29 May 2019
EMA/398153/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Doxolipad

International non-proprietary name: doxorubicin hydrochloride

Procedure No. EMEA/H/C/004110/0000

Note

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

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List of abbreviations

AE	adverse event
ALT (SGPT)	alanine aminotransferase
ANOVA	analysis of variance
ANCOVA	analysis of covariance
aPTT	activated partial thromboplastin time
AST (SGOT)	aspartate aminotransferase
AUC	area under the curve
BE	bioequivalence
BMI	body mass index
BSA	body surface area
CEP	Certificate of Suitability of the EP
CIB	clinical investigator brochure
CIOMS	Council for International Organizations of Medical Sciences
Cl	clearance
CHMP	Committee for Medicinal Products for Human use
Cmax	peak concentration
CPP	Critical process parameter
CQA	Critical Quality Attribute
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
DLS	Dynamic light scattering
DNA	deoxyribonucleic acid
DSPE	distearoyl phosphatidylethanolamine
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDQM	European Directorate for the Quality of Medicines
EOS	end of study
EU	European Union
FD	Free Doxorubicin
FDA	Food and Drug Administration
FMEA	Failure mode effects analysis
GC	Gas Chromatography
GCP	Good Clinical Practice
HCl	hydrochloric acid
HPLC	High performance liquid chromatography
HSPC	hydrogenated soy phosphatidylcholine
ICF	informed consent form
ICH	International Conference On Harmonisation
IMP	Investigational medicinal product
INR	international normalized ratio
IR	Infrared
IRB	institutional review board
IV	intravenous
KF	Karl Fischer titration
ëz	elimination rate constant
LED	Liposomal Encapsulated Doxorubicin
LLOQ	lower limit of quantification max maximum
LPC	lysophosphatidylcholine
MedDRA	Medical Dictionary for Regulatory Activities
MRT	mean residence time
mPEG	methoxypolyethylene glycol
MO	Major objection
MUGA	multi-gated acquisition
NMT	Not more than NYHA - New York Heart Association
PACMP	Post-approval change management protocol
Ph. Eur.	European Pharmacopoeia
PK	pharmacokinetics
PLD	pegylated liposomal doxorubicin
PPE	palmar-plantar erythrodysesthesia

PT	prothrombin time
PT	preferred term
QTPP	Quality target product profile
RA	Risk assessment
RA	regulatory authority
RH	Relative Humidity
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SEC	size exclusion chromatography
SmPC	Summary of Product Characteristics
SUSAR	suspected unexpected serious adverse reaction
SUV	small unilamellar vesicles
T1/2	half-life
TEAE	treatment-emergent adverse event
TLC	Taiwan Liposome Company
TLC	Thin layer chromatography
Doxolipad	generic pegylated liposomal formulation of doxorubicin
Tmax	time to reach peak concentration
TSE	Transmissible Spongiform Encephalopathy
ULN	upper limit of normal
US	United States
UV	Ultraviolet

1. Background information on the procedure

1.1. Submission of the dossier

The applicant TLC Biopharmaceuticals B.V. submitted on 25 April 2017 an application for Marketing authorisation to the European Medicines Agency (EMA) for Doxolipad, 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 10 November 2016. The eligibility to the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 was based on demonstration of significant scientific innovation and interest of patients at Community level.

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

The applicant applied for the following indication:

- 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.

The legal basis for this application refers to:

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

The application submitted is composed of administrative information, complete quality data, a bioequivalence study with the reference medicinal product Adriamycin and appropriate 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 ApS
- Date of authorisation: 12-10-1988
- Marketing authorisation granted by:
  - Member State (EEA) : Denmark
    - National procedure

Medicinal product authorised in the Union/Members State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form: Adriamycin, 2mg/ml, Solution for injection
- Marketing authorisation holder: Pfizer ApS
- Date of authorisation: 12-10-1988
- Marketing authorisation granted by:
  - Member State (EEA): Denmark
    - National procedure

As bioequivalence against the reference medicinal product was not feasible due to the differences in formulation, Caelyx, which contains doxorubicin hydrochloride in a pegylated liposomal formulation, was considered as appropriate comparator to establish quality, non-clinical and clinical comparability.

Information on paediatric requirements

Not applicable

Information relating to orphan market exclusivity

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.

Scientific advice

The applicant received Scientific Advice on the development relevant for the approved indication from the CHMP on 23 September 2010, 19 January 2012 and 17 January 2013. The Scientific Advice pertained to the following quality, non-clinical and clinical aspects of the dossier:

- Proposed specification for the drug product, adequacy of length and design of the stability studies.
- Completeness of the non-clinical programme to support the demonstration of the similarity to Caelyx. In particular, the design of a single biodistribution study in tumour bearing mice and a sub-acute toxicology study in rats.
- A single bioequivalence study *versus* Caelyx in patients with ovarian cancer: acceptability of an open-label, randomised, balanced, two-treatment, two-period, two-sequence cross over study design. The size and inclusion/exclusion of the study population, dose and dosing schedule, blood sampling schedule and wash out period. Acceptability of human comparative pharmacokinetic data, supported by physico-chemical similarity tests to support the demonstration of bioequivalence and marketing authorisation.
- The use of either Caelyx or Doxil as reference liposomal product in the bioequivalence study, to address the issue of global shortage of the reference drug. The conduct of the bioequivalence study outside the EU.

1.2. Steps taken for the assessment of the product

The Rapporteur appointed by the CHMP was:

Rapporteur: Ewa Balkowiec Iskra

The application was received by the EMA on	25 April 2017
The procedure started on	18 May 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on	31 July 2017
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	18 August 2017
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	14 September 2017

The applicant submitted the responses to the CHMP consolidated List of Questions on	25 May 2018
The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on	3 July 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 July2018
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	26 July 2018
The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on	9 November 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	7 December 2018
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	11 December 2018
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Doxolipad on	31 January 2019
The CHMP adopted a report on similarity of Doxolipad with Yondelis and Zejula on (Appendix 1)	31 January 2019

1.3. Steps taken for the re-examination procedure

The Rapporteur appointed by the CHMP was:

Rapporteur: Jayne Crowe

The applicant submitted written notice to the EMA, to request a re-examination of Doxolipad CHMP opinion of 31 January 2019., on	14 February 2019
The CHMP appointed Jayne Crowe as Rapporteur on	28 February 2019
The applicant submitted the detailed grounds for the re-examination (Appendix X of Final Opinion) on	02 April 2019
The re-examination procedure started on	02 April 2019
The Rapporteurs circulated the Joint Assessment Report on the detailed grounds for re-examination to all CHMP members on	3 May 2019
PKWP was consulted to address questions raised by the CHMP and the PKWP responses to the CHMP questions were circulated to all CHMP members on	3 May 2019
Adhoc Expert group was convened to address questions raised by the CHMP on The CHMP considered the views of the Adhoc Expert group as presented in the minutes of this meeting	20 May 2019

The detailed grounds for re-examination were addressed by the applicant during an oral explanation before the CHMP on	27 May 2019
The CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the marketing authorisation	29 May 2019

2. Scientific discussion

2.1. Introduction

Doxolipad (TLC177) contains doxorubicin hydrochloride 2 mg/ml concentrate for solution for infusion. The application is a hybrid application and refers to Adriamycin which contains doxorubicin hydrochloride 2 mg/ml solution for injection. Doxorubicin hydrochloride 2mg/ml solution for injection is authorised in the treatment of acute leukaemia, lymphomas, soft-tissue and osteogenic sarcomas, paediatric malignancies and adult solid tumours, in particular breast and lung carcinomas.

Adriamycin and Doxolipad differ in terms of formulation as Adriamycin contains doxorubicin hydrochloride in a non-liposomal formulation while doxolipad contains doxorubicin hydrochloride in a pegylated liposomal formulation. As bioequivalence against the reference medicinal product was not feasible due to the differences in formulation, Caelyx, which contains doxorubicin hydrochloride in a pegylated liposomal formulation was considered as appropriate comparator to establish with respect to quality, non-clinical and clinical comparability. Caelyx 2 mg/ml concentrate for solution for infusion is a centrally-authorised medicinal product that was authorised on 20 June 1996 under a hybrid application procedure (Article 10(3) Directive No 2001/83).

Two of the four indications of Caelyx were applied for Doxolipad:

"Doxolipad is indicated

- as monotherapy for adult patients with metastatic breast cancer, where there is an increased cardiac risk.

- for treatment of advanced ovarian cancer in adult women who have failed a first-line platinum-based chemotherapy regimen."

The same posology as the one recommended for Caelyx in the treatment of breast and ovarian cancer was proposed for Doxolipad:

Doxolipad 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.

The active substance, doxorubicin hydrochloride, is a well-established cytotoxic anti-cancer agent. It is 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.

This application is based on clinical studies conducted to evaluate the bioequivalence of Doxolipad to Caelyx.

2.2. Quality aspects

2.2.1. Introduction

The proposed finished product was presented as concentrate for solution for infusion containing 2 mg/ml of doxorubicin hydrochloride as active substance encapsulated in liposomes with surface-bound methoxypolyethylene glycol (MPEG).

Other ingredients were:
α-(2-[1,2-distearoyl-sn-glycero(3)phosphooxy]ethylcarbamoyl)-ω-methoxypoly(oxyethylen)-40, sodium salt (MPEG-DSPE), fully hydrogenated soy phosphatidylcholine (HSPC), cholesterol, ammonium sulphate, sucrose, histidine, water for injections, hydrochloric acid and sodium hydroxide.

The proposed product was packaged in Type I glass vial, with a siliconised grey chlorobutyl stopper, and an aluminium seal, containing a deliverable volume of 10 ml (20 mg).

Active substance

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

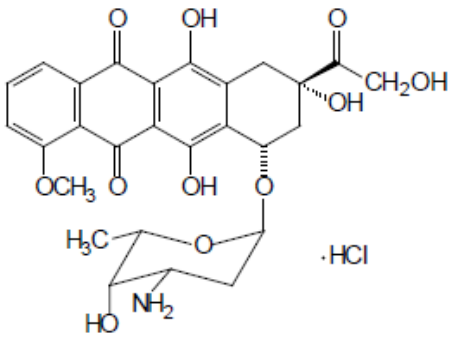


Figure 1: active substance structure

Doxorubicin hydrochloride is a hygroscopic, orange-red crystalline powder. It is soluble in water, normal saline, methanol, acetonitrile and tetrahydrofuran. It is practically insoluble in acetone, benzene, chloroform, ethyl ether and petroleum ether.

Polymorphism has not been observed for doxorubicin hydrochloride.

As there is a monograph of doxorubicin hydrochloride in the European Pharmacopoeia, the manufacturer of the active substance has been granted a Certificate of Suitability of the European Pharmacopoeia (CEP) for this active substance which has been provided within the current Marketing Authorisation Application.

Manufacture

Information on manufacturing, process control and characterisation was not presented. This data was assessed by the EDQM before issuing the CEP. The certificate is still valid according to information on EDQM website.

The container closure system was a glass bottle with polytetrafluoroethylene lined caps. The container closure is in accordance with the current CEP.

Specification

Doxorubicin hydrochloride is manufactured in accordance with the CEP and complies with the specifications of the doxorubicin hydrochloride monograph in the current Ph. Eur.

The active substance specification for doxorubicin hydrochloride, as used by the finished product manufacturer, are summarised in **Error! Reference source not found.** below. It includes tests for description, identification (IR, precipitation), pH, water (KF), assay (HPLC), related substances (HPLC), residual solvents (GC), and bacterial endotoxins (Ph. Eur.).

The compendial analytical procedures used for testing doxorubicin hydrochloride as referenced in specification have been verified to be suitable for their intended use. Validation summaries for the in-house procedure for the determination of residual solvents (GC) used by the finished product manufacturer has been provided.

Satisfactory information regarding the reference standards used for assay testing has been presented.

Batch analysis data on 4 batches of doxorubicin hydrochloride, as tested by the active substance and finished product manufacturers have been provided. They all complied with the proposed specification.

Stability

The stability of the active substance was reviewed and approved by EDQM during granting the CEP. The retest period for the active substance is 3 years if stored in the approved airtight container closure, protected from humidity and excessive heat; the temperature range is equivalent to 1-25^{o}C according to the letter from the active substance manufacturer provided. The storage condition at finished product complies with requirement in the CEP.

2.2.2. Finished medicinal product

Pharmaceutical development

Doxolipad is a concentrate for solution for injection containing a liposomal form of doxorubicin hydrochloride 2 mg/mL, intended for intravenous administration.

Each vial contains the active substance doxorubicin hydrochloride, encapsulated in "stealth" liposome carriers by pegylation of the liposome surface which reduces clearance by mononuclear phagocyte system, and thereby increases blood circulation time. The liposomal suspension consists of small unilamellar vesicles (SUV) with an average size range of 70 – 100 nm.

A schematic representation of a stealth liposome as presented by the applicant is presented in Figure 2.

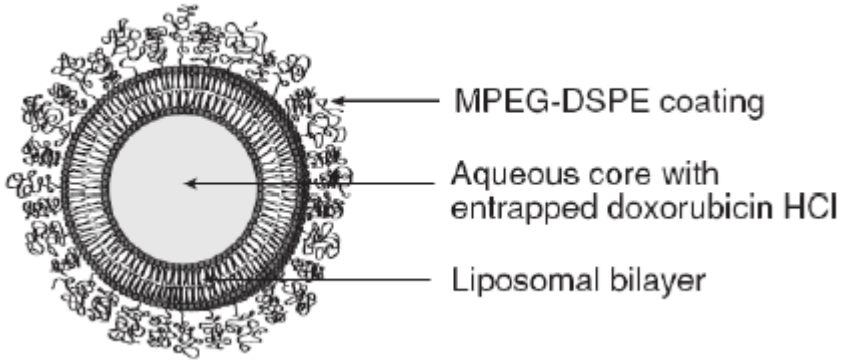


Figure 2. Schematic depiction of a stealth liposome.

The product is a sterile, translucent, red liposomal dispersion presented as 20 mg/10ml in 10 ml single use type I glass vial fitted with chlorobutyl elastomeric closure and sealed with aluminum cap with plastic flip-off top.

This MAA is a hybrid application, with the reference medicinal product being Adriamycin (doxorubicin hydrochloride) 2 mg/ml, solution for injection. Caelyx, which is an existing liposomal formulation of doxorubicin hydrochloride 2 mg/ml, is being used as comparator for the pharmaceutical development and the purposes of comparability.

The composition of Doxolipad is presented in Table 1 below.

Table 1: composition of finished product

Ingredient	Function
Doxorubicin HCl*	Active ingredient
HSPC	Excipient, liposome ingredient
MPEG-DSPE	Excipient, liposome ingredient
Cholesterol	Excipient, liposome ingredient
Ammonium Sulfate	Excipient, ionic gradient
Sucrose	Excipient, osmolality control
Histidine	Excipient, buffer
Hydrochloride acid	Excipient, pH adjustment
Sodium hydroxide	Excipient, pH adjustment
Water for injection	Excipient, solvent

* Equivalent to 1.87 mg Doxorubicin base
HSPC = hydrogenated soy phosphatidylcholine; MPEG-DSPE = N- (carbonylmethoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3 phosphoethanolamine sodium salt

The pharmaceutical development aimed at obtaining a product which was both qualitatively and quantitatively similar to Caelyx, and devising suitable manufacturing controls to reliably and consistently manufacture a product of the same quality and performance profile as Caelyx.

The pharmaceutical development approach consisted on:

- Analysis and characterisation of Caelyx
- Defining quality target product profile (QTPP) based on analysis and characterisation of Caelyx
- Identification of critical quality attributes (CQAs) for the finished product and critical material attributes (CMAs) for the active substance and excipients in relation to the QTPP
- Identification of potential risks for each unit operation (Risk Assessment) and identification of the critical process parameters (CPPs)
- Development of a robust process based on risk assessment (RA)
- Establishment of control strategies
- Manufacture of exhibit batches to validate the control strategies devised

The QTPP for the proposed product encompassed standard quality and regulatory compliance requirements for the parenteral dosage form, as well as standard bioequivalence requirements for generic products.

A list of QTPP elements is provided in Table 2.

Table 2: QTPP for Doxolipad

Category	QTPP elements	Target	Justification
Product design	Dosage form	Liposomal solution	Pharmaceutical equivalence requirement: same dosage form
	Route of administration	Intravenous	Pharmaceutical equivalence requirement: same route of administration
	Dosage strength	2 mg/mL	Pharmaceutical equivalence
Performance	Pharmacokinetic profile	Bioequivalent to reference medicinal product	Bio-equivalence requirement
	Efficacy		
	Toxicity profile (Safety)		
General Quality Requirements for Dosage Form	Appearance	Comparable to reference medicinal product based on characterization studies	Equivalence requirement for physicochemical characterization
	Identification	Confirmed for doxorubicin	General compendia requirement to be concordant with reference standard
	Assay	Comparable to reference medicinal product based on	Equivalence requirement for physicochemical characterization
	Impurities of Doxorubicin	Lower or comparable to reference medicinal product based on characterization studies	Equivalence requirement for physicochemical characterization
	Lipid content	Comparable to reference medicinal product based on characterization studies	Equivalence requirement for physicochemical characterization
	Lipid impurity	Lower or comparable to reference medicinal product based on characterization studies	Equivalence requirement for physicochemical characterization
	pH	Comparable to reference medicinal product based on characterization studies	Equivalence requirement for physicochemical characterization
	Residual solvent (ethanol)	Meets compendia requirements	General compendia requirements to ensure patient safety for the chosen dosage form Equivalence requirement
	Particulate matter	Meets compendia requirements	

	Uniformity	Meets compendia requirements	for physicochemical characterization General compendia requirements to ensure patient safety for the chosen dosage form
	Endotoxin	Meets compendia requirements	
	Extractable volume	Meets compendia requirements	
	Sterility	Meets compendia requirements	
	Osmolaliy	Physiological osmolality	To ensure patient comfort and safety
	Shelf life	At least 20 month shelf life at 2- 8oC	Equivalent to or better than innovator product shelf life
	Container closure system	Container closure system qualified as suitable for this drug product	Achieve the target stability in shelf life or during shipping. Absence of incompatibility and interaction with product formulation.

The composition of the product was defined after characterisation of the EU marketed Caelyx, and comparison against the label claim of the product marketed in the US under the name Doxil. To provide well-defined targets for further product development, potential quality attributes of the finished product were subjected to formal Failure Mode Effect Analysis (FMEA) risk assessment to identify the CQAs. Based on the results of this RA the defined CQAs were: encapsulation ratio, particle size, *in vitro* release, maintenance of liposomal formulation integrity in plasma, phase transition temperature, net charge, internal pH, liposome aggregates, morphology, lamellarity, physical state of active substance, distribution of active substance within liposome (bilayer vs. interior), appearance, identification, assay, impurity (related substance of doxorubicin), lipid content, lipid impurity, pH, residual solvent, particulate matter, uniformity, endotoxin, extractable volume, sterility and osmolality. These were used to guide the pharmaceutical development of Doxolipad to achieve bioequivalence to the product used as comparator Caelyx. The active substance in Doxolipad is doxorubicin hydrochloride and is the same as in Caelyx. The physicochemical attributes of the active substance were described by the applicant (see Table 3). Doxorubicin hydrochloride is soluble in water and there are no specific manufacturing considerations associated with the dissolution of doxorubicin hydrochloride. Due to its acidic pH of in aqueous solution, the inclusion of pH adjustment buffering agent L-histidine in the finished product formulation excipients is used to bring the finished product to physiological pH range, which is consistent with the formulation of Caelyx.

Table 3: Physicochemical properties of doxorubicin hydrochloride

Parameter	Description
Physical description	Doxorubicin hydrochloride is a hygroscopic, orange-red crystalline powder.
Solubility	Doxorubicin hydrochloride is soluble in water, normal saline, methanol, acetonitrile and tetrahydrofuran. It is practically insoluble in acetone, benzene, chloroform, ethyl ether and petroleum ether.
Melting Point	Doxorubicin hydrochloride melts with decomposition at about 205°C.

pH	A 5 mg/mL aqueous solution of Doxorubicin hydrochloride has a pH of between 4.0 and 5.5
pKa	pKa_1 = 5.9; pKa_2 = 8.2; pKa_3 = 10.2; pKa_4 = 13.2
Polymorphism	There is no polymorphism in doxorubicin hydrochloride.

The qualitative composition of the product is the same as Caelyx and consists of fully hydrogenated soy phosphatidylcholine (HSPC), cholesterol and distearoyl-phosphatidylethanolamine (DSPE) conjugated to methoxypolyethylene glycol (MPEG). All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards, when applicable. Details of the manufacture and controls of cholesterol, HSPC and MPEG-DSPE have been provided. The justification of the specifications for the non-compendial excipients HSPC and MPEG-DSPE have been discussed. These were generally acceptable, except of the fact that the specifications for impurities in these two excipients were based on a limit test (TLC). As a result of the concerns raised by the CHMP during the evaluation, the applicant committed to investigate potential quantitative methods in the future. This was accepted since the impurity lysophosphatidylcholine (LPC) is controlled in the finished product using a HPLC-ELSD method, and the impurity lyso-MPEG-DSPE is specifically controlled by the lipid supplier and the finished product manufacturer.

The quantitative composition of doxorubicin HCl and lipid excipients HSPC, cholesterol and MPEG-DSPE in Doxolipad were kept to be identical to Caelyx to obtain the same drug to lipid ratio. Justification of the molecular weight of the MPEG moiety and ratio of constituent fatty acids in HSPC with regards to their intended function and *in vivo* product performance was provided. The differences observed were found to be minor and within the proposed acceptance criteria.

For other excipients, including sucrose and histidine –included to maintain osmolality and pH- and ammonium sulfate, the exact amounts in Doxolipad are not controlled to be quantitatively equivalent to Caelyx. The function of ammonium sulfate is to provide a transmembrane gradient to load doxorubicin into the liposome interior and to retain the drug in the precipitate form to achieve the desired sustained release profile. As such, the applicant claimed that any sufficiently large transmembrane concentration gradient is expected have the same pharmaceutical quality and *in vivo* performance characteristics. Based on the functions of these formulation components, the CQAs affected by ammonium and sulfate content were defined and equivalence of these in Doxolipad and Caelyx was confirmed. Doxolipad was tested to have both equivalent drug encapsulation ratio and in vitro drug release profile as Caelyx. This was supplemented by animal pharmacokinetic and biodistribution studies (see non-clinical section).

The information presented in the original submission with regards to the comparability between Caelyx and Doxolipad was very limited especially in the area of structural characterisation of the liposomes and their impurities. Therefore, a major objection (MO) requesting the applicant to conduct extensive investigations using state of the art characterisation methods to demonstrate with high level of assurance comparability between both products in line the EMA's Reflection Paper on the *Data Requirements for Intravenous Liposomal Products Developed with Reference to an Originator Liposomal Product* (EMA/CHMP/806058/2009) was raised.

Revised physicochemical characterisation and comparison studies presented were and included: appearance, pH, osmolality, drug encapsulation ratio, particle size distribution, zeta potential, characterization of impurities - API related substances, lipid impurities, residual solvents, phase transition temperature, liposome trapped volume, internal pH, comparative stress, MPEG-DSPE molecular weight distribution, PEGlayer thickness, distribution of drug substance, state of drug substance, drug strand width, distance between strands, lamellarity, circularity, bilayer thickness, and *in vitro* drug release.

Batches of Doxolipad used for characterisation studies included the clinical bioequivalence batch and process validation batches at the two proposed commercial scales.

For characterisation of general physicochemical properties, all Doxolipad comparability results were found to be equivalent to Caelyx. There was no difference between both products in terms of appearance, pH, mean particle size and particle size distribution, drug encapsulation ratio, impurity profile and residual solvents. Minor differences were observed for osmolality and zeta potential and the impact of these differences were found to be minimal concluding that they have no impact on the safety and efficacy of the product.

With regards to the characterisation specific to pegylated liposomal doxorubicin, there were no notable differences between Doxolipad and Caelyx in phase transition temperature, internal pH, trapped volume, comparative stress testing under various conditions. Characterisation of the active substance indicated that in both products the active substance is contained within the liposome interior as a precipitate which gives rise to the characteristic "coffee morphology". Quantitative analysis of the doxorubicin precipitate was performed to demonstrate equivalence between both products. Quantitatively, the circularity, lamellarity, and thickness of the liposome bilayer were also found to be equivalent. The only minor differences were observed in the molecular weight distribution of the MPEG-DSPE as well as the corresponding PEG-layer thickness which is a direct consequence of MPEG-DSPE molecular weight. The differences were found to be within the excipient supplier's quality control specifications. Based on the function of MPEG-DSPE being to prolong the *in vivo* circulation time for pegylated liposomes, an *in vitro* release and a pharmacokinetic comparison study in mice were conducted. They concluded that molecular weight ranges encompassing the supplier's specification range have no significant impact on drug performance within the range studied. Further characteristics of the MPEG-DSPE such as stability of conjugation and PEG-layer thickness over product shelf-life were also found to be stable in both products. This indicates the degradation rate of conjugated lipid are minimal and comparable between both products. Liposome integrity in human plasma was also comparable.

Finally, in terms of drug release as measured by *in vitro* methods, both products were found to be equivalent in various conditions tested during method development and the final *in vitro* release method proposed for quality control release testing. The choice of the method conditions and its discriminatory nature have been discussed. The finalized QC dissolution method demonstrated to be discriminatory against changes in composition associated with manufacturing process. For developmental purposes, a comparison of release profiles in plasma was also conducted and results were similarly found to be equivalent. Comparability of the drug release profiles between the test and reference product under different conditions (e.g. temperature, pH and stressed conditions) was also investigated. The results indicated that both products are highly comparable regardless of the *in vitro* release conditions.

The development of Doxolipad manufacturing process consisted of two steps:

1) Development of the overall manufacturing process based on analysis of Caelyx characteristics on multiple batches to identify the most appropriate sequence of process unit operations required to achieve desired product quality attributes.
2) Selection of the operating parameters for each unit operation in respect to the target CQAs.

The choice of sterilisation process was selected in accordance to the decision tree for sterilisation choices for aqueous products described in the annex to the Note for Guidance on Development Pharmaceutics (CPMP/QWP/155/96). In addition to the initial sterilising parameter of 121°C for 15 minutes (standard overkill approach), two additional sterilisation parameters with Fo≧8 minutes were also tested. The suitability of moist heat at 121°C for 15 minutes, as well as Fo ≧ 8 minutes were tested. The results showed significant chemical degradation of the active substance doxorubicin as well as instability of the liposome vesicles as indicated by increase in particle size and size distribution. Sterilisation by moist heat

was therefore deemed unsuitable for Doxolipad. Following the decision tree for aqueous products, filtration through a microbial retentive filter was selected.

The conceptual design of the manufacturing process steps included: lipid compounding, liposome forming, liposome sizing, buffer exchange, additional compounding, drug loading, sterilizing filtration, filling, capping and sealing. For each of the manufacturing process steps (unit operations), the potential process variables, including process equipment material attributes and process parameters, were identified. The product quality attributes relevant to each process step were also identified to allow correlation of the CQAs to the process variables through RA and identification of the development work required as risk mitigation strategies to control the product CQAs.

Based on the initial risk correlation matrix, CQAs that were identified to be risk-correlated with an unit operation were subjected to further RA to identify which of the unit operation's process variables are potentially risk contributors. These were further investigated in development studies for each unit operation to select appropriate operating parameters and/or control strategies.

As mitigation action identified in the initial RA, a hold time stability study was conducted to support the proposed storage of empty liposomes. The results indicated that there is no physicochemical degradation or microbiological growth over the hold time period investigated.

Doxolipad is presented in clear Type I glass vials of 10 ml capacity, closed with 20 mm siliconised-grey chlorobutyl stoppers and sealed with aluminum seals containing plastic flip off top. Specifications for each primary packaging component have been provided. The container and the closure comply with current Ph. Eur. regulatory requirements. The secondary packaging consists of a cardboard box. A summary of data on the suitability of the primary packaging components, including material evaluation, closure integrity and data on extractables/leachables was provided.

To note, the original submission did not include information on an *in vitro* dissolution method and this parameter was not included in the proposed finished specification. This was not acceptable and a MO was raised.The in vitro liposome release test was therefore designed as a quality control method with the understanding that any in-vivo-in-vitro correlations (IVIVC) would be highly unfeasible.

