutrophil count increased). 114 were related to test product and 126 unrelated. 118 were "recovered without sequelae", 84 "not yet recovered", 17 "change in severity", 18 "stable", 2 "converted to SAE" and 1 "recovered with sequelae".

143 of the AEs were still ongoing at the end of the study, the majority related to laboratory abnormalities.

Out of 454 AEs, 15 AEs were infusion related reactions. These infusion related AEs occurred in 8 patients, out of which 2 received test product and were withdrawn (due to chest pain, uneasiness in chest and flushing of face in 1; giddiness, palpitation, and headache in another). Other 4 infusion related reactions occurred with test product, and 2 with reference product; all were resolved with stopping the infusion.

A total of 4 SAEs were reported in the study. Out of 4 SAEs, 2 SAEs (Suspected Neutropenia, Oral candidiasis) occurred after administration of the test treatment and 2 SAEs (disease progression, anaemia) occurred after administration of the reference treatment. The causality assessment was judged as related for 3 SAEs (2 SAEs in test treatment and 1 SAE in reference treatment) and as unrelated for 1 SAE (reference treatment).

No immunological events were reported.

A total of 8 patients discontinued/ were withdrawn after dosing of Period-I and 1 patient was withdrawn after dosing of Period-II.

Four (4) patients discontinued treatment due to AEs, out of two patients (2) in the test group and two patients (2) in the reference group; however, the two (2) patients in test group discontinued due to infusion related reactions, and in the reference group no relation to reference drug seems possible.

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

In support of this application for this hybrid application with reference to Adriamycin, the applicant provided the results of a bioequivalence study and a review of scientific literature on the clinical pharmacokinetics and pharmacodynamics of doxorubicin.

Due to the difference in formulation between Celdoxome pegylated liposomal and Adriamycin, the pivotal clinical study is a bioequivalence study to establish essential similarity between Celdoxome pegylated liposomal and the reference product Caelyx 2 mg/mL concentrate for solution for infusion.

The applicant stated that the trial was conducted in compliance with GCP and GLP requirements.

Concerns raised regarding the quality system of the CRO based on previous GCP inspections, and its extent and relevance in the current application, triggered a GCP inspection. It was considered that all data collected in the trial was considered acceptable to support the marketing authorisation of the product Celdoxome pegylated liposomal.

The presented study was designed as an open-label, two-period, two-way, crossover study in female patients with metastatic breast cancer and who were scheduled to start therapy with doxorubicin hydrochloride (pegylated liposomal) injection (i.e., the study medications were to be administered in a random fashion on the first day of Cycles 1 and 2).

The test and reference products and the mode of administration are 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 breast cancer patients.

The sample size calculations were based on available CRO's data and are consistent with standard methodology. It was estimated that the number of subjects to meet the 80 to 125% bioequivalence range with an alfa error of 0.05 was 76, with a statistical a priori power of at least 92%. With 68 subjects included in the trial, an a priori power of at least 89% was reached (calculations performed by PK assessor).

The concentrations of unencapsulated doxorubicin (non-liposomal, free) and encapsulated doxorubicin in human plasma were determined using two separate LC-MS/MS methods. Both methods met the acceptance criteria for all the validation parameters evaluated, demonstrating acceptable performance.

Statistical methodology used in the study was conducted in line with the Guideline on the investigation of bioequivalence and is considered appropriate.

Regarding the bioequivalence investigation, the submitted study showed bioequivalence for the required metrics for free form and encapsulated form of doxorubicin, as required in the product specific guidance (EMA/CHMP/800775/2017). Celdoxome pegylated liposomal 2 mg/mL concentrate for dispersion for infusion and the Reference product Caelyx 2 mg/mL (doxorubicin concentrate for solution for infusion; Janssen-Cilag International N.V.), are considered bioequivalent.

The application in the claimed indications was also supported by literature data on pegylated liposomal doxorubicin (PLD) in patients with metastatic breast cancer, recurrent ovarian cancer, multiple myeloma and AIDS-related Kaposi's sarcoma, which is acceptable for a "hybrid" application.

Clinical safety

From the 68 female patients with metastatic breast cancer randomised to the single pivotal BE study CEL-LD-202/0927-17, a total of 63 subjects received one dose of Doxorubicin Celdoxome and 65 subjects received one dose of Caelyx. Therefore, most of the patients were exposed to two doses of liposomal doxorubicin 50mg/m^2 28 days apart, with a limited follow up.

The baseline clinical characteristics per group showed a numerical imbalance in ECOG (ECOG 1 in 68.75% of patients in test product vs 83.33% in reference product), and in radiotherapy (no radiotherapy in 40.63% of test product and 58.33% in the reference product). No differences in cancer staging and past chemotherapy were observed. Overall, the demographics and baseline characteristics seem to have no influence in the results.

