28 June 2018
EMA/486480/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Vyxeos

International non-proprietary name: daunorubicin / cytarabine

Procedure No. EMEA/H/C/004282/0000

Note

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

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List of abbreviations

Abbreviation	Definition
7 + 3	7-day continuous infusion of cytarabine at a dose of 100 to 200 mg/m2/day in combination with an anthracycline for 3 days
ADR	Adverse Drug Reaction
AE	Adverse event
ALL	Acute lymphoblastic leukemia
ALT	Serum Alanine Aminotransferase, also s-ALAT
AML	Acute myeloid leukemia
AML-MRC	AML with myelodysplasia related changes
ANC	Absolute neutrophil count
APL	Acute Promyelocytic Leukaemia
Ara-CTP	Cytocine Arabinoside Triphosphate
Ara-U	1-β-d-arabinofuranosyluracil
AS	Active substance
ASMF	Active substance master file
AST	Serum Aspartate Aminotransferase, also s-ASAT
AUC	Area under the plasma concentration time curve
AUC_{last}	Area under the curve from time zero to the last measurable concentration
AUC_{tau}	Area under the plasma concentration time curve for the dosing interval
BLQ	Below Limit of Quantification
BSA	Body Surface Area
BSE	Bovine Spongiform Encephalopathy
CHMP	Committee for Human Medicinal Products
Chol	cholesterol
CI	Confidence interval
CL	Clearance
C_{max}	Maximum Concentration
CMMoL	Chronic Myelomonocytic Leukaemia
CNS	Central Nervous System
CPP(s)	Critical process parameters
CR	Complete remission
CrCL	Creatinine Clearance
CQA	Critical Quality Attributes
CRi	Complete remission with incomplete platelet or neutrophil recovery
CV	Coefficient of Variation
DCM	methylene chloride
DLT	Dose Limiting Toxicity
DNA	DeoxytiboNucleic Acid
DSC	Differential Scanning Calorimetry
DSMB	Data and Safety Monitoring Board
DSPC	distearoylphosphatidylcholine
DSPG(-Na)	Distearoylphosphatidylglycerol (sodium)
ECG	Electrocardiogram
ECHO	Echocardiography
EDTA	Ethylenediaminetetraacetic acid
EFS	Event-free survival
EMA	European Medicines Agency
ER	Exposure Response
FDA	Food and Drug Administration
GC	Gas Chromatography
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HDW	Hemoglobin concentration distribution width
HMA	Hypomethylating Agent

HPLC	High performance liquid chromatography
HRQoL	Health-related Quality of life
HSCT	Hematopoietic stem cell transplant
ICH	International Conference on Harmonisation
ICP-MS	Inductively coupled plasma mass spectrometry
ILS	Increase in life span
IP	Intraperitoneal
IPCs	In-process controls
IR	Infrared
ITT	Intent to Treat Population
IV	Intravenous
LC/fluorescence	High performance liquid chromatography with fluorescence detection
LC/MS	High performance liquid chromatography with mass spectrometry
LC/MS/MS	High performance liquid chromatography with tandem mass spectrometry
LC/UV	High performance liquid chromatography with ultraviolet detection
LVEF	Left ventricular ejection fraction
MCB	Master Cell Bank
MDS	Myelodysplastic syndrome
MLFS	Morphologic Leukemia-Free State
MPN	Myeloproliferative Neoplasm
MPS	Mononuclear phagocyte system
MST	Median Survival Time
MTD	Maximum tolerated dose
MUGA	Multigated acquisition scan
N/A	Not Available or Not Relevant
NMR	Nuclear Magnetic Resonance
OOS	out-of-specification
OS	Overall Survival
PDCO	Paediatric Committee
Ph.Eur.	European Pharmacopoeia
PIP	Paediatric Investigation Plan
PK	Pharmacokinetic
PP(s)	process parameters
PT	Preferred term
Q3Dx3	Once every 3 days for a total of 3 doses
QTcF	Fridericia's corrected QT-interval
QTTP	Quality Target Product Profile
RBC	Red blood cells
RDW	Red cells distribution width
RNA	RiboNucleic Acid
SAE	Serious adverse event
s-Bil	Serum Bilirubine Concentration
SCE	Sister chromatid exchange
s-Crea	Serum Creatinine Concentration
SD	Standard Deviation
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOI	Start of infusion
t-AML	Therapy related AML
TEA	triethanolamine
TEAE	Treatment-emergent adverse event
TL	Total Lipid
TLC	Thin layer chromatography
$t_{1/2}$	Elimination half time
t_{max}	time of the maximum concentration
TSE	Transmissible Spongiform Encephalopathy
ULN	Upper Limit of Normal (Reference Range)
UV	Ultraviolet
USP/NF	United States Pharmacopoeia/National Formulary

V	Volume of distribution
WBC	White blood cell
WCB	Working Cell bank
XR(P)D	X-Ray (Powder) Diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Jazz Pharmaceuticals Ireland Limited submitted on 2 November 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Vyxeos® (daunorubicin:cytarabine) liposome for injection, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004 . The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 23 July 2015.

Vyxeos (daunorubicin:cytarabine) liposome for injection, was designated as an orphan medicinal product EU/3/11/942 on 11 January 2012 in the following condition: treatment of acute myeloid leukaemia.

The applicant applied for the following indication: Vyxeos is indicated as monotherapy for the treatment of adults with high-risk acute myeloid leukaemia (AML) as defined by therapy-related AML (t-AML) or AML with myelodysplasia-related changes (AML-MRC).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that daunorubicin / cytarabine was considered to be a known active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical data and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Vyxeos as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/Find medicine/Human medicines/European public assessment reports. (http://www.ema.europa.eu/ema/index.jsp?curl=/pages/medicines/human/medicines/004282/human_med_002273.jsp&mid=WC0b01ac058001d124)

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0299/2017 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0299/2017 was not yet completed as some measures were deferred.

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 submitted a critical report addressing the possible similarity with authorised orphan medicinal products.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP:

Scientific advice	**date**	**Area**
EMA/CHMP/SAWP/727423/2011	22 September 2011	quality, non-clinical and clinical
EMA/CHMP/SAWP/833734/2015	17 December 2015	non-clinical and clinical

1.2. Steps taken for the assessment of the product

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

Rapporteur: Robert James Hemmings Co-Rapporteur: Tuomo Lapveteläinen

The application was received by the EMA on	2 November 2017
Accelerated Assessment procedure was agreed-upon by CHMP on	14 September 2017
The procedure started on	23 November 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur declared that they had completed their assessment report in less than 80 days	22 January 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Co-Rapporteur declared that they had completed their assessment report in less than 80 days	23 January 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	29 January 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	8 February 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	20 February 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	22 March 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	12 April 2018
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	24 April 2018

The applicant submitted the responses to the CHMP List of Outstanding Issues on	30 April 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	17 May 2018
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on The accelerated assessment procedure was reverted to a standard assessment procedure.	31 May 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	4 June 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	13 June 2018
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Vyxeos on	28 June 2018
The CHMP adopted a report on similarity of Vyxeos with Vidaza, Ceplene, Dacogen, Rydapt and Mylotarg (see Appendix 1) on	31 May 2018

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Vyxeos is intended for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukaemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC).

2.1.2. Epidemiology

AML is the most frequent form of leukaemia, accounting for approximately 25% of all leukaemias in adults in the Western world. The annual crude incidence of AML is 3.7 per 100000, and the number of new cases per year in European Union is estimated to be 18400. The estimated number of cases of AML in 2008 was 53486, and the prevalence after 5 years from diagnosis was 4.1 per 100000 (RARECARE 2016).

The incidence of AML increases sharply with age, ranging from 1.8 cases per 100000 people aged under 65 years of age to 17.6 cases per 100,000 people over 65 years of age. More than half of the subjects with newly diagnosed AML in developed countries are over 65 years of age, with a median age at diagnosis of 67, and AML is more common in men than in women.

Overall, the 5-year survival rate for AML is 19%. The mortality rate strongly correlates with age: 5-year survival rates are 3% to 8% in patients aged 60 years and older compared with 5-year survival rates of up to 50% for younger patients.

2.1.3. Biologic features/Aetiology and pathogenesis

Acute myeloid leukemia is a biologically heterogeneous disease that can be classified into 3 distinct categories based on clinical ontogeny: de novo AML which arises in the absence of an identified exposure or prodromal stem cell disorder (like in MDS). Secondary AML (s-AML) represents transformation of an antecedent diagnosis of myelodysplastic syndrome (AML-MRC) or myeloproliferative neoplasm (MPN), therapy-related AML (t-AML) develops as a late complication in patients with prior exposure to leukaemogenic therapies. Recently the presence several of several mutations (in SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR, or STAG2) were found to be highly specific for the diagnosis of s-AML (1).

High-risk AML constitutes a biologically distinct subset of disease and comprises a sizeable percentage of cases of adult AML. Based on retrospective review of large registries and given the lack of progress or variation in treatment over the past 25 years, a distinct profile of cytogenetic and molecular features can be used to assign risk. Although high-risk disease features cluster among clinical phenotypes, such as among patients over age 60, those with antecedent haematological disorders, and those who have received prior treatment with cytotoxic chemotherapy, high-risk karyotype or the expression of mutated flt3, kit, and other molecular markers explain both the poor response to induction chemotherapy and the high relapse rate previously attributed to clinical variables alone.

2.1.4. Clinical presentation, diagnosis and stage / prognosis

Acute myeloid leukaemia is a form of leukaemia – i.e., cancer of the white blood cells – characterised by infiltration of proliferative, clonal, abnormally differentiated, and occasionally poorly differentiated haematopoietic cells of myeloid lineage in the bone marrow, blood, and other tissues.

In AML, leukaemic blasts replace normal blood cells in bone marrow and peripheral blood, which leads to anaemia, neutropenia, and thrombocytopenia. This is associated with symptoms of fatigue, shortness of breath, disturbed wound healing, infections and bleedings. If left untreated, AML results in death within a few weeks to months.

AMLs are classified according to the World Health Organisation (WHO) classification from 2001, revised in 2008 and in 2016 (2). The classification incorporates morphological criteria, cytogenetic data, molecular genetics, immunophenotype data and clinical information into a diagnostic algorithm to delineate clinically significant disease entities.

The procedures used to diagnose and classify AML are: morphologic assessment of bone marrow specimens and blood smears (with ≥20% blasts in the bone marrow or peripheral blood being diagnostic of AML), analysis of the expression of cell-surface and cytoplasmic markers (by flow cytometry), identification of chromosomal findings (through cytogenetic testing), and screening for selected molecular genetic alterations. Following 2010 ELN recommendations several molecular markers are used as part of standard clinical practice for risk stratification (3):

- Alterations in nucleophosmin-1 (NPM1);
- Alternations in CCAAT/enhancer-binding protein alpha (CEBPA); and
- Alterations in Fms-like tyrosine kinase 3 (FLT3).

In addition to these three mutational screenings which are currently being used in routine practice, screening of several other mutations such as RUNX1, TP53, ASXL1 are proposed in the these current 2017 ELN recommendations.

Prognostic factors in AML can be subdivided into those that are related to the patient and those that are related to the disease. Patient-associated factors (e.g., increasing age, coexisting conditions, and poor performance status) commonly predict treatment-related early death, whereas disease-related factors (e.g., white-cell count, prior myelodysplastic syndrome or cytotoxic therapy for another disorder, and leukaemic-cell genetic changes including alterations in FLT3) predict resistance to current standard therapy.

2.1.5. Management

Treatment should be planned with curative intent whenever possible. Intensive chemotherapy is divided into an induction phase and consolidation. Potential candidates for allogeneic stem cell transplantation (ASCT) must be identified early at diagnosis or during induction. For more than four decades, the combination of cytarabine (100 to 200 mg/m^2/day) by continuous infusion on Days 1 through 7 together with daunorubicin (45 to 60 mg/m^2) on Days 1 through 3 (known as 7 + 3 regimen) has comprised the backbone of induction therapy. For patients who respond to induction therapy, post-remission options comprise haematopoietic stem cell transplantation (HSCT) or consolidation therapy (in the EU often with high-dose cytarabine). Although AML patients who receive HSCT generally have better outcome relatively few patients receive this potentially curative treatment because factors like older age, comorbidities, and toxicity of prior therapy prevent further treatment.

In the EU (European Union), approved agents include decitabine (Dacogen) which is authorised for the treatment of adult patients with newly diagnosed de novo or secondary AML, according to the WHO classification, who are not candidates for standard induction chemotherapy. Azacitidine (Vidaza) is also authorised for the treatment of adult patients who are not eligible for HSCT with AML with 20-30 % blasts and multi-lineage dysplasia, according to WHO classification and AML with >30% marrow blasts according to the WHO classification. In addition, histamine dihydrochloride (Ceplene) is authorised for adult patients with AML in first remission concomitantly treated with interleukin-2 (IL-2). Midostaurin (Rydapt) is authorised in combination with standard daunorubicin and cytarabine induction and high dose cytarabine consolidation chemotherapy followed by midostaurin single agent maintenance therapy for adult patients with newly diagnosed AML who are FLT3 mutation positive. Finally, Mylotarg is authorised in combination therapy with daunorubicin (DNR) and cytarabine (AraC) for the treatment of patients age 15 years and above with previously untreated, de novo CD33 positive AML, except acute promyelocytic leukaemia (APL).

About the product

Vyxeos is a liposomal formulation of a fixed combination of daunorubicin and cytarabine in a 1:5 molar ratio. The 1:5 molar ratio has been shown in vitro and in vivo to maximise synergistic antitumour activity in AML (SmPC, section 5.1).

Daunorubicin has antimitotic and cytotoxic activity, which is achieved by forming complexes with DNA, inhibiting topoisomerase II activity, inhibiting DNA polymerase activity, affecting regulation of gene expression, and producing DNA-damaging free radicals (SmPC, section 5.1).

Cytarabine is a cell cycle phase-specific antineoplastic agent, affecting cells only during the S-phase of cell division. Intracellularly, cytarabine is converted into cytarabine-5-triphosphate (ara-CTP), which is the active metabolite. The mechanism of action is not completely understood, but it appears that ara-CTP acts primarily through inhibition of DNA synthesis. Incorporation into DNA and RNA may also contribute to cytarabine cytotoxicity. Cytarabine is cytotoxic to proliferating mammalian cells in culture (SmPC, section 5.1).

Vyxeos liposomes exhibit a prolonged plasma half-life following intravenous infusion, with greater than 99% of the daunorubicin and cytarabine in the plasma remaining encapsulated within the liposomes. Vyxeos delivers a synergistic combination of daunorubicin and cytarabine to leukaemia cells for a prolonged period of time. Based on data in animals, Vyxeos liposomes accumulate and persist in high concentration in the bone marrow, where they are preferentially taken up intact by leukaemia cells in an active engulfment process. In leukaemia-bearing mice, the liposomes are taken up by leukaemia cells to a greater extent than by normal bone marrow cells. After internalisation, Vyxeos liposomes undergo degradation, releasing daunorubicin and cytarabine within the intracellular environment, enabling the medicinal products to exert their synergistic antineoplastic activity (SmPC, section 5.1).

The applicant applied for the following indication: Vyxeos is indicated as monotherapy for the treatment of adults with high-risk acute myeloid leukaemia (AML) as defined by therapy-related AML (t-AML) or AML with myelodysplasia-related changes (AML-MRC). The recommended indication for approval is: Vyxeos is intended for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukaemia (t-AML) or AMLwith myelodysplasia-related changes (AML-MRC) (see SmPC, section 4.1).

Vyxeos dosing is based on the patient's body surface area (BSA) according to the following schedule: First induction: daunorubicin 44 mg/m^2 and cytarabine 100 mg/m^2 on days 1, 3, and 5; Second induction: daunorubicin 44 mg/m^2 and cytarabine 100 mg/m^2 on days 1 and 3; Consolidation: daunorubicin 29 mg/m2 and cytarabine 65 mg/m^2 on days 1 and 3 (SmPC, section 4.2).

Consolidation therapy is recommended for patients achieving remission who have recovered to absolute neutrophil count (ANC) > 500/µL and the platelet count has recovered to greater than 50,000/µL in the absence of unacceptable toxicity. A subsequent course of consolidation may be administered in patients who do not show disease progression or unacceptable toxicity within the range of 5 to 8 weeks after the start of the first consolidation. Treatment should be continued as long as the patient continues to benefit or until disease progression, up to maximum of 2 consolidation courses (SmPC, section 4.2).

Type of Application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest.

This was based on the therapeutic advantage over conventional 3+7 regimen with a survival benefit in the claimed indication of patients with untreated therapy related AML of with myelodysplasia related changes. A survival benefit over the existing standard of care that has been used over the last 40 years is remarkable for this disease. In addition, it has the potential of reduced long term toxicity associated with anthracyclines although long term data is needed before it can be confirmed.

2.2. Quality aspects

2.2.1. Introduction

The finished product is a sterile lyophilised liposomal product presented as powder for concentrate for solution for infusion containing 44 mg of daunorubicin (as HCl salt) and 100 mg of cytarabine as the active substances. Vyxeos is the first liposomal product containing two active substances as a fixed-combination product.

Other ingredients are: distearoylphosphatidylcholine, distearoylphosphatidylglycerol, cholesterol, copper gluconate, triethanolamine and sucrose, as described in section 6.1 of the SmPC.

The product is available in a 50 mL Ph. Eur. Type 1 colourless glass vial, sealed with a grey chlorobutyl stopper with a Flurotec coating on the product contact surface and sealed with an aluminium flip-off seal, as described in section 6.5 of the SmPC.

2.2.2. Active Substance

Cytarabine

General information

The chemical name of cytarabine is 4-amino-1-[(2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,2-dihydropyrimidin-2-one corresponding to the molecular formula $C_9H_{13}N_3O_5$. It has a relative molecular mass 243.22 g/mol and has the following structure (Figure 1):

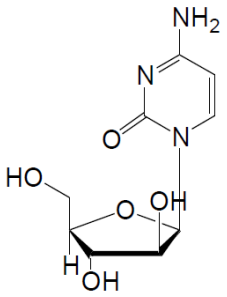


Figure 1. Structure of cytarabine

Cytarabine is the subject of a monograph in Ph. Eur. (0760). Information on the active substance was provided in an active substance master file (ASMF).

The structure of the active substance was elucidated by a combination of elemental analysis, mass spectrometry, NMR spectroscopy, IR spectroscopy and UV spectroscopy. Cytarabine is sufficiently characterised and its structure is adequately elucidated.

Cytarabine appears as white to almost white, crystalline powder. It is non hygroscopic and is freely soluble in water.

Cytarabine has four chiral centres. Cytidine, one of essential starting material, is a synthetic product with four chiral centers. The synthetic process changes the stereochemistry of the pair of hydroxyl groups from cytidine to cytarabine. The stereochemistry of the active substance produced by the manufacturer conforms to β form and was confirmed by NMR.

The crystalline state of cytarabine was investigated by means of Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD). The manufacturing process results always to the same crystalline form.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

Cytarabine is manufactured synthetically using cytidine as a starting material through three chemical steps from cytidine (starting material).

The synthetic steps are followed by purification and crystallisation of the crude cytarabine, drying, pulverising and final packaging. Critical and non-critical process steps and parameters have been identified. The proposed in-process controls (IPCs) have been presented and are justified. The control strategy ensures consistent quality of the active substance. The characterisation of the active substance and its impurities are in accordance with the relevant ICH guidelines.

The potential impurities are controlled in the active substance and intermediate specifications as well as in the in-process control during the manufacturing of the active substance by validated test methods. It has been demonstrated that the impurities are adequately controlled during manufacturing of the active substance. The proposed tests and acceptance criteria for the active substance, the active substance intermediates, and in process controls are considered acceptable.

The active substance is packed in air-tight, polyethylene bag (two ply), and placed in a light resistant tin can (outermost packaging). The primary packaging material is in compliance with the Ph. Eur., and EU Regulation 10/2011, respectively, as supported by evidence provided by the supplier.

Specification

The active substance specification inludes appropriate tests and limits for appearance (visual), identification (UV, IR, HPLC), appearance of solution (Ph. Eur.), specific optical rotation (Ph. Eur.), loss on drying (Ph. Eur.), sulfated ash (Ph. Eur.), chromatographic purity (HPLC) and assay (Ph. Eur.).

The proposed active substance specifications as applied by the finished product manufacturer or the active substance manufacturer comply with the current Ph. Eur. monograph for cytarabine.

The analytical procedures used in the control of the active substance have been satisfactorily described and validated in accordance with the relevant ICH guidelines. Information regarding the reference standards used in the analytical testing is satisfactory.

Batch analysis data for three commercial scale batches of the active substance were provided. The results are within the specifications and confirm consistency of the manufacturing process from batch to batch.

Stability

Stability data on three production scale batches of cytarabine stored in the intended commercial packaging for up to 36 months under long term conditions (25.0 ± 2.0 °C / 60.0 % RH ± 5.0 %) and for up to 6 months under accelerated conditions (40.0 ± 2.0 °C / 75.0 % RH ± 5.0 %) was provided according to the ICH guidelines.

Samples were tested for parameters such as characters, identification (UV, IR, TLC), appearance of solution, specific rotation, related substances, loss on drying and assay. The test methods are stability indicating. All tested parameters consistently meet the required Ph. Eur. specifications under both accelerated and long-term conditions. No significant changes to any of the measured parameters were observed under long term and accelerated conditions and all remained within specification.

According to Ph. Eur. monograph, cytarabine should be protected from light; no formal photostability study was performed, this is acceptable.

The stability results justify the proposed retest period in the proposed container.

Daunorubicin hydrochloride

General information

The chemical name of daunorubicin hydrochloride is (*8S,10S*)-8-acetyl-10-{[(*2R,4S,5S,6S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy}-6,8,11-trihydroxy-1-methoxy-5,7,8,9,10,12-hexahydrotetracene-5,12-dione hydrochloride corresponding to the molecular formula $C_{27}H_{29}NO_{10}$.HCl. It has a relative molecular mass 563.98 g/mol and has the following structure (Figure 2):

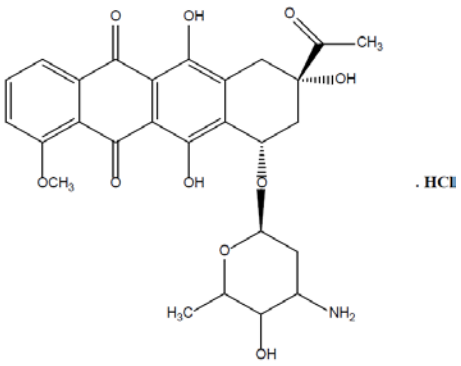


Figure 2. Structure of daunorubicin hydrochloride

Daunorubicin hydrochloride is the subject of a monograph in Ph. Eur. (0662). Information on the active substance was provided in an active substance master file (ASMF).