Early development of the *in vitro* release method was approached from the example of *in vitro* leakage conditions of doxorubicin hydrochloride suggested in the US FDA's Draft Guidance on Doxorubicin Hydrochloride including plasma incubation, pH variation, temperature variation, and mechanical stress by ultrasound. However, limited amount of doxorubicin was released except for ultrasound condition but large variation was observed. Since the aim was to develop a reproducible and robust *in vitro* release method with greater than 85% release of doxorubicin for a complete profiling, these conditions were concluded to be not feasible. An *in vitro* release method was therefore developed, validated, and used for comparison against Caeelyx. The composition and concentration of the release medium were chosen to control pH and to trigger the release of doxorubicin to almost complete release within the study period. pH was chosen to mimic the physiological environment near the tumour site. The proposed *in vitro* release method was demonstrated to be discriminatory against changes in composition associated with manufacturing process. The components of the Caelyx composition relevant for the evaluation of the *in vitro* drug release method were discussed. To be able to distinguish between the method's lack of discriminatory capability for a given composition factor and the possibility that the composition factor has no impact on the rate of drug release, both *in vitro* and *in vivo* release profiles were characterized for the relevant composition factors. Based on the results obtained the discriminatory power of the dissolution method against changes in composition was demonstrated.

In addition, as requested by the CHMP, drug release under different stress conditions such as temperature and pH were investigated as part of the *in vitro* release method development and were showed to be comparable.

The discriminatory power of the in-vitro release method with regards to relevant process parameters was investigated. However, only graphical representation of the results obtained was presented. No indication of the number of batches manufactured under each condition nor statistical evaluation of the results were provided. Therefore the applicant's conclusion that the proposed analytical method is capable of discriminating variations in process parameters was not fully justified. Clarification on the number of batches tested and the statistical evaluation of the results were still pending at the time of opinion.

Adventitious agents

All materials used for this human medicinal product comply with EU regulation (EMA/410/01, rev. 3). The only material used in the manufacturing process of the medicinal product derived from materials of animal and/or human origin is cholesterol. A TSE-Certificate of Suitability and a supplier's TSE statement of compliance with Ph. Eur 5.2.8. have been provided.

Manufacture of the product

The overall manufacturing process for Doxolipad involves two stages:

1) Manufacture of the empty liposome intermediate (Stage 1), and

2) Drug loading and manufacture of the finished product (Stage 2).

Flow charts for the overall manufacturing process, stage 1 and stage 2 including in-process controls have been provided.

The specification for the empty liposome intermediate was presented as requested in the day 120 list of questions

The drug-loaded liposome solution is sterile filtered, then filled into containers and stoppered under aseptic processing conditions. The final capped containers are decontaminated, inspected, labelled, packaged and released according to site procedures.

The description of the manufacturing process and process controls presented in the original submission was very brief and not in line with the guidance provided in the CHMP guideline on manufacture of finished dosage form (EMA/CHMP/QWP/245074/2015). This was revised by the applicant including details of non-critical and critical process parameters, but at the time of CHMP opinion, numerical values for those stirring speeds that were confirmed in the development/validation findings (e.g. liposome forming step) were still missing from the manufacturing process description.

The process is considered to be a non-standard manufacturing process.

The manufacturing process for the empty liposome intermediate was validated. Process validation analyses were conducted during mixing, forming, sizing, diafiltration/recovery and filtration steps of manufacture. These data confirmed that the process is reproducible and consistently produced empty liposomes of the required quality.

A hold time study was also performed on three batches. Samples were analyzed for physicochemical and microbiological properties. The validation studies confirmed that the empty liposome intermediate can be successfully manufactured and that the intermediate can be held under the proposed storage conditions and time. Two studies were conducted to validate manufacture of the finished product batch sizes. A hold time study was performed. In the original submission, validation data from a single batch by the proposed process was presented, which resulted in a MO since the method of manufacture is non-standard and data from at least three batches are to be provided (ref. CHMP process validation guideline). In his response, the applicant provided additional process validation data on two additional batches of finished product, manufactured using the proposed commercial process. Additional data from a batch of a presentation which is not subject to the present application was also provided since it has an

identical formulation and strength. Two of those batches failed pre-filtration bioburden limits (of NMT 10 cfu/100 ml). Investigations conducted by the contract manufacturer concluded that the non-conformance is not related to bioburden analysis (technique, analyst, testing environment or media), manufacturing process, environment, WFI, nor facility or equipment cleaning. The source of bioload is not from the empty liposome component, which is filtered prior to use. The investigation did not conclude a definite root cause. However, doxorubicin hydrochloride active substance was identified as a possible contributing factor since it is not monitored for microbial limits prior to use. As a corrective and preventive action, a post-approval change management protocol (PACMP) to introduce a non-sterilizing filtration step after preparation of the doxorubicin/sucrose solution to reduce bioload of the bulk of doxorubicin/sucrose post-approval was included in the dossier.

Overall, it has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

Product specification

The original specification proposed by the applicant was very limited and did not include special considerations as described in the CHMP reflection paper *on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product EMA/CHMP/806058/2009/Rev.02* as recommended in the scientific advice provided in 2010. In addition, the limits for some parameters were wide and required tightening as per the batch analysis data of Doxolipad and results from the characterization of Caelyx. This concern resulted in a MO raised at day 120 LoQ.

As a result, the applicant revised the finished product release specification based on ICH Q6A, ICH Q3B (R2), the reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058), batch analysis of Caelyx, impact of specification range on product *in vivo* performance in relation to bioequivalence to Caelyx, and Doxolipad batch and stability history. The revised specification included appropriate tests for this kind of dosage form: description (visual), identification (doxorubicin hydrochloride, Cholesterol, HSPC, MPEG-DSPE) (HPLC, UV), pH (Ph. Eur.), assay (doxorubicin hydrochloride, Cholesterol, HSPC, MPEG-DSPE) (HPLC, HPLC-ELSD), encapsulation ratio (SE-HPLC),impurities (HPLC), particle size distribution (DLS), osmolality (osmometry), ethanol (GC), zeta potential (DLS), in vitro release (SEC and HPLC-UV), extractable volume (Ph. Eur.), particulate matter (Ph. Eur.), sterility (Ph. Eur.), bacterial endotoxins (Ph. Eur.).

The revised specification was generally acceptable. However, at the time of opinion, the following points were still outstanding:

-with regards to the proposed particle size specification reported as SPAN instead of d10, d50 and d90, similarity of Doxolipad and Caelyx size distribution by intensity and their Guassian distribution had to be confirmed,

-the limits proposed for assay for cholesterol, HSPC and MPEG-DSPE were not justified considering the results from release and stability data from batches manufactured by new manufacturing process. The applicant was requested to tighten the limits based on the current capability observed and, if appropriate, broaden the specification limits post-approval when he has more supportive data;

-although the limits for two impurities were tightened in the release specification, the limits in both release and shelf-life specifications were still not adequately justified and should be adjusted to the batch data, especially to those manufactured using the proposed commercial process.

The omission of a test for elemental impurities in the finished product specification was adequately justified by the applicant. No metal catalysts are used during the manufacturing process of Doxolipad. In addition, process validation batches tested against the specification limits outlined in ICH Q3D were presented.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay, cholesterol, phospholipid components HSPC and MPEG-DSPE, impurity LPC testing and endotoxins has been presented.

Batch analysis results are provided for several commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data from commercial scale batches of finished product stored for up to 24 months under long term conditions (5±3 ºC) and for up 3 months under accelerated conditions (25±2ºC/ 60±5 RH) according to the ICH guidelines were provided. Samples were packed in the primary packaging proposed for marketing. Samples were stored in the inverted position to stimulate worst case conditions of the product in contact with the rubber closure. Four of these batches were also stored in the upright position under long term conditions.

Samples were tested for description, pH, assay, total phospholipids, encapsulation ratio, related substances, LPC, mean particle size and particle size distribution, osmolality, zeta potential, particulate matter, sterility and bacterial endotoxins. Results from *in vitro* release from three batches were provided following the request from the CHMP. The analytical methods used in the stability studies are the same as the methods used for the release testing and are stability indicating.

The test results met the proposed product release specifications and remained with the proposed limits after 24 month of storage at long term conditions. The results from the same batch stored in the upright and inverted position were comparable. The accelerated stability data, however, showed some out of specification results after 6 month storage.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Although a slight increase in total impurities was observed for the light exposed samples as compared to the dark control, the parameter was well below the specification limit .

Based on available stability data, which was re-assessed based on the revision of the finished product specification during the marketing authorisation review, the proposed shelf-life of 24 months and stored at 2-8 ºC is considered acceptable.

For administration, Doxolipad is diluted in 5% glucose solution for infusion. An in-use stability study was designed following the *EMA Note for Guidance on in-use stability testing of human medicinal products* (EMA/CPMP/QWP/2934/99). It included four batches tested during shelf-life, near end-of shelf life, and post-expiry (25 month). The instructions for reconstitution followed that of Caelyx. For doses < 90 mg, dilute Doxolipad in 250 ml of 5% glucose solution for infusion prior to administration, and for doses ≥90 mg, dilute Doxolipad in 500 ml. Per instruction, the most diluted and highest concentrations were chosen for this study. After dilution and storage for up to 26 hours at 2℃ to 8℃, assay, pH, particle size, and encapsulation ratio were assessed. Both dilutions met the acceptance criteria at all time points, supporting the proposed in-use shelf-life of 24 hours under storage condition at 2℃ to 8 ℃.

Robustness of process for in-use preparation covering different personnel, different lot of mixing bag and infusion set, as well as different lot of diluent 5% glucose injection was also demonstrated.

Based on available stability data, which was re-assessed based on the revision of the finished product specification during the marketing authorization review, the proposed shelf-life of 24 months and stored at 2-8 ºC is considered acceptable. The in-use shelf-life of 24 hours under storage condition at 2℃ to 8 ℃ is also acceptable.

2.2.3. Discussion on chemical, and pharmaceutical aspects

A CEP was presented for the active substance. The original dossier presented by the applicant was not fully in line with the expected characterisation and comparability requirements of liposomal formulations developed with reference to an originator liposomal product as described in the relevant CHMP reflection paper (EMA/CHMP/806058/2009). As a result several major objections were raised during the review, which were addressed by the applicant. Following these revisions of the dossier, the information presented on development, manufacture and control of the finished product is generally acceptable. The results of tests carried out indicate consistency and uniformity of important product quality characteristics. However, at the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product that remained to be addressed:

-Further discussion of the discriminatory nature of the proposed dissolution method against variations in process parameters, including clarification on the number of batches tested and statistical evaluation of the results.

-The description of the manufacturing process and process controls should revised to be in line with CHMP guideline on manufacture of finished dosage form (EMA/CHMP/QWP/245074/2015), e.g. numerical values for those stirring speeds that were confirmed in the development/validation findings (e.g. liposome forming step) should be included.

-The finished product specification should be further revised. Specifically, further justification for the use of SPAN is to be provided; the limits proposed for assay for cholesterol, HSPC, MPEG-DSPE impurities and LPC at release and/or shelf-life should be tightened in accordance with Doxolipad batch analysis data

2.2.4. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is generally considered to be acceptable when used in accordance with the conditions defined in the proposed SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data have been presented to give reassurance TSE safety. However, as indicated above, several other concerns remain to be addressed.

2.2.5. Recommendations for future quality development

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

-The applicant is recommended to investigate potential quantitative methods for the control of impurities in HSPC and MPEG-DSPE.

-In line with the proposed PACMP, the applicant is recommended to introduce a non-sterilising filtration step after preparation of the doxorubicin/sucrose solution to reduce bioload of the bulk of doxorubicin/sucrose.

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. Furthermore the applicant provided the results of a number of non-clinical studies conducted to compare Doxolipad and Caelyx (Table 4).

Table 4: Summary of non-clinical data

Type of Study	Title	Group: Subject No./sex	Schedule / Dose
Pharmacology (non-GLP)	Comparison of antitumor efficacy of Doxisome™ and Caelyx® in C26 murine colon carcinoma model	Control: 7/M Doxisome™ (TLC177): 7/M Caelyx: 7/M	q1w x 3 / 6 mg/kg
	Comparison of antitumor efficacy between Doxisome™ and Caelyx® in ES-2 human clear cell ovarian cancer model	Control: 8/F Doxisome™ (TLC177): 8/F Caelyx: 8/F	q1w x 2 / 6 mg/kg
	Comparison of *in vitro* cytotoxicity of Doxisome™ and Caelyx® in SKOV-3 human ovarian cancer cell line	Ten groups for each of the three test articles in triplicate	0, 0.000256, 0.00128, 0.0064, 0.032, 0.16, 0.8, 4, 20 and 100 µg/ml of each test article - doxorubicin, Doxisome™ (TLC177) or Caelyx®. Incubated 72 hrs.
Safety pharmacology (GLP)	*In vitro* evaluation of the influence of Doxisome™ compared to Caelyx® on human whole blood hemolysis and plasma flocculation	18 groups; 4 control groups; 2 placebo groups; 6 Doxisome™ (TLC177) groups: 2 groups each of 3 concentrations 6 Caelyx® groups: 2 groups each of 3 concentrations	Doxisome™ (TLC177): 0.032, 0.32 and 1 mg/mL final conc. Caelyx®: 0.032, 0.32 and 1 mg/mL final conc.

Type of Study	Title	Group: Subject No./sex	Schedule / Dose
Pharmacokinetics (non-GLP)	Report TLC006QN15002: Pharmacokinetic Profiles of Doxisome™ (Batch B029695XA) and Caelyx® (Lot#EBBS301) in Mice Following Single Dose Intravenous of Doxorubicin HCl Liposome Injection (100 mg Gel)	Doxisome™ (TLC177): 6/M/time point Caelyx®: 6/M/time point	Single dose / 6 mg/kg
	Report TLC006QN16003: The pharmacokinetic profiles of Doxisome™ (Batch B029695XA) and Caelyx® (Lot#EBBS301) in tumor-bearing female mice following single dose intravenous of Doxorubicin HCl liposome injection	4T1 bearing BALB/c mice Doxisome™ (TLC177): 6/F/time point Caelyx®: 6/F/time point	Single dose / 16.7 mg/kg
	Report N42DMR18005: Comparative Pharmacokinetics and Tissue Distribution Study of Doxorubicin and Its Metabolite in Tumor-bearing Female Mouse Following Single Dose Intravenous of Doxorubicin HCl Liposome Injection	4T1 bearing BALB/c mice TLC177: 6/F/time point Caelyx®: 6/F/time point	Single dose/ 16.7 mg/kg
Pharmacokinetics (GLP)	Report TLC006QN15012 - Doxisome™ (Batch B029695XA): Pharmacokinetics and tissue distribution study in tumor-bearing female mouse following single intravenous dosing	Doxisome™ (TLC177): 30/F Caelyx®: Lot A (Lot#EBBS301): 30/F Lot B (Lot#DFZ0P00): 30/F	Single dose / 16.7 mg/kg

Toxicology (non-GLP)	Report N43FSR17001: A single dose toxicity study of Doxisome in SD rat (TLCR020320)	Control: 5/M, 5/F Doxisome™ (TLC177): 4 mg/kg – 5/M, 5/F 8 mg/kg – 5/M, 5/F 12 mg/kg – 5/M, 5/F 15 mg/kg – 5/M, 5/F Caelyx®: 8 mg/kg – 5/M, 5/F	Single dose / Doxisome™ (TLC177): 4, 8, 12 and 15 mg/kg Caelyx®: 8 mg/kg
	Report TLC006QN16006: A 43-day comparative Intravenous cardiotoxicity study following repeated doses of Doxisome and Caelyx® in Wistar Rats with a 42-day recovery period (TLCR020498).	Control: 10/M /group Adriblastina:13/M Doxisome™ (TLC177): 13 /M Caelyx®: 13 /M (5 for dosing phase and 8 for recovery phase)	Placebo: 6xQ1W and 7xQ1W Adriblastina :2 mg/kg, 6xQ1W Doxisome™ (TLC177): 2 mg/kg,7xQ1W Caelyx®: 2 mg/kg; 7xQ1W
Toxicology (GLP)	Report Study T30012001-GN - Doxisome™: A 66-day intravenous toxicity study in rats; 13 doses with a 26-day recovery (GLP)	15 M / 15 F per group (10 for dosing phase and 5 for recovery phase)	Repeated dose: once every 3 days for 13 doses with a 26-day recovery Placebo Caelyx®: 1.0mg/kg Doxisome™ (TLC177):
			0.2mg/kg Doxisome™ (TLC177: 1.0mg/kg Doxisome™ TLC177: 1.5mg/kg

2.3.2. Pharmacology

The pharmacology programme consisted of three primary pharmacodynamic studies (non GLP): one *in vitro* cytotoxicity study in SKOV-3 human ovarian carcinoma cell line and two *in vivo* studies evaluating the anti-tumour effect in murine colon carcinoma model and in human clear cell ovarian carcinoma model respectively.

In addition to the primary pharmacodynamics characterisation, an evaluation of the haemolytic potential of TLC177 in comparison to Caelyx was submitted as a safety pharmacology study (GLP).

Primary pharmacodynamic studies

In Vitro Cytotoxicity Study

Comparison of in vitro cytotoxicity of Doxolipad and Caelyx in SKOV-3 human ovarian cancer cell line (Study No.: TLC006QN13007).

The *in vitro* cytotoxicity profiles of TLC177, Caelyx and non-liposomal doxorubicin were studied using SKOV-3 human ovarian cancer cells. Cells were exposed to various concentrations of test articles in triplicate at concentrations of 0, 0.000256, 0.00128, 0.0064, 0.032, 0.16, 0.8, 4, 20 and 100 μg/ml for 72 h at 37°C. At the end of the incubation period, the cells were assessed for cell viability. The percentage net growth was determined with the Sulforhodamine B assay. The 50% inhibitory concentration (IC50) values are expressed as a percentage of cell viability of the vehicle control groups.

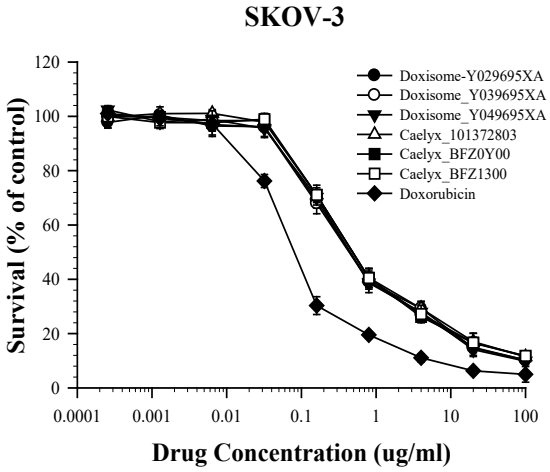


Figure 3: In vitro cell viability of TLC177, Caelyx and doxorubicin in human ovarian cancer cell line –SKOV-3

Table 5: Cytotoxicity (IC_{50}, µg/mL) of TLC177 and Caelyx in SKOV-3 human ovarian cancer cell

IC_{50} (µg/mL)				
TLC177		**Caelyx®**		**Doxorubicin**
Y029695XA	0.388	101372803	0.378	0.069
Y039695XA	0.368	BFZ0Y00	0.380	
Y049695XA	0.377	BFZ1300	0.395	

The growth inhibition curve of TLC177 was similar to that of Caelyx but clearly inferior to that of free doxorubicin. Moreover, the IC50 values of TLC177 and Caelyx were similar. The IC50 value of free doxorubicin (0.069 ug/ml) was almost 5.5-fold lower than that of TLC177 and Caelyx groups. Based on the IC50, TLC177 revealed a similar cytotoxic profile to Caelyx in SKOV-3 cells.

In Vivo Antitumour Efficacy Studies

Comparison of antitumour efficacy of Doxolipad and Caelyx in C26 murine colon carcinoma model (Study No.: N41FSR17004)

Comparison of antitumour efficacy between Doxolipad and Caelyx in ES-2 human clear cell ovarian carcinoma model (Study No.: N41FSR17005)

Two different tumour models were used to compare the anti-neoplastic efficacy of TLC177 with Caelyx.

In a C26 murine colon carcinoma model, male BALB/cByJ mice were IV injected with 6 mg/kg of TLC177 or Caelyx, using a q1w x 3 dosing schedule; while in a ES-2 human clear cell ovarian carcinoma model, female C.B.17-scid mice were IV injected with 6 mg/kg of TLC177 or Caelyx, using a q1w x 2 dosing schedule. Controls received saline. Tumour volume and body weight measurements were performed at least once weekly during the study period. %T/C, tumour doubling time (TDT) and tumour growth delay (TGD) were used for the characterization of therapeutic responses of test articles.

In the C26 murine colon carcinoma model (study No.: N41FSR17004), test article injection was performed when mean tumour volume reached ~270 mm^3.

Both TLC177 and Caelyx treated groups showed a significant delaying response of tumour growth as compared with control group ($p < 0.01$) on day 21, and no difference was found between the TLC177 and Caelyx groups ($p > 0.05$). The mean percentage change of tumour volume for the treated group versus the control group (%T/C) was 20.6% for the TLC177 group and 18.2% for the Caelyx group. The mean TDT (defined as 8 times of the initial tumour volume) was 36.6 days for the TLC177 group and 39.8 days for the Caelyx group while it was 13.9 days for the saline group. The mean tumour growth delay (TGD) was 187.2% after Caelyx treatment and 164.0% in the TLC177 group.

The maximal mean body weight loss in the TLC177 group was 11.6% on day 17 while in the Caelyx group it was 11.7% on day 17.

Table 6: Summary of antitumour efficacy comparison of TLC177 with Caelyx in a C26 murine colon carcinoma model

Treatment	Tumour model	Dosage /Schedule	%T/C (day) [a]	Max. BW change (day) [b]	TDT [c]	TGD [d]
Saline	C26	q1w x 3	-	- 5.2 (7)	13.9 ± 1.4	-
Caelyx		6 mg/kg, q1w x 3	18.2 ± 3.8 (21)	- 11.7 (17)	39.8 ± 3.8	187.2 %
TLC177			20.6 ± 7.0 (17)	- 11.6 (17)	36.6 ± 6.3	164.0 %

a: Percent of tumour volume inhibition (%T/C) is calculated by formula of %T/C = (Tumour volume $_{day\ x}$ - Tumour volume $_{day\ 0})_{treated}$/(Tumour volume $_{day\ x}$ - Tumour volume $_{day\ 0})_{control}$ × 100.

b: Maximum body weight change at indicated day after drug administration.

c: Tumour doubling time (TDT) was calculated by formula of TDT = (day x – day 0) × Ln 8/Ln (Tumour weight day x/ Tumour weight day 0), which day x was the time when tumour volume reached 8-fold as comparing with initial size.

d: Tumour growth delay (TGD) is calculated by formula of TGD = ($TDT_{treatment}$ – $TDT_{control}$)/ $TDT_{control}$ x 100, where $TDT_{treatment}$ and $TDT_{control}$ are the time when tumour volume reached 8-fold big of the initial size for treated and control group, respectively.

(A) (B)

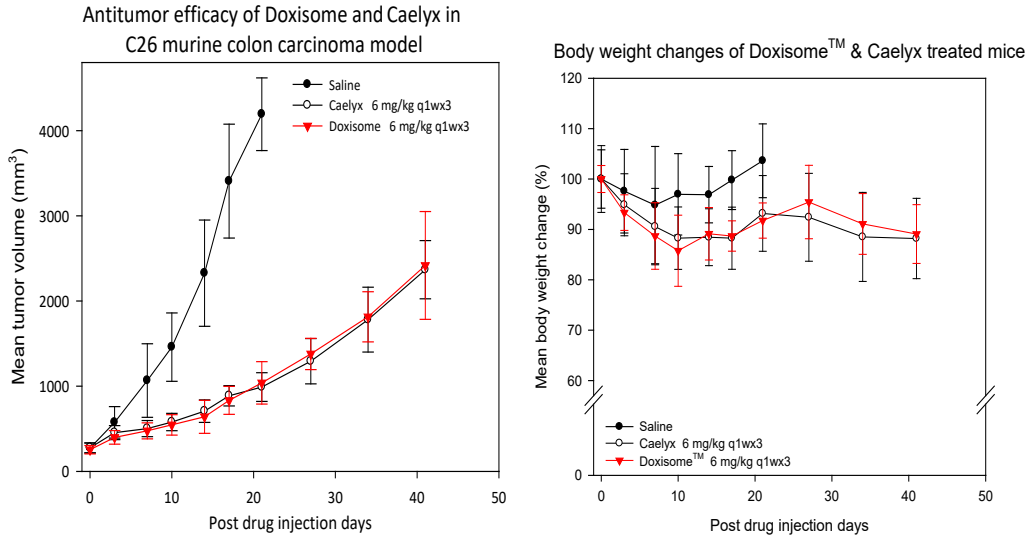


Figure 4: Antitumour efficacy comparison of TLC177 with Caelyx in C26 murine colon carcinoma model

In the ES-2 human clear cell ovarian carcinoma model (study No.: N41FSR17005), test article injection was performed when mean tumour volume reached ~120 mm^3. By applying a q1w x 2 dosing schedule, both TLC177 and Caelyx groups revealed significant growth delay activity on day 19 ($p < 0.01$) as compared with the control group, and no difference was shown between the TLC177 and Caelyx groups ($p > 0.05$). The mean %T/C was 17.4% and 18.4% for the TLC177 and Caelyx treatment groups, respectively. The TDT (defined as 4 times of the initial tumour volume) was 18.1 days in the TLC177 group and 19.0 days in the Caelyx group while in the saline group it was 8.7 days. The TGD was 117.7% following Caelyx treatment and 107.0% in the TLC177 group.

The maximal mean body weight loss in the TLC177 group was 14.9% on day 14 while after Caelyx treatment it was 19.0% on day 17. One of eight mice was found with severe body weight loss (> 20%) and was sacrificed on day 18 in both Caelyx and TLC177 groups.

(A) (B)

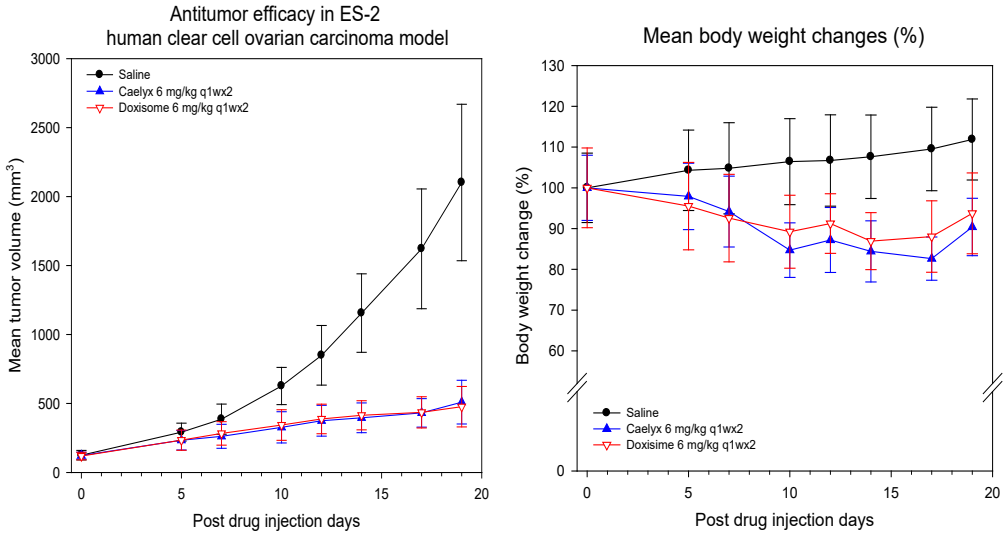


Figure 5: Antitumour efficacy comparison of TLC177 with Caelyx in ES-2 human clear cell ovarian carcinoma model

Table 7: Summary of antitumour efficacy comparison of TLC177 with Caelyx in ES-2 human clear cell ovarian carcinoma model

Treatment	Tumour model	Dosage /Schedule	%T/C (day) [a]	Max. BW change (day) [b]	TDT [c]	TGD [d]
Saline	ES-2	q1w x 2	-	Gained	8.7 ± 1.2	-
Caelyx		6 mg/kg,	18.4 ± 7.4 (19)	-19.0 (17)	19.0 ± 3.3	117.7 %
TLC177		q1w x 2	17.4 ± 6.8 (19)	-14.9 (14)	18.1 ± 5.3	107.0 %

a: Percent of tumour volume inhibition (%T/C) is calculated by formula of %T/C = (Tumour volume $_{day\ x}$ - Tumour volume $_{day\ 0})_{treated}$/(Tumour volume $_{day\ x}$ - Tumour volume $_{day\ 0})_{control}$ × 100.

b: Maximum body weight change at indicated day after drug administration.

c: Tumour doubling time (TDT) was calculated by formula of TDT = (day x – day 0) × Ln 4/Ln (Tumour weight day x/ Tumour weight day 0), which day x was the time when tumour volume reached 4-fold as comparing with initial size.

d: Tumour growth delay (TGD) is calculated by formula of TGD = ($TDT_{treatment}$ – $TDT_{control}$)/ $TDT_{Control}$ x 100, where $TDT_{treatment}$ and $TDT_{control}$ are the time when tumour volume reached 4-fold big of the initial size for treated and control group, respectively.