The incidence and pattern of adverse events per severity and per system organ class were in line with the known safety profile of the medicinal products and the medical condition of the study subjects. There were no unexpected events and there were no significant differences between the AEs for subjects exposed to the test product vs. reference product.

The most common AEs by preferred term were Anaemia, Leukopenia and Neutropenia. Most of the AEs were mild or moderate. The number of SAEs was low, as expected from the known safety profile of liposomal pegylated doxorubicin. There were four (4) SAEs reported during the study, out of which, two (2) SAE were related to test drug and one (1) to reference drug. No deaths had occurred during the study.

The imbalance in the discontinuation cases was also discussed, and the applicant reinforced that the discontinuation was not related to allergy or anaphylactoid reactions.

There were eight patients with infusion related reactions, but not mentioned as immunological events. There seems to be an imbalance in the frequency of infusion reactions: only 2 infusion reactions with reference product, and 6 with the test product (2 withdrawn). The applicant justified that the majority of infusion related reactions were in the first minutes of the infusion, were mild-to-moderate in severity and considered to be allergic-like.

No differences in AEs related to abnormal laboratory values could be observed.

No data was provided pertaining to *Safety related to drug-drug interactions and other interactions*. This is acceptable as the subjects were exposed to the investigational product as a monotherapy.

Overall, it seems there were no major differences in AEs in the Doxorubicin Celdoxome group as compared to Caelyx. Although the numbers are very scarce, and a definitive statistical conclusion is not possible based on the available data, a possible imbalance in infusion related reactions for test product was discussed.

The naming of liposomal formulations has been recognised as a major safety issue; since the liposomal and non-liposomal formulations of the same active substance may have different biodistribution and release properties, medication errors can pose serious risks to the health of patients. In line with requirements for naming of liposomal formulations, the qualifier "Pegylated Liposomal" has been included after the invented name and before the strength in the pharmaceutical form.

2.4.4. Conclusions on clinical aspects

In conclusion, based on the submitted documentation Celdoxome pegylated liposomal is considered bioequivalent to Caelyx.

The safety data from the bioequivalence trial was consistent with the known safety profile of the reference product doxorubicin hydrochloride and the comparator liposomal doxorubicin hydrochloride.

2.5. Risk Management Plan

2.5.1. Safety concerns

None.

2.5.2. Pharmacovigilance plan

Not applicable.

2.5.3. Risk minimisation measures

None.

2.5.4. Conclusion

The CHMP considers that the risk management plan version 0.2 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

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

This centralised procedure concerns a hybrid application for Celdoxome pegylated liposomal 2 mg/ml concentrate for dispersion for infusion with the reference medicinal product Adriamycin 2 mg/ml solution for injection (non-pegylated liposomal formulation). However, since Caelyx contains doxorubicin hydrochloride in a pegylated liposomal formulation, it was considered an appropriate comparator for the purpose of bioequivalence.

The proposed indications for doxorubicin hydrochloride Celdoxome (a pegylated liposomal formulation) are the same as those authorised for Caelyx:

Celdoxome pegylated liposomal is indicated in adults:

- 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.

Celdoxome 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).

This application is based on a single pivotal clinical bioequivalence study to establish essential similarity between Celdoxome pegylated liposomal and the Caelyx 2 mg/mL concentrate for solution for infusion. The presented study was designed as an open-label, two-period, two-way, crossover study in female patients with metastatic breast cancer and who were scheduled to start therapy with doxorubicin hydrochloride (pegylated liposomal) injection (i.e., the study medications were to be administered in a random fashion on the first day of Cycles 1 and 2). The provided comparative bioavailability study is appropriate and also in accordance with the product-specific bioequivalence guidance. Statistical methodology used in the study is considered appropriate.

The submitted study showed bioequivalence for the required metrics for free form and encapsulated form of doxorubicin. Test product Celdoxome pegylated liposomal and the Reference product Caelyx 2 mg/mL (doxorubicin concentrate for solution for infusion; Janssen-Cilag International N.V.) are considered bioequivalent.

A review of bibliographic data was also provided and supports the claimed indications.

The safety data from the bioequivalence trial is consistent with the known safety profile of the reference product doxorubicin hydrochloride and the comparator liposomal doxorubicin hydrochloride.

A benefit/risk balance comparable to the reference product can therefore be concluded.

3.1. Conclusions

The overall benefit/risk balance of Celdoxome pegylated liposomal is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Similarity with authorised orphan medicinal products

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

Outcome

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

Celdoxome pegylated liposomal is indicated in adults:

- 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.

Celdoxome 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).

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 (see Annex I: Summary of Product

Characteristics, section 4.2).

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.