The structure of the active substance was elucidated by a combination IR spectroscopy, UV spectroscopy, ^{1}H- and ^{13}C-NMR spectroscopy, mass spectrometry and differential scanning calorimetry (DSC). The crystalline state of the substance was consistently confirmed using X-Ray Powder Diffraction (XRPD). Daunorubicin hydrochloride is sufficiently characterised and its structure is adequately elucidated.

Daunorubicin hydrochloride appears as orange-red powder, hygroscopic crystalline powder. It is freely soluble in water and methanol, slightly soluble in alcohol, practically insoluble in acetone.

Daunorubicin has six chiral centres. The stereochemistry of the active substance is guaranteed by the fermentation process that is widely known to be highly stereoselective. The fermentation process used employs the strain *Streptomyces peucetius* that is referenced in the Ph. Eur. Monograph 0662. The stereochemistry of the active substance is that provided by the Ph. Eur. Monograph.

The crystalline state of daunorubicin hydrochloride was investigated by means of X-Ray Powder Diffraction technique on three production batches. The diffractograms showed the same crystalline form as that described in literature. The manufacturing process results always to the same crystalline form. However since daunorubicin hydrochloride is solubilised in buffer solution during manufacture of the finished product it is acknowledged that polymorphism of the active substance is not considered as critical attribute for the manufacture of Vyxeos.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

Daunorubicin hydrochloride is manufactured in three main stages. In the first stage, crude daunorubicin hydrochloride (or Intermediate I) is obtained starting from fermentation of *Streptomyces peucetius*. After the fermentation phases, the fermentation broth undergoes a number of hydrolysis, filtration, pH adjustment and extraction and purification steps, which result in crude daunorubicin hydrochloride (or Intermediate I).

In the second stage, crude daunorubicin hydrochloride undergoes a pre-final crystallisation to obtain pre-final crystallization daunorubicin hydrochloride (or Intermediate II).

In the third step, pre-final crystallization daunorubicin hydrochloride is dissolved, recrystallised and sieved to obtain the final active substance.

The Master Cell Bank (MCB) is sufficiently characterised. The preparation of Master Cell Bank and Working Cell bank (WCB) has been clearly reported. The specification for MCB and WCB is presented and is considered satisfactory.

Critical and non-critical process steps and parameters have been identified. The proposed IPCs have been presented and are justified. They include the controls of parameters in the fermentation phases, recovery and synthetic phase.

The control strategy ensures consistent quality of the active substance. The characterisation of the active substance and its impurities are in accordance with the relevant ICH guidelines.

The potential impurities are controlled in the active substance and intermediate specifications as well as in the in-process control during the manufacturing of the active substance by validated test methods. It has been demonstrated that the impurities are adequately controlled during manufacturing of the active substance. The proposed tests and acceptance criteria for the active substance, the active substance intermediates I and II, and in process controls are considered acceptable and justified. It is specified in the Ph. Eur. monograph that the production process of daunorubicin hydrochloride should be designed to eliminate or minimise the presence of histamine. During daunorubicin manufacture it is considered essential to minimise the level of histamine in the final produced daunorubicin hydrochloride in order to avoid unwanted effects from histamine. The ASMF Holder should provide, as post-approval commitment, evidence that the Ph. Eur. monograph recommendation is met.

The active substance is packed in Type III glass bottles, closed with polypropylene cap equipped with polyethylene gasket. The bottle is then put in a transparent polyethylene bag and placed in a cardboard drum and stored at 5 ± 3 °C. The glass bottles and polyethylene gasket used as primary packaging materials are in compliance with the Ph. Eur., and EU Regulation 10/2011, respectively, as supported by evidence provided by the supplier.

Specification

The active substance specification includes appropriate tests and limits for appearance (visual), identification (IR, HPLC), identification of chlorides (Ph. Eur.), crystallinity (microscopy), pH (Ph. Eur.), water content (Ph. Eur.), related substances (Ph. Eur.), residual solvents (Ph. Eur.), assay (Ph. Eur.) and microbiological testing (Ph. Eur.).

The proposed active substance specifications as applied by the finished product manufacturer or the active substance manufacturer comply with the current Ph. Eur. monograph for daunorubicin hydrochloride.

The analytical procedures used in the control of the active substance have generally been satisfactorily described and validated in accordance with the relevant ICH guidelines. Information regarding the reference standards used in the analytical testing is satisfactory.

Batch analysis data for six commercial scale batches of the active substance were provided. The results are within the specifications and confirm consistency of the manufacturing process from batch to batch.

Stability

Stability data on six production scale batches of daunorubicin hydrochloride stored in the intended commercial packaging for up to 12 months under long term conditions (5° ± 3°C) and for up to 6 months under accelerated conditions (25 °C, 60 % RH) was provided according to the ICH guidelines.

Samples were tested for appearance (accelerated condition only), assay, related substances, water content, pH and microbiological testing. The test methods are stability indicating. No significant changes to any of the measured parameters were observed under long term and accelerated conditions and all remained within specification.

According to Ph.Eur. monograph daunorubicin hydrochloride should be protected from light; no formal photostability study was performed, this is acceptable.

The stability results justify the proposed retest period in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Vyxeos is a sterile lyophilised liposome formulation intended for parenteral administration. Each single-use 50 mL vial contains 100 mg cytarabine and 44 mg daunorubicin in a 5:1 molar ratio at a concentration of 100 units per vial, where 1 unit contains 1.0 mg cytarabine and 0.44 mg daunorubicin (as the free base).

The product after reconstitution with 19 mL of sterile Water for Injection is a purple colloidal dispersion of liposomes with daunorubicin and cytarabine encapsulated in the liposomes providing 5 units/mL (5.0 mg/mL cytarabine and 2.2 mg/mL daunorubicin (as the free base)). The liposome membrane is composed of distearoylphosphatidylcholine (DSPC), distearoylphosphatidylglycerol (DSPG) and cholesterol in a 7:2:1 molar ratio.

Vyxeos is a liposomal formulation of cytarabine:daunorubicin intended for the treatment of acute myeloid leukemia. The rationale for developing Vyxeos was based on the principle of ratiometric dosing, in which specific drug:drug ratios provide synergistic activity. The anti-tumor ratio-dependent synergy of cytarabine and daunorubicin was defined in cell-based screening assays *in vitro.* This ratio of the drugs was encapsulated in liposomes. Nano-scale drug delivery vehicles, such as liposomes, are known to prolong the circulation of drugs *in vivo*. The small size (~100 nm diameter) and specific lipid composition of the Vyxeos liposomes result in long circulation times *in vivo*.

The Quality Target Product Profile (QTTP) was reviewed and contained a summary of the quality characteristics that were used to guide pharmaceutical development for Vyxeos in accordance with ICH Q8.

Critical Quality Attributes (CQA)

Based on the QTPP, the critical quality attributes (CQA) to ensure the quality, safety and efficacy of the finished product were identified.

The selection of cytarabine and daunorubicin was based on the established clinical practice which has been the standard first-line induction treatment for acute myeloid leukaemia (AML) for over 40 years.

In addition, a panel of tumour cell lines was utilised to examine drug ratio-dependent synergy for various cytarabine:daunorubicin molar ratios.

Data analysed using the median effect analysis method showed that the cytarabine:daunorubicin 5:1 molar ratio was the most consistently synergistic ratio evaluated, with 53% of cell lines tested displaying synergy and 33% antagonism. The maintenance of the 5:1 molar ratio of cytarabine:daunorubicin during drug release from the liposome is confirmed based on both *in vitro* and *in vivo* evalution in mice and rats.

Since both ASs are water soluble, encapsulation within a liposome carrier was identified as a possible solution to maintain fixed drug ratios *in vivo*. The liposomal membrane composition chosen for the encapsulation of cytarabine and daunorubicin was based on prior formulation experience. Therefore the brief discussion provided on the selection of lipids was accepted. The lipids comprising the liposomes are Distearoylphosphatidylcholine (DSPC), Distearoylphosphatidylglycerol sodium salt (DSPG-Na) and cholesterol (Chol). Both DSPC and DSPG have a relatively high phase transition temperature (~ 55 °C) and the negatively charged lipid (DSPG) is used to help minimise liposome aggregation.

In vitro developmental data relating to a prior floxuridine and irinotecan liposomal formulation was presented to support the chosen lipid ratio of DSPC:DSPG:Chol (70:20:10, mol:mol) instead of new data using cytarabine and daunorubicin. This was considered acceptable because, importantly, the underlying strategy of Vyxeos is for intact liposomes containing the 5:1 molar ratio to be taken up by leukemia cells. Therefore, Vyxeos liposomes are designed for high retention of the drug cargo and low *in vivo* release rates as they circulate in the plasma. Based on PK calculations, at least 99% of the drug in the plasma is encapsulated within Vyxeos liposomes. Subsequent *in vivo* studies confirm the maintenance of the 5:1 molar ratio (cytarabine:daunorubicin).

Based on previous formulation studies performed, different drug loading techniques have been evaluated for the loading of cytarabine and daunorubicin respectively. The excipients used are conventional excipients used for the manufacture of liposomal formulation and are known in the literature for use with anti-cancer agents, therefore the choice of excipients is considered acceptable. There are no novel excipients used in the manufacture of Vyxeos. Diastearoylphosphatidyl choline (DSPC) and distearoylphosphatidylglycerol (DSPG) have been used in different parenteral medicinal products approved globally for many years. No chemical or physical incompatibilities have been noted between cytarabine and daunorubicin or between cytarabine or daunorubicin and the excipients in Vyxeos. Compatibility of cytarabine and daunorubicin with the excipients was confirmed in real time and accelerated stability studies.

Characterisation studies of Vyxeos liposomes were performed. A summary of the characterization studies concerning relevant physicochemical properties for Vyxeos liposome for injection including liposome morphology; lamellarity; characterization of bilamellar structure; surface charge; trapped volume; phase transition temperature were presented. The characterisation of liposomes correlates with observed cryo-EM images pre- and post-drug loading revealing the formation of bilamellar liposomes.

Early Phase 1 and Phase 2 clinical trials were conducted with a slightly different liquid formulation. The change to the lyophilised formulation, which can be stored at 2-8 °C, was made prior to the Phase 3 clinical trials to provide better product stability, ease of commercial distribution and ease of preparation in the clinic. A comparison of the formulations used in the clinical trials programme and container/closures were summarised. The commercial finished product formulation and container/closure system are the same as those used for the Phase 3 clinical studies.

Each manufacturing step has been discussed individually and each unit operation is categorised as either critical or non-critical.

Sufficient information has been presented to demonstrate that stereoisomerisation of either cytarabine or daunorubicin is not possible during the finished product manufacturing process.

Following inspection deficiencies at the ASs manufacturing sites, new manufacturers were identified as alternate commercial manufacturers for cytarabine and daunorubicin. The comparability of the ASs has been discussed in detail and the quality of the ASs sourced from the different manufacturers is considered comparable. A satisfactory comparability investigation for the finished product followed by the second process

performance qualification with a product batch manufactured with the ASs from the new suppliers was also performed.

The *in vitro* release (IVR) assay is one of the critical controls for Vyxeos. An assessment of the relationship of mean particle size with IVR has been performed. It should be noted that IVR / *in vivo* correlations (IVIVC) have not been established. An updated satisfactory method development report of the *in vitro* drug release method has been provided and supported by relevant data during the procedure. The method development report is in line with the EMA "*Reflection Paper on the Data Requirements for Intravenous Liposomal Products Developed with Reference to an Innovator Liposomal Product*" (EMA/CHMP/806058/2009/Rev. 02) and contains a full discussion of the method development strategy, determination of release media and demonstration of the discriminatory nature of the method to formulation and process variants. The proposed *in vitro* drug release method is used for the evaluation of the release characteristics of the two substances, and to discriminate between liposome batches with different formulations or compositions. The discriminatory of the IVR method is demonstrated by varying the formulation and the manufacturing process parameters. Due to the nature of the finished product, a multipoint specification limit / multiple sampling time-points are required for the IVR analysis of both active substances.

The reconstituted product is administered by intravenous (IV) infusion to patients after dilution with either 0.9% Sodium Chloride Injection, or 5% Dextrose in intravenous infusion bags. The physicochemical stability, including the compatibility of Vyxeos liposomes with the diluents, infusion bags and administration sets was evaluated. The results showed that the diluted Vyxeos solution is compatible with representative infusion bags and administration sets.

Vyxeos finished product is packaged in a 50 mL Ph. Eur. Type 1 colourless glass vial, sealed with a 20 mm 4432/50 grey chlorobutyl stopper with a Flurotec coating on the product contact surface and sealed with a 20 mm aluminium flip-off seal. The vial is then packaged into a cardboard carton to protect from light exposure. The primary packaging system has been evaluated with respect to extractables and leachables and taking into account the total number of dosing days. No significant amount of leaching components from the product contacting packaging components was observed.

The declarations from the suppliers of the glass vial and rubber stopper are also provided, confirming compliance with the Ph. Eur. requirements, including Ph. Eur. 3.2.1. *"Glass containers for pharmaceutical use"*, Ph. Eur. 3.2.9. *"Rubber closures for containers for aqueous parenteral preparations for powders and for freeze-dried powders*", and BSE/TSE-free statement.

Manufacture of the product and process controls

The manufacturing process comprises the following main stages: preparation of liposomes, preparation of cytarabine and daunorubicin solutions, liposomes loading with cytarabine and daunorubicin, sterilisation, lyophilisation and packaging. Critical process parameters (CPPs) affecting the finished product critical quality attributes are presented. Ranges or target values for other non-critical process parameters (PPs) are also included. The proposed IPCs for the manufacturing process were established based on historical process performance data from commercial scale batches or in some cases the required finished product characteristics. A summary of the in-process limits, including the action limit and acceptance limit of the identified critical in-process parameters was clearly presented in tabular format.

The proposed in-process limits are generally supported by the relevant batch analysis during in-process testing, but because various batches from development have been pooled together, the calculation mean may be affected. In view of the limited commercial batches currently available, it is acceptable to revise the

IPCs following the manufacture of 26 commercial batches (post-approval commitment). This would provide reassurance of the suitability of the proposed IPCs.

It was not possible to compare batch-to-batch consistency when the hold-time validation studies were only performed on one batch to validate a particular stage of the manufacturing process. Therefore, further validation data are requested to support the proposed hold-times of the intermediate products at different stages of the manufacturing process. The post-approval commitment to provide the data is acceptable.

The validation program for Vyxeos manufacturing process, which is a non-standard process, consisted of qualification of the aseptic filling process at the manufacturing site and validation studies on manufacturing unit operations. A prospective validation study has been performed initially on three commercial scale batches of Vyxeos. One batch failed release testing for one parameter. Following the OOS results some corrective and preventive actions was implemented. A fourth batch was successfully included in the formal validation study. Concerns over the out-of-specifications have been adequately discussed and justified. The relevant specification control limits have been amended accordingly.

Each batch was manufactured in the same manufacturing facility, using the same process and the same equipment as the commercial process.

In conclusion, it has been demonstrated that the manufacturing process is sufficiently robust to provide assurance that finished product of consistent quality, complying with the specification, is produced.

Product specification

The finished product release and shelf life specifications, include appropriate tests and limits for appearance of Lyophilized Cake and Post-Reconstitution Suspension (visual), reconstitution time, water content of the Lyophilized Cake (Ph. Eur.), pH of the reconstituted suspension (Ph. Eur.), particle size (Ph. Eur.), Osmolality (Ph. Eur.), Particulate Matter (Ph. Eur.), identification of cytarabine and daunorubicin (HPLC, UV), cytarabine and daunorubicin assay (HPLC), cytarabine and daunorubicin % encapsulation (HPLC), cytarabine and daunorubicin impurities (HPLC), cytarabine and daunorubicin content uniformity (Ph. Eur.), DSPC assay (HPLC), DSPG-Na assay (HPLC), cholesterol assay (HPLC), Lipid impurities (HPLC), copper assay (atomic absorption), residual solvents (GC), endotoxin (Ph. Eur.), sterility (Ph. Eur.) and in-vitro release.

Data from eight commercial-scale batches, along with data from twelve process development batches of lyophilised finished product form the basis for the justification of specifications for Vyxeos. Batches that were rejected during development were not used for specification setting. The proposed commercial IVR acceptance criteria are based primarily on the commercial scale batches manufactured at the proposed manufacturing site.

A risk assessment was conducted for Vyxeos finished product to identify potential elemental impurities that may be present in the finished product in accordance with ICH Q3D Elemental Impurities in Drug Products. In addition to the elemental impurities risk assessment, Vyxeos finished product was tested for levels of heavy metals via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Based on the outcome of the risk assessment appropriate control strategy is put in place.

The finished product is released on the market following traditional final product release testing. The procedures for analytical methods used are provided. Most of them are compendial and for these methods verification reports were provided. The non-compendial analytical methods were validated according to current ICH guidance. Satisfactory information regarding the reference standards used in the routine analysis of finished product has been presented.

Batch analysis data from commercial-scale batches of lyophilised finished product manufactured to date for use in Phase 3 clinical studies, registration stability studies process validation and commercial batches have been presented. These batches were manufactured at the commercial manufacturing site. In addition, batch analysis data of batches of frozen liquid product, as well as one batch of liquid formulation of Vyxeos, used in Phase I and II development have also been presented as supportive data.

All product batches were tested using the methods, and released against the specifications, in place at the time of test. The results show that the finished product can be manufactured with consistent quality and meeting its specifications.

Stability of the product

Nine batches of Vyxeos, manufactured at the commercial manufacturing facility according to the commercial manufacturing process, have been placed on stability under long term (2 – 8 °C) and accelerated conditions (25 °C/60 % RH) according to the ICH guidelines. All stability batches were of commercial scale and kept in the proposed container closure system. Results are available for up to 42 months stored in long term and up to 24 months at accelerated conditions.

All test vials were stored upright in the container closure system and analysed based on the following parameters: appearance (lyophilised, and post-reconstitution); reconstitution time; cytarabine (identification, assay, % encapsulation and impurities); daunorubicin (identification, assay, % encapsulation and impurities); DSPC/DSPG/cholesterol assay; lipid impurities; pH; liposome particle size; *in vitro* release of cytarabine and daunorubicin; particulate matter and osmolality.

There was no change in appearance of the lyophilised plug or the reconstituted dispersion through 42 months. There were essentially no changes in any of the following: cytarabine and daunorubicin assays, impurities, and encapsulation, lipid assays, pH, osmolality, copper assay, triethanolamine assay, residual water content, particulate matter, endotoxins or sterility, in-vitro release rate. There is a slight trend for the total daunorubicin impurities to increase through 42 months but remains well within the specification limit. However out-of-specification (OOS) results were observed for the *in vitro* drug release and mean particle size of the liposome, both of which are critical parameters for providing quality assurance of the finished product. OOS results were sufficiently justified. Nevertheless, only for three batches were there no OOS were observed for up to 12 months stability testing under all long-term (5 ± 3 °C), accelerated (25 ºC/60% RH) and stress conditions (30 ºC/65% RH or 40 °C/75 % RH).

In view of the limited number of batches that showed full compliance with the finished product specifications, the proposed shelf-life of 48 months is not acceptable; a shelf-life of 24 months can be considered acceptable provided that the Applicant commits to place two additional finished product batches, manufactured using the currently proposed ASs sources, manufacturing process and controls, on stability. Updated stability data should be provided annually until 24 months real time data is available for both batches.

The updated 18-month stability data for the one batch and 9-month long term stability data for a second batch confirms compliance with the proposed finished product specifications for up to the study period of 18- and 9- months, respectively. No significant differences for the tested parameters have been observed for these two batches during stability, which is satisfactory.

Furthermore, stress testing (i.e. photo-stability under ICH photostability conditions, temperature cycling) was also performed to study the effect of heat and light on Vyxeos. The stability of Vyxeos post reconstitution has also been studied in accordance with ICH Q1A guidance. Samples were analysed against the stability

specifications in place at the time of testing. The photostability studies demonstrated that Vyxeos vials should not be exposed to intense light for prolonged periods of time and that the secondary packaging can be considered to provide sufficient protection against light-induced product degradation. Chemical and physical in-use stability has been demonstrated for 4 hours at 2°C to 8°C for the econstituted suspension in the vial and for the diluted infusion solution (SmPC section 6.3).

Short-term stability studies of lyophilised Vyxeos exposed to low and cycling temperatures were also performed. The results demonstrated the product is sensitive to light under ICH photostability conditions, and sensitive to elevated temperatures but not sensitive to temperature cycling within the tested range.

Overall the stability results based on the real-time stability results, a shelf-life of 24 months at storage conditions of 5°C ± 3°C for Vyxeos in the commercial container closure configuration is considered acceptable. According to results from the photostability study and following ICH requirements Vyxeos is sensitive to light. Therefore, the finished product should be protected from prolonged exposure to intense light and kept in the secondary packaging until use.

Adventitious agents

Cholesterol is the only animal derived excipient used in the manufacture of Vyxeos. It is supplied under guarantee from the manufacturer that the source and/or processing used to obtain the cholesterol is in compliance with the "*Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products, EMA/410/01 Rev. 3*". A certificate of suitability from the European Directorate for the Quality of Medicines and Health Care has been provided.

All other excipients/components, including the other lipids, are manufactured from raw materials of plant, petroleum or chemical origin.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The source of both active substances has been changed during development and new manufacturers were identified as alternate commercial suppliers for cytarabine and daunorubicin. The comparability of the ASs has been discussed in detail and the quality of the ASs sourced from the different manufacturers is considered comparable. A satisfactory comparability investigation followed by the second process performance qualification for the finished product manufactured with active substances from the new suppliers was performed. Some issues with a number of development finished product batches not meeting all criteria have been investigated and satisfactory corrective actions and justifications have been provided. The suitability of the in vitro release assay which is one of the critical controls for Vyxeos has been successfully demonstrated. Overall, the results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.2.6. Recommendations for future quality development

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

1. The daunorubicin ASMF Holder should provide by 31st July 2018, as post-approval commitment, evidence that the Ph. Eur. monograph recommendation that the production process of daunorubicin hydrochloride should be designed to eliminate or minimise the presence of histamine is met.
2. All IPC control limits should be reviewed after manufacturing of 26 commercial batches of the finished product.
3. Further satisfactory validation data from two batches are required to support the proposed hold-times relating to the non-standard processing steps.
4. Two additional finished product batches, manufactured using the currently proposed active substance sources, manufacturing process and controls, should be placed on stability.