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were submitted. Reference was made to available literature data.

The EPAR for Caelyx indicates that secondary PD studies of empty liposomes revealed no neurotoxicity signs or adverse behavioural effects in rodents (Caelyx: EPAR– Scientific Discussion). Hypotensive effects characterised as anaphylactic like responses were reported following intravenous administration of empty liposomes in a non-rodent model; while the clinical relevance of this effect of Caelyx is unknown, a statement is included in the Summary of Product Characteristics (SmPC). The standard core battery of safety pharmacology tests were not conducted as Caelyx and its active ingredient, doxorubicin HCl, have well-defined safety and efficacy profiles (Ren et al., 2014, Thorn et al., 2011, Duggan and Keating, 2011, Gabizon et al., 2003), and a detailed understanding of the pharmacodynamic effects.

A concern which was raised with specific regard to pegylated liposomes is an increased potential for haematologic events and thrombogenicity, as a result of either immune-mediated or non-immune mediated reactions (Desai, 2012). Although this effect is not specific to Caelyx, the haemolytic potential of Caelyx and STEALTH placebo liposomes in human blood was assessed in vitro, as well as compatibility with human serum and plasma (Caelyx Product Monograph, Janssen Inc., 2013). Neither Caelyx 1.0 mg/mL nor empty STEALTH liposomes induced haemolysis of human erythrocytes, nor did either cause coagulation or precipitation of human serum or plasma. Lysophosphatidylcholine (LPC) is a degradation product of the phosphatidylcholine component of the liposomes. An additional haemolytic potential study using Caelyx formulations prepared with 0 mg/mL, 0.5 mg/mL, or 0.88 mg/mL LPC caused no haemolysis of rat blood cells. While minimal/no haemolysis was observed in vitro, haemolysis is included as a possibly/probably related adverse event in Caelyx treated AIDS-KS patients (1 – 5% incidence). As this effect may be related to pegylated liposomal formulation, the haemolytic potential of Caelyx in comparison to TLC177 was compared (see safety pharmacology programme).

Safety pharmacology programme

In vitro evaluation of the influence of Doxolipad compared to Caelyx on human whole blood hemolysis and plasma flocculation (Study No.: TLC006QN13006)

This comparative *in vitro* study was conducted to evaluate the influence of TLC177 and Caelyx on human whole blood by evaluating the extent of hemolysis and plasma flocculation (turbidity).

Human whole blood samples were collected in sodium heparin collection tubes on day of analysis, from fasted (for at least 8 hours) female subjects (N=3). Each sample preparation was incubated with the test articles at the final concentration (0, 0.032, 0.32 and 1 mg/ml) in the incubation mixture while being protected from light for 1 hour at 37oC. Negative control was 0.9% NaCl, hemolysis positive control 4% saponin, flocculation positive control (20% Intralipid). Haemolysis was evaluated by determination of whole blood haemoglobin and haematocrit concentration, plasma haemoglobin concentration, plasma haemolytic index and visual macroscopic haemolysis assessment. Flocculation was evaluated by the plasma turbidity index and visual flocculation assessment. No haemolysis effect was observed with TLC177 and Caelyx at concentrations up to 1 mg/ml, with < 0.7% haemolysis detected in both treatment groups. Similarly, no flocculation was observed in any condition.

No significant hemolysis and no flocculation were observed following *in vitro* treatment of human whole blood with TLC177 and Caelyx at final whole blood concentration of 0.032, 0.32 and 1 mg/ml.

Table 8: In vitro hemolysis (%) of TLC177 and Caelyx in human whole blood

Treatment / Parameter		Hemolysis (%) (Mean ± SD)
2	Negative Control (0.9% NaCl)	0.1 ± 0.0
3	Positive Control (4% Saponin)	98.0 ± 0.9
5	TLC177 Placebo	0.1 ± 0.0
6	TLC177 – 0.032 mg/mL	0.2 ± 0.0
7	TLC177 – 0.32 mg/mL	0.4 ± 0.0
8	TLC177 – 1 mg/mL	0.7 ± 0.0
9	Caelyx – 0.032 mg/mL	0.1 ± 0.0
10	Caelyx – 0.32 mg/mL	0.3 ± 0.0
11	Caelyx – 1 mg/mL	0.7 ± 0.0

Table 9: In vitro plasma turbidity index (660 nm/700nm) of TLC177 and Caelyx in human whole blood

Treatment / Parameter		Plasma turbidity index (Mean ± SD)
1	Non-spiked Whole Blood	6 ± 2
2	Negative Control (0.9% NaCl)	2 ± 1
4	Positive Control (20% Intralipid)	121 ± 43
5	TLC177 Placebo	0 ± 0
6	TLC177 – 0.032 mg/mL	0 ± 0
7	TLC177 – 0.32 mg/mL	0 ± 0
8	TLC177 – 1 mg/mL	0 ± 0
9	Caelyx – 0.032 mg/mL	0 ± 0
10	Caelyx – 0.32 mg/mL	0 ± 0
11	Caelyx – 1 mg/mL	0 ± 0

Pharmacodynamic drug interactions

No nonclinical pharmacodynamic drug interaction studies submitted.

2.3.3. Pharmacokinetics

The applicant presented literature data as well as the results of the following studies: two single dose PK studies (TLC006QN15002 and TLC006QN16003) performed in BALB/c mice to detect relevant differences in critical PK parameters of Doxolipad versus Caelyx; two tissue distribution study using tumour bearing female mice (TLC006QN15012, N42DMR18005).

Literature data

Single dose pharmacokinetic studies with Caelyx were performed in rats and dogs, and multiple dose PK studies were conducted in rats, rabbits and dogs (Caelyx EPAR). The plasma pharmacokinetics of Caelyx markedly differ from that of doxorubicin hydrochloride. The plasma concentration of doxorubicin was up to 2000-fold higher in Caelyx-treated animals after intravenous injection of equivalent doses of Caelyx and doxorubicin hydrochloride (Janssen, 2013 – Caelyx Monograph). Despite the higher plasma concentration of doxorubicin after Caelyx treatment, the stability of liposomes and its low rate of doxorubicin release (leakage) in plasma results in very low levels of free doxorubicin hydrochloride in the bloodstream. Direct measurements of the amount of liposomal drug in the plasma showed that more

than 90% to 95% of doxorubicin remains encapsulated in liposomes whilst in systemic circulation (Gabizon et al, 1994; Janssen, 2013– Caelyx Monograph).

Although Cmax and exposure (Area Under the Curve [AUC]) are dose-dependent, the plasma clearance (CL), half-life (T1/2) and volume of distribution (Vd) of Caelyx appeared to be independent of dose, an observation which has been made in both animals and humans (Gabizon et al, 1994). In human, plasma concentration by time data are best fit with a bi-exponential curve, with a relatively short first phase (T1/2 = 1 to 3 hours), and a more prolonged second phase, which represented the majority of the AUC (more than 95%), and a T1/2 ranging from 42 to 46 hours (Gabizon et al, 1994). In contrast, free doxorubicin pharmacokinetics were characterised by biphasic curves with a rapid decline of the initial plasma concentration in the initial rapid distribution phase (half-life of 5 – 10 minutes); the second phase was an elimination and terminal clearance phase with a half-life of 29 hours and a very large volume of distribution (Gabizon et al, 1994).

The long circulation time of Caelyx is primarily dictated by the clearance of the pegylated liposomal carrier. The hydrophilic coating of the pegylated liposomal doxorubicin formulation reduces interactions between various circulating plasma components and the liposome surface, thereby preventing reticuloendothelial system-mediated uptake of the circulating liposomes. As a result, Caelyx exhibits a slower plasma clearance (CL) and a smaller volume of distribution (Vd) which is similar to the total blood volume, and has a longer circulation time in the bloodstream following IV administration compared to free doxorubicin (Duggan and Keating 2011; Gabizon et al, 2003; Working and Dayan, 1996).

The decreased clearance of Caelyx relative to free doxorubicin resulted in at least 60-fold increases in AUC for the liposomal drug, with plasma concentrations of liposome encapsulated doxorubicin several hundred fold greater several hours after injection in liposome formulations as compared to free drug (Gabizon et al, 1994). No evidence of drug accumulation was observed with repeated dosing of dogs treated with 1.0 mg/kg Caelyx every three weeks. Repeat administration of Caelyx to rats was similarly not associated with any alteration in plasma PK (Janssen, 2013– Caelyx Monograph).

With regards to biodistribution, unlike free doxorubicin, pegylated liposomal doxorubicin is associated with low concentrations of doxorubicin in the circulation with limited distribution to the myocardium, resulting in a lower rate of cardiotoxicity compared to free doxorubicin (Theodoulou and Hudis, 2004). The nonclinical PK of Caelyx after single and repeat-dose intravenous injection(s) has been characterised and the results were reported in literature (Gabizon et al, 2003, Gabizon et al, 2002).

Liposomal products administered IV rapidly accumulate in the cells of the reticuloendothelial system, particularly in the liver and spleen, where they are phagocytozed by macrophages of the reticuloendothelial system (Kume et al, 1991). Based on published *in vitro* cytotoxicity study data (Allenet al, 1981), fatty-like lipids in liposomes containing doxorubicin appear to promote uptake into the cancer cells through leaky vessels resulting in greater accumulation in tumours compared to areas that have tight capillary junctions, such as the heart muscle.

Tissue levels of doxorubicin in tumour-bearing mice and in non-tumour-bearing rats and dogs have been investigated (Janssen, 2013 - Caelyx Monograph). In the tumour model studies, tumour AUCs in Caelyx-treated animals ranged from 7-fold higher in a murine C26 colon carcinoma model to 25-fold higher in the human prostatic xenograft model than in mice treated with the same dose of doxorubicin hydrochloride. Tumour and normal tissue levels of doxorubicin continued to rise for at least 24 hours in Caelyx-treated mice, but peaked after 1-4 hours in animals that received doxorubicin hydrochloride, declining rapidly thereafter. Doxorubicin concentrations persisted in the tissues in Caelyx-treated animals, owing to the slower clearance of liposome-associated drug, resulting in significantly higher tissue AUCs. Doxorubicin-associated toxicity, particularly cardiotoxicity is associated with the high peak concentrations of doxorubicin, but not with AUC. Treatment regimens that minimize peak doxorubicin plasma concentrations, but maintain cumulative AUC, are associated with reduced risk of cardiomyopathy

and do not compromise anti-tumour activity. The reduced cardiac tissue concentrations in Caelyx-treated animals correlated well with the observation that Caelyx is less cardiotoxic than doxorubicin hydrochloride in animals.

The higher AUCs in the tissues also did not correlate with increased toxicity, with the exception of cutaneous lesions (Janssen, 2013 - Caelyx Monograph). Doxorubicin concentrations were higher at sites of cutaneous lesions than in normal skin, with levels falling rapidly after treatment stopped and nearing the concentrations found in normal skin by the end of the recovery period. It could not be determined if lesions formed because of increased doxorubicin concentrations, or whether doxorubicin concentration was secondarily increased as a result of extravasation of Caelyx at pre-existing sites of tissue damage. Studies in dogs have demonstrated that the incidence and severity of the cutaneous lesions is related to dose intensity, with lower dose levels associated with decreased lesion formation (Janssen, 2013 - Caelyx Monograph).

Single dose pharmacokinetic studies

Studies TLC006QN15002 and TLC006QN16003 were performed in BALB/c mice.

Non-GLP single dose comparative PK study of TLC177 in Mice (TLC006QN15002)

In Study TLC006QN15002, female BALB/c mice were administered with 6 mg/kg TLC177 or Caelyx single intravenous injection, blood samples were taken via cardiac puncture at 0.25, 4, 48, 96 or 168 hours after dose administration (N = 6 mice per time-point). Plasma liposome encapsulated doxorubicin and free doxorubicin were determined.

For liposomal encapsulated doxorubicin, the compared PK parameters of 90% CI range were 101.44 – 113.18%, 90.27 – 98.09% and 91.12 – 97.95% for C_{max}, AUC_{0-168} and AUC_{inf}, respectively. The upper and lower bounds of each of C_{max}, $AUC_{0-168}$and AUC_{inf} were within the 80 – 125% margin for determination of bioequivalence. For free doxorubicin, the compared PK parameters of 90% CI range were 83.85 – 101.40%, 83.70 – 97.73%, 83.67 – 99.01% for C_{max}, AUC_{0-168} and $AUC_{inf,}$, respectively. The upper and lower bounds of each of C_{max}, AUC_{0-168} and AUC_{inf} were within the 80 – 125% margin for determination of bioequivalence.

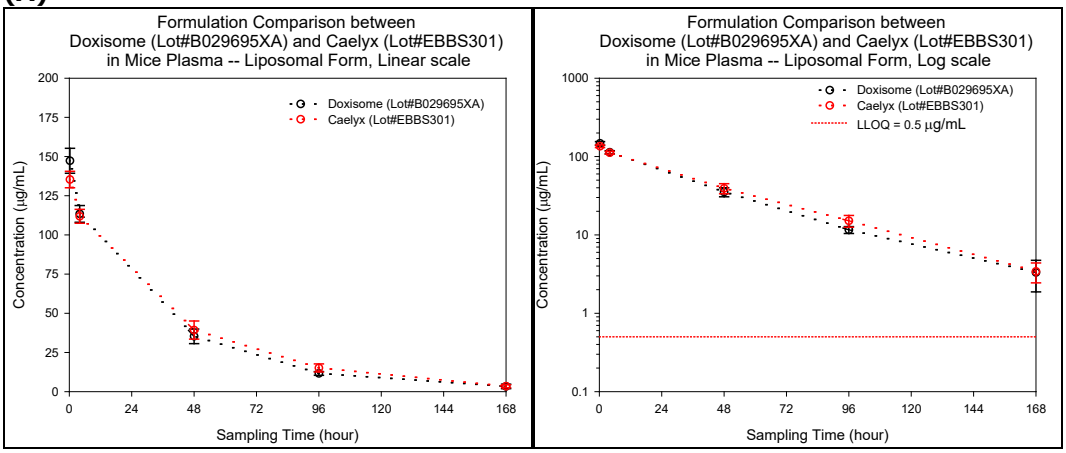
Table 10: Pharmacokinetic parameters of TLC177 and Caelyx after IV injection of single dose of 6 mg/kg to female BALB/c mice

Formulation (Batches)		**TLC177 (B029695XA)**		**Caelyx (EBBS301)**	
Dose (mg/kg)		**6**		**6**	
Analyte		**LED**	**FD**	**LED**	**FD**
T_{max}	(hr)	0.25	0.25	0.25	0.25
C_{max}[a]		145.43 ± 7.28	1682 ± 126.46	135.68 ± 5.78	1825.67 ± 159.38
AUC_{0-168}[a]		5385.69 ± 201.97	63971.07 ± 4160.62	5722.55 ± 185.93	70721.55 ± 4450.83
AUC_{0-inf} [a]		5573.22 ± 138.28	67107.48 ± 5159.45	5900.93 ± 210.15	73665.28 ± 4584.83
AUC_{0-168}/ AUC_{inf}	(%)	96.62 ± 2.1	95.42 ± 2.74	96.99 ± 1.29	96.01 ± 1.94
$T_{1/2}$	(hr)	36.28 ± 8.72	39.36 ± 9.8	34.89 ± 5.87	38.6 ± 8.55
V_d	(mL/kg)	56.3 ± 14.1	5040.7 ± 1034.8	51.0 ± 7.8	4525.4 ± 940.8
CL	(mL/hr/kg)	1.07 ± 0.03	89.56 ± 7.20	1.01 ± 0.04	81.45 ± 5.26
MRT_{last}	(hr)	32.1 ± 2.2	36.2 ± 2.7	35.4 ± 1.4	37.1 ± 1.8

Table 11: Statistical comparison of mice plasma PK parameters

Parameter	Compare TLC177 (B029695XA) to Caelyx (EBBS301)							
	LED				FD			
	Point estimate	90% C.I.		%CV	Point estimate	90% C.I.		%CV
		lower bound	upper bound			lower bound	upper bound	
C_{max}	107.15	101.44	113.18	4.66	92.21	83.85	101.40	8.09
AUC_{0-168}	94.10	90.27	98.09	3.54	90.45	83.70	97.73	6.60
AUC_{inf}	94.47	91.12	97.95	3.07	91.02	83.67	99.01	7.16

(A)



(B)

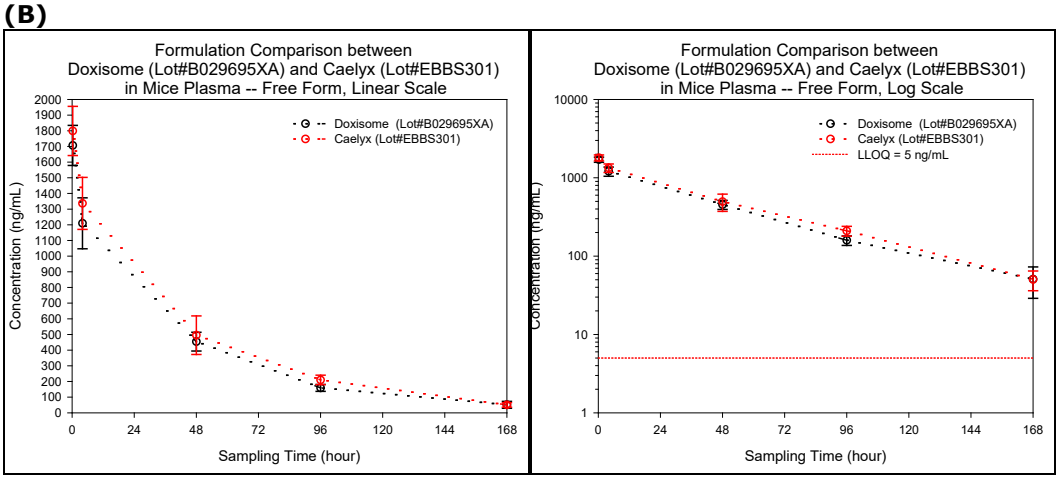


Figure 6: Mean concentration-time plots of plasma doxorubicin for Doxolipad and Caelyx after 6 mg/kg single IV injection infemale BALB/c mice (A) Liposome encapsulated doxorubicin (B) Free doxorubicin

Non-GLP single dose comparative PK study of TLC177 in Mice (TLC006QN16003)

In Study TLC006QN16003, the PK profiles of free doxorubicin, liposome encapsulated doxorubicin, total doxorubicin and doxorubicinol for TLC177 and Caelyx were compared following administration of a single dose of 16.7 mg/kg of TLC177 or Caelyx to female 4T1 murine breast carcinoma tumour-bearing BALB/c

mice. The dose level was selected in line with the GLP biodistribution study. Plasma samples were collected at 0.25, 1, 4, 24, 48, 96, 120, 168, 240, and 336 hours after dose administration.

A statistical assessment was performed to evaluate the bioequivalence between TLC177 to Caelyx. The 90% confidence intervals of the geometric mean ratios (TLC177/ Caelyx) for Cmax, AUC0-t, and AUCinf in the four analytes (total doxorubicin, liposome doxorubicin, free doxorubicin and doxorubicinol) were analysed using an ANOVA model. All the PK parameters were within the bioequivalence acceptance range (90% CI of 80.00 – 125.00%).

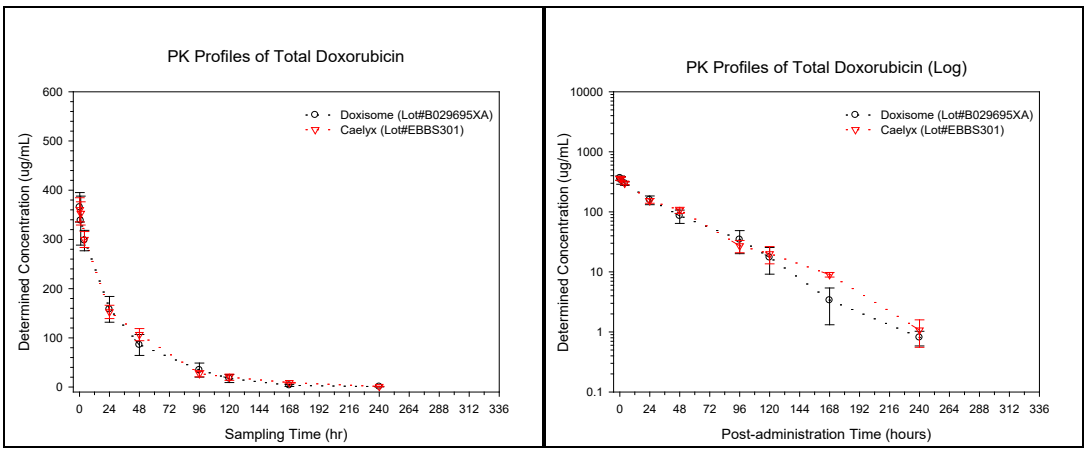


Figure 7: Mean plasma total doxorubicin concentration-time profile of TLC177 and Caelyx after IV injection of single dose of 16.7 mg/kg to female tumour-bearing BALB/c mice

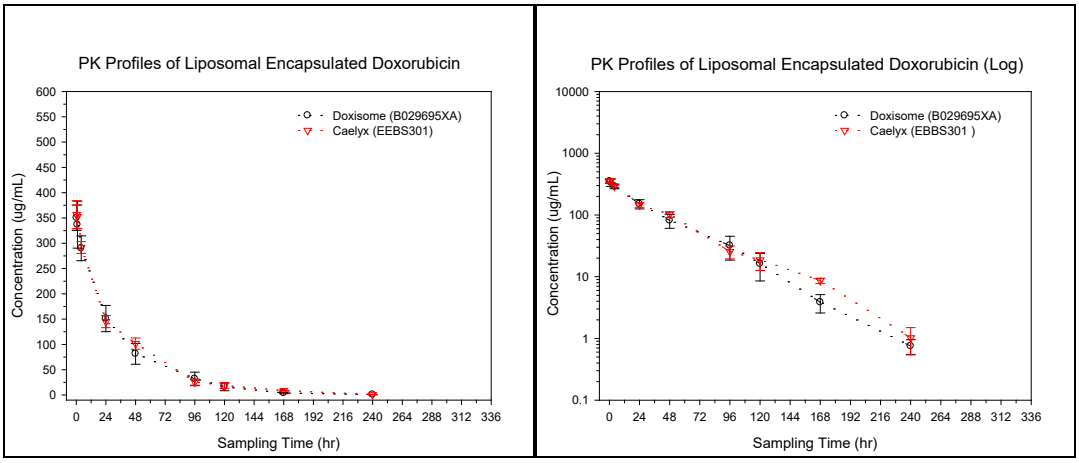


Figure 8: Mean plasma liposome encapsulated doxorubicin concentration-time profile of TLC177 and Caelyx after IV injection of single dose of 16.7 mg/kg to female tumour-bearing BALB/c mice

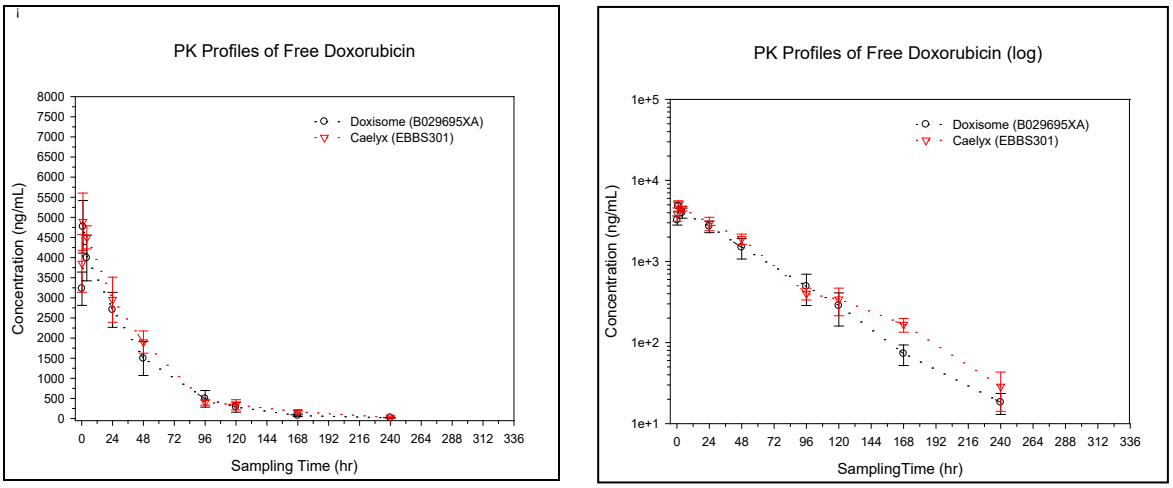


Figure 9: Mean plasma free doxorubicin concentration-time profile of TLC177 and Caelyx after IV injection of single dose of 16.7 mg/kg to female tumour-bearing BALB/c mice

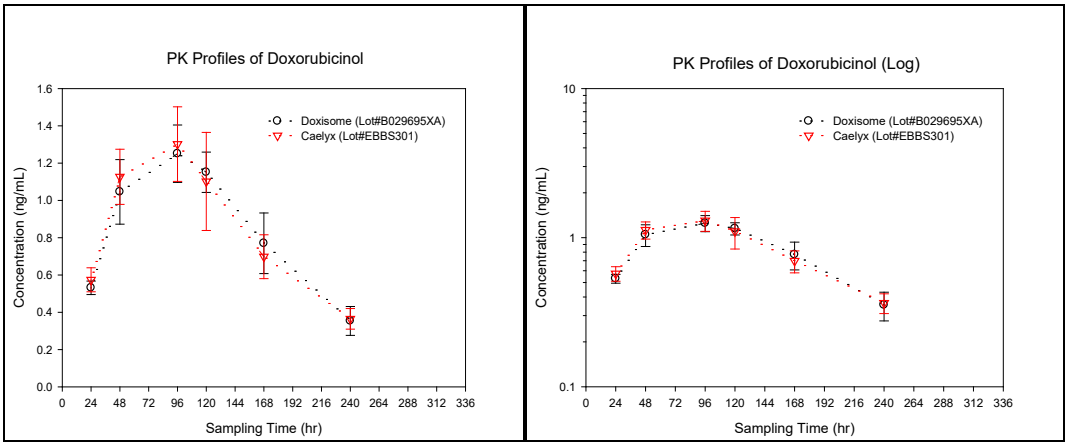


Figure 10: Mean plasma doxorubicinol concentration-time profile of TLC177 and Caelyx after IV injection of single dose of 16.7 mg/kg to female tumour-bearing BALB/c mice

Table 12: Pharmacokinetic parameters of TLC177 and Caelyx after IV injection of single dose of 16.7 mg/kg to female tumour-bearing BALB/c mice

Formulation(Batches)		**TLC177(B029695XA)**			
Analyte		**TD**	**LED**	**FD**	**DXR-OL**
T_{max}	(hr)	0.25	0.25	1	96
C_{max}[a]		365 ± 12.2	350 ± 10.2	4770 ± 266	1.25 ± 0.0629
AUC_{0-168}[a]		13000 ± 473	12400 ± 461	203000 ± 8580	202 ± 5.65
AUC_{0-inf}[a]		13000	12500	204000	238
AUC_{0-t}/ AUC_{inf}	(%)	99.8	99.8	99.6	85.0
$T_{1/2}$	(hr)	26.5	27.3	30.7	69.9
V_d	(mL/kg)	49.1	52.9	3620	7080000
CL	(mL/hr/kg)	1.28	1.34	81.9	70200
MRT_{inf}	(hr)	38.1	37.6	40.5	145

Formulation(Batches)		Caelyx (EBBS301)			
Analyte		**TD**	**LED**	**FD**	**DXR-OL**
T_{max}	(hr)	0.25	0.25	1	96
C_{max}[a]		361 ± 9.99	356 ± 11.3	4890 ± 290	1.30 ± 0.0816
AUC_{0-168}[a]		13800 ± 268	13200 ± 241	235000 ± 6990	203 ± 6.27
AUC_{0-inf}[a]		13800	13300	236000	242
AUC_{0-t}/ AUC_{inf}	(%)	99.7	99.7	99.4	83.6
$T_{1/2}$	(hr)	28.0	28.1	33.1	75.4
V_d	(mL/kg)	48.6	51.0	3380	7490000
CL	(mL/hr/kg)	1.21	1.26	70.8	68900
MRT_{inf}	(hr)	41.3	40.9	43.4	147

a: C_{max} and AUC units are µg/mL and µg/mL*hr for LED and TD, while units are ng/mL and ng/mL*hr for FDand DXR-OL, respectively.

Table 13: Statistical comparison of mice plasma PK parameters

Sample	Parameter	Point estimate	90% CI* lower bound	90% CI* upper bound
FD	C_{max} (ng/mL)	96.0	83.6	110
	AUC_{0-t} (hr·ng/mL)	86.5	80.8	92.6
	AUC_{inf} (hr·ng/mL)	86.7	80.9	92.9
LED	C_{max} (µg/mL)	99.2	91.5	108
	AUC_{0-t} (hr·µg/mL)	93.7	88.1	99.5
	AUC_{inf} (hr·µg/mL)	94.0	88.4	100
TD	C_{max} (µg/mL)	102	93.9	110
	AUC_{0-t} (hr·µg/mL)	93.7	88.0	99.9
	AUC_{inf} (hr·µg/mL)	94.1	88.3	100
DOX-OL	C_{max} (ng/mL)	95.2	87.7	103
	AUC_{0-t} (hr·ng/mL)	99.9	92.9	107
	AUC_{inf} (hr·ng/mL)	98.1	89.6	108

Bioequivalence assessment result of C_{max} was calculated by non-grouping method; Bioequivalence assessment results of AUC_{0-t} and AUC_{inf} were calculated by grouping method.

* The 90% confidence intervals (CIs) for the ratios of geometric mean (Doxolipad/ Caelyx) analyzed using ANOVA model. The data were logarithmic-transformed prior to analysis. The acceptable 90% CIs for bioequivalence were within 80.00%-125.00%

Biodistribution studies

Study TLC006QN15012

This was a GLP bio-distribution study characterised the PK and tissue distribution of TLC177 in comparison to Caelyx (two batches) in 4T1 murine breast carcinoma tumour bearing BALB/c female mice when administered as a single IV bolus 16.7 mg/kg injection.

At 15 minutes (plasma only), 1, 4, 24, 48 96, 120, 168, 240, and 336 hours post administration of the test article, 3 animals/time-point/dose group were sacrificed. Plasma exposure to doxorubicin, in terms of Cmax and AUClast, following a single IV dose of TLC177 and Caelyx were different with almost 2-fold

differences. The bioavailability of doxorubicin formulated as TLC177 relative to batches of Caelyx ranged from 106% to 146%. Additional PK parameters such as terminal half-life, volume of distribution at steady state and clearance where also different.

Two fold differences were also found in tissue exposure to doxorubicin and the metabolite doxorubicinol, for Cmax and AUClast figures. The highest levels of doxorubicin (AUClast) were measured in tumour and spleen and the lowest was in the skin and heart across both formulations. Doxorubicin AUClast was also notably higher than doxorubicinol values for both formulations.

In tissues, doxorubicin was quantifiable up to 336 hours after dosing of all three formulations. Doxorubicinol was generally detectable up to at least 240 hours after dosing of all three formulations with the exception of heart where Tlast was 168 hours after Caelyx (Lot A) or Caelyx (Lot B) formulations. After single intravenous administration of doxorubicin formulated as Doxolipad, Caelyx (Lot A) or Caelyx (Lot B), doxorubicin plasma systemic exposure (either in terms of Cmax and AUClast) were different (almost 2-fold). CL was not comparable across formulation ranging between 1.26 to 1.83 mL/hr/kg with a Vss, ranging between 52.7 to 103 mL/kg. Plasma doxorubicin t½, when calculated, was approximately 27 hours after dosing of Caelyx (Lot A) or Caelyx (Lot B) while was 42 hours after Doxolipad administrations. The plasma doxorubicin MRT were generally difficult to compare among Doxolipad, Caelyx (Lot A) or Caelyx (Lot B) administration, with values in the range approximately between 42 and 56 hours. The relative bioavailability (Frel), evaluated in plasma, of doxorubicin formulated as Doxolipad versus Caelyx (Lot A) or versus Caelyx (Lot B) was approximately 146% and 106%, respectively. Following intravenous administration doxorubicin formulated as Doxolipad, Caelyx (Lot A) or Caelyx (Lot B), doxorubicin and doxorubicinol tissues systemic exposures (as mean Cmax and AUClast) were generally not comparable (2-fold of differences) between formulations at the same dose. In terms of total amount Cmax and AUClast, Doxorubicin and doxorubicinol tissues systemic exposures were generally not comparable (2-fold of differences) among formulations at the same dose. The highest doxorubicin systemic exposure (as AUClast) was measured in tumour (ranging between 1800000 to 2120000 ng*h) and the lowest one in the heart (ranging between 60000 to 74900 ng*h), across formulations. Doxorubicin AUClast was always significantly higher than doxorubicinol values irrespective of formulation. In details the ratio (as percentage across tissues and formulations) of doxorubicinol/doxorubicin AUClast was approximately 0.6 % with the exception in tumour where the ratio was approximately 0.3%.

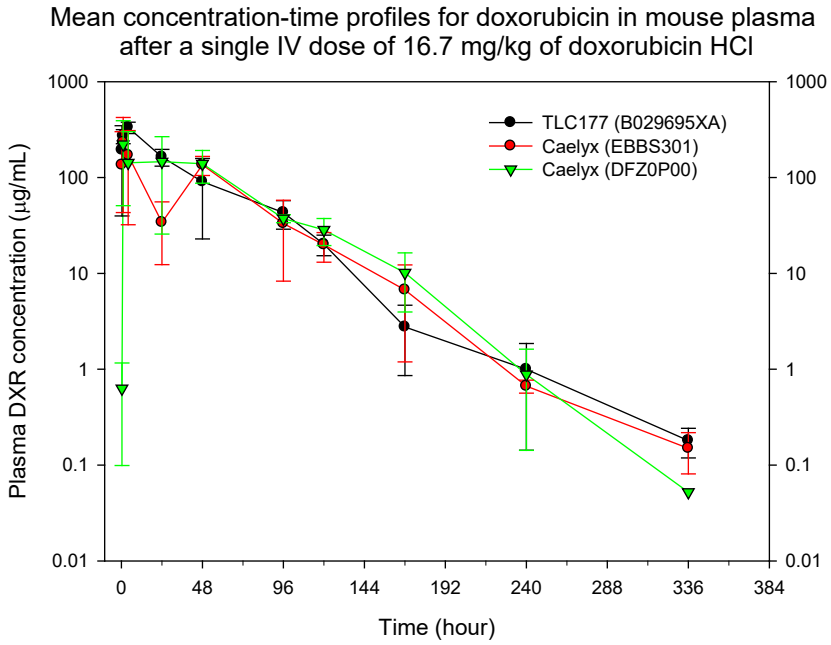


Figure 11: Mean plasma doxorubicin concentration-time profiles after single dose of 16.7 mg/kg administration in GLP bio-distribution study

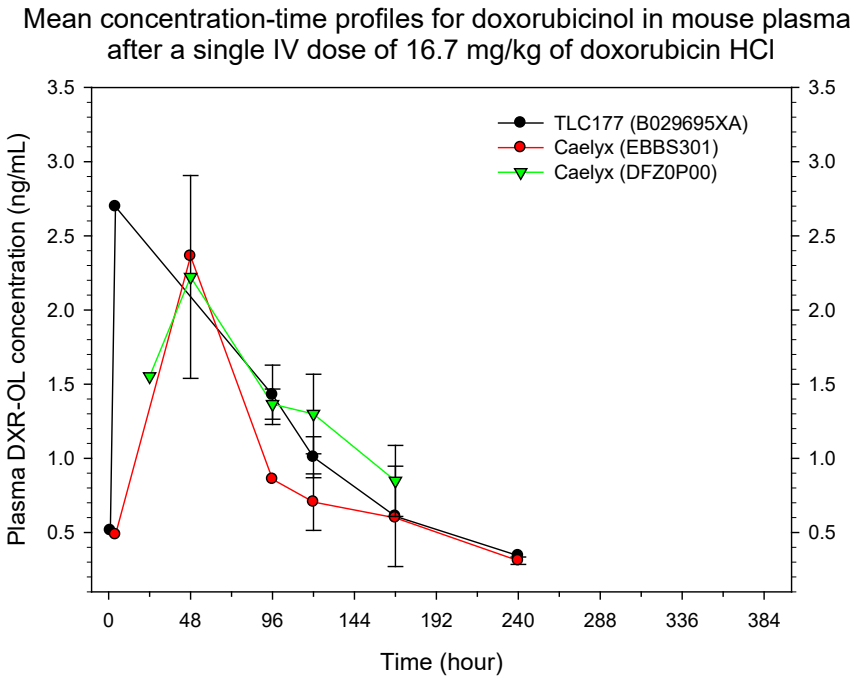


Figure 12: Mean plasma doxorubicinol concentration-time profiles after single dose of 16.7 mg/kg administration in GLP bio-distribution study

Table 14: Plasma PK parameters for doxorubicin in GLP bio-distribution study

Parameter	Formulation of Doxorubicin		
	Caelyx (EBBS301)	**Caelyx (DFZ0P00)**	**TLC177 (B029695XA)**
AUC_{0-t} [a] (µg.hr/mL)	9140± 2254.16	12600± 3768.23	13300± 2651.98
C_{max} (µg/mL)	233± 189.99	222± 170.62	333± 45.05
T_{max} (hr)	1.00	1.00	4.00
T_{last} (hr)	336	240	336
t″ (hr)	27.35	27.04	42.46
V_{ss} (mL/kg)	103	71.4	52.7
CL (mL/hr/kg)	1.83	1.33	1.26
MRT_{last} (hr)	56.14	53.26	41.56
Frel%[b]			146* 106#

a. AUC_{0-t} = the area under the plasma concentration time curve (AUC) from the start of dosing to the last quantifiable time point which it was 336 hours for Caelyx (EBBS301) or Doxolipad and 240 hours for Caelyx (DFZ0P00).

b. Frel% = relative bioavailability (%), calculated by comparing plasma doxorubicin systemic exposure (AUC_{0-last}) obtained after dosing of Doxolipad with values determined after dosing of Caelyx (EBBS301) where * is shown or Caelyx (DFZ0P00) where # is shown.

Table 15: Tissue PK parameters for doxorubicin and doxorubicinol in GLP bio-distribution study

Tissue/ Organ	Formulation	Doxorubicin				Doxorubicinol				AUC_{0-t} Doxorubicinol/ AUC_{0-t} Doxorubicin
		AUC_{0-t} (ng*hr)	C_{max} (ng)	T_{max} (hr)	T_{last} (hr)	AUC_{0-t} (ng*hr)	C_{max} (ng)	T_{max} (hr)	T_{last} (hr)	
Heart	Caelyx (EBBS301)	60000	578 ± 133.31	48	336	411	2.73 ± 0.18	48	168	0.007
	Caelyx (DFZ0P00)	69800	571 ± 174.19	48	336	457	3.09 ± 0.19	120	168	0.007
	TLC177 (B029695XA)	74900	1080 ± 923.63	4	336	536	3.20 ± 0.33	96	240	0.007
Kidney	Caelyx (EBBS301)	921000	5420 ± 1423.43	48	336	5560	24.8 ± 5.53	120	336	0.006
	Caelyx (DFZ0P00)	1210000	7050 ± 3070.79	48	336	5820	28.9 ± 3.22	120	336	0.005
	TLC177(B029695XA)	1050000	6330 ± 3702.37	4	336	6060	31.5 ± 1.05	120	336	0.006
Liver	Caelyx (EBBS301)	1080000	20800 ± 17630.52	4	336	6730	58.0 ±	48	240	0.006

Tissue/ Organ	Formulation	Doxorubicin				Doxorubicinol				AUC_{0-t} Doxorubicinol/ AUC_{0-t} Doxorubicin
		AUC_{0-t} (ng*hr)	C_{max} (ng)	T_{max} (hr)	T_{last} (hr)	AUC_{0-t} (ng*hr)	C_{max} (ng)	T_{max} (hr)	T_{last} (hr)	
							4.13			
	Caelyx (DFZ0P00)	1310000	13400 ± 10780.65	24	336	8750	54.0 ± 13.45	48	240	0.007
	TLC177(B029695XA)	1390000	28200 ± 5369.37	4	336	7580	50.7 ± 9.53	96	240	0.005
Skin	Caelyx (EBBS301)	105000	1050 ± 570.41	120	336	602	6.06 ± 3.42	120	240	0.006
	Caelyx (DFZ0P00)	197000	1330 ± 674.90	96	336	1170	6.63 ± 2.22	120	240	0.006
	TLC177 (B029695XA)	136000	992 ± 278.86	120	336	695	6.17 ± 1.47	120	240	0.005
Spleen	Caelyx (EBBS301)	422000	4050 ± 3429.25	4	336	2040	11.0 ± 3.26	120	336	0.005
	Caelyx (DFZ0P00)	508000	3900 ± 3293.63	24	336	2040	12.6 ± 3.33	120	336	0.004
	TLC177 (B029695XA)	504000	4870 ± 921.32	24	336	1940	10.1 ± 4.37	120	336	0.004
Tumour	Caelyx (EBBS301)	1800000	12200 ± 5898.49	120	336	4790	27.9 ± 17.3	120	336	0.003
	Caelyx (DFZ0P00)	2120000	13500 ± 4340.07	120	336	5110	30.5 ± 14.4	120	336	0.002
	TLC177 (B029695XA)	2060000	16300 ± 1706.93	96	336	5340	29.6 ± 23.6	240	336	0.003

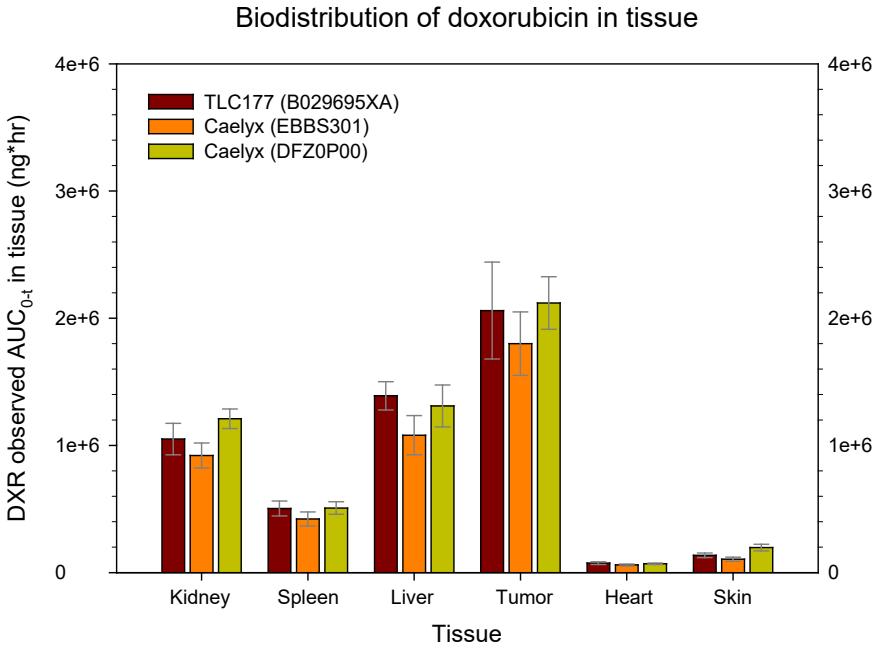


Figure 13: Comparison of observed doxorubicin AUC0-t in mice tissues

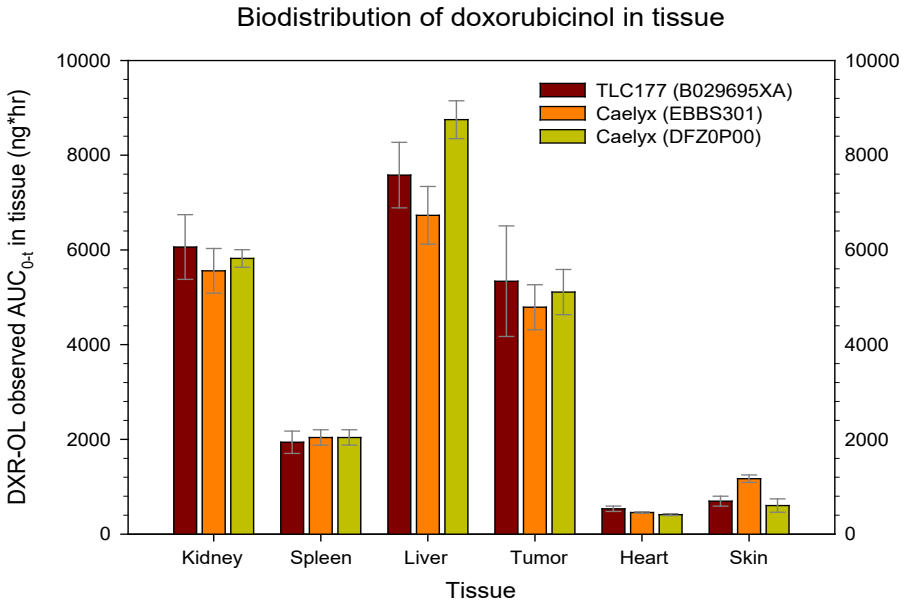


Figure 14: Comparison of observed doxorubicinol AUC0-t in mice tissues

Study N42DMR18005

Study N42DMR18005 (non-GLP) characterised the PK and tissue distribution of TLC177 in comparison to Caelyx in 4T1 murine breast carcinoma tumour bearing BALB/c female mice when administered as a single IV bolus 16.7 mg/kg injection.

Animals were randomised to treatment when tumour sizes reached between 70.4 and 271.6 mm3. At 15 minutes (plasma only), 1, 4, 24, 48, 72, 96, 120, 168, 240, 336, and 408 hours post administration of the test article and reference article, 6 animals/time-point/dose group were sacrificed; sampling time-points were selected based on a half-life of Caelyx of approximately 15 -20 hours and anticipated peak concentration in tumours between 2 – 3 days following drug administration. Plasma samples were

analysed according to validated methods in line with ones used in Study TLC006QN16003. As for tissue samples, all were analyzed using an Ultra Performance Liquid Chromatography coupled with tandem mass spectrometer (UPLC-MS/MS) for the determination of doxorubicinol and an Ultra Performance Liquid Chromatography coupled with fluorescence detector (UPLC-FLD) for the determination of doxorubicin. PK analyses included individual and mean total doxorubicin and doxorubicinol tissues concentrations and doxorubicinol to doxorubicin concentration ratios (as AUC_{last}). PK parameters including (where data allow) but not limited to, maximum observed liposome encapsulated doxorubicin and free doxorubicin plasma or tissue concentration (C_{max}), time to C_{max} (T_{max}), and the area under the plasma or tissue concentration-time curve from t=0 to the last quantifiable time-point (AUC_{last}) were evaluated.

Plasma exposure to liposome encapsulated doxorubicin and free doxorubicin, in terms of C_{max} and AUC_{last}, following a single IV dose of TLC177 and Caelyx were comparable with differences less than 2-fold. The bioavailability (T/R ratio) of liposome encapsulated doxorubicin and free doxorubicin were 98.29% and 95.33%, respectively. Additional PK parameters such as terminal half-life, volume of distribution at steady state and clearance also support differences of <2-fold between the formulations.

Tissue exposure to doxorubicin and the metabolite doxorubicinol, were also similar between TLC177 and Caelyx with mean C_{max} and AUC_{last} figures being <2-fold in difference. The highest levels of doxorubicin (AUC_{last}) were measured in tumour and spleen and the lowest was in the liver and heart. Doxorubicin AUC_{last} was also notably higher than doxorubicinol values for both formulations.

Table 16: Plasma PK parameters for liposome encapsulated doxorubicin and free doxorubicin in non-GLP bio-distribution study

Formulation (Batches)		**TLC177 (D049695XA)**		**Caelyx (GLZT800)**	
Dose (mg/kg)		**16.7**			
Analyte		**LED**	**FD**	**LED**	**FD**
T_{max}	(hr)	0.25	0.25	0.25	1
Cmax [a]		358.80±4.2960	3859.4±214.74	346.80±9.0658	3911.1±302.76
AUC0-t [a]		13791±226.77	199230±3928.6	14031±193.59	208990±4670.9
AUC0-inf [a]		13803	199670	14050	209450
AUC0-t/ AUCinf	(%)	99.9	99.8	99.9	99.8
$t_{1/2}$	(hr)	21.8	24.4	25.3	26.7
Vd	(mL/kg)	38.1	2946.5	43.4	3065.4
CL	(mL/hr/kg)	1.21	83.6	1.19	79.7
MRT_{last}	(hr)	38.1	45.7	36.4	42.6
T/R ratio (%) [b]		98.29%	95.33%		

[a] C_{max} and AUC units are µg/mL and µg/mL*hr for LED while units are ng/mL and ng/mL*hr for FD, respectively.
[b] T/R ratio (%) = relative bioavailability (%), calculated by comparing plasma doxorubicin systemic exposure ($AUC_{0\text{-}last}$) obtained after dosing of TLC177 with values determined after dosing of Caelyx.

Table 17: Tissue PK parameters for doxorubicin and doxorubicinol in non-GLP bio-distribution study

Tissue/ Organ	Formulation	Doxorubicin				Doxorubicinol				AUC_{0-t} Doxorubicinol/ AUC_{0-t} Doxorubicin
		AUC_{0-t} (hr*µg/mL)	C_{max} (µg/mL)	T_{max} (hr)	T_{last} (hr)	AUC_{0-t} (hr*ng/mL)	C_{max} (ng/mL)	T_{max} (hr)	T_{last} (hr)	
Heart	Caelyx (GLZT800)	51.085 ± 1.2880	0.35269 ± 0.022108	24	336	21 ± 4.68 7.5036	1.9578 ± 0.067670	72	168	0.004
	TLC177 (D049695XA)	57.114 ± 1.1178	0.37575 ± 0.026320	24	336	22 ± 8.38 7.2821	2.2331 ± 0.16125	72	168	0.004
Kidney	Caelyx (GLZT800)	331.18 ± 9.8980	1.3127 ± 0.073729	48	408	17 ± 78.8 30.185	7.9164 ± 0.16552	96	408	0.005
	TLC177 (D049695XA)	381.77 ± 11.549	1.7469 ± 0.12041	48	408	19 ± 29.7 36.254	8.6525 ± 0.19885	96	408	0.005
Liver	Caelyx (GLZT800)	127.31 ± 2.1674	1.6426 ± 0.079414	4	336	99 ± 0.55 43.756	7.1562 ± 0.91972	72	336	0.008
	TLC177 (D049695XA)	134.06 ± 3.6039	1.9892 ± 0.044020	4	336	10 ± 03.9 26.031	6.1244 ± 0.58080	72	336	0.007
Skin	Caelyx (GLZT800)	159.72 ± 5.3015	1.0014 ± 0.095749	48	408	51 ± 5.60 28.661	3.4926 ± 0.25678	96	336	0.003
	TLC177 (D049695XA)	176.28 ± 4.8744	1.1558 ± 0.10197	72	408	51 ± 6.46 18.661	3.2661 ± 0.19278	72	240	0.003
Spleen	Caelyx (GLZT800)	709.21 ± 12.641	6.7621 ± 0.24521	24	408	26 ± 61.4 119.77	12.478 ± 0.95230	96	408	0.004
	TLC177 (D049695XA)	727.74 ± 11.479	6.9646 ± 0.18988	24	408	27 ± 44.3 88.313	12.755 ± 1.1573	96	408	0.004
Tumour	Caelyx (GLZT800)	772.42 ± 31.382	4.4321 ± 0.37153	24	408	20 ± 95.3 89.594	9.3974 ± 0.70319	120	408	0.003
	TLC177 (D049695XA)	774.99 ± 38.819	4.9380 ± 0.32324	48	408	19 ± 30.8 163.49	7.8298 ± 1.4530	120	408	0.002

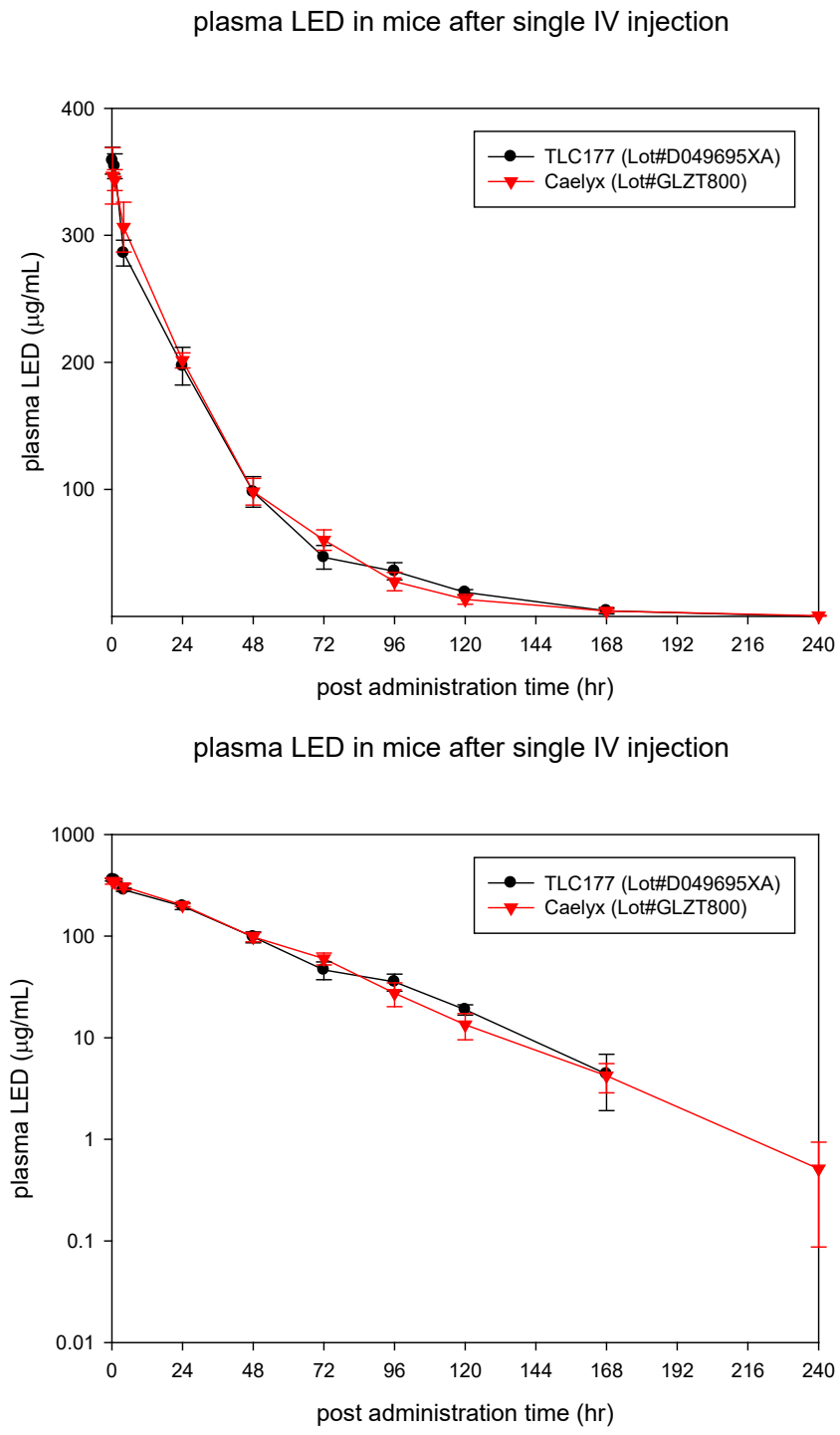


Figure 15: Mean plasma liposome encapsulated doxorubicin concentration-time profile of TLC177 and Caelyx in mice after single dose of 16.7 mg/kg administration in non-GLP bio-distribution study