2.3. Non-clinical aspects

2.3.1. Introduction

Single dose toxicity studies conducted with Vyxeos comprised a non-GLP single ascending dose study, in dogs, and two GLP-compliant single dose studies followed by a 14-day observation period, in rats and dogs.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro assessment of cytarabine:daunorubicin combinations for synergy

Synergism with respect to cytotoxicity was more evident at higher cytarabine:daunorubicin molar ratios than one, and antagonist cytotoxic effects were observed in a larger proportion of cells at ratios lower than one. (Study FR-051212TH). A molar ratio of 5:1 cytarabine:daunorubicin showed the greatest degree of synergy while minimizing the degree of antagonism in a majority of tumour cell lines.

Anti-tumour efficacy of Vyxeos against P388 Murine Lymphocytic Leukaemia in BDF-1 Mice (Study SR050705)

In Study SR050705, CPX-351, non-liposomal (free) cytarabine and daunorubicin, and the free drug cocktail (cytarabine:daunorubicin) were administered intravenously on Days 1, 4, 7 at the MTD and at dose-matched levels in the P388 murine lymphocytic leukaemia model.

With increasing dosage of daunorubicin, %ILS values increased, whereas cytarabine showed minimal dose dependence in the dosage range of 400 to 1000 mg/kg. Administration of the free drug cocktail at the MTD of 600:9 mg/kg cytarabine:daunorubicin was moderately effective with an MST of 29.5 days and %ILS of 293.3%, while treatment with higher doses of the free drug cocktail was not tolerated. Overall, the anti-tumour activity of CPX-351 administered at 12.5:5 mg/kg (in the first experiment) was approximately 3-fold greater than the activity of the free drug (cytarabine, 1000 mg/kg) or the free drug cocktail administered at the MTD.

Anti-tumour Efficacy of Vyxeos against WEHI-3B Murine Myelomonocytic leukaemia cells implanted in CD-1 nude mice (Study SR190805)

Administration of free cytarabine:daunorubicin cocktail at doses ≥ MTD of 600:9 mg/kg was associated with high toxicity compared with animals treated with 12:5.3 mg/kg . Mice treated with had a longer MST and larger %ILS than ratio-matched free drug cocktail at matched doses and at the MTD.

Anti-tumour efficacy of Vyxeos in the CCRF-CEM Human Acute T-Lymphoblastic Leukaemia Xenograft Model: Dose-Matched and at MTD Levels (Study SR250805)

Vyxeos and the 5:1 molar ratio-matched non-liposomal free drug cocktail (cytarabine:daunorubicin) were administered at dose-matched and at MTD levels in SCID/Rag2M mice implanted with CCRF-CEM human acute T-lymphoblastic leukaemia cells. Vyxeos had potent and dose-dependent anti-tumour activity against CCRF-CEM human acute T-lymphoblastic leukaemia. Maximum anti-tumour effect occurred at a dose of 12.5:5 mg/kg with 5/5 animals surviving until the end of the study.

Anti-tumour efficacy of Vyxeos in the CCRF-CEM Human Acute T-Lymphoblastic Leukaemia Xenograft Model: Ratio-Matched and Dose-Pushed Levels (Study SR060905)

Vyxeos and non-liposomal free drug cocktail (cytarabine:daunorubicin) were administered at ratio-matched and dose-pushed levels to SCID/Rag2M mice. Vyxeos had potent and dose-dependent anti-tumour activity against CCRF-CEM human acute T-lymphoblastic leukaemia. Maximum effect occurred at a dose of 10:4.4 mg/kg (MST and %ILS values of 79.5 days and 134% respectively with a p-value of 0.0008). Administration of Vyxeos at the highest dose of 12:5.3 mg/kg was not tolerated and was associated with significant mortality and adverse effects.

Vyxeos efficacy was also compared with the free drug cocktail administered at ratio-matched and dose-pushed levels. Treatment with the ratio-matched free cocktail at 25:11 mg/kg was associated with significant body weight loss, high toxicity scores, and marked adverse events. Dose escalation of the dose-pushed free drug cocktail to 600:9 mg/kg resulted in unacceptable bodyweight loss and toxicity. Vyxeos at 6:2.64 mg/kg and at 10:4.4 mg/kg had respectively a longer MST and a higher %ILS than dose-pushed free drug cocktail at the MTD of 300:4.5 mg/kg (respectively 59.5 day MST and 75% ILS for Vyxeos at 6:2.64 mg/kg and 79.5 day MST and 134% ILS for Vyxeos at 10:4.4 mg/kg compared to a 53.5 day MST and 57% for the free drug cocktail at 300:4.5 mg/kg with a respective p-value of 0.066 and 0.0123.

Anti-tumour Efficacy of Vyxeos in the HL-60B Human Acute Promyelocytic Leukaemia Xenograft Model (Study SR160905)

In Study SR160905, Vyxeos and non-liposomal free drug cocktail (cytarabine:daunorubicin) were administered to SCID/[CB17SC-M] mice implanted with HL-60B human acute promyelocytic leukaemia cells. The dose regimen consisted of IV injections on Days 8, 11, and 14 after implantation of HL-60B leukaemia cells.

Vyxeos exhibited dose-dependent anti-tumour activity against HL-60B human acute promyelocytic leukaemia xenografts, producing a maximum effect at the highest dose of 5:2 mg/kg (MST and %ILS values of 43.0 days and 43% respectively with a p-value of 0.0006). Treatment with a lower dose of Vyxeos at 2.5:1 mg/kg was less effective (MST and %ILS values of 35.0 days and 17%, respectively with a p-value of 0.006). No animals survived to Day 45 in either of the Vyxeos-treated groups. However, comparison of survival time between Vyxeos groups treated at 5:2 mg/kg and 2.5:1 mg/kg with that of control saline-treated group demonstrated statistically significant anti-tumour activity for both Vyxeos doses at p=0.0006 and p=0.006, respectively.

Treatment of mice with Vyxeos at 5:2 mg/kg compared to animals treated with the ratio-matched free cocktail at the same dose resulted in a longer MST (43 days compared to 33 days) and % ILS (43% compared to10%). Dose escalation of the ratio-matched free drug cocktail to the MTD level of 10:4 mg/kg did not result in a survival benefit, with the MST value remaining comparable to that of controls.

The survival effect of Vyxeos at 5:2 mg/kg resulted in a longer MST and higher %ILS than mice treated with the ratio-matched free drug cocktail at matched dose of 5:2 mg/kg (43.0 days MST and 43% ILS for Vyxeos at 5:2 mg/kg compared to a 33.0 days MST and 10% ILS for the ratio-matched free drug cocktail at matched dose of 5:2 mg/kg) and at the MTD of 10:4 mg/kg (43.0 days MST and 43% ILS for Vyxeos at 5:2 mg/kg compared to a 27.0 days MST and -10% ILS for the ratio-matched free drug cocktail at matched dose of 10:4 mg/kg) with p-values at 0.0007 and 0.0012, respectively. CPX-35l anti-tumour activity was also greater than the dose-pushed free drug cocktail (a ratio of 200:3; p=0.0009).

Anti-tumour Efficacy of Vyxeos Compared with Individual Liposomal Agents Against P388 Murine Lymphocytic Leukaemia Cells Implanted in BDF-1 Mice (Study SR250505)

Study SR250505 was a compilation of data from 2 individual experiments (NC003CT312 and NC003CT325) in the IP-implanted P388 murine lymphocytic leukaemia model. The dose regimen consisted of multiple IV injections administered Q3Dx3 on Days 1, 4, and 7, beginning 1 day after tumour cell implantation. After a 60-day period, surviving animals were re-inoculated to rule out the possibility of an immunological tumour rejection.

Animals in group treated with the high dose of Vyxeos (10:5 mg/kg) survived for 60 days, representing a >7-fold increase over the control (saline) median survival time. The studies showed that Vyxeos had potent anti-tumour activity against P388 syngeneic murine lymphocytic leukaemia. The anti-tumour activity of Vyxeos was shown to be dose-dependent, producing the greatest effect at a dosage level of 10:5 mg/kg. Vyxeos dose-dependent anti-tumour effect was consistent with animal survival at Day 60. Treatment of mice with Vyxeos at 10:5 mg/kg resulted in a longer MST and higher %ILS than individual liposomal drugs administered at dose-matched levels that produced a relatively moderate activity (>60 days MST and >675%

ILS for Vyxeos at 10:5 mg/kg compared to a 22 days MTS and 175% ILS for Liposomal Cytarabine at 10 mg/kg and a 16 day MST and 100% ILS for Liposomal Daunorubicin at 5 mg/kg with a p-value <0.0001).

Surviving mice re-inoculated with P388 tumour cells had an MST of 8 days, identical to that of saline-control mice.

Anti-tumour Efficacy of Vyxeos Compared with Free Drug Cocktail and Individual Liposomal Agents Against L1210 Murine Lymphocytic Leukaemia Cells Implanted in BDF-1 Mice (Study SR220705)

In Study SR220705, Vyxeos, and individual liposomal cytarabine and liposomal daunorubicin were administered at dose-matched and/or MTD levels in BDF-1 mice implanted with L1210 murine lymphocytic cells. The dose regimen consisted of multiple IV injections administered Q3Dx3 on Days 1, 4, and 7, beginning 1 day after tumour cell implantation. Vyxeos had potent and dose-dependent anti-tumour activity against Ll210 syngeneic murine lymphocytic leukaemia cells. A robust anti-tumour effect was observed at the highest dose of Vyxeos used in this study (12.5:5 mg/kg) (MST and %ILS values of >64 days and >814% respectively with a p-value of 0.0009). The Vyxeos dose-dependent anti-tumour effect was consistent with the survival rate.

Mice treated with Vyxeos had a longer MST and higher %ILS than those treated with the ratio-matched free drug cocktail at both the matched dose level as well as at the MTD (>64 days MST and >814% ILS for Vyxeos at 12.5:5 mg/kg compared to a 12 day MST and 71% ILS for the free cocktail administered at the

same dose (12.5:5 mg/kg)with a p-value of 0.0012). Furthermore, all mice treated with the 12.5:5 mg/kg dose of Vyxeos survived until the end of the study (9-fold increase in MST vs. saline control), whereas none of the mice treated with the free drug cocktail at the same ratio survived until Day 64.

Mice treated with Vyxeos had a longer MST and higher %ILS than mice treated with individual liposomal drugs administered at the MTD (>64 days MST and >814% ILS for Vyxeos at 12.5:5 mg/kg compared to a 43.5 day MTS and 521% ILS for Liposomal Cytarabine at 15 mg/kg and a 19 day MST and 171% ILS for Liposomal Daunorubicin at 10 mg/kg with a respective p-value of 0.0566 and 0.0005). Likewise, the anti-tumour efficacy of Vyxeos over the individual liposomal drugs was also reflected by an increased incidence of survival at Day 64.

Anti-tumour Efficacy of Vyxeos Compared with Individual Liposomal Agents Following a Delayed Q3Dx3 IV Administration Against P388 Murine Lymphocytic Leukaemia in BDF-1 Mice (Study SR221105)

In Study SR221105 Vyxeos, liposomal daunorubicin, and liposomal cytarabine were administered at dose-matched and MTD levels to BDF-1 mice implanted with P388 syngeneic murine lymphocytic leukaemia cells. The dose regimen consisted of multiple IV injections administered on Days 4, 7, and 10, beginning 4 days after IP inoculation of P388 leukaemia cells.

Vyxeos had potent and dose-dependent anti-tumour activity against the P388 murine lymphocytic leukaemia which correlated with survival at Day 64. Mice treated with Vyxeos had a longer MST and higher %ILS than the individual liposomal drugs administered at dose-matched levels and at their MTD (38 days MST and 443% ILS for Vyxeos at 10:4.4 mg/kg compared to a 20.0 day MTS and 186 % ILS for Liposomal Cytarabine at 10 mg/kg and a 10 day MST and 43% ILS for Liposomal Daunorubicin at 4.4 mg/kg with a respective p-value of 0.0006 and 0.0012).

Anti-tumour Efficacy of Vyxeos Compared with Individual Liposomal Agents Against WEHI-3B Murine Myelomonocytic Leukaemia Cells Implanted in CD-1 Nude Mice (Study SR011205)

In Study SR011205, Vyxeos, liposomal daunorubicin, and liposomal cytarabine were administered at dose-matched and MTD levels to CD-1 nude mice implanted with WEHI-3B murine myelomonocytic leukaemia cells. The dose regimen consisted of multiple IV injections administered on Days 1, 4, and 7, beginning 1 day after IP inoculation of tumour cells. Vyxeos had potent and dose-dependent anti-tumour activity against the WEHI-3B murine myelomonocytic leukaemia, producing a maximum effect at a Vyxeosdose of 12:5: 3 mg/kg (MST and %ILS values of >102 days and >343% respectively with a p-value of 0.0008). The dose-dependent anti-tumour effect of Vyxeos was consistent with an increase in survival. Mice treated with the mid- and high dose of Vyxeos demonstrated a >5-fold increase in MST compared with the control (saline).

Mice treated with Vyxeos had a longer MST and higher %ILS than mice treated with the individual liposomal drugs administered at dose-matched levels in WEHI-3B tumour-bearing mice (>102 days MST and >343% ILS for Vyxeos at 12:5.3 mg/kg compared to a 24.5 day MTS and 7.0 % ILS for Liposomal Cytarabine at 12 mg/kg and a 38.5 day MST and 67% ILS for Liposomal Daunorubicin at 5.3 mg/kg with a respective p-value of 0.0018 and 0.1006). The anti-tumour effect was consistent with an increased incidence of survival at Day 102.

Anti-tumour Efficacy of Liposomal Cytarabine:Daunorubicin at Different Drug to Drug Molar Ratios: Optimal Drug Ratio Identification in P388 Xenografts (Study SR090905)

In study SR090905 three liposomal cytarabine:daunorubicin formulations at various fixed drug:drug molar ratios were compared with Vyxeos. Therapeutic treatment consisted of IV injections on Days 1, 4, 7 after IP tumour cell implantation. In a P388 murine lymphocytic leukaemia model, anti-tumour activity of Vyxeos

(5:1 molar ratio) was dose dependent producing maximum effect at the highest dose tested correlating with the survival at Day 63 (p=0.001 vs. control). Vyxeos at 10:4 mg/kg (0.8 MTD) resulted in a comparable anti-tumour activity to that of liposomal cytarabine:daunorubicin administered at 11.6:1 molar ratio administered at 15:3 mg/kg (MTD). Mice treated with Vyxeos (5:1) had a longer MST and higher %ILS than formulations at 3:1 or 1:1 molar ratio administered at their respective MTD (>63 days MST and 563% ILS for Vyxeos (5:1 molar ratio) compared to a 47.0 day MTS and 395 % ILS for formulation at 3:1 molar ratio and a 44.0 day MST and 358% ILS for ILS for formulation at 1:1 molar ratio with a respective p-value of 0.1895 and 0.0563).

Secondary pharmacodynamic studies

Following IV bolus doses of Vyxeos or a non-liposomal cocktail of cytarabine and daunorubicin (Study CT832), bone marrow exposures to cytarabine and daunorubicin were higher and more sustained with Vyxeos than with the non-liposomal formulations, half-lives in bone marrow for cytarabine and daunorubicin with Vyxeos were at least twice as long as those in plasma suggesting bone marrow as the site of retention for Vyxeos.

A Rag2 CCRF-CEM xenograft model was used to assess distribution to bone marrow in Study SR150105. Following a full course (Q3Dx3) of treatment with either Vyxeos or free drug cocktail, peak bone marrow levels of cytarabine or daunorubicin ranged from 2- to 14-fold higher in Vyxeos treated mice than in free drug-treated mice. Concomitantly, the bone marrow AUC values for both drugs were higher for Vyxeos treated mice than those treated with the free drug cocktail.

Off-target activity of Vyxeos on normal bone marrow cell lines was evaluated in study SR150105. Vyxeos was less cytotoxic in normal bone marrow cells. Intracellular accumulation of cytarabine and daunorubicin were 9 and 2 times higher in CCRF-CEM cells than in normal bone marrow cells. Similarly, the uptake of liposomal lipid in CCRF-CEM cells was 2 times greater than that in normal bone marrow cells.

The time-dependent intracellular accumulation of daunorubicin from Vyxeos liposomes was examined using fluorescence microscopy. In CCRF-CEM cells and chronic myelogenous leukemia cells, daunorubicin was observed to accumulate within the nucleus, and fluorescently labeled lipid in the Vyxeos liposomes was visible on the plasma membrane, surrounding the nucleus and throughout the cytoplasm. Concomitantly, plasma membrane-associated liposomes decreased over time.

Safety pharmacology programme

No safety pharmacology studies have been submitted (see discussion on non- clinical aspects).

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies have been submitted (see discussion on non- clinical aspects).

2.3.3. Pharmacokinetics

Validated bioanalytical methods based on high-performance liquid chromatography and tandem mass spectrometry (LC/MS/MS) were used to measure plasma concentrations of cytarabine and AraU, daunorubicin and daunorubicinol in rats and dogs in GLP toxicology studies.

For the non-GLP studies, qualified methods based on high-performance liquid chromatography with ultraviolet light, fluorescence, or mass spectrometry (LC/UV, LC/fluorescence, and LC/MS, respectively) were used to measure concentrations of total and free cytarabine and daunorubicin in plasma and bone marrow.

The general PK of cytarabine and daunorubicin associated with Vyxeos was evaluated in mice, rats, and dogs and the results are displayed in Table 1.

Table 1 PK parameters for cytarabine and daunorubicin in mice, rats, and dogs

	Mice [a]		Rats [b]		Dogs [c]	
PK Parameter	**CPX-351**	**NL**	**CPX-351**	**NL**	**CPX-351**	**NL**
Cytarabine						
Dose (mg/kg)	12	600	5	NA	2	2
AUC_{last} (µg/mL·h)	2233	399	1438	NA	380	3.29
DN-AUC	186	0.665	288	NA	190	1.64
t_{last} (h)	38	4	48	NA	48	4
CL (mL/h/kg)	5.24	1503	3.49	NA	5.26	587
V_{ss} (mL/kg)	48.1	592	29.4	NA	49.4	693
Terminal $t_{½}$ (h)	6.58	0.35	6.94	NA	5.55	0.86
Daunorubicin						
Dose (mg/kg)	5.3	9	2.2	5	0.88	1
AUC_{last} (µg/mL·h)	872	1.001	546	3.60	195	0.076
DN-AUC	165	0.111	248	0.72	222	0.076
t_{last} (h)	38	4	48	NA	24	NA
CL (mL/h/kg)	6.0	6869	4.15	1424	4.49	12633
V_{ss} (mL/kg)	49.2	18253	37.8	50836	29.8	261810
Terminal $t_{½}$ (h)	5.74	1.99	6.46	25.4	4.59	21.8

[a] Mouse data are from single-dose study CT832. CPX-351 was dosed at 12 units/kg; non-liposomal formulations were dosed at 600 mg/kg for cytarabine and 9 mg/kg for daunorubicin.

[b] Rat data are from study 1005-0361; mean data from males and females on Day 1. CPX-351 was dosed at 5 mg/kg. Data for non-liposomal daunorubicin were obtained from literature at 5 mg/kg (Zhang et al. 2014).

[c] Dog data are from study 1005-0372; mean data from males and females on Day 1. Both CPX-351 and non-liposomal cytarabine were dosed at 2 mg/kg. Data for non-liposomal daunorubicin were obtained from literature at 1 mg/kg (Oosterbaan et al. 1984).

Note: PK parameters are shown for single-dose intravenous administration.

CL=clearance; NL=non-liposomal; PK=pharmacokinetic; $t_{½}$=half-life; V_{ss}=volume of distribution at steady state; DN-AUC=dose-normalized AUC, calculated by AUC/dose.

Cytarabine and daunorubicin tissue distributions were evaluated in two studies in which the 14C-labeled drugs were administered as components of Vyxeos or as non-liposomal formulations (Studies XBL14628 and XBL13687). The results are displayed in Table 2.

Table 2 Tissue-to-Plasma AUClast Ratios for 14C-Cytarabine and 14C-Daunorubicin in Rats (Studies XBL14628 and XBL13687)

	Tissue-to-Plasma AUC_{last} Ratio			
	CPX-351		Non-liposomal	
Tissue	Cytarabine	Daunorubicin	Cytarabine	Daunorubicin
Plasma	1.00	1.00	1.00	1.00
Spleen	0.44	3.28	1.50	131.46
Adrenal cortex	0.24	0.57	1.15	90.87
Adrenal gland	0.26	0.64	1.13	91.87
Adrenal medulla	0.39	1.06	1.17	102.54
Urinary bladder wall	0.29	0.07	1054.5	17.62
Bone marrow	0.21	0.97	0.47	66.08
Lung	0.21	0.35	1.15	36.65
Kidney	0.15	0.39	2.81	49.61
Kidney cortex	0.14	0.37	2.15	45.55
Kidney medulla	0.16	0.42	4.23	57.41
Heart	0.12	0.31	1.31	29.30
Liver	0.11	0.54	1.14	27.43
Lymph node	0.09	0.73	1.27	70.16
Thymus	0.05	0.27	2.74	50.39
Pancreas	0.04	0.23	1.24	113.35
Pituitary gland	0.04	0.25	1.09	120.91
Esophagus wall	0.03	0.09	1.90	20.70
Stomach wall	0.03	0.14	1.22	11.44
Brain (whole)	0.01	0.01	0.28	0.95

AUClast=area under the curve up to the last measurable concentration

Study CT832 assessed bone marrow targeting in in female CD-1 nude mice. The purpose was to determine the bone marrow uptake profiles of cytarabine and daunorubicin associated with administration of Vyxeos and non-liposomal formulations (cytarabine and daunorubicin in saline). The mice received an IV bolus dose of Vyxeos at 12 units/kg or a combination of 600 mg/kg cytarabine and 9 mg/kg daunorubicin as the non-liposomal formulations. Following administration of Vyxeos, both cytarabine and daunorubicin showed sustained retention in bone marrow; time of the maximum concentration (t_{max}) was 1 and 2 hours for cytarabine and daunorubicin, respectively, and levels of cytarabine and daunorubicin decreased very gradually and were still detectable at 24 hours post-dose. Following administration of the non-liposomal formulations, both cytarabine and daunorubicin distributed rapidly into bone marrow; t_{max} was 0.25 and 0.5 hours for cytarabine and daunorubicin, respectively. Levels of cytarabine decreased quickly and were below the limit of detection at 4 hours post-dose. Levels of daunorubicin remained relatively constant through 24 hours post-dose.

Bone marrow exposures to cytarabine and daunorubicin were higher and more sustained with Vyxeos than with the non-liposomal formulations. Dose-normalised AUCs were more than 2-times higher with Vyxeos administration, suggesting greater efficiency in delivering cytarabine and daunorubicin to the bone marrow.