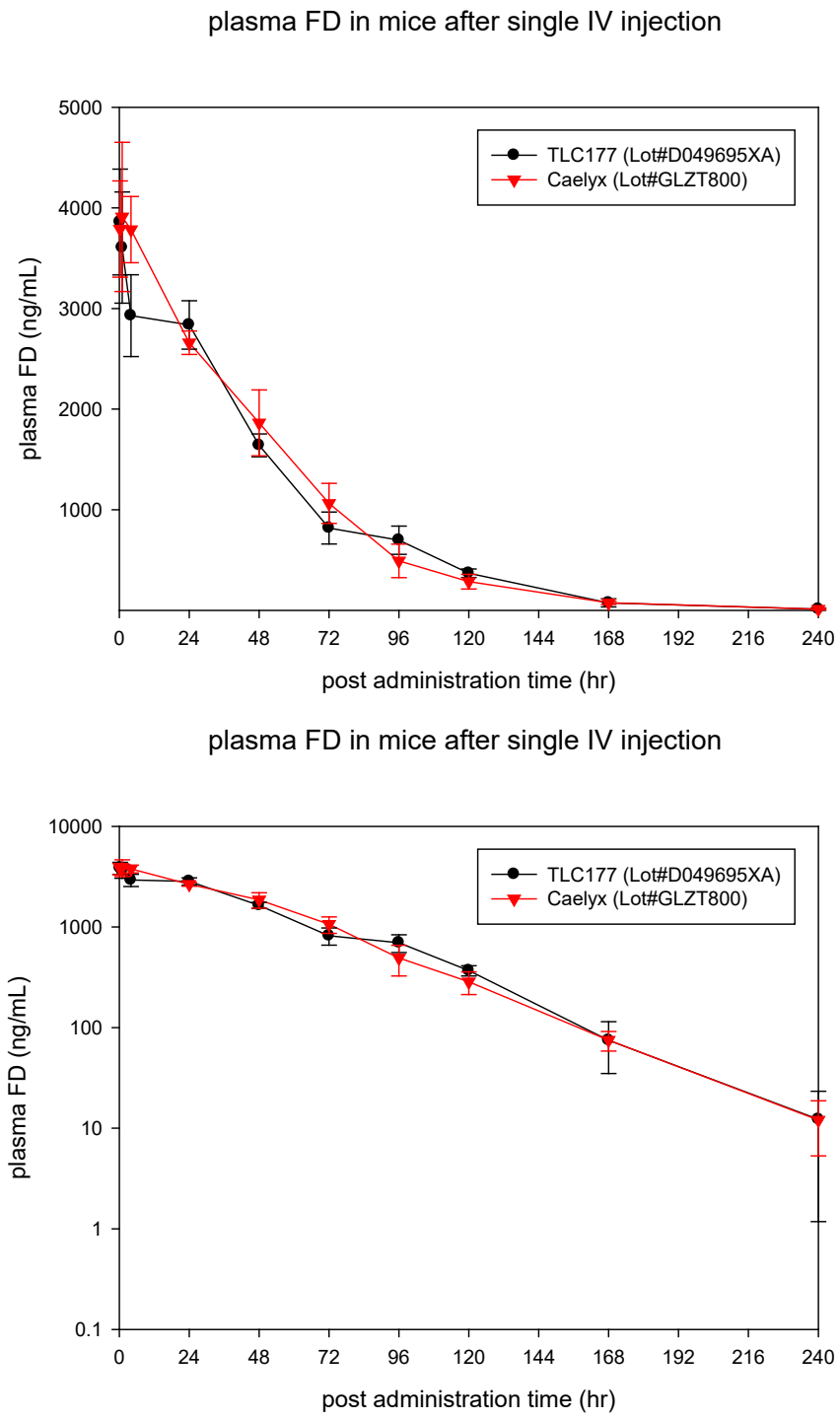


Figure 16: Mean plasma free doxorubicin concentration-time profile of TLC177 and Caelyx in mice after single dose of 16.7 mg/kg administration in non-GLP bio-distribution study

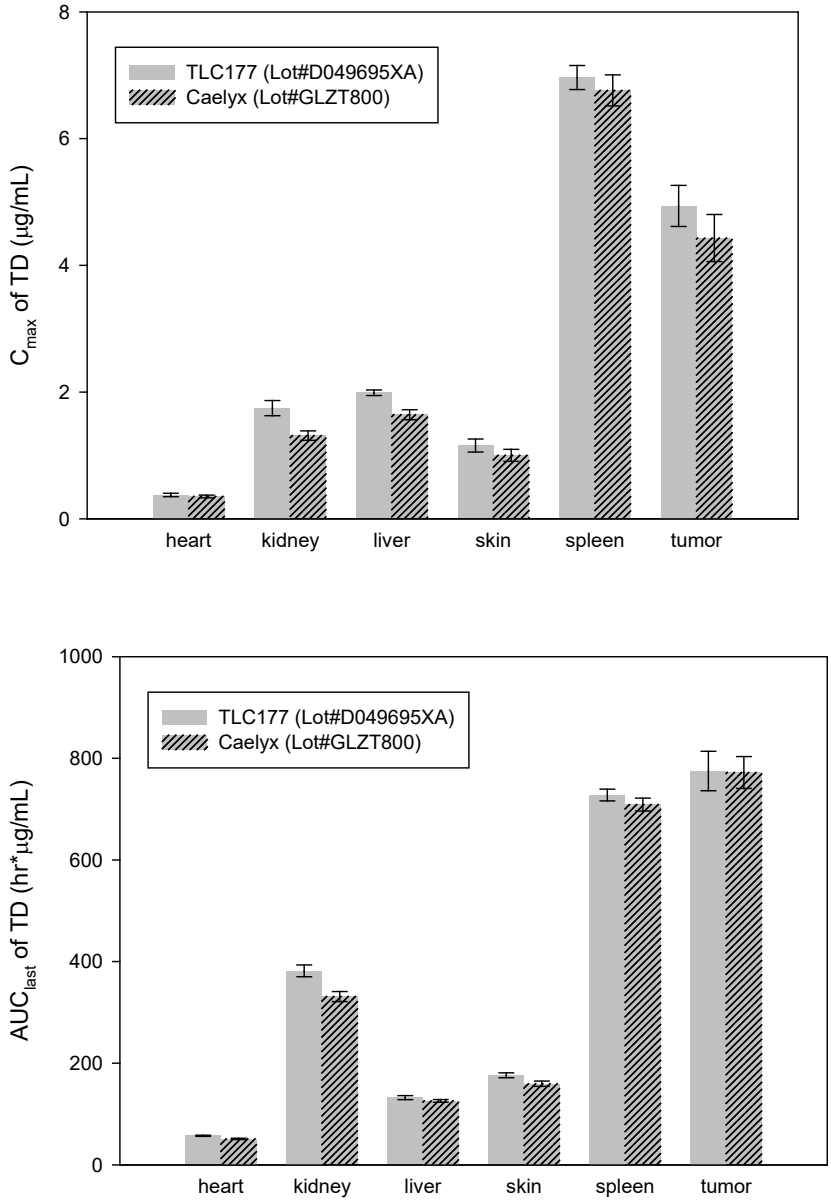


Figure 17: Comparison of observed total doxorubicin (TD) C_{max} and AUC_{last} in mice tissues in non-GLP bio-distribution study

2.3.4. Toxicology

Single dose toxicity

Study Title: a single dose toxicity study of Doxolipad in SD RAT (Report number: N43FSR17001)

A non-GLP compliant intravenous (IV) comparative single dose toxicity study with TLC177 and Caelyx was conducted in SD rats including a 14-day observational period. Mortality, clinical observation, body weight, organ weight and gross examination were evaluated.

Mortality: Following administration, two male animals of the 12 mg/kg TLC177 group died on Day 14 and three males of the 15 mg/kg TLC177 group died on Day 13 or 14. No deaths were found in the other groups.

Clinical Signs: Skin lesions such as red spot, reddish skin, desquamation, scab or alopecia of hind limbs, eyes or nose were observed in males at dose levels of 4 mg/kg and higher and in females at 8 mg/kg of TLC177. In the Caelyx treatment groups (8 mg/kg), skin lesions were also observed in both genders. Rough hair, sensitive to touch, hypoactivity, hunchback, chromodacryorrhea and stained unformed feces were observed at dose levels of 12 and 15 mg/kg of TLC177.

Body Weight: Body weight losses were dose-related.

Gross Pathology: In the TLC177 treatment groups lesions in the gastrointestinal tract were found in male rats at 12 mg/kg (1/3 and 2/2 of scheduled sacrificed and unscheduled dead animals, respectively) and 15 mg/kg (1/2 and 3/3 of scheduled sacrificed and unscheduled dead animals, respectively) and in one female at a dose level of 8 mg/kg (1/5 of scheduled sacrificed animals). There was one male (1/5) with dark reddish contents in the jejunum and ileum in the Caelyx group.

Organ Weight: Statistically significant and dose related decreases in absolute organ weights and organ weight to brain weight ratioof liver, heart, kidney, testes, spleen and thymuswere observed in TLC177 treated male animals and statistically significant and dose related decreases of heart, kidney, spleen and thymus were observed in TLC177 treated female animals, respectively. At a dose of 8 mg/kg, both TLC177 and Caelyx groups showed decreased organ weights in male liver, heart, kidney, spleen and thymus and in female spleen and thymus when compared to saline control group. There were statistically significant difference between TLC177 and Caelyx groups in male kidney and female spleen. These differences are minimal when the organ weight variations and the deviations of the dose dependent trend of the two organs from 4 to 15 mg/kg are considered. Therefore it was concluded that, TLC177 and Caelyx showed a similar change in organ weights at 8 mg/kg.

The maximum tolerated doses of TLC177 were 8 and 15 mg/kg in male and female rats, respectively, at a single dose intravenous injection. Based on the results of mortality, clinical signs, body weight change, gross findings and organ weight changes at 8 mg/kg, it is concluded that TLC177 and Caelyx show a similar toxicity profile.

Table 18: Summary of single dose toxicity study N43FSR17001
Test Article: TLC177

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Rats/SD	IV	Saline Caelyx : 8mg/kg TLC177: 4mg/kg TLC177: 8mg/kg TLC177: 12mg/kg TLC177: 15mg/kg	Male and Female/ 5/group	Male: 8 mg/kg Female: 15 mg/kg	Male: 12 mg/kg; Female: >15 mg/kg	Mortality was observed in males at TLC177 doses of 12 and 15 mg/kg. No death in females was found. Target organs are heart, liver, kidney, spleen, testes and thymus in males; heart, kidney, spleen, and thymus in females. Similar toxic profile including the mortality, body weight change and	N43FSR17001

Test Article: TLC177

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
						organ weight changes is observed in TLC177 or Caelyx treatment groups at 8 mg/kg.	

Repeat dose toxicity

One non-GLP and one GLP compliant repeated dose toxicity studies were conducted.

Study Title: A 43-Day Comparative Intravenous Cardiotoxicity Study Following Repeated Doses of Doxolipad and Caelyx in Wistar Rats with a 42-Day Recovery Period. (Report number: TLC006QN16006)

A comparative IV toxicity study (non-GLP compliant) compared the cardiotoxic effects of Adriblastina (non-liposomal preparation of doxorubicin), TLC177 and Caelyx in the Wistar rat following once weekly doses of 2 mg/kg for 7 weeks followed by a 42-day recovery period. Male Wistar rats were chosen due to a higher sensitivity to doxorubicin with regard to cardiotoxicity (Moulin et al, 2015).

Mortality, body weight, clinical observation, serum biochemistry, gross pathology, organ weight and cardiac histopathology were evaluated. For analysis of the cardiac biomarker cTroponin I (cTnI), blood samples during treatment period were collected 3 hours post the final doses (on Day 36 or Day 43) and on the scheduled sacrifice days after the recovery period (on Day 66 or Day 86). In the Adriblastina group, the blood samples from moribund and euthanized animals were collected on Days 41, 57, 57 and 62. The blood samples were centrifuged at 3,500 g at 4°C for 5 minutes for collection of serum and then freezed at -20°C till analysis by an automated chemiluminescence immunoanalyser, the SIEMENS ADVIA Centaur system (O'Brien et al, 2006). All the analytical procedures were performed in accordance with the package insert (ADVIA Centaur XP - Siemens Healthineers Global - Siemens Healthcare). The ADVIA Centaur TnI-Ultra assay uses a three-site sandwich immunoassay through direct chemiluminometric technology. Calibrators were prepared following the preparation guide. Then, the system automatically performs procedures with the arranged calibrators, quality control and serum samples. First, 100 µL of sample was dispensed into a cuvette and followed by dispensing 100 µL of Binary Lite Reagent (which contains a polyclonal goat anti-troponin I antibody labeled with acridinium ester and 2 biotinylated mouse monoclonal anti-troponin I antibodies) plus 50 µL of ancillary reagent (which is to reduce nonspecific binding) and incubated for 2.75 minutes at 37°C. Then, 150 µL of Solid Phase Reagent (which is magnetic latex particles conjugated with streptavidin) was dispensed into the cuvette and incubated for 5 minutes at 37°C. After cuvette wash, it dispensed 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction. Then, the results was reported. The ADVIA Centaur TnI-Ultra assay measures troponin I concentrations up to 50 ng/mL (µg/L) with a minimum detectable concentration (analytical sensitivity) of 0.006 ng/mL (µg/L).

Mortality/Moribundity: The recovery animals (8/group) previously treated with Adriblastina were terminated early in Week 10 following a 4-week recovery period due to their poor condition (7/8 animals of the recovery animals of Adriblastina group were found dead or moribund), and a concurrent control group (5 males) was killed at the same time. In addition, there was an unexpected death of TLC177 group during blood sampling on day 43.

Body Weight: The body weights were significantly decreased in the Adriblastina group, TLC177 or Caelyx groups compared to their concurrent saline control groups from days 15, 8 or 8, respectively, to the end of study. There was no statistical significance between the TLC177 and Caelyx groups in body weight.

Clinical signs: Tail wounds were observed in the Adriblastina group during the treatment period; tail necrosis, abdominal distention, hunched posture, emaciation, hypoactivity, feces stain, dyspnea, pale mucous membrane and piloerection were noted during the recovery period. In the TLC177 and Caelyx groups, skin lesions and hair loss were observed during treatment period and teeth damage was observed during the recovery period. The clinical signs in the TLC177 and Caelyx groups were comparable.

Cardiac Biomarker cTroponin I: With respect to the serum levels of the cardiotoxic biomarker cTnI , no significant differences were found in between the Adriblastina, TLC177 or Caelyx groups when compared to their saline control groups during the treatment period. However, the Adriblastina group showed higher cTnI concentration (0.043±0.014 ng/mL) compared to its control group (0.010±0.006 ng/mL) on SD66 during the recovery period. Although there was a statistically increase of cTnI in the Caelyx group on Day 86 compared to its saline control group, the low level which was below the normal range, 0.03 ng/mL (Review of Qualification Data for Cardiac Troponins. 2011,U.S. FDA), is not considered cardiotoxic.

Gross Pathology: Decreased size of thymus, spleen, testis, epididymis, prostate, and seminal vesicles, enlarged kidney, pale adrenal, liver, and kidney, ascites and pleural effusion were noted in Adriblastina treated animals. Decreased size of thymus, spleen, testis and epididymis were also noted in TLC177 and Caelyx treated animals during the treatment period.

Organ Weight: Reduced absolute heart weight and heart weight to body weight ratio were noted in groups of Adriblastina, TLC177 and Caelyx during treatment and recovery period. There was no statistically significant difference between TLC177 and Caelyx groups over the study period.

Histopathology: Treatment-related microscopic changes were observed in Adriblastina treated animals, including myocardium vacuolation in 6 out of 13 animals and myocarditis in one animal. There were no microscopic findings in the heart indicative of cardiotoxicity in TLC177 or Caelyx -treated animals.

In conclusion, based on the results of mortality, body weight, clinical observation, cTnI serum concentration, gross pathology, organ weight and cardiac histopathology, TLC177 and Caelyx showed similar toxicity profiles. With respect to cardiotoxicity, a high cTnI level, decreased heart organ weight, myocardium vacuolation and myocarditis was observed in the Adriblastina group at a cumulative dose of 12 mg/kg. For TLC177 and Caelyx groups, there were similar decreases of heart organ weights but a lack of heart microscopic effects at a cumulative dose of 14 mg/kg.

Table 19: Summary of repeat-dose toxicity study TLC006QN16006
Test Article: TLC177

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Rat/ Wistar	IV	36 days/once weekly saline (1) and adriblastinawith 30-day recovery period 43 days/onc	Saline Control (1) Saline Control (2) Adriblastina 2 mg/kg TLC177 2 mg/kg Caelyx 2 mg/kg	10 M/control group 13 M/treated group (5/group dosing phase and 5 or 8/group for recovery phase)	Not applicable	Heart toxicity was compared for adriblastina, TLC177 and Caelyx . 7/8 animals dosed previously with adriblastina died in the recovery phase; 3 were found dead and 4 were moribund; the group was terminated early in Week 10. Microscopic changes of	TLC006QN16006

Test Article: TLC177

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
		e weekly saline (2), TLC177 and Caelyx with 42-day recovery period				vacuolation and myocarditis were seen in the heart of recovery phase animals treated previously with adriblastina once weekly for 6 doses. No heart pathology was seen at the end of the dosing phase. No pathology changes were seen in the heart of animals dosed with TLC177 or Caelyx at 2 mg/kg at the end of dosing or recovery phases.	

Study Title: DOXOLIPAD TM: A 66-DAY INTRAVENOUS TOXICITY STUDY IN RATS; 13 DOSES WITH A 26-DAY RECOVERY (GLP) (Report number: T30012001-GN)

A GLP compliant repeated dose toxicity study was conducted to evaluate the toxicity of Doxolipad (TLC177) or Caelyx in SD rats when given once every 3 days for 13 doses and followed by a 26-day recovery period. Animals received intravenous administration of control article (Placebo for Doxorubicin HCl liposome injection: Batch No. PB-KJ2617-2), test article (TLC177: Lot No. Y049695XA) or reference article (Caelyx : Batch No. BFZ1300) by bolus injection.

Toxicity was evaluated by clinical signs, body weight, food consumption, ophthalmology, clinical pathology (hematology, coagulation, serum biochemistry and urinalysis), and pathology (organ weight, gross and histopathological examinations).

Mortality and Moribundity: Although females tolerated the dosing (once every 3 days) of TLC177 up to 1.5 mg/kg for 13 dosings, males could not tolerate the dosing at 1.5 mg/kg after the 10th dose.Mortality and moribundity were observed in 53% of Group 4 males (8 out of 15 males: 6 males during dosing and 2 males during recovery phases).

Clinical Signs: Skin lesions (characterized as wound, swelling, papules on skin, scars and scales etc.) were firstly noted on Day 16 in females at a dose of 1.0 mg/kg TLC177, on Day 17 at 1.5 mg/kg TLC177 in both sexes or at 1.0 mg/kg Caelyx in females. No skin lesions were observed at 0.2 mg/kg TLC177. The number of animals showing skin lesions and the number of affected skin sites increased with the number of doses. Reversibility of skin lesions was noted in some locations of the skin.

In addition to skin lesions, aggressiveness was observed at doses of 1.0 mg/kg TLC177and higher in both sexes and at 1.0 mg/kg Caelyx in males; piloerection and hunched posture were observed at 1.0 mg/kg TLC177 or Caelyx and at 1.5 mg/kg TLC177 in both sexes; dehydration was noted at 1.5 mg/kg TLC177 and at 1.0 mg/kg Caelyx in males; emaciation, prostrate, tip toe walk most likely due to skin lesion(s) around hind paw(s) and tremors were noted at 1.5 mg/kg TLC177 in males.

Body Weight: Dose-related reductions in body weight were observed in the TLC177 treatment groups(Table 5).The effects were stronger in males compared to females. TLC177 and Caelyx at equal doses showed similar effects during the study period. Reversibility of the reduced body weight was observed during the recovery period with exception of males at 1.5 mg/kg TLC177. Liposomal

doxorubicin-related reduced food consumption was noted in males (TLC177 [1.0 and 1.5 mg/kg] and Caelyx [1.0 mg/kg]) and females (TLC177 [1.5 mg/kg] and Caelyx [1.0 mg/kg]), which were fairly correlated with body weight data.

Ophthalmology: Swelling of the eyelid and corneal opacity were observed at 1.0 mg/kg in females and at 1.5 mg/kg in both sexes of the TLC177 treatment groups. Swelling of the eyelid (both sexes) and corneal opacity (females) was also observed at 1.0 mg/kg in the Caelyx group. Since all animals that displayed corneal opacity had swelling of eyelid, corneal opacity was possibly caused by scratching of eyelid.

Haematology: Reduced red blood cells, hemoglobin, hematocrit, reticulocytes, reduced white blood cells, lymphocytes, eosinophils and basophils, and increased platelets and monocytes were observed at 1.0 mg/kg TLC177 and Caelyx and 1.5 mg/kg TLC177 in both sexes. Changes in haematology parameters relative to the control groups are shown in Table 6.

Reversibility of these changes was noted during the recovery period with exception of males of the 1.5 mg/kg TLC177 group. The recovery period (26-day) was not sufficient for the complete recovery of RBC (in both sexes) and PLT (in males).

Coagulation: Significantly increased fibrinogen, compared to the control group, was observed in both sexes at 1.5 mg/kg TLC177 and in males at 1.0 mg/kg Caelyx on Day 40. Although there was a statistically significantly shortened PT (Prothrombin Time) in males at 1.5 mg/kg TLC177 (-14%) and in both sexes of the Caelyx group (-11% and -3% of male and female, respectively), the biological significance of the shortened PT is minimal considering the small degree of changes and the lack of changes in APTT (Activated Partial Thromboplastin Time). All of the PT and fibrinogen changes were reversible, and the reversibility of males at 1.5 mg/kg TLC177could not be assessed since data could not be collected on Day 66.

Serum Chemistry: Elevation of potassium, reduced creatinine, decreased total protein, decreased albumin and triglyceride were observed at a dose of 1.0 mg/kg in TLC177 and Caelyx treatment groups (males or females) and at 1.5 mg/kg of TLC177 group (both sexes). Reduced creatinine, total protein, albumin and triglyceride were attributed to the decrease of food consumption.

Slight increases of ALP, AST and γ-GT were noted on Day 37/40 in both sexes; while these changes were considered to be liposomal doxorubicin-related. They were not considered to be toxicologically significant in the absence of histological changes. All of the changes noted on Day 37/40 were reversible with exception of the changes noted at 1.5 mg/kg TLC177 in males based on the data from a single recovery male from this group (ID 1120451032), which displayed increased ALT, AST, γ GT, BUN, K, CHO and UA and reduced TP, ALB. The changes in ALT, AST and γ GT were correlated with liver changes observed in histopathology. These changes were considered to be a consequence of prolonged reduced food consumption and associated reduced body weight gain.

Urinalysis: There was no change identified in urinalysis.

Gross Pathology: Alopecia and/ or discoloration of the skin were noted at 1.0 and 1.5 mg/kg of TLC177 and 1.0 mg/kg of Caelyx in both sexes. Decreased thymus and spleen (TLC177 [1.0 and 1.5 mg/kg] and Caelyx [1.0 mg/kg] in both sexes); and decreased testes size (TLC177 [1.0 and 1.5 mg/kg] and Caelyx [1.0 mg/kg] in males) were noted.

Organ weight: Reduced thymus and spleen weights were noted at 1.0 and 1.5 mg/kg TLC177 and 1.0 mg/kg Caelyx in both sexes. Reduced testes weight was noted in all three TLC177-treated groups (0.2 mg/kg and higher) and the Caelyx group. Reduced ovary weight was noted in females at 1.5 mg/kg TLC177; however, this change was not considered to be toxicologically significant due to a lack of histological changes.

Histopathology: Target organs of toxicity included skin, thymus, spleen, bone marrow, testis, epididymis and prostate.

Skin ulceration and/ or inflammation, decreased cellularity (depletion) of the thymic cortex/medulla, bone marrow erythroid/myeloid lineages and splenic white pulp, depletion of testicular germ cells and single cell necrosis/apoptosis of the epididymal epithelia were observed at 1.0 and 1.5 mg/kg TLC177 and 1.0 mg/kg Caelyx. Prostate inflammation was observed in males at 1.5 mg/kg TLC177.

By Day 66, thymic and splenic depletion were fully recovered in all animals except for the single surviving male at 1.5 mg/kg TLC177, and bone marrow depletion was recovered except for a single female and the single surviving male at 1.5 mg/kg TLC177. Skin changes observed in females of the TLC177 groups at 1.0 and 1.5 mg/kg and the Caelyx group were recovered at the end of study period. The testicular, prostatic and epididymal changes worsened on Day 66 in all TLC177 groups and the Caelyx group. Sertoli cell-only seminiferous tubules associated with Leydig cell hyperplasia were noted in all male TLC177 and Caelyx treated animals except control males necropsied on Day 66, a single recovery male at 1.5 mg/kg TLC177 found dead on Day 44 and a terminal male at 1.5 mg/kg TLC177 on Day 37. These findings were considered the sequelae of the primary treatment effect, testicular germ cell depletion. The prostatic changes were still observed by the end of study at 1.0 and 1.5 mg/kg TLC177 and 1.0 mg/kg Caelyx. Additionally, severe hepatocellular degeneration/swelling with cytoplasmic eosinophilic globule/microvesicular vacuolation, correlating with increased ALT, AST and γ-GT, was observed in the single surviving male at 1.5 mg/kg TLC177 on Day 66 and was considered be the effect of prolonged altered nutritional status by reduced food consumption. These changes may be a delayed effect of TLC177.

Histopathologic examination did not reveal any changes in hearts in all treatment groups. Based on the above observations, the MTD level for TLC177 was 1.0 mg/kg for males, and 1.5 mg/kg for females. According to the severity of observed effects, the STD10 for TLC177 was between 0.2 mg/kg and 1.0 mg/kg in both sexes. And the NOAEL of TLC177 was below 0.2 mg/kg in males and 0.2 mg/kg in females. The TLC177-related changes at 1.0 mg/kg were comparable to those noted at 1.0 mg/kg Caelyx. Cardiotoxicity, a well-known doxorubicin-related toxicity, was not identified in either form of liposomal doxorubicin.

Table 20: Summary of repeat-dose toxicity study T30012001-GN
Test Article: TLC177

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Rat/SD	IV	66 days/ q3d*13	Placebo Caelyx: 1.0mg/kg TLC177: 0.2mg/kg TLC177: 1.0mg/kg TLC177: 1.5mg/kg IV injection	15M/ 15F (10 for dosing phase and 5 for recovery phase)	MTD: 1.0mg/kg for male; 1.5mg/kg for female NOAEL: 0.2 mg/kg for females and <0.2 mg/kg for males	No adverse effect levels of TLC177 were at 0.2 mg/kg in female and were below 0.2 mg/kg in male. Adverse effects included dermatologic toxicity, body weight and food consumption decreases, reduced RBCs and WBCs, corneal opacity, loss in thymus, spleen, bone marrow cellularity and sex organs. The changes were reversible with exception that testes, epididymis and prostate changes progressed during the recovery period. These changes observed at 1.0 mg/kg TLC177 were similar to those observed in Caelyx® treated group at the same dose level. Cardiotoxicity was not identified in TLC177 nor in Caelyx®. treated groups.	T30012001-GN

Genotoxicity

No genotoxicity studies were submitted (see discussion on non-clinical aspects).

Carcinogenicity

No carcinogenicity studies were submitted (see discussion on non-clinical aspects).

Reproduction toxicity

No reproductive and developmental toxicity studies were submitted with TLC177.

The potential developmental toxicity of Caelyx was evaluated in rats and rabbits (Janssen Inc. 2013). In the first study, intravenous bolus injections of Caelyx 0.1, 0.5, or 1.0 mg/kg was 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 centers in the fetuses. No adverse effects were seen in dams or fetuses in the placebo liposome or Caelyx 0.1 mg/kg groups.

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.

The Caelyx SmPC contains the appropriate advisory information in the fertility, pregnancy and lactation section. The same advisory information will be included in TLC177 SmPC.

Local tolerance

No studies specifically assessing the local tolerance of TLC177 or comparing it to that of Caelyx were submitted.

Other toxicity studies

The potential for the product to induce complement activation-related pseudoallergy (CARPA) was investigated by *in vitro* and *in vivo* immune reactogenicity assays.

In vitro complement activation study (study TLC006QN15011)

Doxisome and Caelyx, as well as negative and positive controls, were each incubated with 10 human sera. SC5b-9, as an indicator of reactogenicity, was then measured with Quidel's SC5b-9 EIA kit. The results showed that both Doxisome and Caelyx tended to cause complement activation. The SC5b-9 levels induced by Doxisome and Caelyx were 7.8±4.7 µg/ml and 4.9±1.3 µg/ml respectively. Statistical analysis was conducted using:

· Kruskal-Wallis's nonparametric test followed by Dunn's multiple comparison *post-hoc* test

· a one-way ANOVA test followed by Sidak's multiple comparison *post-hoc* test.

· an unpaired t-test (with Welch's correction).

The p values between Doxisome and Caelyx in Dunn's multiple comparison *post-hoc* test, Sidak's multiple comparison *post-hoc* test and the unpaired t-test were >0.9999, 0.7692 and 0.0835 respectively. All the three methods indicated there to be no significant difference between the two products.

In vivo immunotoxicity reactivity studies comparing Doxisome and Caelyx (study TLC006QN15009)

This *in vivo* study to investigate the comparability of reaction induction by Doxisome and Caelyx was conducted in pigs (Rudolf Urbanics. 2015). A total of ten pigs were randomized to Doxisome or Caelyx groups. The Doxisome group animals were then administered sequentially, as intravenous boluses, 5% dextrose (negative control), 0.1 mg/kg of Doxisome (based on phospholipid dose), 1.0 mg/kg of Doxisome, and Zymosan A (positive control) - with appropriate recovery intervals between administrations. The Caelyx group animals were subjected to the same procedure, except for replacement of test article of Doxisome with Caelyx. All of the animals were monitored / measured for the following parameters: Pulmonary Arterial Pressure (PAP), Systemic Arterial Pressure (SAP), Heart rate (HR), ECG (Einthoven's lead II), Respiratory rate, End-tidal CO2 (et CO2), Tissue oxygen saturation (SpO2 %), Haematology (such as white blood cell (WBC), platelet Thromboxane B2 (TxB2) and skin reaction. Skin reaction PAP and SAP were observed to change after injections of the test article (i.e. Doxisome or Caelyx). The average AUC values of PAP and SAP were observed to be smaller following the high dose injections as compared with the low dose injections. WBC and platelet Max Change (%) measurements exhibited similar trends to the cardiovascular reaction changes after low and high doses. The maximal alterations were observed during the first 1 to 3 minutes post injection. The AUC results of

PAP and SAP, along with the Max Change (%) for WBC and platelets are tabulated below. Data for Doxisome and Caelyx were compared by a two-tailed independent sample t-test.