The $t_{½}$ values were more than 2-times longer with Vyxeos. In addition, the $t_{½}$ values for cytarabine and daunorubicin in bone marrow with Vyxeos were at least twice as long as the $t_{½}$ in plasma. The cytarabine:daunorubicin molar ratio in bone marrow was maintained close to the desired synergistic ratio of 5:1 (range: 2:1 to 6:1) for the first 24 hours. In contrast, the molar ratio of cytarabine:daunorubicin with the non-liposomal formulations changed rapidly over the first 4 hours post-dose, reflecting no control of the desired molar ratio in bone marrow.

A single-dose metabolism study was performed in rats to determine the metabolite profile of 14C-daunorubicin associated with administration of Vyxeos and a non-liposomal presentation (daunorubicin in saline) in plasma, urine, faeces, and bile. Parent daunorubicin was the only prominent circulating entity across the two formulations, accounting for approximately 91% of the total circulating radioactivity for Vyxeos and approximately 78% for the non-liposomal formulation. In the Vyxeos treatment, the only other radioactive component that accounted for at least ~3% of the plasma AUC for total radioactivity was daunorubicinol, which amounted to approximately 4% to 5%. The daunorubicinol metabolite:parent ratio based on plasma AUC was approximately 0.05 for Vyxeos.

A single-dose vivo metabolism study was performed in rats to determine the metabolite profile of 14C-cytarabine associated with administration of Vyxeos and a non-liposomal presentation (cytarabine in saline) in plasma, urine, feces, and bile. Plasma profiling showed that parent cytarabine was the only prominent circulating entity across the two formulations, accounting for approximately 97% of the total circulating radioactivity for Vyxeos and approximately 86% for the non-liposomal formulation. The only other radioactive component that accounted for at least ~1% of the plasma AUC for total radioactivity was AraU, which amounted to approximately 0.6% to 0.9% with Vyxeos and approximately 8% for the non-liposomal formulation.

Single-dose 14C mass balance and biliary excretion studies were performed in both intact and bile duct-cannulated male and female Sprague-Dawley rats (Studies XBL14629 and XBL13700). The results are displayed in Table 3 and Table 4.

Table 3 Excretion of 14C-Cytarabine with Administration of Vyxeos or Non-Liposomal Formulation in Rats (Study XBL14629)

	Percent of Administered Dose (0-96 hours)				
Group	Bile	Urine	Feces	Cage Rinse/Wash	Total
CPX-351, BDC, Mean	0.40	83.61	0.80	11.61	96.42
NL, BDC, Male	0.66	90.18	0.88	8.47	100.19

NOTE: Values are means (n=7 for Vyxeos; n=3 for NL).
BDC=bile duct-cannulated; NL=non-liposomal formulation in saline.

Table 4 Excretion of 14C-Daunorubicin with Administration of Vyxeos or Non-Liposomal Formulation in Rats (Study XBL13700)

	Percent of Administered Dose					
Group	Bile	Urine	Feces	Cage Rinse/Wash	Carcass	Total
CPX-351, Intact, Mean	ND	9.32	76.14	2.56	2.28	90.30
CPX-351, BDC, Mean [b]	45.52	6.76	3.08	1.32	ND	56.69
NL, BDC, Male [b]	61.57	20.44	4.93	2.09	ND	89.03

a Excretion was determined over 336 hours after dosing; values are means (n=8). b Excretion was determined over 96 hours after dosing; values are means (n=7 for Vyxeos; n=4 for NL).
BDC=bile duct-cannulated; ND=not determined; NL=non-liposomal formulation in saline.

2.3.4. Toxicology

Single dose toxicity

An overview of the single-dose toxicity studies is displayed in Table 5.

Table 5 Single-Dose Toxicity studies

Species/ Strain	ROA (Vehicle/ Formulation)	Dose Ratio Cytarabine: Daunorubicin (mg/kg)	No. per Sex per Group	Observed Max. Nonlethal Dose (Cytarabine: Daunorubicin [mg/kg])	Approx. Lethal Dose (mg/kg)	Noteworthy Findings	Study No.
Rat/ Sprague-Dawley	IV 60-minute infusion (liposomal formulation)	0:0 (saline) 5: 2.2 10: 4.4 15: 6.6 20: 8.8	N=10/dose 5M/5F	20:8.8	NA	• No mortality, drug-related clinical signs, changes in body weight, coagulation, clinical chemistry, or urinalysis • Compared with saline control values, a dose-dependent trend was observed in plasma copper levels collected on Day 15, but the trend was not statistically significant. • Slight to moderate decrease in WBC, RBC and lymphocytes in 15:6.6 and 20:8.8 mg/kg doses • Drug-related increase in spleen and liver extramedullary hematopoiesis	1004-3331
Dog/ Beagle	IV 60-minute infusion (liposomal formulation)	Single ascending dose given once each week at: 1: 0.44 2: 0.88 3: 1.32 4: 1.76	N=1F	4:1.76	NA	• Decreased food consumption and activity after 3:1.32 mg/kg dose. No clinical signs after 4:1.76 mg/kg dose.	3004-3322
Dog/ Beagle	IV 60-minute infusion (liposomal formulation)	0:0 (saline) 0:0 (vehicle) 1.5: 0.66 3.0: 1.32 6.0: 2.64	N=2/dose, 1M/1F	1.5: 0.66	3.0-6.0	• Poor condition led to premature termination of animals in the 6.0:2.64 mg/kg dosing group and the female in the 3.0:1.32 mg/kg dosing group between days 8-10. • The 3.0:1.32 and 6.0:2.64 mg/kg groups displayed decreased food consumption and body weight, retching, vomiting, diarrhea, loss of activity, dehydration, and pale gums. • Blood samples for copper concentration analysis were collected once prior to treatment and on Days 2 and 15 (or earlier in pre-terminal sacrificed animals). Compared to predose, blood copper concentrations on Day 2 were elevated. • Animals terminated early had marked lymphoid hypocellularity, spleen atrophy, glandular necrosis, GI tract hemorrhage, decreased WBC, and decreased platelets. • No treatment-related gross or microscopic findings in vehicle or 1.5:0.66 mg/kg dosing groups.	1004-3342

Repeat dose toxicity

A 2-Cycle IV Infusion Toxicity Study followed by a 28-Day Recovery Period in Sprague-Dawley Rats (Study 1005-0361)

The purpose of this study was to evaluate the toxicity and toxicokinetic profile of Vyxeos when administered as 2 cycles (for a total of 3 doses/cycle) and to assess the reversibility of any test article-related effects following a 28-day recovery period. Test article or saline control was administered to 10 animals/sex (main study group) by IV infusion for a period of 1 hour at a dose rate of 20 mL/kg/h, as 2 cycles, on Days 1, 3 and 5, and on Days 22, 24 and 26. The control (Group 1) and high-dose (Group 4) groups included 5 additional rats per sex to serve as recovery groups.

Mortality and poor condition leading to preterminal sacrifice, which were considered related to the test article, occurred at mid and high dose levels of Vyxeos. In Group 3 (mid dose), 8 of 10 male rats and 7 of 10 female rats died or were sacrificed early between Days 3 and 33; in Group 4 (high dose) all rats died or were sacrificed early between Days 8 and 16. Clinical signs preceding death included decreased grooming, decreased activity, dehydration, laboured respiration, and changes in the consistency/colour of faeces. The underlying histopathological changes that lead to the unscheduled sacrifices or preterminal deaths were mostly due to marked or severe hematopoietic hypocellularity of the bone marrow of the sternum and femur, as well as marked or severe lymphoid hypocellularity/atrophy of the spleen and thymus. There was also glandular and cryptal epithelium necrosis of the large and small intestinal mucosa. Other treatment-related changes, such as mild to marked haemorrhage in different tissues (testes, epididymis, adrenals, heart) and minimal to marked bacterial deposits in tissues such as the lungs, kidneys, bone marrow, and heart were considered secondary to the cytotoxic effects on the bone marrow.

When compared with the control animals, there were no apparent drug-related clinical signs in Group 2 (low dose) and the 4 unscheduled terminations (1 male and 3 females) of this group were considered to be related to surgical catheterization and not test-article related. Drug-related reductions in body weight gain were noted in Group 2 (low dose) and surviving Group 3 (mid dose) animals, concomitant with reductions in food consumption. There were no ophthalmoscopy, coagulation, clinical chemistry, or urinalysis effects related to the administration of Vyxeos. Compared with controls, no neurologic effects of Vyxeos were evident in Group 2 (low dose) rats; interpretation of neurologic assessment of rats in Group 3 (mid dose) was complicated by the poor clinical condition of the few remaining animals in that group and no definitive conclusion could be drawn. When compared with mean control values obtained on Day 34 (7 days after the last dose), a moderate drug-related reduction in WBC counts was noted in Group 2 (low dose). In addition, slight drug-related decreases in RBCs and associated parameters, including platelet counts, were also noted. Among the 5 surviving Group 3 (mid dose) rats, 4 animals showed marked reductions in white and RBCs, platelets, haemoglobin, and reticulocytes.

When compared to control values, dose-related reductions in absolute and relative thymic and spleen weight, and prostate (for males only) were noted in Vyxeos -treated animals. A dose-related increase in absolute and relative adrenal weight was noted in Vyxeos -treated animals.

Minimal to severe drug- and dose-related histological changes included hematopoietic hypocellularity of sternal and/or femoral bone marrow, lymphoid hypocellularity/atrophy of the spleen and/or thymus, and lymphoid hypocellularity/atrophy with or without sinusoidal erythrocytosis/hemorrhage of various lymph nodes.

A 2-Cycle IV Infusion Toxicity Study in Beagle Dogs followed by a 28-Day Recovery Period (Study 1005-0372)

The purpose of this study was to evaluate the toxicity and toxicokinetic profile of Vyxeos when administered as 2 cycles (for a total of 3 doses/cycle) and to assess the reversibility of any test article-related effects following a 28-day recovery period. The test and control articles were administered by IV infusion (except as noted for Group 6 where the commercial formation of daunorubicin HCl was given as a slow IV bolus). Infusions were delivered over a 1-hour period at a dose rate of 20 mL/kg/h as 1 or 2 cycles. The first cycle was administered on Days 1, 3, and 5 to animals of all groups. The second cycle was administered on Days 22, 24, and 26 to Groups 1 (control), 2 (vehicle), 3 (low dose), and 6 (comparative control). Due to the number of deaths and/or preterminal sacrifices in the high-dose Group 5 animals, the remaining animals in this group were sacrificed on Day 11. Subsequently, an additional haematology investigation was performed on all remaining animals prior to the start of the second cycle of dosing. After evaluation, the second cycle of dosing was not administered to surviving Group 4 (mid dose) animals. The surviving Group 4 animals were re-assigned as recovery animals to replace the animals from Group 5 (high dose) that were sacrificed early.

No significant electrocardiography findings were noted. Following the first dosing cycle, all dogs in the high dose Vyxeos group (Group 5) were either found dead (2 males) or were sacrificed due to poor condition between Days 7 and 10. Two dogs in the mid-dose group (Group 4, 1 male and 1 female) were sacrificed in poor condition on Day 12. The probable cause of death in the dogs found dead and the main underlying affliction of those that underwent unscheduled sacrifice was considered to be severe bone marrow hypocellularity and/or moderate to severe enteric (duodenum, jejunum, ileum, cecum, colon, and rectum) cryptal/glandular necrosis and lymphoid atrophy of gut-associated lymphoid tissue.

Decreases in WBCs were evident in animals in Groups 3 (low dose) and 6 (comparative control) at Day 33 [6 days after the last dose of Vyxeos or the non-liposomal free drugs]. These reductions were the result of marked decreases in absolute and relative neutrophil, monocyte, and eosinophil counts. In addition, mild to moderate decreases in hemoglobin, hematocrit, and platelet counts were noted in Groups 3 and 6. Group 4 (mid dose) animals, having received only 1 cycle of drug treatment, showed mean haematology values similar to those of the control (Group 1) on Day 33 (with the exception of RDW and HDW). Following the 22-day recovery period (Day 56), the haematological values for Group 4 animals were comparable to those of control animals, including RDW and HDW.

Drug-related severe hematopoietic hypocellularity of sternal and/or femoral bone marrow was observed in all animals in the mid- and high-dose groups (Groups 4 and 5; 2 and 3 units/kg, respectively) that were sacrificed early. In addition mild to severe cryptal necrosis of the small intestinal mucosa (with or without cryptal epithelial hyperplasia or hypertrophy, cryptal dilation, villous atrophy, GALT lymphoid atrophy and mucosal hemorrhage) as well as marked to severe glandular necrosis of the large intestinal mucosa were noted in these animals. Moderate to severe splenic lymphoid necrosis and/or atrophy of the white pulp, moderate to marked thymic lymphoid cortical hypocellularity, and mild to marked lymphoid atrophy of the mandibular, mediastinal, mesenteric, or tracheobronchial lymph nodes were also present in the animals sacrificed early. Following the recovery period, there were no drug-related histopathological changes noted in the surviving dogs in the mid-dose group (Group 4; 2 units/kg) with the exception of 1 dog showing moderate splenic lymphoid necrosis.

The plasma toxicokinetics of liposome-encapsulated cytarabine and daunorubicin (Vyxeos) and their respective metabolites, uracil arabinoside and daunorubicinol, were evaluated and confirmed a dose-dependent increase in exposure to the test article.

Genotoxicity

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

Cytarabine was genotoxic in cultures of normal proliferating human lymphocytes during late G2-prophase, *i.e.*, when cells performed post-replication DNA repair. The frequency of chromosomal aberrations during metaphase increased with increasing drug concentration. The genotoxic damage was associated with a block in cell cycle progression at the G2 checkpoint, however, this block was consistently overridden before DNA repair was complete (checkpoint adaptation). Due to the inhibitory effects of cytarabine on post-replication DNA repair processes, activation of the G2 checkpoint blockade promotes the development of genomic instability instead of preventing it (4).

In mutagenicity studies, the effects of Ara-C on the chromosomes of human leukocytes were tested (5). Ara-C (10-5 M) induced chromosome aberrations in human leukocytes and the effects consisted of gaps and open breaks.

In an *in vitro* transformation study, exponentially growing HF (secondary Syrian Golden hamster fetal) cells and H43 (rat) cells were exposed to various doses of cytarabine for 2 to 24 hours (6). Transformed effects were induced by various doses (10-3 to 10-7 M) of Ara-C.

The effect of Ara-C was tested for the induction of micronuclei in mouse bone marrow erythrocytes (7). Ara-C was clastogenic at 12.5 mg/kg IP and 25 mg/kg PO.

The cytogenetic effects of Ara-C were studied in *in vivo* and *In vitro* test systems (8). Male mice received either single or daily x5 IP doses of Ara-C at 0, 160, 440, 660, and 870 µg. Animals were sacrificed at different time intervals after single and multiple doses. Ara-C exhibited a strong mitodepressive effect in mouse bone marrow cells. The effect was more intense in the multiple-dose than single-dose treatment. Cytarabine caused a dose-dependent increase in structural abnormalities, polyploidy, and SCEs with both schedules. Ara-C caused sperm-head abnormalities after single- and multiple-dose administration. *In vitro*, Ara-C induced dose-dependent structural abnormalities in human leukocytes and in SCE analysis a significant effect was noted for all doses from 24-72 hour exposure.

Carcinogenicity

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

Published literature suggests that cytarabine is not carcinogenic in rats (9)(10).

Berger conducted a long-term study of carcinogenic effect of Ara-C in Sprague-Dawley rats. Rats received either daily IP doses of 5 and 25 mg/kg or pulse doses of 500 mg/kg x 3/week for 72 weeks. Ara-C did not reduce the life-expectancy of the animals. Overall tumor incidence (malignant/benign) was slightly higher in male rats treated with 25 mg/kg/day x 5 (38%), 5 mg/kg/day x 5 (35%) and 500 mg/kg x 3/week (87%) and female rats treated with 500 mg/kg x3/week (50%) than control.

Weisburger tested mice and rats given IP doses of 125 and 250 mg/kg Ara-C 3 times a week for 6 months and observed for 1 year. Forty-eight of 50 female rats survived 18 months. Eighteen of these 49 (38%) rats had tumors and 6/18 tumors were malignant (10 breast, 3 pituitary, 2 adrenal). If the level of significance is computed for control vs treated rats with mammary tumors, its p-value (0.156) is not significant (Fisher's exact test). Thirteen of 49 male rats that survived had tumors and only 3/13 were malignant. Only 10/50 female and 13/50 male mice survived 18 months after IP doses of 62 and 125 mg/kg Ara-C. Tumor incidences were 2/10 (20%) in female mice and 6/13 (46%) in male mice. However, tumor incidence is not

an appropriate parameter for assessing carcinogenicity. There is no basis for concluding Ara-C is carcinogenic under the conditions of this experiment.

The IARC Working Group (IARC 2000) conducted a carcinogenic evaluation of cytostatics (antineoplastic medicinal products), including daunorubicin, and classified daunorubicin under Group 2B – Drugs which are possibly carcinogenic to humans (generally, limited human evidence, but absence of animal evidence).

Published literature reports that daunorubicin may be carcinogenic. It can cause renal and mammary tumors in rats (11).

Multiple mammary tumors, mostly adenocarcinomas, were observed in 64% of female Sprague-Dawley rats administered a single IV dose of 10 mg/kg daunorubicin. The mean induction time in females was 80 days. Single mammary tumors, also mostly adenocarcinomas, were observed on average 91 days after injection in 37% of the males (12).

In another study in female Sprague-Dawley rats, a single IV injection of 10 mg/kg daunorubicin resulted in the development of mammary tumors in 11 of 24 (46%) of the animals within 8 months after injection. Tissue distribution studies found that the liver, kidney, heart, and intestines had higher daunorubicin concentrations than mammary tissue in the first 24 hours after injection. A comparison of the *in vitro* metabolism of daunorubicin in mammary tissue and hepatocytes demonstrated approximately 70% of the daunorubicin was metabolised in 90 minutes by hepatocyte homogenates, whereas no metabolism was seen with mammary tissue homogenates. In addition, daunorubicin injected directly into rat mammary gland was neither metabolised nor released into the systemic circulation. These results suggest that retention of daunorubicin in the mammary tissue may explain the preferential induction of mammary tumors in rats intravenously administered daunorubicin (13).

Reproduction Toxicity

No reproductive and developmental toxicity studies were submitted with Vyxeos (see discussion on non-clinical aspects).

Toxicokinetic data

An overview of toxicokinetic data from repeat dose studies is presented in Table 6.

Table 6 Toxicokinetic data from repeat dose studies

Cytarabine + daunorubicin dose level (mg/kg)	Sampling day	Animal AUC_{0-t} (ng.h/ml) (M/F)		Animal C_{max}(ng/ml) (M/F)	
		Cytarabine	Daunorubicin	Cytarabine	Daunorubicin
Two-cycle repeated dose toxicity study in Sprague-Dawley rats (Study 1005-0361)					
5 + 2.2 (low dose)	1	1598082 /278387	444989 / 646256	107317 /103533	46480 / 45867
	5	1555565 / 1153976	1313338 541134	107867 / 99150	42200 / 44720
10 + 4.4 (mid dose)	1	3135371 / 3099705	1348911 /1386307	212833 / 215667	91160 / 92080
	5	3963012 / 3178525	1569630 /1218737	203333 /185667	87200 / 77467
Two-cycle repeated dose toxicity study in Beagle dogs (Study 1005-0372)					

1 + 044 (low dose)	1	132589 / 151259	72553 / 79546	13600 / 12933	9017 / 9013
	5	108907 / 121993	59938 / 66837	12300 / 12900	7987 / 8867
2 + 0.88 (mid dose)	1	364713 / 395024	178378 /211504	28100 / 26500	17933 / 20200
	5	455831 / 435680	188908 /203138	26233 / 24700	16367 / 18200
2 + 0.88 (comparator)	1	3018 / 3557	BLQ*	1710 / 2150	BLQ
	5	3022 / 3093	BLQ	1730 / 1780	BLQ
3 + 1.32 (high dose)	1	531756 /698313	304374 /400135	37133 / 40867	26667 / 30000
	5	911629 / 902906	430334 /457412	45867 / 39500	27833 / 28067

* BLQ= bellow the limit of quantification

Local Tolerance

No studies on local tolerance were submitted with Vyxeos (see discussion on non-clinical aspects).

Other toxicity studies

Toxicological evaluation of copper as an excipient of Vyxeos

Vyxeos contains copper gluconate, which plays a role in the retention of daunorubicin inside the liposome. The amount of copper gluconate in the to-be-marketed formulation of Vyxeos is 5 mg/mL for the reconstituted product, of which approximately 14% is copper. The maximum exposure to copper in patients under the currently proposed dosing regimen is 42.0 mg per induction dose and 27.3 mg per consolidation dose. A patient would theoretically have 319 mg maximum lifetime exposure to copper (5 total induction doses [100 units/m^2] and 4 total consolidation doses [65 units/m^2]). A lifetime exposure of 319 mg copper exceeds safe doses reported in the literature for either oral or parenteral administration of copper.

Whole blood concentrations of copper were measured in several single- and repeat-dose nonclinical toxicology studies with the to-be-marketed Vyxeos formulation. In all cases, copper was administered as a component of Vyxeos. In single dose Study 1004-331, copper was administered at 0.5 to 2 mg/kg and blood samples for copper determinations were collected approximately 14 days after administration of the single dose. In repeat-dose Study 1005-0361, copper was administered at 0.5 to 1.5 mg/kg, and blood samples were collected approximately 7 or 28 days after the last dose for all animals except in instances of early termination. Whole blood copper concentrations increased relative to baseline in a dose-dependent manner. In the repeat-dose study, copper concentrations remained higher than baseline for 24 hours from 7 to 9 days after the last dose of Vyxeos administration; the highest copper level was observed in an animal that was sacrificed on Day 3, and corresponded to about 8 times the baseline concentration. Whole blood concentrations of copper concentrations returned to baseline several days to a few weeks after the last dose.

Dogs also received copper as a component of Vyxeos. In single dose Study 1004-3342, copper was administered at 0.2 to 0.8 mg/kg and blood samples for copper determinations were collected predose and approximately 2 and 15 days after administration of the single dose. In repeat-dose Study 1005-0372, copper was administered at 0.1 to 0.3 mg/kg, and blood samples were collected approximately 1 or 3 weeks after the last dose for all animals except in instances of early termination. On Day 2 of the single dose study, whole blood copper concentrations were increased relative to baseline in a dose-dependent manner; at the

highest dose level of 0.8 mg/kg, copper concentrations were 6 times higher than baseline. In both studies, copper concentrations returned to baseline within 8 to 15 days after the last dose.