Cardiovascular parameters

	AUC of PAP (min*mmHg)		AUC of SAP (min*mmHg)	
Dose (mg/kg)	0.1	1.0	0.1	1.0
Doxisome	58.98 ± 64.70	21.37 ± 10.56	104.19 ± 68.40	53.41 ± 22.59
Caelyx	98.63 ± 59.30	25.60 ± 14.48	402.66 ± 533.40	48.60 ± 12.98
P value	0.34	0.61	0.25	0.69

Haematology parameters

	Max WBC Change (%)		Max Platelet Change (%)	
Dose (mg/kg)	0.1	1.0	0.1	1.0
Doxisome	20.44 ± 30.92	-3.29 ± 10.59	21.97 ± 38.75	1.50 ± 19.52
Caelyx	6.77 ± 35.02	3.93 ± 8.80	11.14 ± 21.96	3.51 ± 14.80
P value	0.53	0.28	0.60	0.860

The results indicated there to be no statistically significant differences between the two liposomal products in terms of the 4 most sensitive parameters. A composite score of the cardiovascular, respiratory, blood cell count, skin reaction and ECG changes was also performed with no significant difference observed between the two products when evaluated by a non-parametric Mann-Whitney U test. [U value was 7.5 which is greater than the two tailed asymptotic significance of 0.295].

2.3.5. Ecotoxicity/environmental risk assessment

No Environmental Risk Assessment was submitted. This was justified by the applicant as the introduction of Doxolipad manufactured by TLC Biopharmaceuticals B.V. 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. Thus, the ERA is expected to be similar and not increased.

2.3.6. Discussion on non-clinical aspects

The mechanism of action of doxorubicin is reported in literature. It is thought to be via intercalation into DNA and disruption of topoisomerase mediated DNA repair, which results in DNA damage and cell death. It is also proposed that doxorubicin hydrochloride generates free radicals damaging cellular membranes, DNA and proteins[1,2]. The applicant presented primary pharmacodynamic studies results to bridge the pharmacodynamic profile of Doxolipad and Caelyx formulations. This can be accepted given the doxorubicin long history of use in humans and this is in line with the scientific advice given to the applicant (EMA/CHMP/SAWP/3655/2013).

The pharmacodynamics of TLC177 was studied in both *in vitro* and *in vivo* systems to demonstrate the bio-similarity with Caelyx. The cytotoxic profile of TLC177 was comparable to Caelyx when incubated in SKOV-3 human ovarian cancer cells at 37°C for 72 hours. It has been known that the cytotoxic effect of liposome- encapsulated drugs is mediated mostly by the extracellular release of the encapsulated drug (Allen et al, 1981). Therefore, the results suggested that TLC177 has similar releasing profile with Caelyx without any batch to batch variation. The anti-tumour efficacy of TLC177 and Caelyx was evaluated in

[1] Gewirtz, D.A. (1999). "A critical evaluation of the mechanisms of action proposed for the antitumour effects of the anthracycline antibiotics adriamycin and daunorubicin." Biochem Pharmacol 57(7): 727-741
[2] Thorn CF, Oshiro C, Marsh S, et al. Doxorubicin pathways: pharmacodynamics and adverse effects. *Pharmacogenet Genomics*. 2011;21(7):440-6.

both C26 murine colon carcinoma and ES-2 human clear cell ovarian carcinoma models. By assessing %T/C, TDT and TGD, high comparability in the anti-tumour efficacy was shown between TLC177 and Caelyx in both studies, and the selected drug dosages were reasonably tolerated. Although the studies were not GLP and the reports had to be updated to adequately present the methods and results, they may be considered as supportive information.

No specific secondary pharmacodynamic studies comparing TLC177 and Caelyx were submitted. Secondary PD studies of empty liposomes revealed no neurotoxicity signs or adverse behavioural effects in rodents (EPAR Caelyx). Hypotensive effects characterised as anaphylactic like responses were reported following intravenous administration of empty liposomes in a non-rodent model. While the clinical relevance of this effect is unknown, a statement is included in the Summary of Product Characteristics (SmPC) of Caelyx and should also be included in the SmPC of Doxolipad.

The standard core battery of safety pharmacology tests were not conducted as Caelyx and its active ingredient, doxorubicin hydrochloride, have well-defined safety and efficacy profiles (Ren et al., 2014, Thorn et al., 2011, Duggan and Keating, 2011, Gabizon et al., 2003). The non-clinical programme was designed to include studies which may identify clinically relevant differences between TLC177 and Caelyx. One concern which has been raised with specific regard to pegylated liposomes is an increased potential for haematologic events and thrombogenicity as a result of either immune-mediated or non-immune mediated reactions (Desai, 2012). Therefore, a comparative *in vitro* study to evaluate the influence of TLC177 and Caelyx on human whole blood by evaluating the extent of haemolysis and plasma flocculation (turbidity) was conducted. No obvious haemolysis effect and plasma flocculation were observed with TLC177 and Caelyx at concentrations up to 1 mg/ml.

No specific nonclinical pharmacodynamic drug interaction studies were submitted. The applicant provided bibliographic data on this topic (not shown in this report) which is considered acceptable for a hybrid application.

With regards to pharmacokinetics data, the studies performed to determine the pharmacokinetic properties and tissue distribution of TLC177 in comparison to Caelyx were designed in accordance with EMA/CHMP/806058/2009/Rev.2 and the Scientific Advice received (EMA/CHMP/SAWP/3655/2013). During development of TLC177 the mouse model was considered appropriate for PK studies, consistent with the characterisation of Caelyx PK in tumour bearing mice. Since the clinical dose range of Caelyx was from 20 to 50 mg/m^2, 6 mg/kg and 16.7 mg/kg were selected as the low and high doses for the TLC177 mice PK studies based on the mouse allometric scaling (km) factor of 3 (factor for converting mg/kg dose to mg/m^2 dose).

Non-GLP single dose comparative PK study of TLC177 in Mice (TLC006QN15002) analysis of PK parameters was done using grouping method since the study utilised a destructive measurement technique, as necessitated by the limited blood volume available from the study species of mice in line with other studies design (Gunnarsdottir et al. (2003) and Burade et al. (2017)). For calculation of pharmacokinetic parameters, two means were taken; one of which was determined through a grouping method by the randomized sorting by the number of each animal from low to high for the pre-defined number at each time point, and with the other mean determined by sparse sampling computation. A comparison of the pharmacokinetic results calculated by the grouping method to those obtained by sparse sampling computation showed a high degree of consistency between one another (data not shown). Due to the limitations of sparse sampling computation (except for Cmax), the assessment of bioequivalence can only be considered using calculations from the grouping method. On review of the bioequivalence results for Cmax and AUC0-∞, the bioequivalence results with small inter-subject variation based on parallel design, were observed to meet bioequivalence criteria (80.00% to 125.00%).

In the non-GLP single dose comparative PK study of TLC177 in Mice (TLC006QN16003), results of AUC0-t and AUCinf were also calculated by grouping mice from different time points. The comparison of the

pharmacokinetic results calculated by grouping method with those calculated by sparse sampling computation indicated a high degree of consistency between calculation methods. Therefore this approach is not considered to impact on the study results. Due to the limitations of sparse sampling computation, except for Cmax, the bioequivalence assessment was calculated using the grouping method only. All the PK parameters were within the bioequivalence acceptance range (90% CI of 80.00 – 125.00%) in this study.

In terms of biodistribution, results from Study TLC006QN15012 have not demonstrated that TLC177 and Caelyx have similar distribution profiles; representing the distribution of doxorubicin in systemic circulation, tissues (kidney, spleen, liver, tumour, heart and skin), and the releasing characteristics of liposomes. All parameters were significantly different between TLC177 and Caelyx over the time course tested. Concentrations of doxorubicinol, the major metabolite of doxorubicin implicated in causing cardiotoxicity, were very low within tissues with the highest levels measured in the kidney and the lowest levels in the heart. In this study, large inter-animal variability and batch variations was observed in each group because of small animal number. The applicant provided further data using confidence interval, t-test and data variability analyses (data not shown) supporting the fact that the differences observed are the results of high data variability and small sampling number.

Due to the wide range of data variation observed in study TLC006QN15012, the applicant submitted an additional non-GLP study, N42DMR18005, that doubled the sample size to n=6 for each treatment group at every time point. In study TLC006QN15012 the pharmacokinetic profiles in plasma were performed on total doxorubicin and doxorubicinol, while in study N42DMR18005 the pharmacokinetic profile of liposome encapsulated doxorubicin and free doxorubicin in plasma were investigated with two more time points at 72 hour and 408 hour post-dose being added. The results showed high similarity in distribution profiles of doxorubicin and doxorubicinol between TLC177 and Caelyx. The Cmax and AUClast ratios for TLC177 to Caelyx for doxorubicin and doxorubicinol were all less than 2.00 (range from 0.83 to 1.33). These results were consistent to the observations from the TLC006QN15012 study.

When focusing on the target of concern (heart) and target of interest (tumour), which has direct implication to the safety and efficacy equivalence respectively, the distribution of doxorubicinol in the heart and the distribution of doxorubicin in the tumour were equivalent for TLC177 and Caelyx in study TLC006QN15012 as well as in line with previous pharmacology studies. Overall, the similarity of the biodistribution pattern was verified in study N42DMR18005 by increasing the sample size.

Consistent across all toxicity studies, the TLC177 and Caelyx findings were comparable in body weight loss and organ weight loss. Overall, the findings from the organs assessed in the biodistribution study were compatible to the observations made in the toxicity studies. In addition, TLC177 and Caelyx findings were comparable for cardiotoxicity, clinical signs and mortality.

According to the results from the single dose and the two repeat dose toxicity studies, TLC177 and Caelyx were considered comparable with regard to their toxicity profiles. However, due to the sensitivity of toxicology studies, these data are not considered to provide definitive evidence of similarity between TLC177 and Caelyx. In accordance with the Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 02), it should be considered to investigate the potential for the product to induce complement activation related pseudoallergy (CARPA) by *in vitro* and *in vivo* immune reactogenicity assays such as complement and/or macrophage/basophil activation assays and studies in sensitive animal models. Minor details can influence the complement activation process and it is difficult to predict the complement activating capability of liposome formulations based on structural analysis. Complement activation may need to be experimentally quantitated for each liposome preparation (Szebeni & Storm, 2015). The absence of such studies should therefore be adequately justified. To evaluate the effect, both *in vitro* complement activation and *in vivo* immunotoxicity reactivity studies

comparing Doxolipad and Caelyx were submitted (studies TLC006QN15011 and TLC006QN15009). The results showed that both Doxolipad and Caelyx tended to cause complement activation. Statistical analysis was conducted using three different methods which indicated there to be no significant difference between the two products. In order to provide additional assurance of the comparability between Doxolipad and Caelyx, an *in vivo* study was also conducted in pigs which are considered the best animal model for mimicking human infusion reactions to liposomes in terms of both kinetics and spectrum of symptoms (Rudolf Urbanics. 2015). The results indicated there was no statistically significant difference between the two liposomal products in terms of the 4 most sensitive parameters. A composite score of the cardiovascular, respiratory, blood cell count, skin reaction and ECG changes was also performed with no significant difference observed between the two products when evaluated by a non-parametric Mann-Whitney U test. In conclusion, both the *in vitro* and *in vivo* studies performed demonstrated there to be no significant differences between the test articles (i.e. Doxolipad or Caelyx) associated with the risk of causing CARPA.

The applicant was also requested to discuss the relevance of any potential differences based on data on comparative biodistribution in tissues to the clinical setting. The applicant argued that the non-clinical studies were also predictive of outcomes of the pivotal human bioequivalence study, as follows: the induction of CARPA hypersensitivity reactions and the absence of haemolysis in the non-clinical studies were mirrored in the alleviated hypersensitivity and minimized adverse event profiles observed in the clinical trial; the equivalent PK parameters (including Cmax, AUC0-t and AUC0-∞) of Doxolipad and Caelyx in the non-clinical PK study for total doxorubicin, encapsulated doxorubicin and doxorubicinol were also seen in the clinical trial; Non-clinical tissue distribution studies in tumour-bearing mice showed accumulation of doxorubicin in tumours, consistent with scintigraphic data from radiolabeled liposome studies and tumour biopsy studies in Caelyx (Doxil)-treated patients; The equivalence observed in the in vitro and in vivo non-clinical studies was reflected in the pivotal human bioequivalence clinical study in the similarity of the safety adverse events profile, particularly in terms of drug product-induced hypersensitivity and anaemia.

No reproductive and developmental toxicity studies were submitted with TLC177 considering the toxicological profile of doxorubicin hydrochloride and Caelyx are well characterised and additional studies were not considered suitable for determination of comparability of TLC177 with Caelyx. This is consistent with the recommendations in the Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA/CHMP/806058/2009/Rev. 2).

With regards to the ERA, doxorubicin is already used in existing marketed products and no significant increase in environmental exposure is anticipated with doxolipad. Therefore doxorubicin is not expected to pose a risk to the environment. The SmPC of Doxolipad should reflect that any unused medicinal product or waste material should be disposed of in accordance with local requirements.

2.3.7. Conclusion on the non-clinical aspects

The non-clinical studies provided are considered adequate to support a hybrid application. The presented data indicated comparability of biodistribution, pharmacokinetics and toxicological properties of Doxolipad and Caelyx. The totality of the non-clinical study results showed that Doxolipad and Caelyx were similar in non-clinical aspects.

2.4. Clinical aspects

2.4.1. Introduction

This is an application for a concentrate for solution for infusion containing doxorubicin hydrochloride as pegylated liposomal formulation. To support the marketing authorisation application the applicant

conducted one bioequivalence study (Study TLC177.6) with cross-over design. This study was the pivotal study for the application.

Formal scientific advice by the CHMP was given for this medicinal product. For the clinical assessment Guideline on the Investigation of Bioequivalence CPMP/EWP/QWP/1401/98 Rev.1) in its current version is of particular relevance.

GCP

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.

Clinical studies

Table 21: Tabular overview of clinical studies

Study/Sponsor	Design	Population	Reference Drug	Location	Subjects number	Test Dosage
TLC177.0/ TLC	Randomized, open-Label, two- period, two-way, crossover, BE Study	Advanced Carcinoma of the Ovary	Caelyx	Taiwan	N=3* * Terminated early due to reference drug supply shortage	50 mg/m^2
DOX-BE-001/ TLC	Randomized, open-Label, two- period, two-way, crossover, BE Study	Advanced Carcinoma of the Ovary	Doxil	EU, Taiwan, US	No enrollment* * Terminated due to reference drug supply shortage	50 mg/m^2
TLC177.2/ Teva Pharm USA	Randomized, open-Label, two- period, two-way, crossover, BE Study	Advanced Carcinoma of the Ovary	Caelyx	US, Taiwan	N=37* * Terminated early due to reference drug supply shortage	50 mg/m^2
TLC177.6/ TLC	Randomized, open-Label, two- period, two-way, crossover, BE Study	Advanced Carcinoma of the Ovary	Caelyx	EU: Poland, Bosnia and Herzegovina, Croatia	N=52	50 mg/m^2

2.4.2. Pharmacokinetics

The applicant provided a summary of available PK data on Caelyx as well as the results of one bioequivalence study in support of the application.

The PK profile of Caelyx after IV infusion in humans over the dose range of 10 mg/m2 to 60 mg/m2 was best described by a two compartment non-linear model with zero order input and Michaelis- Menten elimination and is comparable to that in animals. In humans, the mean intrinsic clearance of Caelyx was 0.030 L/h/m2 (range 0.008 to 0.152 L/h/m^2) and the mean central volume of distribution was 1.93 L/m^2

(range 0.96-3.85 L/m^2) approximating the plasma volume. The apparent half-life ranged from 24-231 hours, with a mean of 73.9 hours (Caelyx SmPC).

Bioequivalence study

Study TLC177.6

Study TLC177.6 was a randomized open-label crossover study of bioequivalence and safety of doxorubicin hydrochloride liposome injection formulations in patients with advanced carcinoma of the ovary.

Methods

Study design

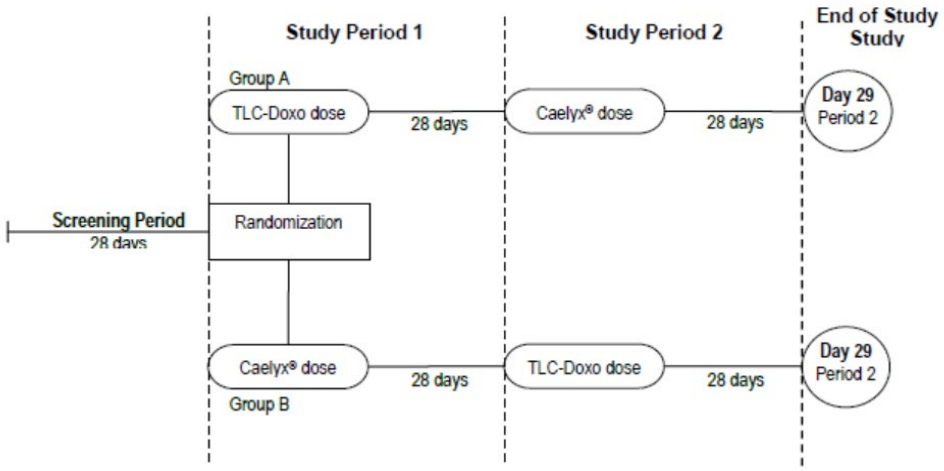


Figure 18: Study design of TLC177.6

Study objectives

The primary objective was the assessment of the bioequivalence of two doxorubicin hydrochloride liposome injection formulations: Doxolipad 2 mg/ml as test product and Caelyx 2 mg/ml, manufactured by Ben Venue Laboratories, Inc., as the Reference Drug by conducting a pharmacokinetic analysis on free doxorubicin (FD) and encapsulated doxorubicin (LED).

The secondary objective was to analyse concentrations of doxorubicinol and total doxorubicin in plasma and to assess the safety of a single dose of Doxolipad.

Study centers:	8 active centers in Europe: n=5 Poland; n=2 Bosnia and Herzegovina; n=1 Croatia An additional 3 centers were initiated in Poland and 1 center in Bosnia and Herzegovina, but these sites did not enroll subjects and thus were not active sites.	
Clinical phase:	Phase I study of bioequivalence	
Study period:	**First subject enrollment:**	28 OCT 2014
	Last subject, last visit:	24 FEB 2016

Blood sampling schedule

Blood sampling for pharmacokinetic assessments was to be performed on Day 1 of each study period immediately prior to the start of the infusion (pre-dose, 0.0 hours), 20 minutes and 40 minutes after the start of the infusion, immediately after infusion is completed, and at 10 min, 20 min, 40 min, 1.0 hr, 4.0 hr, and 8.0 hr after completion of the infusion.

Subjects were also to return to the study site on Days 2, 3, 5, 8, 11, and 15 of the study period for PK samples to be collected at the 24, 48, 96, 168, 240, and 336 hour post-dose time points.

Deviation from the nominal sample time window was to be recorded in a deviation log. All PK samples were to be considered evaluable as long as the actual collection times were recorded.

Test and reference products

Test drug/agent: Doxolipad (doxorubicin hydrochloride [HCl] liposome injection).

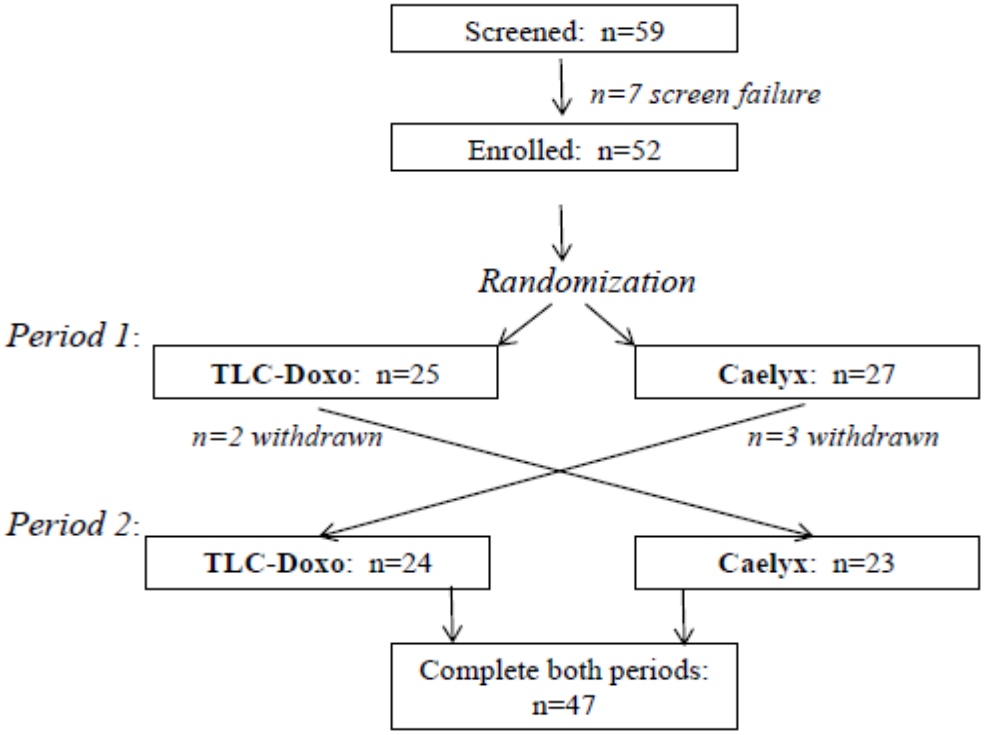
Bulk batch number(s): B029695XAA, B029695XAD, and B029695XAC manufacturing date 27/06/2014 exp. date 02/2016
Dose: A single dose; 50 mg/m^2
Route of administration: Intravenous infusion over 60-90 minutes

Reference drug: Caelyx (doxorubicin HCl liposome injection) manufactured by Janssen-Cilag Ltd
Bulk batch number(s): EBBS301 exp. date 09/2015 and EFBS000 exp. date 01/2016
Dose: A single dose; 50 mg/m^2
Route of administration: Intravenous infusion over 60-90 minutes

The patient should be fasting starting 2 hours prior to drug administration until 4 hours post-administration. If the patient is unable to fast due to their condition, they can be given a light snack (low-fat/low-calorie).

Population studied

Participant flow



Study population

Table 22: Summary of study population

Variable / Status	Total (N=52)
Safety population	
Included	52 (100.0%)
PK population	
Excluded	5 (9.6%)
Included	47 (90.4%)
BE population	
Excluded	22 (42.3%)
Included	30 (57.7%)

Abbreviations: PK = pharmacokinetic; BE = bioequivalence
Source: Table 14.1.3

The Bioequivalence population comprised 30 (57.7%) subjects who received both doses of study medication and whose not-recommended concomitant medications were delivered in an identical manner during both treatment periods. A total of 22 (42.3%) subjects were excluded from the BE analysis: 13 were excluded for receiving asymmetrical delivery of concomitant medications; 4 were withdrawn for undergoing procedures that were not performed during both treatment periods; 2 were withdrawn for disease progression or death, 2 were withdrawn for AEs, and 1 was withdrawn by sponsor's decision.

Protocol deviation

There were two major deviations reported from TLC177.6 study. One subject has been enrolled although her neutrophil count in screening was 1.2 x 10^9/L which was in violation of criteria #4 and indicated inadequate bone marrow function; Subject 07-02's drug was administrated 20 minutes shorter than

expected (dosing time was 67 minutes however time was 87 minutes). The remaining deviations were categorized as minor. These deviations were numerous and were primarily associated with missed laboratory assessments or errors in the time window for these assessments.

Baseline characteristics

In the safety population (n=52), the mean age was 58.6 years and all subjects were white. Mean weight was 71.0 kg and mean body mass index (BMI) was 27.1 (kg/m2). The majority of subjects had an ECOG performance status of 0 (75.0%). The mean time from ovarian cancer diagnosis was 3.33 years (median, 2.28 years) and the disease stage at diagnosis was most commonly Stage III, 65.4%. The mean time elapsed from disease progression was 0.59 years (median, 0.09 years). Per protocol, all subjects had experienced progression following a prior platinum-based chemotherapy. The majority of subjects had not previously received doxorubicin, 86.5%.

Analytical methods

Analytical sample preparation

At the time-points listed previously, blood samples were collected in tubes containing DMSO, a cryoprotectant employed to retard liposome fracture during storage and freeze-thaw operations. The blood samples were separated by centrifugation immediately after being collected and the plasma component was decanted, frozen, and shipped to the central analytical laboratory for future bio-analysis.

Analytical Report AN-81814 for Determination of Total, Free and Liposomal Encapsulated Doxorubicin plus Doxorubicinol in Human Plasma present the objectives for the determination of Total Doxorubicin (TD), Free Doxorubicin (FD), Liposomal Encapsulated Doxorubicin (LED) and Doxorubicinol in human plasma derived from a clinical study by HPLC-Fluorescence and HPLC-MS/MS.

For the main study No. TLC177.6 all samples were delivered deep-frozen in dry ice to pharm-analyt, were checked while deep-frozen and stored at nominal -70 to -85 °C pending preparation for analysis.

Adequate Validation Reports on HPLC Method for the determination of Total Doxorubicin, Free doxorubicin, and Liposomal encapsulated doxorubicin in human plasma and in human whole blood were presented.

Additional document New validation of an HPLC-MS/MS method for the determination of doxorubicinol in human plasma was presented (dated 23.05.2016).

Pharmacokinetic variables

The following parameters were evaluated for free doxorubicin, liposome-encapsulated doxorubicin, doxorubicinol and total doxorubicin in human plasma:

- Peak concentration (Cmax)
- Time to reach peak concentration (Tmax)
- Area under the plasma concentration-time curve from time zero to time of last quantifiable concentration (AUC0-t)
- Area under the plasma concentration-time curve from time zero to infinity (AUC0-∞)
- Elimination rate constant (λz)
- Volume of distribution (Vd)
- Clearance (Cl)

- Terminal elimination half-life (T½)
- Mean residence time (MRT)
- Ratio of AUC0-t to AUC0-∞.

The following plasma PK parameters were computed using WinNonlin Professional, version 6.3.

Statistical methods

Study design methods and statistical analysis carried out in the study were established in SAP version 4.0 (dated: 15 April 2016). All statistical analyses were performed using SAS for Windows (Version 9.3 or higher).

The PK parameters (AUC0-inf, AUC0-t, RAA, overall false discovery rate, AUC0-48, AUC48-t, Cmax, tmax, t1/2, Vz, CL, MRT, and λz) for test and reference formulations were summarized by mean, geometric mean, minimum, median, maximum, standard deviation, and coefficient of variation. The evaluation of bioequivalence was determined by comparing test formulation to reference formulation. The following null vs. alternative hypothesis was tested using two one-sided tests for PK parameters (AUC0-t, AUC0-inf, and Cmax): H0: $\mu T/\mu R < 0.8$ or $\mu T/\mu R > 1.25$ vs. H1: $0.8 \leq \mu T/\mu R \leq 1.25$, Where μT is the geometric mean of the test formulation and μR is the geometric mean of the reference formulation. The level of significance for tests of both endpoints was set to 0.05. The two one-sided tests at a level of significance of 0.05 is equivalent to the comparison of the 90% confidence interval (CI) for the ratio of geometric means (test/reference), with the acceptance limits of bioequivalence 0.8000 - 1.2500. In order to demonstrate bioequivalence, the lower bound of 90% CI must be ≥ 0.8000 and the upper bound must be ≤ 1.2500 when rounded to four decimal places. Bioequivalence of test and reference formulations was demonstrated if the 90% CI for ratios of the geometric means (test/reference) for Cmax, AUC0-t, and AUC0-inf were within the acceptance interval 0.8000-1.2500 (80.00% -125.00%) with regards to encapsulated and free doxorubicin. For the sensitivity analysis, bioequivalence was also analyzed for total doxorubicin and doxorubicinol but without any confirmatory consequences.