Copper levels in plasma, tissues, urine, and feces were used for mass balance calculations. Twelve days after single dose administration of Vyxeos in female rats, the total copper levels indicated that 2% of the total copper was associated with plasma and tissues and 85% was associated with feces and urine.

Toxicological evaluation of cobalt as an elemental impurity in Vyxeos drug product

Administration of Vyxeos induction and consolidation doses may result in exposure to approximately 4.2 and 2.7 µg/day cobalt, respectively, leading to an absolute maximum total exposure of approximate 31.8 µg cobalt for any patient over a lifetime (i.e., 5 induction and 4 consolidation doses). Potential exposure to the 4.2 µg/day exogenous cobalt can transiently exceed the *ICH Q3D guideline* parenteral PDE threshold (5 µg/day).

The acute oral rat no observed effect level of 175-mg/kg and the 90-day oral rat no observed adverse effect level of 3-mg/kg/day results in a > 70,000-fold and 1251-fold safety margin, respectively. There is a 2571-fold safety margin at the clinical dose of 150 mg/day cobalt (approximately 1 mg/kg/day) that resulted in polycythemia following short-term (22 days) ingestion. The safety margin calculations were based on the most conservative estimate of bioavailability (18%) relative to the approximate 4.2-µg daily dose of cobalt that a patient could be exposed to following administration of a 60-mL induction dose.

Peak blood cobalt concentrations as high as 0.117 µg/mL were not associated with clinically significant changes in haematological, thyroid, cardiac, or neurological parameters in healthy individuals following oral administration for 90 days. Theoretical maximum concentrations would be 0.0009 µg/mL, assuming 100% bioavailability of the approximate 4.2 µg daily dose of cobalt, given a 4800-mL human blood volume. This result in a 130-fold safety margin for the 4.2-µg/day cobalt dose administered during a Vyxeos induction dose.

2.3.5. Ecotoxicity/environmental risk assessment

Screening for Persistence, Bioaccumulation, and Toxicity

The octanol-water partition coefficient (log P) of cytarabine, was determined by the Shake Flask Method *(Liboiron, 2017).* The log P of cytarabine at 21°C, determined for 3 different water:octanol ratios from 0.5 to 2 using the shake flask method, was determined to be -2.02 ± 0.04. This result is comparable to the calculated log P estimate of -2.27 for cytarabine. For daunorubicin, the experimental value of log P reported in the literature is 1.83 *(EPA, 2017),* compared with the estimated value of 1.79. The experimental log P values for the drug substances cytarabine, -2.02, and daunorubicin, 1.83, are below 4.5; therefore, further screening is not necessary for persistence, bioaccumulation, and toxicity.

Calculation of the Predicted Environmental Concentration

The $PECS_{URFACEWATER}$ value is not above 0.01 µg/L for both drug substances, therefore, the phase II environmental-fate and effect analysis is not required.

2.3.6. Discussion on non-clinical aspects

Vyxeos is a liposomal formulation of a fixed combination (5:1 molar ratio) of the antineoplastic drugs cytarabine and daunorubicin hydrochloride. Vyxeos was designed to enhance the anti-tumour efficacy of cytarabine and daunorubicin combination therapy by encapsulating both compounds within a drug carrier that maintains the synergistic 5:1 molar ratio for extended times after injection. The 5:1 molar ratio of cytarabine:daunorubicin HCl has been shown to demonstrate the most optimal synergy against tumour cells synergy against tumour cells in vitro whereas reduced synergy or antagonism was observed at other ratios. Superiority of Vyxeos over free cytarabine:daunorubicin HCl cocktail treatment in anti-leukaemic efficacy was observed in a number of animal leukaemia models. In addition, Vyxeos liposomes were shown to be able to maintain the 5:1 molar ratio of cytarabine:daunorubicin HCl in plasma for an extended period of time compared to the free drug cocktail. Furthermore, within bone marrow that contained both normal and leukaemic cells, Vyxeos was shown to be preferentially taken up by the leukaemic cell population, however, the mechanism whereby this occurs is currently unknown.

Consistent with ICH S9, no stand-alone safety pharmacology studies were conducted with Vyxeos. Safety pharmacology parameters were included as part of the repeat-dose toxicology studies in rat and dog. Although the safety pharmacology assessment was confounded by several toxicities, no unexpected CNS or CVS safety signals were evident. Overall, the safety pharmacology assessment did not identify toxicities other than those already well known for cytarabine and daunorubicin. In view of the known toxicities of both compounds, further non-clinical testing is not warranted.

No stand-alone nonclinical pharmacodynamic drug interactions studies have been conducted with Vyxeos. The risks of pharmacodynamic drug interactions are expected to be similar to those described in the SmPCs and literature of the referenced medicinal products (DepoCyte and DaunoXome) and, by inference, the respective active ingredients, cytarabine and daunorubicin.

The PK studies were performed in mice, rats, and dogs and involved investigations of single- and repeat-dose PK, tissue distribution, metabolism, and excretion of Vyxeos following IV administration. The nonclinical species, strains, drug doses, and routes of administration were comparable with those used in the nonclinical pharmacology and toxicology programmes. The same liquid formulation used in the pharmacology and toxicology studies was also used in the nonclinical PK studies.

Cytarabine:daunorubicin molar ratios in animal plasma were maintained near the desired 5:1 molar ratio for at least 24 hours after IV administration. This was attributed to high retention of the drug cargo within the liposomes. *In vivo* release from Vyxeos liposomes is deduced to be very slow, because only a very small fraction (<1%) of the total drug concentration in the circulation exists as free drug.

Due to high retention of drug cargo within Vyxeos liposomes, when cytarabine and daunorubicin are administered to animals as components of Vyxeos, the liposomes appear to govern their tissue distribution and rates of elimination therefore whilst the non-liposomal drugs have markedly different CL, V, and t½, Vyxeos causes these PK parameters to converge. High and sustained plasma concentrations are achieved with Vyxeos because CL is approximately 100-times lower than those of the non-liposomal drugs. The volume of distribution is approximately the same as the blood volume, because the liposomes are mostly confined to the vascular space.

Upon repeat-dose administration of Vyxeos in animals, there were no major deviations from dose proportionality or time linearity for cytarabine or daunorubicin. Very good allometric relationships indicate that excretion, metabolism, and distribution data in animals are likely to translate directly to humans.

Tissue distribution data for cytarabine and daunorubicin suggested that Vyxeos liposomes are slowly taken up by the mononuclear phagocyte system (MPS) in several tissues, particularly spleen. Intracellular accumulation of Vyxeos is higher within leukaemic than normal bone marrow cells (9.5-fold higher for cytarabine; 2.2-fold higher for daunorubicin), indicating selective targeting. After internalization by leukaemia cells, Vyxeos liposomes undergo degradation, releasing the drug cargo within the intracellular environment and enabling the drugs to exert their antineoplastic activity. There were different patterns of tissue distribution for 14C-cytarabine and 14C-daunorubicin associated with Vyxeos versus the non-liposomal formulations.

The major metabolism and biotransformation pathways in humans are similar to those in animals and qualitatively similar for Vyxeos versus non-liposomal drugs the only difference being that the rates of excretion are slower with Vyxeos.The presence of the major human metabolites (AraU and daunorubicinol) in rats and dogs suggested suitability of these animal models to predict Vyxeos safety in humans.

The toxicology profile of Vyxeos is sufficiently characterised based on the data and information generated in the Vyxeos toxicology studies and is comparable with the known toxicological effects of cytarabine and daunorubicin. No unexpected or novel toxicities were noted for Vyxeos, beyond those reported for cytarabine or daunorubicin.

Repeat-dose toxicity of Vyxeos was tested in two-cycle intravenous infusion toxicity studies with 28 day recovery periods conducted in rats and dogs. Adverse effects of Vyxeos occurred at all tested dose levels (low to null safety margins as based on systemic exposures) and were generally consistent with those known for non-liposomal daunorubicin and/or cytarabine, comprising mainly findings of gastrointestinal and hematological toxicity. Although central nervous system (CNS) and cardiovascular system parameters were included in these studies, given the observed morbidity and mortality, there was insufficient information to arrive at an integrated assessment of safety pharmacology of Vyxeos. Vyxeos contains daunorubicin, which is known for its profound cardiotoxicity potential, and cytarabine, which is known to be associated with CNS toxicities(SmPC, section 5.3).

Carcinogenicity, mutagenicity, and reproductive toxicity studies have not been conducted with Vyxeos(SmPC, section 5.3). This is acceptable since cytarabine and daunorubicin are well known compounds and their toxicological properties are well characterise and described in the SmPC.

A high incidence of mammary tumours was observed about 120 days after a single intravenous dose of daunorubicin in rats (at about 1.7 times the human dose on a mg/m^2 basis). Daunorubicin was mutagenic in *in vitro* tests (Ames assay, V79 hamster cell assay), and clastogenic *in vitro* (CCRF CEM human lymphoblasts) and *in vivo* (SCE assay in mouse bone marrow) tests (SmPC, section 5.3).

Cytarabine was mutagenic in *in vitro* tests and was clastogenic *in vitro* (chromosome aberrations and SCE in human leukocytes) and *in vivo* (chromosome aberrations and SCE assay in rodent bone marrow, mouse micronucleus assay). Cytarabine caused the transformation of hamster embryo cells and rat H43 cells in *vitro* (SmPC, section 5.3).

Cytarabine was clastogenic to meiotic cells (SmPC, section 5.3).

Both cytarabine and daunorubicin, tested separately, showed teratogenic and embryotoxic effects in animal studies. Furthermore, daunorubicin caused testicular atrophy and total aplasia of spermatocytes in the seminiferous tubules in dogs and cytarabine, sperm-head abnormalities in mice. A single dose of cytarabine in rats, administered on day 14 of gestation, reduced prenatal and postnatal brain size and caused permanent impairment of learning ability (SmPC, section 5.3).

Based on findings in animals, male fertility may be compromised by treatment with Vyxeos (SmPC, section 4.6).

Each vial contains 100 mg of copper gluconate, which corresponds to 14 mg of elemental copper. Vyxeos should only be used in patients with a history of Wilson's disease or other copper-related disorder if the benefits outweigh the risks. Vyxeos should be discontinued in patients with signs or symptoms of acute copper toxicity (SmPC, section 4.4).

The amounts of cobalt that patients will receive following the therapeutic administration of Vyxeos, is unlikely to pose an unacceptable risk to the intended patient population.

While cytarabine is not a carcinogen, daunorubicin is a possible carcinogen, hence, Vyxeos may be associated with a carcinogenic potential. Both daunorubicin and cytarabine are genotoxic, therefore, Vyxeos may be associated with a genotoxic risk (SmPC, section 5.3).

Environmental risk assessment has shown that Vyxeos is not anticipated to have the potential to be persistent, bioaccumulative, or toxic to the environment (SmPC, section 5.3).

2.3.7. Conclusion on the non-clinical aspects

Overall, the non-clinical documentation submitted was considered adequate. The relevant information has been included in the SmPC (sections 4.4, 4.6, 5.1, 5.3).

2.4. Clinical aspects

2.4.1. Introduction

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.

- Tabular overview of clinical studies

Table 7 Clinical studies in the clinical development program of Vyxeos

Study Number, Status	Type of Study	Phase; Study Design	Primary Objective(s)	Number and Age (mean [range]) of Subjects	Treatment; Route of Administration, Dose, and Duration of Treatment
CLTR0305-101 Complete	Safety and PK	Phase 1; OL, single-arm, dose escalation	To determine the recommended Phase 2 dose of CPX-351 (defined as MTD in protocol) that can be given to subjects with advanced hematologic malignancies	31 male subjects and 17 female subjects with confirmed AML, ALL or MDS; 60 (23 to 81)	CPX-351, IV (90 min infusion) Dose escalation by cohort: Cohort 1: 3 units/m^2 Cohort 2: 6 units/m^2 Cohort 3: 12 units/m^2 Cohort 4: 24 units/m^2 Cohort 5: 32 units/m^2 Cohort 6: 43 units/m^2 Cohort 7: 57 units/m^2 Cohort 8: 76 units/m^2 Cohort 9: 101 units/m^2 Cohort 10: 134 units/m^2
Study Number, Status	**Type of Study**	**Phase; Study Design**	**Primary Objective(s)**	**Number and Age (mean [range]) of Subjects**	**Treatment; Route of Administration, Dose, and Duration of Treatment**
CLTR0308-204 Complete	Safety and Efficacy	Phase 2b OL, Parallel-arm, Rand, MC, Standard-therapy controlled	To estimate the rate of response and confirm the safety of CPX-351 compared to 7 + 3 as first line therapy in elderly patients with standard or high risk AML	78 male subjects and 48 female subjects with newly diagnosed or secondary AML; 68 (60 to 77)	CPX-351, IV (90 min infusion) *1st Induction:* 100 units/m^2/day, Days 1, 3 and 5 *2nd Induction:* 100 units/m^2/day, Days 1 and 3 *Consolidation (up to 2 courses):* 100 units/m^2/day, Days 1 and 3 7 + 3, IV (control) *1st Induction:* Cytarabine 100 mg/m^2/day, 7-day continuous infusion Daunorubicin 40-60 mg/m^2/day, Days 1, 2 and 3 *2nd Induction:* Cytarabine 100 mg/m^2/day, 5-day continuous infusion Daunorubicin 40-60 mg/m^2/day, Days 1 and 2 *Consolidation (up to 2 courses):* *(Investigator's choice)* Recommended: Cytarabine 100-200 mg/m^2/day for 5-7 days ± anthracycline or Cytarabine 1.0-1.5 g/m^2/day for 4-6 days

Study Number, Status	Type of Study	Phase; Study Design	Primary Objective(s)	Number and Age (mean [range]) of Subjects	Treatment; Route of Administration, Dose, and Duration of Treatment
CLTR0308-205 Complete	Safety and Efficacy	Phase 2b OL, Parallel-arm, Rand, MC	To estimate the efficacy of CPX-351 when compared to intensive salvage therapy in patients with AML in first relapse.	57 male subjects and 68 female subjects with pathologically confirmed relapsed AML; 50 (20 to 66)	CPX-351, IV (90 min infusion) *1st Induction:* 100 units/m^2/day, Days 1, 3 and 5 *2nd Induction:* 100 units/m^2/day, Days 1 and 3 *Consolidation (up to 2 courses):* 100 units/m^2/day, Days 1 and 3 Salvage Therapy (control) (Investigator's choice of intensive salvage therapy included:) *1st Induction*[a] • Mitoxantrone / Etoposide based regimens such as "MEC" • 7 + 3 • Cytarabine ± anthracycline ± additional agents: *2nd Induction* Second inductions were administered according to local practice. *Consolidation (up to 2 courses)* Investigators chose an appropriate consolidation regimen according to local practice.
CLTR0310-206 Complete	Safety, Efficacy, and PK	Phase 2 OL, Single-arm, MC	To assess the effects of CPX-351 on cardiac repolarization following the first induction cycle as determined by QTcF	14 male subjects and 12 female subjects with pathological confirmation of AML with high cytogenetic risk, secondary AML, refractory AML or ALL, or MDS; 65 (37 to 80)	CPX-351, IV (90 min infusion) *1st Induction:* 100 units/m^2/day, Days 1, 3 and 5 *2nd Induction:* 100 units/m^2/day, Days 1 and 3 *Consolidation (up to 4 courses):* 65 units/m^2/day, Days 1 and 3
Study Number, Status	**Type of Study**	**Phase; Study Design**	**Primary Objective(s)**	**Number and Age (mean [range]) of Subjects**	**Treatment; Route of Administration, Dose, and Duration of Treatment**
CLTR0310-301 Ongoing	Safety, Efficacy and PK	Phase 3 OL, parallel-arm, Rand, MC, standard-therapy controlled	To confirm the efficacy of CPX-351 compared with 7 + 3 as first line therapy in elderly patients (60 to 75 years old) with high risk (secondary) AML.	190 male subjects and 119 female subjects with newly diagnosed AML; 68 (60 to 75)	CPX-351, IV (90 min infusion) *1st Induction:* 100 units/m^2/day, Days 1, 3 and 5 *2nd Induction:* 100 units/m^2/day, Days 1 and 3 *Consolidation (up to 2 courses):* 65 units/m^2/day, Days 1 and 3 7 + 3 / 5 + 2, IV (control) *1st Induction:* Cytarabine 100 mg/m^2/day, 7-day continuous infusion Daunorubicin 60 mg/m^2/day, Days 1, 2 and 3 *2nd Induction:* Cytarabine 100 mg/m^2/day, 5-day continuous infusion Daunorubicin 60 mg/m^2/day, Days 1 and 2 *Consolidation (up to 2 courses):* Cytarabine 100 mg/m^2/day, 5-day continuous infusion Daunorubicin 60 mg/m^2/day, Days 1 and 2

Abbreviations: AML = acute myeloid leukemia; AE = adverse event; ALL = acute lymphocytic leukemia; CI= continuous infusion; D= days; IV = intravenous; MC = multicenter; MDS = myelodysplastic syndrome; OL = open-label; PK = pharmacokinetic; QTcF = QT interval with Fridericia's correction; Rand = randomised;

[a] In Study 205, the decision of which salvage therapy to prescribe was up to the investigator. Recommended dosing regimens were as follows: *1st induction*: High Dose Ara-C ± Anthracycline: Cytarabine: 6-36 g/m^2 Total Dose (eg, 3 g/m^2 x 12

doses; 1.5 g/m2 continuous infusion x 4 days; 2 g/m2 Q12h x 8 doses); "7 + 3": Cytarabine: 100- 200 mg/m2 CI x 7 days; Daunorubicin: 45 to 60 mg/m2 days 1-3 or equivalent; Mitoxantrone / Etoposide based regimens such as "MEC": Mitoxantrone 8 mg/m2/day IV + Etoposide 100 mg/m2/day IV + Cytarabine 1000 mg/m2/day IV on days 1-5.

2.4.2. Pharmacokinetics

Data on clinical pharmacokinetics are provided from three clinical studies as indicated in Table 9.

Table 8 Tabular summary of clinical pharmacology studies

Clinical Study	Title	Formulation	Induction Dose (Number of Subjects with PK Data)	PK Samples
Study 101	Phase 1 Study of CPX-351 Liposome Injection in Subjects with Advanced Hematologic Malignancies	Liquid	3 units/m^2 (n=1) 6 units/m^2 (n=1) 12 units/m^2 (n=2) 24 units/m^2 (n=3) 32 units/m^2 (n=3) 43 units/m^2 (n=4) 57 units/m^2 (n=3) 76 units/m^2 (n=3) 101 units/m^2 (n=13) 134 units/m^2 (n=6)	Plasma samples were collected at the following times (relative to the start of the infusion) during the first induction: **Day 1**: Prior to dosing, during the infusion at 45 min (or the mid-point of the infusion) and 90 min (or at EOI), then at 2, 4, 6, 8, 12 and 24 h relative to the SOI. **Day 3:** prior to dosing, during the infusion at 45 and 90 min. **Day 5:** Prior to dosing, during the infusion at 45 min (or the mid-point of the infusion) and 90 min (or at the EOI), then at 2, 4, 6, 8, 12, 24, 48, 72, 96, and 168 h relative to the SOI
Study 206	An Open Label Phase 2 Pharmacokinetic and Pharmacodynamic Assessment of the Potential for QTc Prolongation Following First Induction Treatment with CPX-351 Liposome Injection in Acute Leukemias and MDS Subjects	Lyophilized	100 units/m^2 (n=26)	The following plasma samples were collected after the first induction. **Day 1**: pre-dose, 45 min, 1.5, 2, 4, 6, 8, and 24 h after SOI on Day 1 **Day 5:** pre-dose, 45 min, 1.5, 2, 4, 6, 8, 24, 28, 48, 72, 96, 168, 216, 336, and 384 h after the SOI on Day 5
Study 301	Phase 3, Multicenter, Randomized, Trial of CPX-351 Liposome Injection versus Cytarabine and Daunorubicin in Subjects 60-75 years of Age with Untreated High Risk (Secondary) AML	Lyophilized	100 units/m^2 (n=132[†])	Subjects randomized to CPX-351 had samples drawn for population PK. Five plasma samples per subject were taken during the first induction, all times were relative to the start of Day 1 infusion. CPX-351 subjects were randomized to one of two PK sampling schedules: **Schedule 1 Day 1**: 45 min, 3 h, 8 h, Day 3 prior to dosing (48 h (+/- 6 h)) and on Day 7 (168 h (+/- 6 h)) or **Schedule 2: Day 1**: EOI, 2 h, 6 h, Day 5 prior to dosing (96 h (+/- 6 h)) and on Day 7 (168 h (+/- 6 h)).

[†] One subject was excluded due to missing/erroneous sampling date and/or time records.
Urine PK samples were collected from some of the subjects Study 206 as described in 2.2.2
AML, acute myeloid leukemia; EOI, end of infusion; h, hours; MDS, myelodysplasia syndrome; min, minutes; QTc, interval between the start of Q wave to the end of the T wave.
Source: Study 101 Protocol, Study 206 Protocol, and Study 301 Protocol; and (for sample sizes) Population PK Report

2.4.3. Methods

The bioanalytical methods for cytarabine, daunorubicin and their respective metabolites were based on liquid chromatography with tandem mass spectrometry (LC-MS/MS) among the studies.

Absorption

Vyxeos is administered intravenously, therefore absorption is not applicable. The plasma PK of total daunorubicin following administration of Vyxeos is presented in Table 10.

Table 9 Plasma PK of Total Daunorubicin Following Administration of Vyxeos

Clinical Study	CPX-351 Dose (units/m^2)	PK Interval	No. Subjects	C_{max} (µg/mL)	AUC_{48h} (µg.h/mL)	$t_{1/2}$ (h)	CL (L/h)	Vss (L)
Study 101	3	Day 1	1	6.94	216	28.2	0.0177	0.727
		Day 5	1	3.77	113	31.1	0.0497	2.17
	6	Day 1	1	2.23	28.7	9.67	0.346	4.58
		Day 5	1	2.11	29.6	9.54	0.346	4.37
	12	Day 1	2	5.88 (13.9%)	131 (73.8%)	17.3 (103.7%)	0.204 (79.3%)	3.2 (30.4%)
		Day 5	2	7.89 (54.0%)	192 (121.7%)	23.3 (126.8%)	0.4 (114.5%)	3.48 (68.4%)
	24	Day 1	3	12.9 (43.5%)	330 (33.4%)	38.8 (43.1%)	0.101 (60.4%)	4.72 (25.4%)
		Day 5	3	14.7 (36.7%)	433 (50.7%)	31.9 (8.7%)	0.13 (42.4%)	6.62 (39.6%)
	32	Day 1	3	16.6 (24.1%)	482 (28%)	40.7 (26.9%)	0.0817 (53.6%)	4.38 (31.1%)
		Day 5	3	25.3 (29.8%)	742 (26.3%)	35.1 (11.1%)	0.089 (28.9%)	4.69 (34.1%)
	43	Day 1	4	20.0 (2.8%)	575 (24.2%)	54.6 (79.4%)	0.074 (54.2%)	4.16 (12.9%)
		Day 5	3	31.7 (16.2%)	984 (43.9%)	35.8 (20.6%)	0.0871 (46.0%)	3.92 (21.7%)
	57	Day 1	3	20.3 (13.7%)	601 (14.3%)	63.6 (83.9%)	0.07 (38.7%)	5.31 (31%)
		Day 5	3	33.9 (3.0%)	1082 (8.2%)	37.2 (18.0%)	0.0863 (17.1%)	4.83 (21.1%)
	76	Day 1	3	29.1 (35%)	813 (59.3%)	43.3 (98.9%)	0.218 (107.9%)	7.31 (47%)
		Day 5	3	39.9 (54.1%)	1102 (62.3%)	28.4 (30.5%)	0.236 (82.0%)	9.58 (67.9%)
	101	Day 1	13	42.6 (19.6%)	1158 (33.2%)	42.5 (62.2%)	0.119 (56.8%)	5.48 (36.9%)
		Day 5	13	64.6 (36.0%)	1851 (50.5%)	36.9 (66.4%)	0.131 (41.2%)	5.88 (38.3%)
	134	Day 1	6	62.6 (15%)	1896 (15.0%)	52.2 (44.6%)	0.0677 (50.9%)	4.36 (27.6%)
		Day 5	6	89.1 (4.8%)	2973 (13.8%)	46.0 (35.2%)	0.0809 (14.1%)	5.36 (46.3%)
Study 206	100	Day 1	26	46.0 (27.3%)	1110 (31.0%)	NA	NA	NA
		Day 5	26	62.2 (33.7%)	1900 (44.3%)	40.4 (24.2%)	0.131 (60.2%)	7.11 (49.2%)
Study 101	3	Day 1	1	3.30	91.5	16.2	0.0234	0.553
		Day 5	1	1.06	24.0	12.9	0.103	2.09
	6	Day 1	1	1.15	14.8	8.69	0.295	3.54
		Day 5	1	1.03	15.9	8.27	0.283	3.31
	12	Day 1	2	2.92 (20.8%)	61.4 (73.1%)	13.0 (78.8%)	0.195 (67.2%)	2.64 (19.1%)
		Day 5	2	3.83 (39.5%)	74.0 (109.9%)	14.2 (114.8%)	0.305 (99.2%)	2.56 (60.2%)
	24	Day 1	3	6.50 (38.1%)	135 (21.4%)	19.3 (19.3%)	0.135 (27.4%)	3.49 (21.1%)
		Day 5	3	6.41 (24.6%)	151 (30.5%)	20.6 (24.6%)	0.15 (32.8%)	4.8 (33.3%)
	32	Day 1	3	8.54 (15.8%)	230 (16.4%)	17.9 (38.8%)	0.0939 (17.5%)	2.97 (25.1%)
		Day 5	3	9.06 (25.2%)	257 (35.1%)	20.3 (28%)	0.118 (39.7%)	7.35 (64%)
	43	Day 1	4	11.2 (20.4%)	288 (35.8%)	24.2 (43.6%)	0.0984 (54.6%)	2.79 (15.8%)
		Day 5	3	14.8 (27.2%)	395 (46.3%)	26.7 (58.7%)	0.102 (60.3%)	4.21 (39.7%)
	57	Day 1	3	14.3 (8.7%)	377 (5.3%)	32.4 (33.7%)	0.0706 (13.6%)	3.19 (35.1%)
		Day 5	3	17.6 (10.5%)	474 (1.9%)	35.0 (8.2%)	0.0878 (27%)	3.59 (21.9%)
	76	Day 1	3	18.2 (33%)	446 (42.7%)	16.8 (43.2%)	0.172 (53.5%)	3.67 (35.2%)
		Day 5	3	20.6 (45%)	444 (47.7%)	19 (8.9%)	0.206 (53.1%)	5 (42%)
	101	Day 1	13	24.8 (18.3%)	553 (27.6%)	22.1 (31.2%)	0.133 (31%)	3.82 (32.7%)
		Day 5	13	30.2 (20.5%)	667 (31.4%)	25.2 (46.1%)	0.143 (32.8%)	4.55 (36.5%)
	134	Day 1	6	37.8 (14.5%)	954 (12.7%)	26.2 (21.6%)	0.0816 (20.9%)	3.01 (36.8%)
		Day 5	6	44.7 (5.8%)	1204 (17.3%)	37.0 (31.5%)	0.0879 (10%)	4.06 (34.7%)
Study 206	100	Day 1	26	23.1 (28.5%)	499 (28.9%)	NA	NA	NA
		Day 5	26	26.0 (32.7)	637 (38.4%)	31.5 (28.5%)	0.163 (53.3%)	6.64 (36.8)

NOTES: Values reported as mean (%CV). Values may differ from clinical study reports due to rounding.
AUC_{48h}, area under the curve from t = 0 to 48 h postdose; C_{max}, maximum observed concentration; $t_{1/2}$, terminal elimination half-life; CL, clearance; V_{ss}, steady-state volume of distribution; NR, not reported. NA, not available

Distribution

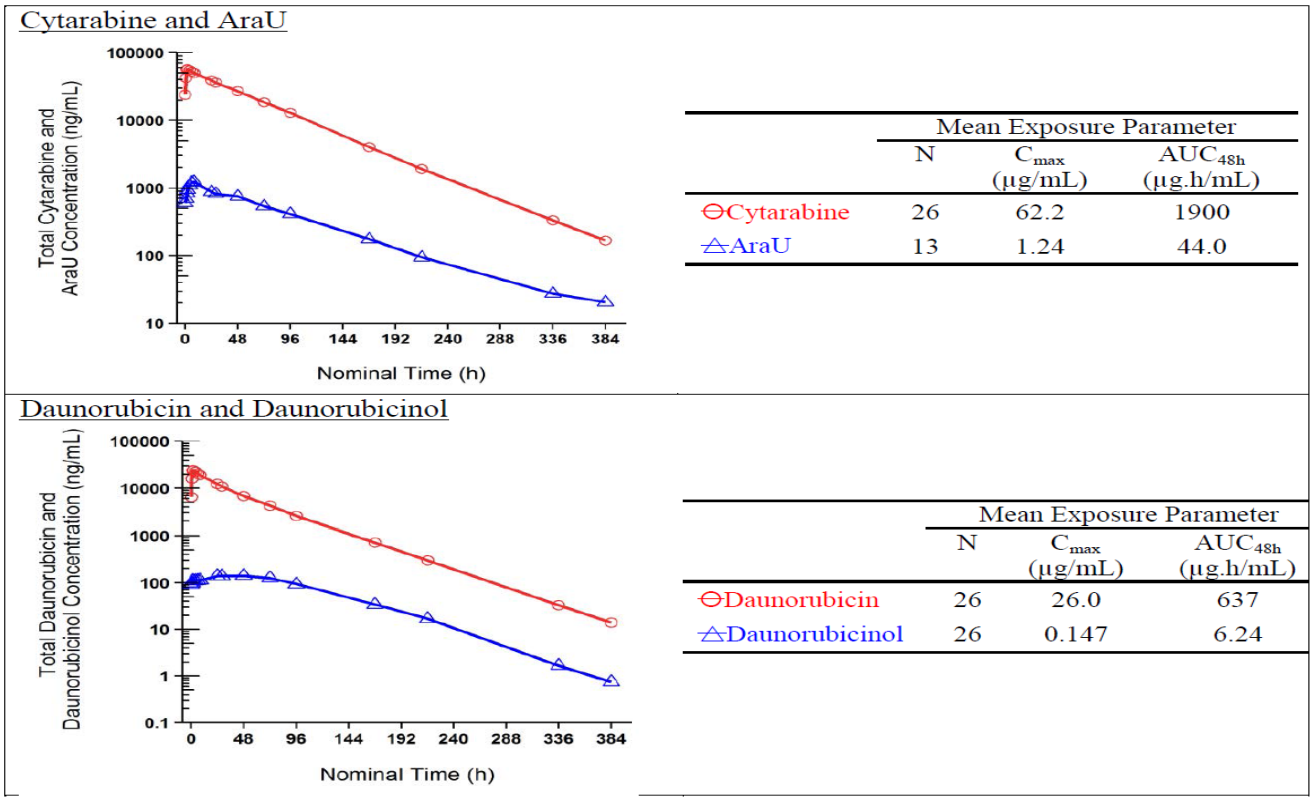
The volume of distribution (%CV) for daunorubicin is 6.6 L (36.8%) and cytarabine is 7.1 L (49.2%). Plasma protein binding was not evaluated (SmPC, section 5.2).

Elimination

Based on published data, cytarabine to Ara-U and daunorubicin to daunorubicinol are important systemic metabolism pathways in humans, and Ara-U and daunorubicinol are major human metabolites. To assess these pathways with Vyxeos, data on the plasma PK of Ara-U and daunorubicinol were obtained from 13 – 26 patients who received Vyxeos at 100 units/m^2 on Days 1, 3, and 5 in Study 206. Plasma concentration-time profiles show that plasma concentrations of the metabolites were considerably lower than those of the parent drugs (~40-60% for daunorubicinol:daunorubicin and ~80% for AraU:cytarabine.

Figure 3). Median T_{max} of Ara-U and daunorubicinol after the Day 5 dose were 8.0 h and 26.2 h, respectively, i.e. substantially later than T_{max} of the parent drugs. The mean AUC_{last} metabolite/parent ratio was 3.22% for Ara-U/cytarabine and 1.79% for daunorubicinol/daunorubicin. These ratios are lower than the ratios typically reported for the non-liposomal products, ~40-60% for daunorubicinol:daunorubicin and ~80% for AraU:cytarabine.

Figure 3 Mean Plasma Concentration-Time Curves and Exposure Parameters for Total Cytarabine and Daunorubicin and their Major Metabolites on Day 5 (Study 206)



	Mean Exposure Parameter		
	N	C_{max} (µg/mL)	AUC_{48h} (µg.h/mL)
Cytarabine	26	62.2	1900
AraU	13	1.24	44.0

	Mean Exposure Parameter		
	N	C_{max} (µg/mL)	AUC_{48h} (µg.h/mL)
Daunorubicin	26	26.0	637
Daunorubicinol	26	0.147	6.24

AraU, 1-β-d-arabinofuranosyluracil; AUC_{48h}, area under the curve from t = 0 to 48 hours postdose; C_{max}, maximum observed concentration; N, sample size

Figure 4 Plasma PK Parameters for Cytarabine and Daunorubicin when Administered as Vyxeos or as Non-liposomal Formulations

PK Parameter	CPX-351		Non-liposomal	
	Cytarabine	Daunorubicin	Cytarabine	Daunorubicin
V (L)	7.11	6.64	138[a]	1364[a]
CL (L/h)	0.131	0.163	272[a]	129[a]
$t_{1/2}$ (h)	40.4	31.5	1-3[b]	18.5[c]

NOTES: Values reported are means.

V, volume of distribution; CL, clearance; $t_{1/2}$, terminal half-life

Total urinary recovery over one dosing interval for cytarabine plus Ara-U averaged 70.7% (33.8%). The mean renal clearance of cytarabine was 0.00167 (0.00102) L/h and of Ara-U was 3.81 (2.02) L/h (Table 11).

Table 10 Urinary excretion parameters for cytarabine and Ara-U

	Cytarabine			AraU			Total
Statistic	Ae (mg)	Cumulative % Dose Recovered (%)	Renal Clearance (L/h)	Ae (mg)	Cumulative % Dose Recovered (% of Dose)	Renal Clearance (L/h)	Percent Recovered (%)
N	6	6	6	6	6	6	6
Mean	2.22	1.11	0.00167	136	69.6	3.81	70.7
SD	0.748	0.354	0.00102	56.6	33.9	2.02	33.8
CV%	33.6	31.7	60.9	41.6	48.7	52.9	47.8
Geo Mean	2.11	1.06	0.00140	128	64.2	3.38	65.4
Geo CV%	38.6	35.9	75.7	38.5	44.1	58.8	43.1
Median	2.23	1.15	0.00168	112	53.1	3.67	45.0
Min	1.11	0.576	0.000581	92.5	43.9	1.88	54.5
Max	3.35	1.67	0.00334	237	132	6.92	133

Ae = Amount Excreted in mg.

Total urinary recovery over one dosing interval for daunorubicin plus daunorubicinol averaged 9.00% (3.21%). The mean renal clearance of daunorubicin was 0.00610 (0.00284) L/h and of daunorubicinol was 1.13 (0.738) L/h (Table 12).

Table 11 Urinary excretion parameters for daunorubicin and daunorubicinol

	Daunorubicin			Daunorubicinol			Total
Statistic	Ae (mg)	% Dose Recovered (%)	Renal Clearance (L/h)	Ae (mg)	% Dose Recovered (%)	Renal Clearance (L/h)	Percent Recovered (%)
N	6	6	6	6	6	6	6
Mean	2.77	3.19	0.00610	5.00	5.80	1.13	9.00
SD	0.756	0.912	0.00284	1.79	2.49	0.738	3.21
CV%	27.3	28.6	46.5	35.8	42.9	65.3	35.7
Geo Mean	2.70	3.09	0.00554	4.78	5.46	0.936	8.60
Geo CV%	25.8	27.8	51.4	32.1	37.9	77.2	32.6
Median	2.39	2.82	0.00565	4.61	5.24	0.913	6.03
Min	2.14	2.40	0.00330	3.55	3.63	0.388	7.88
Max	4.00	4.35	0.00967	8.42	10.6	2.24	15.0

Ae = Amount Excreted in mg

Dose proportionality and time dependencies

Table 12 Cytarabine PK parameters Vyxeos Dose (3 – 134 units/m^2) for Days 1 and 5

Dose (units/m^2) and Day	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{0\text{-last}}$ $AUC_{0\text{-tau}}$ (ng*hr/mL)	$AUC_{0\text{-inf}}$ $AUC_{0\text{-tau}}$ (ng*hr/mL)	$t_{1/2}$ (hr)	Vz (mL/m^2)	Vss (mL/m^2)	CL (mL/hr/m^2)	C_{max}/Dose [(ng/mL) /(mg/kg)]	$AUC_{0\text{-last}}$/Dose $AUC_{0\text{-tau}}$/Dose [(ng*hr/mL) /(mg/kg)]	$AUC_{0\text{-inf}}$/Dose $AUC_{0\text{-tau}}$/Dose [(ng*hr/mL) /(mg/kg)]
24 Day 1	12897	3.83	330179	599560	38.8	2397	2367	50.4	537	13757	24982
24 Day 5	14733	2.33	432519	432519	31.9	2880	3267	64	614	18022	18022
32 Day 1	16600	2	481688	888748	40.7	2232	2213	41	519	15053	27773
32 Day 5	25333	3.17	741802	741802	35.1	2319	2364	45	792	23181	23181
43 Day 1	19975	1.75	575294	1392494	54.6	2457	2452	44	465	13379	32384
43 Day 5	31700	3	983820	983820	35.8	2515	2263	50.5	737	22880	22880
57 Day 1	20267	6	601127	1631171	63.6	3067	3220	46	356	10546	28617
57 Day 5	33933	4.5	1081929	1081929	37.2	2868	2977	52.9	595	18981	18981
76 Day 1	29133	2.33	813020	1693635	43.3	3269	3206	105	383	10698	22285
76 Day 5	39900	5.83	1101673	1101673	28.4	4290	4364	111	525	14496	14496
101 Day 1	42577	2.42	1158444	2276888	42.5	2750	2739	62.5	422	11470	22543
101 Day 5	64608	3.02	1851089	1851089	36.9	2876	2925	67.3	640	18328	18328
134 Day 1	62567	2.42	1896454	4216357	52.2	2398	2389	37.1	467	14153	31465
134 Day 5	89117	2.58	2972656	2972656	46	2939	2896	45.9	665	22184	22184
p: Treatment	<0.001	0.657	<0.001	<0.001	0.834	0.875	0.707	0.618	0.378	0.460	0.618
p: Day	<0.001	0.834	<0.001	0.004	0.117	0.250	0.039	0.004	<0.001	<0.001	0.004
p: Interaction	0.547	0.570	0.633	0.986	0.996	0.940	0.400	0.986	0.547	0.633	0.986

* Note – Due to limited sample size Group 1-3 (i.e., 3, 6, and 12 units/m^2 CPX-351) were excluded from statistical comparisons.

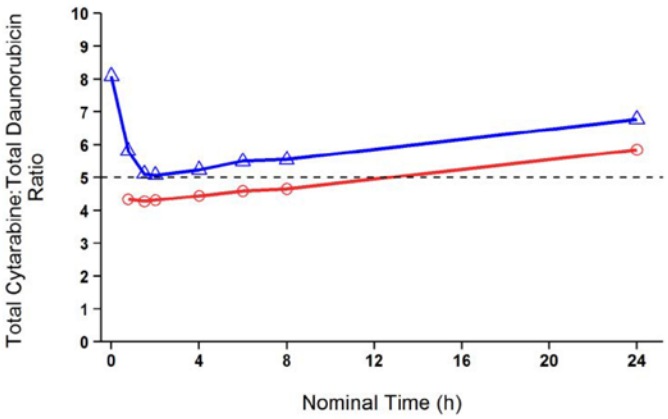
Table 13 Daunorubicin PK parameters Vyxeos Dose (3 – 134 units/m^2) for Days 1 and 5

Table 8. Statistical Comparison* of Daunorubicin Pharmacokinetic Parameters between Day 1 and Day 5 Following Administration of 3, 6, 12, 24, 32, 43, 57, 76, 101, and 134 units/m^2 CPX-351 Every 48 Hours by Intravenous Infusion over 90 Minutes to Patients with Advanced Hematologic Malignancies

Dose (units/m^2) and Day	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{0\text{-last}}$ $AUC_{0\text{-tau}}$ (ng*hr/mL)	$AUC_{0\text{-inf}}$ $AUC_{0\text{-tau}}$ (ng*hr/mL)	$t_{1/2}$ (hr)	Vz (mL/m^2)	Vss (mL/m^2)	CL (mL/hr/m^2)	C_{max}/Dose [(ng/mL) /(mg/kg)]	$AUC_{0\text{-last}}$/Dose $AUC_{0\text{-tau}}$/Dose [(ng*hr/mL) /(mg/kg)]	$AUC_{0\text{-inf}}$/Dose $AUC_{0\text{-tau}}$/Dose [(ng*hr/mL) /(mg/kg)]
24 Day 1	6503	3.83	135013	165360	19.3	1835	1753	67.2	614	12737	15600
24 Day 5	6410	1.67	151297	151297	20.6	2274	2374	74.2	605	14273	14273
32 Day 1	8543	2.67	229618	298098	17.9	1195	1506	47.7	606	16285	21142
32 Day 5	9057	1.83	257151	257151	20.3	1725	3734	59.4	642	18238	18238
43 Day 1	11248	1.88	287905	411360	24.2	1687	1639	58.3	595	15233	21765
43 Day 5	14833	2.33	395338	395338	26.7	1775	2437	58.8	785	20917	20917
57 Day 1	14333	2.67	376944	590219	32.4	1949	1894	44.3	571	15018	23515
57 Day 5	17633	2.5	473848	473848	35	2671	2188	53	703	18878	18878
76 Day 1	18200	2.5	446045	534360	16.8	1641	1596	79	545	13355	15999
76 Day 5	20567	1.67	444427	444427	19	2611	2217	94.8	616	13306	13306
101 Day 1	24831	2.19	552527	725034	22.1	1997	1912	68.4	559	12444	16330
101 Day 5	30185	1.87	666640	666640	25.2	2413	2269	72.9	680	15014	15014
134 Day 1	37750	2.13	953673	1316510	26.2	1700	1634	45.8	640	16164	22314
134 Day 5	44733	2.33	1204275	1204275	37	2575	2205	50.2	758	20411	20411
p: Treatment	<0.001	0.673	<0.001	<0.001	0.080	0.082	0.901	0.222	0.707	0.247	0.222
p: Day	0.001	0.044	0.002	0.004	0.093	0.001	<0.001	0.004	0.001	0.002	0.004
p: Interaction	0.580	0.835	0.644	0.947	0.865	0.696	0.225	0.947	0.580	0.644	0.947

* Note – Due to limited sample size Group 1-3 (i.e., 3, 6, and 12 units/m^2 CPX-351) were excluded from statistical comparisons.

Figure 5 Mean cytarabine:daunorubicin molar ratio vs. Time on Days 1 and 5



	Mean Molar Ratios from 0.75 to 24 Hours Postdose	
	N	Range (min – max)
Day 1	26	4.28 to 5.85
Day 5	26	5.07 to 6.77

NOTES: Ratios were computed by comparing total concentrations of cytarabine:daunorubicin (in molar units) at each PK sample time for individual subjects; the reported ranges correspond to lowest to highest mean ratios from 0.75 to 24 hours after the start of the infusion
N, sample size

Special populations

Table 14 Subjects participating in population PK assessments from study 301, by age category

	Age 65 to 74 n / N (%)	Age 75 to 84 n / N (%)	Age 85+ n / N (%)
Subjects participating in PK Trials (N = 131)	90/131 (68.7)	9/131 (6.9)	0/131 (0)

Based on the covariate analyses, age, sex, race, body weight, body mass index, and white blood cell count do not appear to account for significant sources of variability in PK parameters for total cytarabine or daunorubicin.

Descriptive statistics for cytarabine AUC from time 0 to 48 h after the Day 5 dose (AUC_T) across renal impairment and bilirubin categories in the analysis population are summarised in Table 16.