Results

Table 23: Summary of pharmacokinetic parameters for free doxorubicin after IV infusion of a single dose of 50 mg/m² TLC-Doxo and Caelyx to subjects who completed both periods of the study

Parameters	TLC-Doxo			Caelyx		
	N	Mean	SD	N	Mean	SD
C_{max}, ng/mL	47	1340	1740	46	1150	1430
T_{max}, h	47	49.35	50.86	46	37.71	28.99
AUC_{0-t}, h•ng/mL	47	143000	163000	46	110000	109000
AUC_{0-inf}, h•ng/mL	39[a]	121000	143000	38[a]	103000	72400
AUC_{0-48}, h•ng/mL	47	31300	31800	46	29300	26900
AUC_{48-t}, h•ng/mL	47	112000	136000	46	81000	94300
λ_z, 1/h	39[a]	0.0114	0.00434	38[a]	0.0107	0.00346
V_z, L	39[a]	116	65.8	38[a]	124	70.0
CL, L/h	39[a]	1.24	0.780	38[a]	1.26	0.839
$t_{1/2}$, h	39[a]	68.57	23.67	38[a]	71.20	23.35
MRT, h	39[a]	113	28.7	38[a]	116	31.1
RAA, %	39[a]	95.5	3.41	38[a]	94.9	4.04

Abbreviations: SD = standard deviation; AUC = area under the curve; λ_z = elimination rate constant; V_z = volume of distribution during the terminal elimination phase, calculated as [Dose /(λ_z * AUC_{0-inf})] (computed for parent only); CL = Total plasma clearance calculated as [Dose/AUC_{0-inf}] (computed for parent only); $t_{1/2}$ = half-life; MRT = mean residence time; RAA = (AUC_{0-t}/ AUC_{0-inf})*100; C_{max} = peak concentration; R^2 = coefficient of determination.

Table 24: Summary of pharmacokinetic parameters for liposome-encapsulated doxorubicin after IV infusion of a single dose of 50 mg/m² TLC-Doxo and Caelyx to subjects who completed both periods of the study

Parameters	TLC-Doxo			Caelyx		
	N	Mean	SD	N	Mean	SD
C_{max}, µg/mL	47	41.5	5.41	46	42.1	6.17
T_{max}, h	47	3.14	1.79	46	2.78	1.34
AUC_{0-t}, h•µg/mL	47	4540	1210	46	4730	1160
AUC_{0-inf}, h•µg/mL	47	4840	1410	46	5120	1430
AUC_{0-48}, h•µg/mL	47	1570	228	46	1580	227
AUC_{48-t}, h•µg/mL	47	2970	1040	46	3150	1020
λ_z, 1/h	47	0.0104	0.00531	46	0.00964	0.00601
V_z, L	47	2.01	0.433	46	2.09	0.575
CL, L/h	47	0.0200	0.00686	46	0.0185	0.00558
$t_{1/2}$, h	47	75.67	22.17	46	83.06	23.39
MRT, h	47	110	30.6	46	118	32.4
RAA, %	47	94.6	3.36	46	93.0	4.36

Abbreviations: SD = standard deviation; AUC = area under the curve; λ_z = elimination rate constant; V_z = volume of distribution during the terminal elimination phase, calculated as [Dose /(λ_z * AUC_{0-inf})] (computed for parent only); CL = Total plasma clearance calculated as [Dose/AUC_{0-inf}] (computed for parent only); $t_{1/2}$ = half-life; MRT = mean residence time; RAA = (AUC_{0-t}/ AUC_{0-inf})*100; C_{max} = peak concentration; R^2 = coefficient of determination.

Table 25: Summary of 90% confidence intervals between test and reference in pivotal study TLC177.6

PK Parameter	Test (TLC-Doxo)		Reference (Caelyx®)		(TLC-Doxo / Caelyx®)		Intra-subject Variability %CV
	N	GM	N	GM	GMR (%)	90% CI [LB, UB]	
Total Doxorubicin (TD)							
C_{max}, µg/mL	30	37.7	29	37.5	100.54	[98.17 , 102.97]	5.32
AUC_{0-t}, h•µg/mL	30	3950	29	4120	95.89	[90.97 , 101.07]	11.7
AUC_{0-inf}, h•µg/mL	30	4320	29	4420	97.78	[91.73 , 104.23]	14.3
Liposome Encapsulated Doxorubicin (LED)							
C_{max}, µg/mL	30	40.4	29	40.6	99.31	[96.91 , 101.76]	5.43
AUC_{0-t}, h•µg/mL	30	4340	29	4490	96.49	[92.03 , 101.17]	10.6
AUC_{0-inf}, h•µg/mL	30	4590	29	4830	94.90	[91.04 , 98.93]	9.26
Free Doxorubicin (FD)							
C_{max}, ng/mL	30	737	29	624	118.09	[93.58 ,	55.4

PK Parameter	Test (TLC-Doxo)		Reference (Caelyx®)		(TLC-Doxo / Caelyx®)		Intra-subject Variability %CV
	N	GM	N	GM	GMR (%)	90% CI [LB, UB]	
						149.03]	
AUC_{0-t}, h•ng/mL	30	84900	29	73800	115.16	[95.93 , 138.24]	42.4
AUC_{0-inf}, h•ng/mL	24	75300	23	74000	101.84	[84.44 , 122.83]	37.2
Doxorubicinol							
C_{max}, ng/mL	22	2.60	26	2.61	99.46	[94.83 , 104.32]	8.95
AUC_{0-t}, h•ng/mL	22	638	26	652	97.91	[93.96 , 102.03]	7.72
AUC_{0-inf}, h•ng/mL	3	NA	5	NA	NA	NA	15.6

Bioequivalence results for partial AUCs for the encapsulated doxorubicin were provided (see **Table 26**).

Table 26: Encapsulated doxorubicine bioequivalence including partial AUC results

	(Doxolipad / Caelyx)		Intra-subject Variability %CV
PK Parameter	GMR (%)	90% CI	
C_{max}	99.31	[96.91,101.76]	5.43
AUC_{0-t}	96.49	[92.03,101.17]	10.6
AUC_{0-inf}	94.90	[91.04,98.93]	9.26
AUC_{0-48}	99.98	[97.65,102.37]	5.26
AUC_{48-t}	94.97	[88.11,102.36]	16.8
V_z, L	98.14	[94.54,101.89]	8.33
CL, L/h	105.37	[101.08,109.84]	9.26

Conclusions

As Doxolipad contains the active ingredient doxorubicin hydrochloride in a pegylated liposomal formulation, Caelyx, doxorubicin hydrochloride (pegylated liposomal formulation), 2 mg/ml concentrate for solution for infusion was considered as the appropriate comparator for use in clinical comparability investigations.

To support the application, the applicant submitted TLC177.6 bioequivalence study. This was a randomized, open-label crossover study of doxorubicin hydrochloride Liposome injection formulations in patients with advanced carcinoma of the ovary. Study TLC177.6 was designed, conducted and the data analysed in line with the CHMP Scientific advices overall. At the time, the applicant was warned against subtle formulation differences that may modify efficacy due to specific cell interactions and distribution characteristics. The requirement for establishing bioequivalence for both the encapsulated and non-encapsulated doxorubicin was discussed. The importance of the sample size calculation was emphasized, to ensure enough patients data could be analysed to demonstrated pharmacokinetic equivalence if the formulation were truly equivalent.

Overall, the study design has been considered appropriate to estimate PK parameters. A wash-out period has been considered adequate as well as sampling period. The analytical methods were validated and considered acceptable. To establish bioequivalence, for pharmacokinetics analysis ANOVA model was conducted and this was considered appropriate.

In Study TLC177.6, doxolipad met the bioequivalence acceptance criteria for Cmax and AUCs (AUC0-t and AUC0-inf) with regards to liposome encapsulated doxorubicin (Cmax (96.91%-101.76%); AUC0-t (92.03% - 101.17%); AUC0-inf (91.04%-98.93%)), total doxorubicin (Cmax (98.17% - 102.97%); AUC0-t (90.97%-101.07%); AUC0-inf (91.73%-104.23%)), and doxorubicinol (Cmax (94.83% - 104.32%); AUC0-t (93.96% - 102.03%)).

The free form of doxorubicin met the bioequivalence acceptance criteria for AUC0-inf (84.44% - 122.83%) between Doxolipad and Caelyx. However, the acceptance criteria were not met for Cmax

(93.58% - 149.03%) and AUC0-t (95.93% - 138.24%) at a 90% CI. Therefore, bioequivalence of free (un-encapsulated) doxorubicin between the test and reference product has not been established.

Partial AUCs are also required for the encapsulated drug to ensure profile shape comparability. These data were provided by the applicant during the oral explanation and appeared to show bioequivalence.

The applicant argued that anti-tumour efficacy is related to tissue doxorubicin concentrations, and not to free doxorubicin plasma concentrations, and minimizing free doxorubicin in plasma is important to improve drug product safety, and PEGylated liposomal doxorubicin products minimize free doxorubicin to a negligible fraction of total doxorubicin in circulation. However, the CHMP considered that both the encapsulated and un-encapsulated drug should be analysed as a basis for pharmacokinetic comparability between two liposomal doxorubicin products.

Generally, bioequivalence is focussing on formulation differences rather than efficacy/safety of any compound. Conceptually, this is because bioequivalence investigations compare products that contain the same amount of the same active drug substance; and the reference already demonstrated efficacy and safety of that particular amount of drug. More specifically liposomal formulations may be considered as having even two actives, i.e. the encapsulated and the un-encapsulated drug. Liposomal formulations have a major impact on the in vivo pharmacokinetic (PK) and pharmacodynamic (PD) properties, since some or all of the following may occur to varying degrees:

• the active substance release rates from liposomes can affect PK and PD and therefore the safety and efficacy profile of the medicinal product

• an entrapped active substance may not be biologically available and may be protected from degradation, in addition to metabolism whilst it is entrapped in the liposome

• the PK of the encapsulated substance may be controlled by the PK of the carrier (i.e. the liposomal formulation) which is influenced/determined by the physicochemical properties of the liposomes, by the physico-chemical state of the encapsulated drug substance and by interactions between the components of the liposome and the biological environment.

In addition, the clearance of the liposomal active substance is dependent on the clearance of the liposomal carrier itself, the rate of release of entrapped drug from the liposomal carrier, and the clearance and metabolism of un-encapsulated drug upon its release.

Furthermore the un-encapsulated doxorubicin does constitute a relatively low fraction of total in circulation but can be reliably quantified.

For these reasons the encapsulated drug plus the un-encapsulated compound are considered most relevant to reflect biopharmaceutic product performance and detect possible formulation differences if they are there.

Overall, similarity in terms of efficacy and safety between Doxolipad and Caelyx is not considered sufficiently established due to the fact that bioequivalence of free (un-encapsulated) doxorubicin between Doxolipad and Caelyx has not been established (see also discussion on clinical aspects).

2.4.3. Pharmacodynamics

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

2.4.4. Clinical efficacy

No efficacy studies were submitted which was considered acceptable as no such studies are required for this type of application.

2.4.5. Clinical safety

TLC177 was developed to have two of the same indications and posology as Caelyx. According to the Summary of Product Characteristics (SmPC) of Caelyx (SmPC of Caelyx, 2017), the approved posology for specific clinical indications is different in metastatic breast cancer, advanced ovarian cancer, progressive multiple myeloma and AIDS-related Kaposi's sarcoma. For breast and ovarian cancer, the dosing schedule is 50 mg/m^2 once every 4 weeks. For the treatment of multiple myeloma, Caelyx is administered at a dose of 30 mg/m^2 on day 4 of the bortezomib 3-week regimen and for AIDS-related Kaposi's sarcoma at a dose of 20 mg/m^2 every two-to-three weeks.

The patients with advanced carcinoma of the ovary from three bioequivalence studies were included in the safety population. An integrated report of the safety data from TLC177.0, TLC177.2 and TLC177.6 was generated because those studies had the same design with actual patient enrolment. Information regarding the incidence, intensity and relationship of adverse events (AEs) observed compared to Caelyx was summarised.

Patient exposure

There were 92 subjects randomized in these three studies, including 3 subjects from TLC177.0 study, 37 subjects from TLC177.2 study, and 52 subjects from TLC177.6 (main) study. In total, 84 subjects received Doxolipad treatment and 85 subjects received Caelyx and the average dose of drug exposure was 86.88±9.64 mg for Doxolipad and 86.99±10.03 for Caelyx. In TLC177.6, doxorubicin had been given in seven patients as previous chemotherapy regimen with median cumulative dose of 160 mg/m^2.

Adverse events

The most common (≥10%) TEAEs observed by preferred term in Doxolipad group was anaemia (16.67%), followed by nausea (10.71%), fatigue (10.71%) and vomiting (10.71%); and the most common (≥10%) TEAEs observed by preferred term in Caelyx group was nausea (16.47%), followed by anaemia (15.29%), urinary tract infection (10.59%). For both Doxolipad and Caelyx groups, most of the TEAEs were mild, except for anaemia and urinary tract infection (UTI).

There were fewer AEs, TEAEs and SAEs in TLC177 group of patients as compared to Caelyx. Most of AEs were of mild or moderate grade. In the TLC177 population there were less number of episodes of febrile neutropenia, cardiac disorders, mouth ulcerations but more drug hypersensitivity and palmar-plantar erythrodysaesthesia episodes.

There was only one subject who received Doxolipad at Period 1-Day 1 from TLC177.6 study experienced an AE of drug hypersensitivity which led to premature discontinuation. This AE was judged as definitely related to study treatment by investigator and resolved without sequelae.

Serious adverse event/deaths/other significant events

There were only 15 severe AEs from 11 subjects and 4 life-threatening AEs from 3 subjects in the Doxolipad group. In the Caelyx group, there were 13 severe AEs from 8 subjects and 2 life-threatening AEs from 2 subjects. There were 5 SAEs from 2 subjects (2/84, 2.38%) in the Doxolipad group and 10 SAEs from 8 subjects (8/85, 9.41%) in the Caelyx group were reported. Only one subject interrupted drug due to AE (neutropenia) in the Caelyx group, and one subject discontinued drug due to AE (drug hypersensitivity) in the Doxolipad group. There was one death (disease progression with ascites) reported among three studies.

Laboratory findings

No new safety signal in laboratory findings for Doxolipad as compared to Caelyx was detected in the analysed three studies population.

Discontinuation due to adverse events

Only one subject interrupted drug due to AE (neutropenia) in Caelyx group, and one subject discontinued drug due to AE (drug hypersensitivity) in Doxolipad group. This AE was judged as definitely related to study treatment by investigator and resolved without sequelae.

2.4.6. Post marketing experience

No post-marketing data are available. The medicinal product has not been marketed in any country.

2.4.7. Discussion on clinical aspects

The indications applied for Doxolipad are the same as two indications approved for Caelyx and are as follows: "Doxolipad is indicated as monotherapy for adult patients with metastatic breast cancer, where there is an increased cardiac risk; for treatment of advanced ovarian cancer in adult women who have failed a first-line platinum-based chemotherapy regimen."

A summary of the literature with regard to clinical efficacy and safety data of doxorubicin hydrochoride (pegylated liposomal form) was provided and was accepted by the CHMP. This is in accordance with the relevant guideline and additional clinical studies were not considered necessary.

The applicant also presented safety data from 92 subjects randomized in three bioequivalence studies, including 3 subjects from TLC177.0 study, 37 subjects from TLC177.2 study, and 52 subjects from TLC177.6 (main) study. In total, 84 subjects received Doxolipad treatment and 85 subjects received Caelyx. The two compared groups of patients exposed to the investigational products were homogenous in term of number, age, indication, disease status and drug exposure.

The most common (≥10%) TEAEs observed by preferred term in Doxolipad group was anaemia (16.67%), followed by nausea (10.71%), fatigue (10.71%) and vomiting (10.71%); and the most common (≥10%) TEAEs observed by preferred term in Caelyx group was nausea (16.47%), followed by anaemia (15.29%), urinary tract infection (10.59%). For both Doxolipad and Caelyx groups, most of the TEAEs were mild, except for anaemia and urinary tract infection (UTI).

There were less AEs, TEAEs and SAEs in TLC177 group of patients as compared to Caelyx. Most of AEs were of mild or moderate grade. In the TLC177 population there were fewer episodes of febrile neutropenia, cardiac disorders, mouth ulcerations but more drug hypersensitivity and palmar-plantar erythrodysaesthesia episodes.

There was one subject who received Doxolipad at Period 1-Day 1 from TLC177.6 study experienced an AE of drug hypersensitivity which led to premature discontinuation. There were less number of AEs, TEAEs and SAEs in TLC177 group of patients as compared to Caelyx. Most of AEs were of mild or moderate grade. In the TLC177 population there were less number of episodes of febrile neutropenia, cardiac disorders, mouth ulcerations but more drug hypersensitivity and palmar-plantar erythrodysaesthesia episodes. No new safety signal in laboratory findings for Doxolipad as compared to Caelyx was detected in the analysed three studies' population.

Overall, no new safety issues emerged from the presented pivotal study. Due to small number of patients no statistical differences could be documented between the studied product and comparator.

2.4.8. Conclusions on clinical aspects

Proving equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator liposomal product is considered a step-wise approach which in addition to the pharmacokinetic study also takes account of quality and non-clinical comparison, where appropriate. Similarity in terms of efficacy and safety between Doxolipad and Caelyx cannot be considered established due to the fact that bioequivalence of free (un-encapsulated) doxorubicin between Doxolipad and Caelyx has not been established.

2.5. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	• Cutaneous lesions • Myelosuppression • Secondary malignancies (haematological and oral) • Cardiac toxicity
Important potential risks	• Off-label use • Foetotoxicity • Interstitial lung disease • Urinary tract infection • Irreversible changes in testes, epididymis and prostate changes • Renal failure
Missing information	• Use in paediatric population

Pharmacovigilance plan

No additional pharmacovigilance activities are proposed.

Risk minimisation measures

No additional risk minimisations measures are proposed.

Conclusion

The CHMP and PRAC, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application (see Clinical Pharmacology section), the risk management plan cannot be agreed.

2.6. Pharmacovigilance

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.

2.7. Product information

Due to the concerns discussed above (see discussion on Clinical Pharmacology) a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed.

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 application concerns a hybrid version of doxorubicin hydrochloride for a different pharmaceutical form (concentrate for solution for infusion; pegylated liposomal formulation) with reference to Adriamycin (solution for injection; non pegylated liposomal formulation). Caelyx, which contains doxorubicin hydrochloride in a pegylated liposomal formulation was considered as appropriate comparator to establish quality, non-clinical and clinical comparability. The indications applied for are the same as two indications approved for Caelyx and are as follows: "Doxolipad is indicated as monotherapy for adult patients with metastatic breast cancer, where there is an increased cardiac risk; for treatment of advanced ovarian cancer in adult women who have failed a first-line platinum-based chemotherapy regimen."

A CEP was presented for the active substance. The information presented on development, manufacture and control of the finished product is generally acceptable. However, at the time of the CHMP opinion, there were a number of unresolved quality issues having no impact on the Benefit/Risk ratio of the product that remained to be addressed, i.e. demonstration of the discriminatory nature of the proposed dissolution method against variations in manufacturing process parameters settings, level of detail in the manufacturing process description for the finished product and further revision of the finished product specification (justification of the use of SPAN for PSD, and revision of the limits proposed for assay for cholesterol, HSPC, MPEG-DSPE impurities and LPC at release and/or shelf-life in accordance with Doxolipad batch analysis data). In addition, the applicant is strongly advised to introduce a non-sterilising filtration step after preparation of the doxorubicin/sucrose solution to reduce bioload of the bulk of doxorubicin/sucrose in line with the PACMP presented. He should also consider investigating potential quantitative methods for the control of impurities in HSPC and MPEG-DSPE.

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

Proving equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator liposomal product is considered a step-wise approach which in addition to the pharmacokinetic study also takes account of quality and non-clinical comparison, where appropriate. The bioequivalence study forms the pivotal basis of this application. This was a randomised open-label crossover study of bioequivalence and safety of doxorubicin hydrochloride liposome injection formulations in patients with advanced carcinoma of the ovary. The study design was considered adequate to evaluate the bioequivalence of this formulation and was in line with the respective European requirements. The choice of dose, sampling points, overall sampling time as well as wash-out period was considered adequate. The analytical method was validated. The pharmacokinetic and statistical methods applied were adequate.

The study showed that doxolipad met the bioequivalence acceptance criteria for Cmax and AUCs (AUC0-t and AUC0-inf) with regards to liposome encapsulated doxorubicin, total doxorubicin, and doxorubicinol, except for the AUC0-inf of doxorubicinol as its 90% CI of geometric mean could not be estimated. The free form of doxorubicin met the bioequivalence acceptance criteria for AUC0-inf (84.44% - 122.83%) between Doxolipad and Caelyx. However, the acceptance criteria were not met for Cmax (93.58% - 149.03%) and AUC0-t (95.93% - 138.24%) at a 90% CI. Therefore, bioequivalence of free (un-encapsulated) doxorubicin between Doxolipad and Caelyx has not been established.

In conclusion, similarity in terms of efficacy and safety between Doxolipad and Caelyx has not been sufficiently established as in the submitted bioequivalence study the 90% confidence intervals for free (un-encapsulated) doxorubicin GMR AUC0-t and Cmax were not within the 80.00-125.00% standard bioequivalence criteria. Therefore, it is not possible to establish a positive benefit -risk balance for Doxolipad.

4. Recommendation

Similarity with authorised orphan medicinal products

The CHMP is of the opinion that Doxolipad is not similar to Zejula and Yondelis within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy for Doxolipad as monotherapy for patients with metastatic breast cancer, where there is an increased cardiac risk and for treatment of advanced ovarian cancer in women who have failed a first-line platinum-based chemotherapy regimen, the CHMP considers by consensus that similarity between the above mentioned medicinal product and Caelyx, a liposomal formulation of doxorubicin hydrochloride, is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the marketing authorisation for the above mentioned medicinal product. The CHMP considers that:

- Similarity in terms of efficacy and safety between Doxolipad and Caelyx, which was considered an appropriate comparator to establish quality, non-clinical and clinical comparability, has not been sufficiently established as it remains that in the submitted pharmacokinetic bioequivalence study, the 90% confidence intervals for free (un-encapsulated) doxorubicin GMR AUC0-t and Cmax were not within the 80.00-125.00% standard bioequivalence criteria. Therefore, it is not possible to establish a positive benefit risk balance for Doxolipad.

5. Re-examination of the CHMP opinion

Following the CHMP conclusion that Doxolipad was not approvable considering similarity in terms of efficacy and safety between Doxolipad and Caelyx has not been sufficiently established, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

Detailed grounds for re-examination submitted by the applicant

The applicant presented in writing and at an oral explanation detailed grounds for re-examination which are summarised below.

Ground 1: Bioequivalence of free doxorubicin between Doxolipad and Caelyx

Doxolipad Pharmacological / Therapeutic effect – Mechanism of action

Liposomes containing doxorubicin are very well characterized preclinically and clinically with several thousand research articles published. The wealth of literature has made the physicochemical parameters, pharmacokinetics, biodistribution, toxicology and therapeutic effects of doxorubicin liposomes very well established in both preclinical models and in clinic.

- Similar to Caelyx, Doxolipad is a concentrate for solution for infusion administered by IV and is therefore bioavailable.
- Encapsulation in liposomes modifies the pharmacokinetics and biodistribution of doxorubicin by substantially enhancing circulation half-life and by lowering renal clearance and vascular adsorption (Gabizon 1994, Harrington 2001, Gabizon 2003). Doxorubicin encapsulated in liposomes therefore has a substantially longer half-life as long as it remains encapsulated. Doxolipad and Caelyx provide identical circulation half-life of the total amount of doxorubicin in patients as measured by the total doxorubicin on plasma from patients. Furthermore, it is well established that the tumour accumulation of doxorubicin liposomes is proportional to blood circulation half-life and that this correlates with the therapeutic effects pre-clinically and clinically (Gabizon 1988, Gabizon 1994)
- Liposomes induce greater doxorubicin accumulation in tumours compared to free drug (Gabizon 1994, Laginha 2005)
- Liposomes slowly release doxorubicin keeping systemic drug concentration low (Gabizon 1994, Laginha 2005)
- Liposomes improve doxorubicin therapeutic effect by reducing systemic peak free doxorubicin concentrations (which is determined by the metabolite doxorubicinol) while enhancing doxorubicin AUC within the tumour (Gabizon 1994, Amantea 1997, Laginha 2005)

Therefore, a small difference in free doxorubicin concentrations in blood between Caelyx and Doxolipad will not alter the drug's therapeutic effect. More than 95% of the doxorubicin is encapsulated in the liposomes at all times after intravenous infusion until the blood concentration of liposomal doxorubicin is <5% of injected dose.

Bioequivalence clinical study

TLC177.6, the pivotal BE study of Doxolipad (TLC-Doxo) against Caelyx is designed as an open-label, two-period, two-way, crossover study of the BE and safety of single dose of TLCDoxo and Caelyx in patients with advanced ovarian cancer (**Figure 18**). The study design and methods are described in section 2.4.2 of this report.

Doxolipad met the BE acceptance criteria for Cmax and AUCs (AUC0-t and AUC0-inf) for liposome-encapsulated doxorubicin, total doxorubicin, and doxorubicinol, with the exception of the AUC0-inf of doxorubicinol as its 90% CI of geometric mean could not be estimated.

The levels of total doxorubicin measured in the BE clinical study were valid and within the accepted range. The applicant referred to results of the BE study previously presented, in particular to the mean plasma concentrations and PK parameters of liposome-encapsulated doxorubicin, total doxorubicin, free doxorubicin and doxorubicinol respectively (bioequivalence population).

The free form of doxorubicin met the BE acceptance criteria for AUC0-inf (84.44% - 122.83%) between Doxolipad and Caelyx although the acceptance criteria were not met for Cmax and AUC0-t at a 90% CI.

A summary of 90% confidence intervals between test and reference in pivotal study TLC177.6 is presented in **Table 25**.

Therefore, whereas BE for one component of the PK study (free doxorubicin) was not met, the similarity of Doxolipad with reference to Caelyx was established overall. Free doxorubicin plasma concentration is however very challenging to measure in the presence of liposome doxorubicin in the blood, as further described below, and it should be stressed that measurement of free doxorubicin has not been carried out successfully in the original clinical literature on liposomal doxorubicin either (Gabizon 1994, Amantea 1997).

In addition, a small difference in free doxorubicin plasmatic concentrations between Caelyx and Doxolipad will not alter the therapeutic effect of Doxolipad with respect to the reference product (refer to the section above).

Separation and analysis method limitations

The separation of free doxorubicin from liposomal doxorubicin is required to measure free doxorubicin in plasma samples. The applicant noted that the separation, isolation and analysis methods were considered adequate by the Agency. The Applicant has showed that free doxorubicin is separated and measured adequately in plasma.

However, measurements of free plasmatic doxorubicin in patients are difficult to achieve and results are variable. This variability can be explained by:

- Measurement of a free form in the presence of a much more abundant encapsulated form: In the PK parameter analysis, the liposome-encapsulated doxorubicin showed a Cmax of 40.7 and 41.4 ug/ml for Doxolipad and Caelyx, respectively; whereas free doxorubicin Cmax was measured at 1.23 and 0.951 ug/mL for Doxolipad and Caelyx, respectively. TLC used the solid phase extraction (SPE) centrifugation method for the separation of doxorubicin components, which is considered to only create very low perturbations of the liposomes due to the speed of separation (1 min). However, the process of separating and analyzing the doxorubicin components in human plasma, i.e. liposome-encapsulated vs free doxorubicin, is challenged by potential small variations during sample handling before analysis, as well as during the separation method itself in the presence of plasma. Liposome-encapsulated doxorubicin is present at an approximately 40 times higher concentration in the patient plasma compared to free doxorubicin, at all measurement times. This creates a very high demand on the separation method for providing sufficient control to make it reproducible for measuring free doxorubicin in plasma.

  Even a very small (1-2%) release of doxorubicin from the liposomes in plasma, during sample handling and pre-analytical steps (e.g. during centrifugation), would dramatically influence the measurement of free doxorubicin concentration. At Cmax for Caelyx where liposome-encapsulated doxorubicin is 41.4 ug/mL and free doxorubicin is 0.951 ug/ml, a 2% release of doxorubicin from liposomes would correspond to a 41.4*0.02=0.828 ug/ml over-estimation of the free doxorubicin concentration, which represents 87% of the measured concentration of the free drug. This means that during the handling and separation of liposome-encapsulated doxorubicin from free doxorubicin in plasma, a variation in free drug release as low as 1% would lead to approximately 50% variation in the measurement results when comparing Cmax of Doxolipad and Caelyx. For further reference, if a similar calculation is carried out with AUC0-t of Caelyx liposome-encapsulated doxorubicin vs free doxorubicin, then a 2% release from liposomes would correspond to 103% of the total measured free doxorubicin. In addition, similar calculations on the Doxolipad patient group lead to the same conclusion, that free doxorubicin cannot be reliably measured within the CI necessary to fully meet the BE for free

doxorubicin as it is not feasible to eliminate such small variations associated with sample handling and separation steps.