Table 15 Descriptive statistics of AUC_T for cytarabine among renal impairment and bilirubin categories

Statistics	AUCtau (ng·h/mL)				
	Renal Impairment Categories (Creatinine Clearance)			Bilirubin Categories	
	Normal (≥ 90 mL/min)	Mild (60 - 89 mL/min)	Moderate (30 - 59 mL/min)	<1.2 mg/dL	1.2 – 3 mg/dL
N	69	63	24	141	15
Arithmetic Mean	1760000	2000000	2140000	1940000	1650000
SD	896000	986000	865000	949000	697000
CV%	50.8	48.9	40.5	48.9	42.2
Geometric Mean	1520000	1730000	1960000	1680000	1510000
Geometric CV%	65.4	66.0	46.3	65.1	46.7
Median	1630000	1820000	2280000	1820000	1390000
Minimum	202000	197000	907000	197000	543400
Maximum	4300000	5020000	3920000	5020000	3030000
Lower 95% CI	1550000	1760000	1790000	1790000	1300000
Upper 95% CI	1970000	2240000	2480000	2100000	2000000

The analysis population consisted of all subjects in the PK population who were treated with 100 units/m^2 CPX-351 on Days 1, 3 and 5. This corresponded to subjects in the PK population who were enrolled in studies CLTR0310-206 and CLTR0310-301.
AUCtau: AUC from time 0 to 48 hours after the Day 5 dose, CI: confidence interval, CV% coefficient of variability, N: number of subjects, SD: Standard deviation.

Descriptive statistics for daunorubicin AUC from time 0 to 48 h after the Day 5 dose (AUC_{0-T}) across renal impairment and bilirubin categories in the analysis population are summarised in Table 17.

Table 16 Descriptive statistics of AUC_T for daunorubicin among renal impairment and bilirubin categories

Statistics	AUCtau (ng·h/mL)				
	Renal Impairment Categories (Creatinine Clearance)			Bilirubin Categories	
	Normal (≥ 90 mL/min)	Mild (60 - 89 mL/min)	Moderate (30 - 59 mL/min)	<1.2 mg/dL	1.2 – 3 mg/dL
N	69	63	24	141	15
Arithmetic Mean	584000	655000	684000	635000	562000
SD	256000	249000	225000	255000	201000
CV%	43.8	38.1	32.9	40.1	35.9
Geometric Mean	527000	604000	647000	580000	528000
Geometric CV%	50.8	44.6	36.2	47.7	38.2
Median	531000	643000	723000	596000	531000
Minimum	123000	188000	370000	123000	240000
Maximum	1220000	1160000	1073000	1220000	975000
Lower 95% CI	524000	593000	595000	593000	460000
Upper 95% CI	644000	716000	774000	677000	663000

The analysis population consisted of all subjects in the PK population who were treated with 100 units/m^2 CPX-351 on Days 1, 3 and 5. This corresponded to subjects in the PK population who were enrolled in studies CLTR0310-206 and CLTR0310-301.
AUCtau: AUC from time 0 to 48 hours after the Day 5 dose, CI: confidence interval, CV% coefficient of variability, N: number of subjects, SD: Standard deviation.

Pharmacokinetic interaction studies

No *in vitro* or *in vivo* studies on pharmacokinetic drug interactions have been submitted.

Pharmacokinetics using human biomaterials

N/A

2.4.4. Pharmacodynamics

Mechanism of action

No clinical pharmacodynamic studies were submitted.

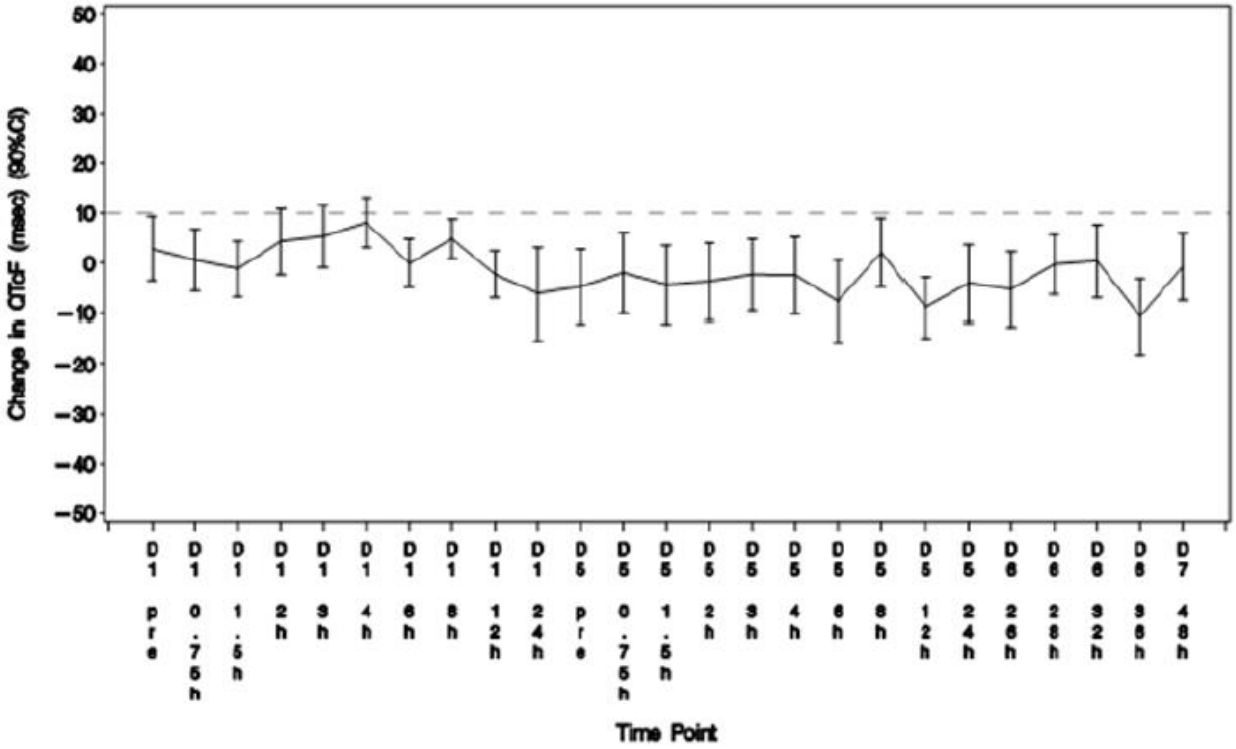
Primary and Secondary pharmacology

No primary pharmacology studies were submitted.

The effect of Vyxeos on cardiac repolarization was determined following the first induction (100 units/m^2 on Days 1, 3 and 5) in Study 206. ECG intervals at selected time points were obtained as the averages from triplicate recordings for all time points. Each ECG was interpreted using digital, on-screen software allowing the placement of electronic markers at the beginning and end of each ECG interval. The primary endpoint was to assess the mean change between time-matched measurements of Fridericia's corrected QT-interval (QTcF) following the first induction dose of Vyxeos. Bazett's corrected QT-interval (QTcB) was also tested for comparison.

A total of 26 patients were enrolled in the study. Twenty-four patients had ECG and PK data; however, not all patients had data at all time-points reducing the number available for comparisons. The largest change from baseline, upper limit of the one-sided 95% CI of the ΔQTcF was 12.3 msecs at 4 hours post-SOI (start of infusion) on Day 1 (mean ± SD, 8.0 ± 11.69 msecs). At 3 hours post-SOI, the UB 95% CI was 10.7 msecs (mean ± SD, 5.3 ± 15.17 msecs), but it was not statistically significant. No other change from baseline 95% CI crossed was greater than 10 msecs (Figure 6). No patients had QTcF changes from baseline greater than 60 msecs and no QTcF values were greater than 500 msecs.

Figure 6 Mean (90% CI) change from baseline and placebo in QTcF duration by time point



Abbreviations: CI = confidence interval; ECG = electrocardiogram; QTcF = Fridericia's corrected QT-interval.
Note that the upper limit of the 2-sided 90% CI is the same quantity as the upper limit for a 1-sided 95% CI.

Results of analyses using QTcB were comparable to those using QTcF (data not shown).

2.4.5. Discussion on clinical pharmacology

The pharmacokinetics of daunorubicin and cytarabine administered as Vyxeos were investigated in adult patients who received a dose of daunorubicin 44 mg/m2 and cytarabine 100 mg/m2 administered as a 90-minute intravenous infusion on days 1, 3, and 5. The pharmacokinetics of each medicinal product was based on total plasma concentrations (i.e., encapsulated plus unencapsulated medicinal product). Following the dose administered on day 5, the mean (% coefficient of variation [CV]) maximum plasma concentrations (Cmax) for daunorubicin was 26.0 (32.7%) mcg/mL and cytarabine was 62.2 (33.7%) mcg/mL. The mean (%CV) area under the curve (AUC) during one dosing interval for daunorubicin was 637 (38.4%) mcg.h/mL and cytarabine was 1900 (44.3%) mcg.h/mL (SmPC, section 5.2).

When daunorubicin and cytarabine are administered as components of Vyxeos, the liposomes appear to govern their tissue distribution and rates of elimination; therefore, while the non-liposomal medicinal products have markedly different clearance (CL), volume of distribution (V), and terminal half-life (t1/2) Vyxeos causes these pharmacokinetic parameters to converge (SmPC, section 5.2).

The accumulation ratio was 1.3 for daunorubicin and 1.4 for cytarabine. There was no evidence of time-dependent kinetics or major departures from dose proportionality over the range of 1.3 mg/3 mg per m^2 to 59 mg/134 mg per m^2 (0.03 to 1.3 times the approved recommended dosage) (SmPC, section 5.2).

Age, sex, race, body weight, body mass index, and white blood cell count do not have a clinically important effect on the exposure of total daunorubicin or cytarabine after adjusting dose by body surface area(SmPC, section 5.2).

Insufficient pharmacokinetic data were collected in paediatric patients to draw conclusions (SmPC, section 5.2).

The pharmacokinetics of Vyxeos in patients aged > 85 years has not yet been evaluated. No data are available(SmPC, section 5.2).

The pharmacokinetics of total daunorubicin and cytarabine were not altered in patients with bilirubin ≤ 50 µmol/L. The pharmacokinetics in patients with bilirubin greater than 50 µmol/L is unknown(SmPC, section 5.2).

Based on a population pharmacokinetic analysis using data from clinical studies in patients, no significant difference in clearance of daunorubicin or cytarabine was observed in patients with pre-existing mild to moderate renal impairment (30 mL/min ≤ creatinine clearance [CrCL] ≤ 59 mL/min for moderate, and 60 mL/min ≤ creatinine clearance [CrCL] ≤ 89 mL/min for mild)) compared to patients with baseline normal renal function (CrCL ≥ 90 mL/min). The potential effects of severe renal impairment (CrCL 15 mL/min to 29 mL/min, C-G) and end stage renal disease on the pharmacokinetics of daunorubicin and cytarabine administered as Vyxeos are unknown (SmPC, section 5.2).

Vyxeos can be considered an optimised liposomal formulation of the standard 7+3 daunorubicin and cytarabine combination chemotherapy with the advantage of a synergy anti-tumour activity between cytarabine and anthracycline and the delivery of these two anti-neoplastic substances at a high cargo to the target leukaemic bone marrow.

Anthracyclines are considered to be among the most active drugs in AML treatment but their use have been limited by the cumulative dose related cardiac toxicity. Vyxeos is associated with a lower cumulative dose of daunorubicin compared to 7+3 and there is no evidence of having a meaningful clinical effect on cardiac repolarization. However, long term safety data would still be needed to confirm any advantage with regards to cardiac toxicity, of no concern for this submission given the proposed patient population has very poor prognosis. Concurrent use of cardiotoxic agents may increase the risk of cardiotoxicity. Use of Vyxeos in patients who have previously received doxorubicin increases the risk of cardiotoxicity. Vyxeos should not be administered in combination with other cardiotoxic agents unless the patient's cardiac function is closely monitored (SmPC, section 4.5).

No interaction studies have been performed with Vyxeos. The delivery of daunorubicin and cytarabine in the Vyxeos liposomal formulation is anticipated to reduce the possibility of interactions, because systemic free-drug concentrations of daunorubicin and cytarabine are much lower than when administered as the non-liposomal formulation (SmPC, section 4.5).

Hepatotoxic medicinal products may impair liver function and increase the toxicity. Since daunorubicin is metabolised by the liver, changes in hepatic function induced by concomitant therapies may affect metabolism, pharmacokinetics, therapeutic efficacy, and/or the toxicity of Vyxeos. Monitor hepatic function more frequently when Vyxeos is coadministered with hepatotoxic agents (SmPC, section 4.5).

2.4.6. Conclusions on clinical pharmacology

The PK of Vyxeos has been reasonably well investigated. The relevant information has been included in the SmPC (sections 5.2).

2.5. Clinical efficacy

2.5.1. Dose response study

Study CLTR0310-101

This was a Phase 1, single arm, dose escalation study in subjects > 18 years with AML (multiply relapsed, refractory, or with first CR duration of ≤6 months), acute lymphocytic leukemia (ALL), or high risk MDS.

The primary objective was to determine the recommended Phase 2 dose of Vyxeos (defined as maximum tolerated dose [MTD]) that could be given to patients with advanced hematologic malignancies. Secondary objectives were to evaluate the safety and dose limiting toxicities, PK parameters, and assess preliminary efficacy.

Subjects were to receive up to 2 inductions and 1 consolidation. Vyxeos was administered by 90 minute IV infusions on Days 1, 3, and 5 for each induction and on Days 1 and 3 for each consolidation. Dose escalation was initiated at 3 units/m^2 and a 2-phase accelerated dose escalation schedule was employed with early escalation by dose doubling and late escalation by 33% increments. Dose expansion occurred at the declared MTD. At every dose level subjects were monitored for signs of antileukemic activity.

A total of 48 subjects were enrolled in the study, received at least 1 dose, and comprised the safety population. Two subjects were excluded from the efficacy evaluable analysis set (N = 46). The majority of subjects (60.4%) terminated treatment due to progressive disease or lack of response; 7 subjects (14.6%) completed the study. Two subjects discontinued the study due to AEs.

Most subjects were male (64.6%), white (85.4%) and the mean (SD) age was 59.1 (15.34) years. Most subjects (89.6%) had AML; 3 subjects had ALL, and 2 subjects had MDS. At enrolment, the mean (SD) time since diagnosis was 11.4 (7.93) months.

A total of 48 subjects were exposed to Vyxeos at doses from 3 units/m^2 to 134 units/m^2. Subjects had 1 to 10 exposures and cumulative dose of 9 to 804 units/m^2. Most subjects (26/48, 54%) were treated at the higher dose levels (ie, 101 and 134 units/m2). Vyxeos at a dose of 134 units/m^2 was administered to 6 adults, and dose-limiting toxicities were observed in 3 subjects (hypertensive crisis, reduced left ventricular ejection fraction in the clinical context of sepsis, and prolonged cytopenia).

Therefore, the MTD in this study and the dose recommended for Phase 2 was 101 units/m^2 administered by a 90 minute IV infusion on Days 1, 3, and 5 for each induction course and on Days 1 and 3 for each consolidation course in subjects able to tolerate intensive chemotherapy.

Table 11. Actual Administered Dose Levels and Number of Patients

Dose				Patients Entered	
Level	CPX-351 units/m^2	Cytarabine mg/m^2	Daunorubicin mg/m^2	Escalation Phase	Extension Phase
1	3	3	1.32	1	
2	6	6	2.64	1	
3	12	12	5.28	2	
4	24	24	10.6	4	
5	32	32	14.08	4	
6	43	43	18.92	4	
7	57	57	25.08	3	
8	76	76	33.44	3	
9	101	101	44.44	6	14
10	134	134	58.96	6	

The key efficacy findings were that responses were achieved in multiple subjects, most with prior exposure to cytarabine and daunorubicin. No clinical responses were observed in any patient receiving less than 32 units/m^2 of CPX 351. Neither of the two patients with high-risk MDS responded.

Forty-three patients with AML were treated and 41 patients completed one induction course of therapy. Two patients received only two of the planned three doses before adverse events led to treatment discontinuation. Ten of 43 AML patients cleared their leukaemias, including one patient with multiple relapses who achieved complete responses with incomplete platelet recovery (CRp) after treatment at 32 units/m^2 following two induction courses.

Complete responses (CR) were achieved after single induction course at dose level as low as 43 units/m^2. At the MTD dose level, aplasia was observed in 13 of 20 patients leading to 5 CR, 4 of which lasted longer than 6 months (including two consolidated with stem cell transplant). There were 7 CR among 22 AML patients receiving Vyxeos as first salvage, 1 CR and 1 CRp among the 11 patients given second salvage, and 1 CR among the 10 treated with third or greater salvage. Among the first salvage patients there were 5 patients with primary induction failure (PIF) 1 of whom achieved CR.

Not unexpectedly, Vyxeos showed substantially greater anti-leukemic efficacy among younger first relapse patients, with 4 of 7 (57.1%) patients aged 18-60 achieving CR compared with only 2 of 11 (18.2%) patients older than 60. Among the 18 first relapse patients, CR was achieved in 6, and of these patients the remission achieved following Vyxeos was longer than the initial remission in 2 patients (12 vs 16+ months in patient 03-018 at the 101 units/m^2 dose level, and 2 vs 5.4 months in patient 03-006 at the 32 units/m^2 dose level).

DLTs in this study were hypertensive crisis, prolonged cytopenia and congestive heart failure.

The cardiotoxic potential of daunorubicin has been appreciated for at least two decades and cardiac toxicity is a well-known side effect of anthracycline therapy, so it is unsurprising that this was observed as a DLT. The predominant AEs in this study are related to myelosuppression and its consequences, infections with opportunistic organisms during the myelosupressed period. At the recommended Phase 2 dose, 101units/m^2, there does not seem to be an unusual predisposition to observe the classic cardiovascular consequences of anthracycline exposure, which are largely related to cumulative doses. Cardiac events were observed, including one patient treated at the Phase 2 recommended dose.

The declared MTD in this study and the dose recommended for Phase 2 was 101 units/m^2 administered by a 90 minute IV infusion on Days 1, 3, and 5 for each induction course and on Days 1 and 3 for each consolidation course in patients able to tolerate intensive chemotherapy.

2.5.2. Main study

Study CLTR0310-301

Methods

This was a phase III, multicentre, randomised, trial of Vyxeos (cytarabine: daunorubicin) liposome injection versus cytarabine and daunorubicin in patients 60 -75 years of age with untreated high risk (secondary) AML study.

Study Participants

Inclusion criteria

- Age 60 to 75 years at the time of diagnosis of AML.
- Pathological diagnosis of AML according to WHO criteria (at least 20% blasts in peripheral blood or bone marrow).
- Documentation of antecedent hematologic disorder: t-AML: prior cytotoxic or radiation therapy for an unrelated disease; $_{MDS}$AML: MDS prior to diagnosis of AML; $_{CMMoL}$AML: CMMoL prior to diagnosis of AML; $_{de\ novo}$AML with FISH or cytogenetic changes linked to MDS per WHO criteria.
- ECOG performance status of 0-2.
- Laboratory values: serum creatinine < 2.0 mg/mL.; serum total bilirubin < 2.0 mg/dL; serum ALT or AST < 3 times the upper limit of normal.
- Cardiac ejection fraction ≥ 50% by ECHO or MUGA.
- Subjects with second malignancies in remission were eligible if there was clinical evidence of disease stability for ≥ 6 months off cytotoxic chemotherapy, documented by imaging, tumor marker studies at screening. Subjects on long-term non-chemotherapy treatment such as hormonal therapy were eligible.

Exclusion criteria

- Except for CMMoL, history of MPN (essential thrombocytosis or polycythemia vera, or idiopathic myelofibrosis) prior to the diagnosis of AML, or combined MDS/MPN
- Acute promyelocytic leukemia [t(15;17)] or favourable cytogenetics, including t(8;21) or inv 16 if known at the time of randomization
- Active central nervous system (CNS) leukemia.
- Active (uncontrolled, metastatic) second malignancies.
- Prior treatment for induction therapy of AML; only hydroxyurea was permitted for control of blood counts. For example, a subject with MDS that changes HMA dose and schedule after the diagnosis of AML was excluded. AML-type therapy, such as cytarabine alone (> 1g/m^2/day) or cytarabine plus an anthracycline as well as prior HSCT were also excluded.
- Administration of any therapy for MDS must have been completed by 2 weeks before the first study treatment; in the event of rapidly proliferative disease, the use of hydroxyurea was permitted until 24

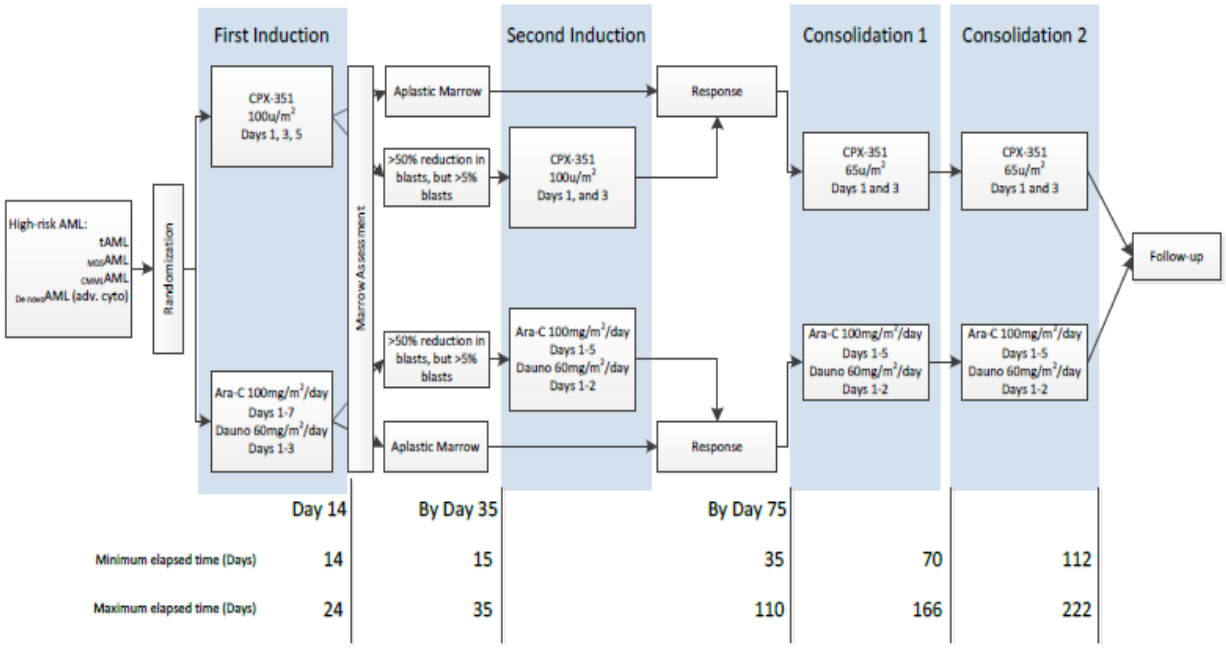
hours before the start of study treatment. Toxicities associated with prior MDS therapy must have recovered to grade 1 or less prior to start of treatment.