It is therefore difficult and challenging to carry out reproducible measurements of free doxorubicin in the patient plasma. Currently, there are no alternative methods to make the separation of doxorubicin more reliable. The method used in the present study is state-of-the-art in the field.

- Patient characteristics: cancer patients with comorbidity and other ongoing treatments (access to healthy volunteers is not possible for this study) will also lead to data variability;

The ratio of Cmax reaching 149% in the upper limit of its 90% CI (instead of 144%) does not constitute a risk tipping the balance on the negative side, given the small amount of free doxorubicin compared to the encapsulated quantity that circulates.

Therefore, the applicant considered that BE was not obtained for free doxorubicin because of technical issues in measuring free doxorubicin in patient plasma rather than an actual pharmacological difference between Doxolipad and Caelyx.

For these reasons, the primary metabolite of doxorubicin, i.e. doxorubicinol, is considered a preferred alternative for estimating the free drug fraction by the applicant.

Metabolite measurement

Doxorubicinol is a better surrogate to measure free drug than directly measuring free doxorubicin as liposomes do not contain doxorubicinol that can leak and perturb measurement results.

Doxorubicinol is very well established as the most important metabolite of doxorubicin formed from reduction of ketone 13-C. Even though doxorubicinol's Cmax is substantially lower than doxorubicin's, mean AUC0-inf values for doxorubicinol and doxorubicin have been reported to be similar in cancer patient plasma upon IV administration of free doxorubicin (298 (±91.1) and 566 (±103) (ug.h/L) (±StdDev), respectively) (Joerger 2005).

Notably, the Agency, in the 2012 Scientific advice on non-clinical aspects acknowledged that the challenges associated with the separation and analysis of free doxorubicin could be overcome by measuring total doxorubicin and doxorubicinol concentrations: *"It is recognized that the extraction procedure could disrupt the liposomes and hence only total doxorubicin may reliably be measured. If every effort to develop a method that measures free and encapsulated doxorubicin in tissue has failed, the feasibility of measuring total doxorubicin and its main metabolite doxorubicinol in tissues and free, encapsulated and total drug in plasma should be considered. (…) Nevertheless, doxorubicinol could be determined as a measure of liberated doxorubicin but also as a control for unexpectedly high release"* (EMA/CHMP/SAWP/3655/2013).

Thus, with the technical difficulties presented by measuring free doxorubicin in patient plasma in the presence of liposome-encapsulated doxorubicin, the high correlation between doxorubicinol and doxorubicin makes doxorubicinol an appropriate surrogate for doxorubicin in the clinical study for PK parameter measurements.

Conclusions

The **guideline on liposomal IV product** (EMA / CHMP / 806058/2009 / Rev. 02) states "***Similarity should be demonstrated*** *for the total, encapsulated and unencapsulated drug.* ***Generally,*** *the 90% confidence intervals of Cmax, AUC0-inf and AUC0-t ratios should be within 80 - 125%.*"

Here, the 90% CIs are within these limits for the total form and the encapsulated form, and for the metabolite doxorubicinol (AUC0-inf for doxorubicinol is technically not measurable).

In addition, the AUC0-inf of the free form fall within the BE 90% CI BE margins and the amount of the free form is very small (<2% of the total) as discussed above. The 90% Cis are therefore **"generally**" in the desired intervals, except Cmax and AUC0-t of the free form. Similarity of Doxolipad is therefore demonstrated and sufficient, provided safety is ensured.

Ground 2: Toxicity and safety of Doxolipad

Introduction

i. Low risks associated with liposomal doxorubicin

Liposomal formulations allow a greatly reduced systemic exposure to doxorubicin and therefore limit the know cardiotoxicity of free doxorubicin (Gabizon 2004). In the present study TLC177.6, the amount of free drug (Cmax) was measured to be within an acceptable range for which there is no safety concern. In addition, the Cmax of free doxorubicin is potentially overestimated due to leakage associated with the separation step, as described above.

In addition, the 90% CIs of Cmax and AUC0-t of the free form reach 149% and 138%, respectively, are close to, or even within, the interval that could be accepted for a product with high intraindividual variability. In the present study, intraindividual variability equals 55% for Cmax and 42% for AUC0-t. However, a 0.70-1.44 interval is often accepted for Cmax of highly variable products.

ii. Low risks associated with Doxorubicinol

Toxicologically, doxorubicinol is the most important metabolite of doxorubicin. Notably, plasmatic doxorubicinol concentrations are highly correlated to free doxorubicin concentrations (Joeger 2005) and doxorubicinol has been found to be more cardiotoxic than doxorubicin (Olson 1988).

However, in the present clinical study, doxorubicinol levels were low and close to the detection level.

Non-clinical safety

The standard array of safety pharmacology tests was not conducted as Caelyx and doxorubicin hydrochloride (its active ingredient) have well-characterized safety and efficacy profiles.

However, one single dose toxicity and two repeat dose toxicity studies were conducted in rats. Based on the results of mortality, clinical signs, body and organ weight change, cTroponin I (cTnI) concentration, gross findings and cardiac histopathology, it was concluded that Doxolipad and Caelyx show a similar toxicity profile.

With respect to cardiotoxicity in particular, a high cTnI level observed in conjunction with a decreased heart weight, myocardium vacuolation and myocarditis was observed at a cumulative dose of 12 mg / kg for both Doxolipad and Caelyx groups. Therefore, Doxolipad and Caelyx findings were comparable with respect to cardiotoxicity, clinical signs and mortality.

According to the results from the single dose and the two repeat dose toxicity studies, Doxolipad and Caelyx were considered similar with respect to their toxicity profiles.

Clinical safety

Doxolipad was developed to have the same indications and posologies as Caelyx. For breast and ovarian cancer, the dosing schedule is 50 mg/m^2 once every 4 weeks. The patients with advanced carcinoma of the ovary from three BE studies (TLC177.0, TLC177.2, TLC177.6) were included in the safety population. In total, 84 subjects received Doxolipad treatment and 85 subjects received Caelyx. The average dose of drug exposure was 86.88 mg (±9.64) for Doxolipad and 86.99 mg (±10.03) for Caelyx. As of today, no post-marketing data is available as Doxolipad has not been marketed in any country.

There were fewer adverse events (AE) in the Doxolipad group of patients as compared to Caelyx. Most AEs were of mild or moderate grade. In the Doxolipad population there were fewer episodes of febrile neutropenia, cardiac disorders, mouth ulcerations but more cases of drug hypersensitivity and palmar-plantar erythrodysesthesia (PPE) episodes. According to the safety data of Doxolipad, no patient discontinued treatment due to PPE, stomatitis or myelosuppression.

Overall, no new safety issues emerged from the presented pivotal study.

Conclusion

Therefore, Doxolipad does not present any concerns with respect to toxicity and safety.

Applicant's overall conclusion on the benefit/risk balance of Doxolipad

Benefits

Doxolipad is generic product of the originator pegylated liposomal doxorubicin with an identical formulation and similar manufacturing process. First, pegylated liposomal doxorubicin is a highly effective anti-tumour agent. In addition, similarity can be established between Doxolipad and Caelyx as shown by the BE of total doxorubicin and liposomal doxorubicin levels in patients' plasma. Second, Doxolipad significantly reduces safety concerns with respect to cardiotoxicity as compared to other doxorubicin formulations.

Risks

Palmar-plantar erythrodysesthesia (PPE), stomatitis/mucositis/nausea and myelosuppression are known to be caused by pegylated liposomal doxorubicin, as a cytotoxic agent. However, the observed safety profiles of Doxolipad and Caelyx were also comparable. Overall, no new safety issues emerged from the presented pivotal study. The risk management plan and post marketing safety studies will ensure a close safety follow-up.

Conclusion on the benefit/risk ratio

Doxolipad was shown to be very comparable to Caelyx with respect to total doxorubicin levels and liposomal doxorubicin levels in plasma. Less than 5% of the doxorubicin dose was found as free doxorubicin in blood at any time point after infusion, and the Cmax of free doxorubicin was approximately 2% of the liposome-encapsulated doxorubicin for both Doxolipad and Caelyx.

The ratio of Cmax reaching 149% in the upper limit of its 90% CI (instead of 144% which would probably be accepted without any discussion – refer to point above, section 6.a.i) does not constitute a risk affecting the benefit / risk ratio, given the small amount of free doxorubicin compared to the encapsulated quantity that circulates. This shows that the systemic exposure to the infused doxorubicin is minimal for both Doxolipad and Caelyx, which provides safety to the patient.

Based on the PK profile of Doxolipad and Doxorubicin, and with reference to the relationship between PK and bio-distribution which is very well described in the literature for Caelyx, it is reasonable to conclude that the tumour accumulation of the drug will be the same for Doxolipad and Caelyx, and therefore that the treatment benefit will be the same for Doxolipad as for Caelyx.

Doxolipad therefore provide a positive benefit to risk relationship as a treatment substitute for Caelyx.

Additional expert consultation

Ad-hoc expert group consultation

Following a request from the applicant at the time of the re-examination, the CHMP convened an Ad-Hoc expert Group inviting the experts to provide their views on the CHMP grounds for refusal, taking into

account the applicant's response. The views of the experts on the questions posed are presented below.

1. The experts are invited to provide their views on the CHMP grounds for refusal, in particular on the need to show bioequivalence for the un-encapsulated doxorubicin, in view of the grounds for re-examination submitted by the applicant.

The experts were overall in agreement with the CHMP grounds for refusal. Bioequivalence of free (un-encapsulated) doxorubicin has not been established as main PK parameters (AUC0-t and Cmax) were not within 80.00-125.00% (90% CI) standard bioequivalence criteria. Showing bioequivalence for the un-encapsulated doxorubicin is needed to ensure comparable performance of the liposomal preparations. This reflects current standards for establishing bioequivalence of this liposomal doxorubicin formulation with the innovator product, as stated in the CHMP guideline ('Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance; EMA/CHMP/800775/2017). This guideline in turn reflects the need to demonstrate bioequivalence for both the encapsulated and the un-encapsulated doxorubicin in the context of using the results of the comparative study to bridge the safety and efficacy between the test and reference formulations.

Although the technical difficulties of reliably measuring concentrations of un-encapsulated doxorubicin are acknowledged, it is unclear if the study was designed taking adequate power calculations into account. Furthermore, from the data presented it appeared that important sources of variability were not well-controlled in terms of laboratory practices, at least in one centre, nor did the company provide sufficient evidence that those sources of variability were properly investigated.

2. Can the experts elaborate on the contribution of encapsulated doxorubicin as compared to un-encapsulated doxorubicin to biopharmaceutic drug performance in-vivo, including pharmacokinetics, tumour uptake and safety?

There is a vast literature on the pharmacokinetics, biodistribution, toxicology and pharmacodynamic effects of doxorubicin. There are clinical data showing differences in efficacy and safety (e.g., substantial reduction in cardiotoxicity) for encapsulated doxorubicin as compared to free doxorubicin.

Whereas the expert group agreed that the anti-tumoural effects (efficacy) are driven by encapsulated doxorubicin this was not clear for the adverse effects, e.g. cardiotoxicity. Whereas uptake of liposomes in the macrophages of the RES may be important, toxicity in other tissue may depend on either free or encapsulated doxorubicin or both. It was agreed that the demonstration of bioequivalence for un-encapsulated doxorubicin *in vivo* provides important information about the release behaviour of the liposomes and is therefore considered a critical PK parameter for the whole bioequivalence exercise.

3. In practice, can un-encapsulated doxorubicin concentrations be reliably measured in plasma i.e. are assays available to this effect?

Although the technical difficulties are acknowledged, related especially to liposomal destabilisation during the processing of samples, a reliable measurement is considered possible based on the experience of the experts.

In addition, release of doxorubicin from the liposomes by and during sample preparation should occur for both test product and reference product in a similar way, i.e. increase variability but not result in differences between test and reference product.. The observed differences in the point estimates (e.g., Cmax GMR = 118.09%; AUC 0-t= 115.16%) cannot be attributed solely to problems of handling of samples in different centres.

4. The views of the experts are sought on the contribution of demonstration of bioequivalence for the metabolite (doxorubicinol) to the overall comparability exercise.

The approach presented is of scientific interest although doxorubicinol concentration cannot be considered as an established surrogate for doxorubicin concentration. Thus, this analysis could provide some corroborative information but cannot be used to establish bioequivalence for un-encapsulated doxorubicin, which should be measured directly.

PKWP consultation

The CHMP has also recommended a PKWP consultation and the PKWP was invited to address the same questions as listed above. The views of the PKWP are presented below.

1. The experts are invited to provide their views on the CHMP grounds for refusal, in particular on the need to show bioequivalence for the un-encapsulated doxorubicin, in view of the grounds for re-examination submitted by the applicant.

This comment refers to the final conclusion on the bioequivalence study comparing Doxolipad and Caelyx. Accordingly, the CHMP summarized: "Similarity in terms of efficacy and safety between Doxolipad and Caelyx, which was considered an appropriate comparator to establish quality non-clinical and clinical comparability, has not been sufficiently established as it remains that in the submitted pharmacokinetic BE study, the 90% confidence intervals for free (un-encapsulated) doxorubicin GMR AUC0-t and Cmax were not within the 80.00-125.00% standard BE criteria. Therefore, it is not possible to establish a positive benefit risk balance for Doxolipad".

This conclusion is basically in line with the current product-specific EMA guideline on liposomal doxorubicin (https://www.ema.europa.eu/en/documents/scientific-guideline/pegylated-liposomal-doxorubicin-hydrochloride-concentrate-solution-2-mg/ml-product-specific-bioequivalence-guidance_en.pdf) that reflects the PKWP current thinking specific to liposomal doxorubicin and as such is an update on the earlier and more general 'Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product' (EMA/CHMP/806058/2009/Rev. 02). The product specific guideline requires bioequivalence for encapsulated and un-encapsulated doxorubicin within usual acceptance criteria as the PKWP considers that when it comes to bioequivalence, plasma levels of both are required to provide reassurance on the behaviour of the liposome (e.g. if the liposomes 'leaks' then this will be reflected in the plasma concentration of un-encapsulated doxorubicin and similarly if release from the liposome is impaired) and therefore on similar efficacy and safety when compared with those of the reference product.

The company's arguments in the grounds for re-examination are not agreed regarding the assessment of failed bioequivalence results for the free (un-encapsulated) compound. Employing the bioequivalence approach means to compare the biopharmaceutical performance of products in vivo, i.e. we are interested in ensuring the same biopharmaceutical behaviour in a few subjects to extrapolate to all potential patients and the clinical relevance of the differences cannot be assessed in a few subjects. Using this relatively simple and straightforward concept for complex liposomal formulations requires particularly careful assessment of in vivo release processes including e.g. partial AUCs. It is therefore highly questionable whether it can be argued that exceeding acceptance criteria are likely clinically irrelevant since the outcome is on similarity rather than on a range of clinically relevant differences which has no definition.

2. Can the experts elaborate on the contribution of encapsulated doxorubicin as compared to un-encapsulated doxorubicin to biopharmaceutic drug performance in-vivo, including pharmacokinetics, tumour uptake and safety?

In short: it is agreed, well known and widely published that the liposomal formulation of doxorubicin determines its pharmacokinetics and hence its efficacy and safety, i.e. distribution is confined to the vascular fluid, plasma clearance is slowed down, and uptake through leaky tumour sites increased. The safety is improved since the free drug concentration is relatively low, and substantially reduced cardiotoxicity is undisputed. A difference in behaviour of liposomes between different formulations that results in an increased concentration of un-encapsulated doxorubicin is therefore particularly relevant for safety.

3. In practice, can un-encapsulated doxorubicin concentrations be reliably measured in plasma i.e. are assays available to this effect?

Potential challenges are acknowledged regarding the correct sample processing for the purpose of reliable bioanalytical measurements, in particular rupture of liposomes. However, available data indicate that quantification is possible. It is also the case that a number of liposomal doxorubicin products have been approved in the US by demonstrating bioequivalence for both encapsulated and un-encapsulated doxorubicin within usual acceptance criteria.

4. The views of the experts are sought on the contribution of demonstration of bioequivalence for the metabolite (doxorubicinol) to the overall comparability exercise.

The general reflection paper on liposomal 'generics' mentions that measurement of a metabolite might facilitate the assessment and comparison of active substance release rate from the liposomal formulation. However, this does not seem the case with liposomal doxorubicin since plasma concentrations of doxorubicinol are substantially lower than un-encapsulated drug concentrations with tmax after approximately 150 h. Hence, metabolite concentrations are likely not a sensitive means to compare the 'active substance release rate from the liposomal formulation' as required, all the more the un-encapsulated drug is considered quantifiable.

Overall conclusion on grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant and considered the views of the Ad-hoc expert group and PKWP.

Concerning clinical ground 1: Bioequivalence of free doxorubicin between Doxolipad and Caelyx

It is acknowledged that the general pharmacological and therapeutic effects of PEG-liposomal-encapsulated doxorubicin have been well described in the literature. It is also agreed, that liposomal doxorubicin has particularly modified pharmacokinetics leading to improved efficacy and safety as compared to the non-liposomal drug. However, this does not address the question of similar biopharmaceutic product performance of a 'generic' product as compared to a reference product which is considered a step-wise approach based on pharmaceutical product comparability as the first step.

Employing the bioequivalence approach is considered a biopharmaceutic tool in order to compare the quantitative product performance irrespective of clinical contributions of relevant single entities. Of note, the availability after i.v. administration represents the availability of the liposomal formulation rather than the drug substance. Contrary to products where the active substance is in the form of a simple solution, liposomal medicinal products have formulation and manufacturing-specific distribution characteristics after intravenous administration. Therefore, for complex formulations like liposomes, comparing the biopharmaceutic product performance *in vivo* has to be addressed in the most sensitive way. Demonstrating bioequivalence of un-encapsulated doxorubicin is considered part of this biopharmaceutic comparability approach and needed to ensure comparable performance of the liposomal preparations.

The trial design was a two-period, two-way crossover study which is an acceptable design of trial for standard bioequivalence studies with a within-subject CV% of $\leqslant$ 30. For bioequivalence studies with a

higher CV% such as the current study where the intra-subject variability, %CV for the free doxorubicin was 55.4%, 42.4% and 37.2% for Cmax, AUC0-t and AUC0-inf, the bioequivalence margins of 80-125% may be widened with a replicate design study (3 period or 4 period crossover). As the applicant has not performed such a replicate design study the bioequivalence margins of 80-125% apply. In addition, widened margins would only apply to Cmax and not AUC (bioequivalence was not demonstrated for Cmax and AUC0-t for free (un-encapsulated) doxorubicin).

According to the study protocol, the primary objective was the assessment of the bioequivalence of two doxorubicin hydrochloride liposome injection formulations: Doxolipad 2 mg/ml as test product and Caelyx 2 mg/ml as the Reference Drug by conducting a pharmacokinetic analysis on free doxorubicin (FD) and encapsulated doxorubicin (LED).

The secondary objective was to analyse concentrations of doxorubicinol and total doxorubicin in plasma and to assess the safety of a single dose of Doxolipad.

The primary endpoints were met for encapsulated doxorubicin, however for free doxorubicin only the end-point for AUC0-inf was contained within the bioequivalence limits of 80-125% while the upper limits were breached for Cmax and AUC0-t. This is not in line with current standards since bioequivalence should be demonstrated for free (un-encapsulated) and encapsulated doxorubicin in the context of using the results of the comparative study to bridge the safety and efficacy between the test and reference formulations. Un-encapsulated drug concentrations must be achieved by means of appropriate bioanalytical methods.

Potential challenges are acknowledged regarding the correct sample processing for the purpose of reliable bioanalytical measurements of the unencapsulated doxorubicin due to quantities being only a small proportion of the total and rupture of liposomes during sample processing. However, available data indicate that quantification is possible.

However, the comparison of concentrations of metabolite between products cannot replace comparisons for encapsulated drug plus the un-encapsulated drug. In the case of liposomal doxorubicin, the encapsulated drug plus the un-encapsulated compound are considered most relevant to better reflect biopharmaceutic product performance and to detect possible formulation differences if they are there.

In principle, bioequivalence should be evaluated based upon measured concentrations of the parent compound, rather than a metabolite. The reason for this is that Cmax of a parent compound is usually more sensitive to detect differences between formulations in absorption rate than Cmax of a metabolite. This reflects current standards for establishing bioequivalence as reflected in the CHMP Guideline on the Investigation of Bioequivalence Doc. (Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **)).

This approach is not related to the pharmacological or toxicological activity of parent compound or metabolite but it is related to the sensitivity in detecting differences between formulations. In addition, doxorubicinol concentrations are substantially lower than un-encapsulated drug concentrations with tmax after approximately 150 h and the assessment of doxorubicinol is considered as less sensitive than the assessment of the un-encapsulated drug.

In line with the study protocol of TLC177.6 study, the analysis of doxorubicinol was considered only as a secondary objective. Doxorubicinol was a secondary parameter to confirm no major deviations and will be presented with descriptive statistics only.

Therefore, the comparison of concentrations of metabolite between products cannot replace comparisons for encapsulated drug plus the un-encapsulated drug. In the case of liposomal doxorubicin, the encapsulated drug plus the un-encapsulated compound are considered most relevant to better reflect biopharmaceutic product performance and to detect possible formulation differences if they are there.

Overall, the 90% confidence intervals for encapsulated doxorubicin, Cmax and AUC were within the 80-125% standard bioequivalence criteria. However, the 90% confidence intervals for the free (un-encapsulated) doxorubicin, Cmax and AUC were not within the standard bioequivalence criteria, 80-125%. Therefore, the bioequivalence of free (un-encapsulated) doxorubicin has not been demonstrated between Doxolipad and Caelyx and it is not possible to conclude that Doxolipad is sufficiently similar to Caelyx with regard to efficacy and safety.

Concerning clinical ground 2: Toxicity and safety of Doxolipad

In general, it can be agreed that liposomal formulations allow a greatly reduced systemic exposure to doxorubicin and therefore limit the know cardiotoxicity of free doxorubicin. A more favourable profile in terms of cardiotoxicity as compared to conventional formulations of doxorubicin was shown in studies submitted with the Caelyx marketing authorisation application (see Caelyx EPAR). However, the safety of Doxolipad can be only considered comparable to safety of Caelyx if bioequivalence between Doxolipad and Caelyx is shown.

The applicant has highlighted that a 70-144% confidence interval is often accepted for Cmax of highly variable products. This theoretically could be accepted however such a widening should be requested prospectively. In addition, as the applicant has not performed such a replicate design study the bioequivalence margins of 80-125% apply. Furthermore, widened margins only apply to Cmax and not AUC (bioequivalence was not demonstrated for Cmax and AUC0-t for free (un-encapsulated) doxorubicin).

One requirement for two products to be claimed as bioequivalent is that the test and reference products have the same qualitative and quantitative composition. Two other criteria should be met for liposomal products, first that comparable amount of the encapsulated product is reaching the tissues and second that the release from the encapsulated product is comparable. Since it is known that the safety and efficacy of liposomal doxorubicin is influenced by its tissue distribution and a limiting factor of doxorubicin is cardiotoxicity caused by doxorubicinol, the applicant conducted one single and two repeat-dose toxicity studies in rats.

Looking at the heart, doxorubicin concentration collected from TLC177 as the test product, showed an enormous inter-animal variability with higher variability in the Cmax results than in the AUClast results. This is owed to the inter-animal variability of Cmax relying on the average of three animals while the inter-animal variability of AUClast relied on the average of three animals per time point averaged over the duration of the study. Nevertheless, further analysis through the use of confidence intervals, t-test and data variability analyses, suggested a similarity with respect to distribution and by increasing the sample size, the similarity of the heart tissue distribution confirmed this outcome.

For further determination of comparability, tissues of particular importance, meaning those where doxorubicin exerts its pharmacological action, such as kidney, spleen, liver, tumour (here: 4T1 breast cancer cells), heart and skin, were analysed. Looking at the data provided, differences could be observed. However, these variations were within an acceptable range (less than 2-fold).

Evaluating the doxorubicinol levels from two performed studies revealed the following: doxorubicinol heart mean Cmax ranged from 2.23 to 3.22 ng/mL for the TLC177 treatment group and 1.96 to 3.66 ng/mL for Caelyx treatment groups. Doxorubicinol heart mean AUClast ranged from 228 to 513 hr·ng/mL for the TLC177 treatment groups and 215 to 517 hr·ng/mL for Caelyx treatment groups. These values were close to each other and therefore indicate similarity.

The values of doxorubicin and doxorubicinol exposure in plasma and tissue obtained in the non-clinical studies between TLC177 and Caelyx differ less than 2-fold. From a non-clinical point of view, both products can be regarded as sufficiently similar. However, the non-clinical data is not considered to be robust enough/conclusive, alone, to demonstrate similarity between Doxolipad and Caelyx.

In terms of clinical aspects, the safety of Doxolipad was examined in three BE studies (TLC177.0, TLC177.2, TLC177.6). There were fewer adverse events (AE) in the Doxolipad group of patients as compared to Caelyx. However, safety data from these studies are very limited and the fact that no new safety issues emerged from the presented pivotal bioequivalence study is insufficient to claim comparable safety profile between the innovator and generic product. The results of the PK analysis are what ultimately determines if such a bridge can be made.

With respect to safety, it is considered that AUC for the free level of doxorubicin is an important parameter. Furthermore, as highlighted by the experts, whereas uptake of liposomes in the macrophages of the RES may be important, toxicity in other tissue may depend on either free or encapsulated doxorubicin or both.

Overall, the demonstration of bioequivalence for un-encapsulated doxorubicin *in vivo* provides important information about the release behaviour of the liposomes and is therefore considered a critical PK parameter for the whole bioequivalence exercise. As bioequivalence was not shown for the un-encapsulated doxorubicin, the bridge to comparable safety or efficacy cannot be concluded.

6. Benefit-risk balance following re-examination

Proving equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator liposomal product is considered a step-wise approach which in addition to the pharmacokinetic study also takes account of quality and non-clinical comparison, where appropriate. The bioequivalence study, which was a randomized open-label crossover study of bioequivalence of Doxolipad versus Caelyx in patients with advanced carcinoma of the ovary, forms the pivotal basis of this application.

The aim of the bioequivalence study is to use the primary PK endpoints falling within pre-specified limits to provide assurance on a lack of formulation differences rather than to directly assess the efficacy and safety of the test versus the reference products or to support conclusions about clinical relevance.

Importantly, the primary endpoints of the bioequivalence study evaluating Doxolipad versus Caelyx were met for encapsulated doxorubicin, however for free doxorubicin only AUC0-inf was contained within the bioequivalence limits of 80-125% while the upper limits were breached for Cmax and AUC0-t. Therefore, bioequivalence of free (un-encapsulated) doxorubicin between Doxolipad and Caelyx has not been established.

Although the potential technical difficulties of reliably measuring concentrations of un-encapsulated doxorubicin are acknowledged, it is unclear if the study was designed using adequate power calculations. Furthermore, from the data presented it appeared that important sources of variability were not well-controlled in terms of laboratory practices, at least in one centre, nor did the company provide sufficient evidence that those sources of variability were identified.

In addition, the observed differences in the point estimates for the unencapsulated doxorubicin (i.e., Cmax GMR = 118.09%; AUC 0-t= 115.16%) cannot be attributed solely to problems of handling of samples in different centres.

Demonstration of bioequivalence for the un-encapsulated doxorubicin is needed to ensure comparable performance of the liposomal preparations. A difference in behaviour of liposomes between different formulations that results in an increased concentration of un-encapsulated doxorubicin is particularly relevant for safety.

In conclusion, comparable efficacy and safety between Doxolipad and Caelyx cannot be established because the bioequivalence of free (un-encapsulated) doxorubicin between Doxolipad and Caelyx has not been demonstrated.

Therefore, it is not possible to establish a positive benefit risk balance for Doxolipad.

7. Recommendations following re-examination

Based on the arguments of the applicant and all the supporting data on safety and efficacy, the CHMP re-examined its initial opinion and in its final opinion concluded by consensus that similarity between the Doxolipad and Caelyx, a liposomal formulation of doxorubicin hydrochloride, is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the marketing authorisation for the above mentioned medicinal product.

The CHMP considers that:

- Similarity in terms of efficacy and safety between Doxolipad and Caelyx, which was considered an appropriate comparator to establish quality, non-clinical and clinical comparability, has not been sufficiently established as it remains that in the submitted pharmacokinetic bioequivalence study, the 90% confidence intervals for free (un-encapsulated) doxorubicin GMR AUC0-t and Cmax were not within the 80.00-125.00% standard bioequivalence criteria. Therefore, it is not possible to establish a positive benefit risk balance for Doxolipad.