- Any major surgery or radiation therapy in 4 weeks.
- Prior cumulative anthracycline exposure of greater than 368 mg/m^2 daunorubicin (or equivalent).
- Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent obtaining informed consent.
- Myocardial impairment of any cause (e.g., cardiomyopathy, ischemic heart disease, significant valvular dysfunction, hypertensive heart disease, and congestive heart failure) resulting in heart failure by New York Heart Association Criteria (Class III or IV staging).
- Active or uncontrolled infection; subjects with an infection receiving treatment could be entered into the study provided the subject was afebrile and hemodynamically stable for ≥ 72 hours.
- Current evidence of invasive fungal infection (blood or tissue culture); subjects with recent fungal infection must have had subsequent negative cultures to be eligible; known HIV (new testing not required) or evidence of active hepatitis B or C infection (with rising transaminase values).
- Hypersensitivity to cytarabine, daunorubicin or liposomal products.
- History of Wilson's disease or other copper-metabolism disorder.

Treatments

The study comprised 2 phases: a Treatment Phase and a Follow-up Phase.

Figure 7 Study Design (Study CLTR0310-301)



For the treatment phase subjects were to receive up to 2 inductions and up to 2 consolidations with either Vyxeos or cytarabine and daunorubicin given as a 7+3. The number of inductions and consolidations received depended on response confirmed by bone marrow assessment.

The follow-up phase began 30 days after the completion of the last induction or consolidation course, and was to last until death or 5 years after randomization.

The initial induction course began within 24 hours of randomization.

Each unit of Vyxeos contained 1 mg cytarabine and 0.44 mg daunorubicin base in liposomes.

First induction:

- Vyxeos: 100 units/m^2 by 90-minute IV infusion on Days 1, 3, 5
- 7+3: cytarabine 100 mg/m^2/day on Days 1 through 7 by continuous infusion
  daunorubicin 60 mg/m^2/day on Days 1, 2, and 3

Second induction:

- Vyxeos 100 units/m^2 by 90-minute IV infusion on Days 1 and 3
- 5+2: cytarabine 100 mg/m^2/day on Days 1 through 5 by continuous infusion
  daunorubicin 60 mg/m^2/day on Days 1 and 2

Consolidation:

- Vyxeos 65 units/m^2 by 90-minute IV infusion on Days 1 and 3
- 5+2: cytarabine 100 mg/m^2/day on Days 1 through 5 by continuous infusion
  daunorubicin 60 mg/m^2/day on Days 1 and 2

Doses in both treatment groups could be delayed due to toxicities or hypersensitivity reactions. Any doses missed or delayed due to these events were administered as soon as the subject recovered from the event.

Post remission therapy with bone marrow transplant was permitted either in place of or after postremission chemotherapy and was administered according to local practice.

Permitted Therapies

- Premedication for nausea and vomiting per institutional policy
- Subjects were not routinely pre-medicated for hypersensitivity or for potential infusion-related reactions prior to the first infusion of the first treatment course. If a subject developed a hypersensitivity reaction, then the subject should have been pre-medicated at all subsequent infusions.
- Prophylactic use of anti-infectives was highly recommended during the period of neutropenia until the absolute neutrophil count (ANC) returned to 500/µL or greater. The choice of anti-infectives was determined according to institutional protocol.
- Growth factors were allowed according to institutional protocol

Objectives

The primary objective was to confirm the efficacy of Vyxeos compared with 7+3 as first line therapy in elderly patients (60 to 75 years old) with high risk (secondary) AML.

The secondary objectives were to confirm the safety of Vyxeos and improvement in the rate of leukemia-free state, post induction response, remission duration, event-free survival, and overall best response rate. It also included assessment of serum copper elevations, population pharmacokinetics of Vyxeos and pharmacoeconomic.

Outcomes/endpoints

The primary endpoint was overall survival (OS) defined as the time from randomisation to death from any cause.

Secondary

- Event-free-survival (EFS): time from randomization until the date of induction treatment failure (persistent disease), relapse from CR or CRi or death from any cause, whichever came first.

- Response: subjects who achieved CR or CRi during the treatment phase.

- Best response: subjects who completed the induction(s) with a response of CRi but were upgraded to CR during or after consolidation

- Remission duration: time from the date of achievement of a remission (CR or CRi) until the date of relapse or death from any cause. For subjects whose best response was upgraded from CRi to CR, the remission duration for CR+CRi analyses were calculated from the date of CRi to the date of relapse or death.

- Morphologic Leukemia-free State: bone marrow blasts < 5% and the absence of Auer rods and/or extramedullary disease. All randomised subjects with at least 1 evaluable post-randomization bone marrow assessment performed on or after Day 14 after the last induction were assessed for MLFS.

- The number and percentage of subjects transferred for HSCT after induction

- Medical resource use to identify costs associated with planned and any unplanned treatment/support.

Criteria used for assessment of response:

· CR: bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; ANC > 1.0 x 10^9/L (1000/µL); platelet count > 100 x 10^9/L (100,000/µL); independence from red cell transfusions.

· CRi: all the CR criteria except for residual neutropenia (< 1.0 x 109/L [1000/µL]) or thrombocytopenia (< 100 x 109/L [100,000/µL]).

Subject's response to induction was made on the first day when all criteria for CR or treatment failure were met. Response was assessed locally and decisions about starting post-remission chemotherapy or referral for transplant were made per local institutional standards prior to independent review of response. An independent hematopathologist evaluated response assessments by reviewing bone marrow reports, data on peripheral blood and transfusion reports. The responses reported in this study are those confirmed by the independent hematopathologist.

The independent confirmation of induction response was completed earlier than assessment of overall survival or other secondary endpoints. To minimise the potential for bias, the analysis of induction response with independent confirmation was prospectively incorporated as a final analysis into the study protocol and was initiated only after subjects had completed randomization and all study treatments.

In addition, molecular mutation data for CEBPA, FLT3, and NPM1 were obtained via a central or local laboratory prior to the primary endpoint analysis.

Sample size

The study was designed to enrol 300 subjects so that a minimum of 270 subjects would be evaluable for the primary endpoint. It was anticipated that up to 10% of subjects would be ineligible or withdraw consent. All sample size and power justifications were based on evaluable subjects only.

Assuming a median OS of 0.526 years in the 7+3 treatment group, 236 deaths were required for final analysis of OS with 93.7% power and a 1-sided significance level (α = 0.025) to detect a hazard ratio of 0.635 between the 2 treatment groups.

This study was designed to have 93.7% power for the primary endpoint to ensure that a sensitivity analysis of OS, which would censor subjects at the start of transplant, was powered at 90% for the same hazard ratio (0.635).

Randomisation

At registration, patients were randomised to receive Vyxeos (Study Arm A) or cytarabine + daunorubicin (7+3 regimen) (Study Arm B) in a 1:1 ratio.

Randomization was stratified according to Age (60-69 vs 70-75) and AML type (t-AML vs MDS AML with prior treatment with hypomethylating agents vs MDS AML without prior treatment with hypomethylating agents, vs de novo AML with karyotype characteristic of MDS vs CMMoL AML).

Blinding (masking)

This was an open label study.

Statistical methods

Analysis populations:

- Intent-to-Treat (ITT): all randomised subjects who were assigned to a treatment group. It constituted the primary efficacy population.
- Safety population: all subjects who received at least 1 dose of a study medication
- Per protocol population (PP): subjects who were a subset of the ITT population, met all inclusion/exclusion criteria, received at least 1 dose of a study medication, and have their AML diagnosis and type confirmed by an independent pathologist. The analysis of transfer to HSCT was performed on this study population.

- Morphologic leukemia-free state (MLFS): subjects who were a subset of the PP population and had at least 1 evaluable post-randomization bone marrow assessment performed on or after Day 14 after the last induction.

Analyses:

Primary analysis: OS (ITT population). Subjects who did not die at last follow-up were censored on the date they were last known to be alive. Subjects were to be followed for up to 5 years.

Sensitivity analyses: because of potential imbalances in use of HSCT, a sensitivity analysis was performed after censoring subjects at the start of HSCT, so that survival potentially attributable to HSCT could be removed.

Subgroup analyses on time to event endpoints (remission duration and EFS) to assess whether the treatment effect differs according to the stratification factors.

Exploratory analyses to determine which prognostic factors had a significant effect on the primary and secondary endpoints. The prognostic factors used were: sex, ECOG performance status, karyotype, white blood cell (WBC) category, platelet category, hemoglobin category, bone marrow blast count, and FLT3 mutation.

Method of analysis

A stratified log-rank test was used to compare the Vyxeos and 7+3 treatment groups. In addition, the distribution of overall survival was estimated in each treatment group with the Kaplan-Meier methodology. The hazard ratio and overall survival at different time points, along with corresponding confidence intervals, were reported. Exploratory multivariate analyses were performed to assess the treatment effect adjusting for key prognostic factors using the Cox proportional hazard regression model.

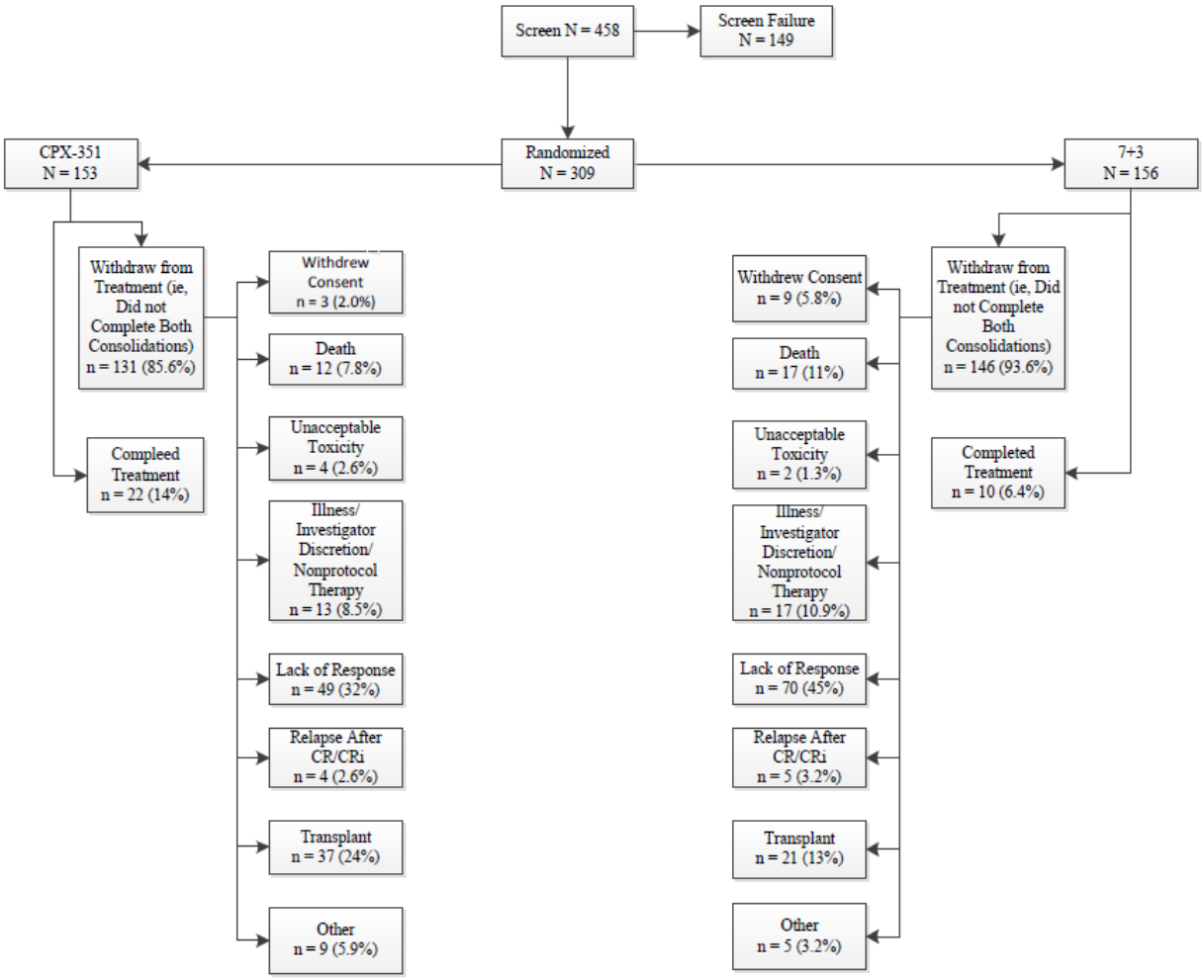
Timing of analysis

The pre-planned analysis of induction response (CR+CRi) was performed after all subjects had been enrolled and completed induction and consolidation treatments. This analysis was reviewed by the DSMB to allow decisions for initiating other clinical studies of Vyxeos. The use of response information did not bias the conduct of the study because all subjects were randomised, had completed all study treatments, and had been followed long enough to recover from hematopoietic effects of treatment and because the remaining data to be collected on each subject consisted only of relapse and survival information. These analyses did not affect the conduct of the trial or the alpha of the primary endpoint. All other analyses were performed after the endpoint for the primary analysis had occurred. The analysis for the primary endpoint was performed after 236 deaths had occurred. The number of deaths was based on the alternative hypothesis.

Results

Participant flow

Figure 8 Disposition of subjects (ITT population-Study CLTR0310-301)



Recruitment

Conduct of the study

Protocol amendments

The initial protocol (Version 1.0) was issued on 22 May 2012. It was subsequently amended 4 times: on 12 March 2013 (Version 2.0), 17 September 2013 (Version 2.1, never issued, changes incorporated in Version 2.2), 9 October 2013 (Version 2.2), and 4 November 2013 (Version 2.3).

Protocol amendments of clinical relevance were (Amendment version 2.0): Increased the sample size to 300 to provide greater power. Consequently, the analysis of the primary endpoint was to be performed after 236

deaths occurred; The mandatory second induction should occur only if it was safe to do so; Added an optional consolidation schedule (intermediate dose cytarabine, 1.5 g/m^2 twice daily on days 1, 3 and 5) for subjects that had exceeded 500 mg/m^2 lifetime exposure to daunorubicin or its equivalent; The assessment of early deaths was changed so that the assessment by the DSMB was conducted after the first 75 subjects had been evaluated for induction mortality

Protocol compliance

A total of 9 subjects (4 in the Vyxeos and 5 in the control) violated at least 1 of the inclusion criteria and 17 subjects (5 in the Vyxeos and 12 in the control) failed at least 1 exclusion criterion. A total of 9 protocol deviations were categorised as major deviations, 4 subjects in the Vyxeos and 5 in the control. In the Vyxeos arm two subjects had a delay of 1 week after randomization before they received the investigational product, a site delayed reporting an SAE for 1 subject who died, and 1 subject received 100 units/m^2 instead of 65 units/m^2 for consolidation. In the control, 2 subjects were assigned the wrong AML subtype, 1 subject received idarubicin instead of daunorubicin for 1 treatment course, 1 subject had a delay in receiving daunorubicin, and 1 subject failed to sign the consent form for Amendment 2.

Baseline data

The baseline demographics and disease characteristics are presented in Table 18.

Table 17 Baseline demographics and disease characteristics (ITT population-Study CLTR0310-301)

	CPX-351 N = 153	7 +3 N = 156
Age, years, median (range)	68 (60, 75)	68 (60, 75)
60 to 69, n (%)	96 (63)	102 (65)
70 to 75, n (%)	57 (37)	54 (35)
Sex, n (%)		
Male	94 (61)	96 (62)
Female	59 (39)	60 (38)
Race, n (%)		
American Indian or Alaska Native	1 (1)	0
Asian	6 (4)	2 (1)
Black	7 (5)	6 (4)
White	128 (84)	139 (89)
Other	11 (7)	8 (5)
Multiple	0	1 (1)
ECOG Performance Group, n (%)		
PS 0	37 (24)	45 (29)
PS 1	101 (66)	89 (57)
PS 2	15 (10)	22 (14)
Extra medullary disease, n (%)	5 (3)	5 (3)
AML subtype, n (%)		
CMMol to AML	11 (7)	12 (8)
De Novo AML	41 (27)	37 (24)
MDS with HMA to AML	50 (33)	55 (35)
MDS without HMA to AML	21 (14)	19 (12)
tAML	30 (20)	33 (21)
Genetic Mutations, n (%)		
FLT3 positive mutation	22 (14)	21 (14)
NPM1 positive mutation	13 (9)	12 (8)
CEBPA mutated	12 (8)	5 (3)
Cytogenetic risk assessment, n (%)		
Better–risk	7 (5)	5 (3)
Intermediate–risk	64 (45)	58 (40)
Poor–risk	72 (50)	83 (57)

Abbreviations: AML = acute myeloid leukemia; CMMoL = chronic myelomonocytic leukemia; ECOG = East Coast Oncology Group; MDS = myelodysplastic syndrome; HMA = hypomethylating agents; PS = performance status; tAML = therapy-related AML.

Numbers analysed

Table 18 Summary of the number of subjects in each analysis population (Study CLTR0310-301)

Analysis Population	Number of Subjects	
	CPX-351	7+3
ITT	153	156
Safety	153	151
Per Protocol	145	135
Morphologic Leukemia-free State	126	119

Outcomes and estimation

Primary endpoint: Overall survival

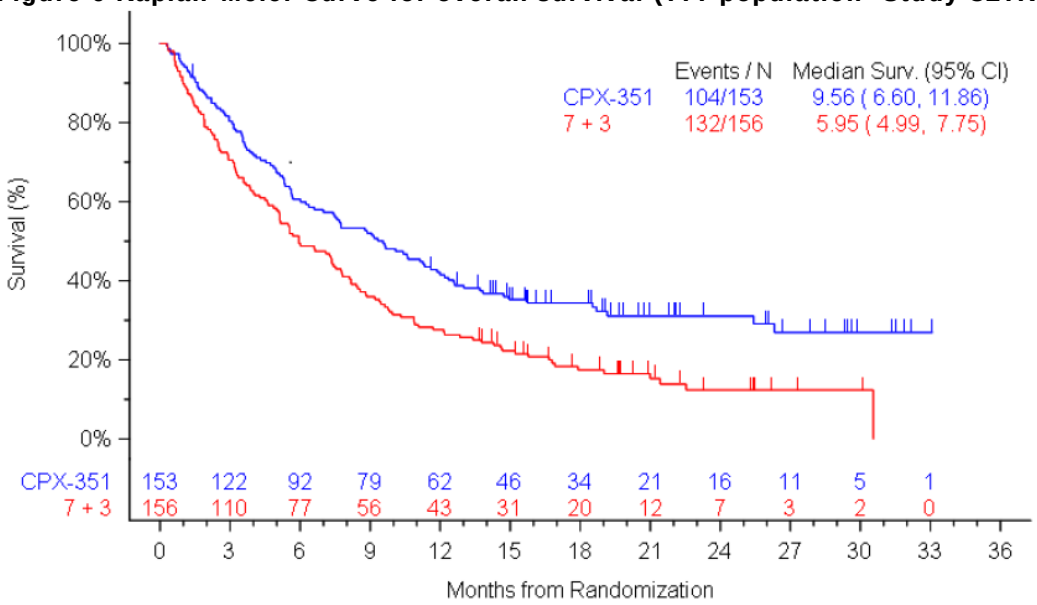
The results of the primary endpoint OS are presented in Table 20 and Figure 9.

Table 19 Primary Endpoint Analysis: Overall Survival (ITT Analysis Population-Study CLTR0310-301)

	CPX-351 (N=153)	7 + 3 (N=156)
Survived	49 (32.0)	24 (15.4)
Median Survival in Months (95% Conf. Int.)	9.56 (6.60, 11.86)	5.95 (4.99, 7.75)
Hazard Ratio (95% Conf. Int.)	0.69 (0.52, 0.90)	
p-value (1-sided)	0.003	

Note: P-value from stratified log-rank test
Note: Hazard ratio are calculated with the 7 + 3 arm as the reference group.

Figure 9 Kaplan-Meier Curve for overall survival (ITT population- Study CLTR0310-301)

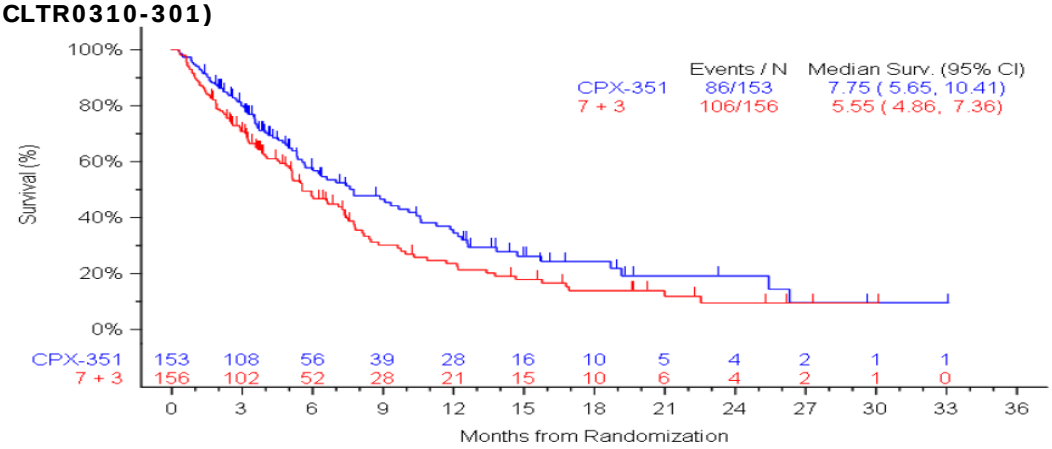


Sensitivity analyses

In a sensitivity analysis that censored OS data at HSCT in the ITT population, a Kaplan-Meier analysis showed that subjects in the Vyxeos treatment group had median survival 7.75 months compared to 5.55 months with those in the 7+3 treatment group (HR=0.81, for log rank test, 1-sided p = 0.082,).

Figure 10).

Figure 10 Kaplan-Meier Curve for overall survival, sensitivity analysis (ITT population-Study CLTR0310-301)

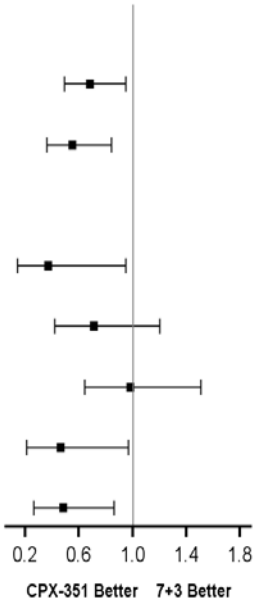


In the per protocol population when censored OS data at HSCT in the ITT population, subjects in the Vyxeos group had 7.75 months median survival compared with 5.55 months in the control arm (HR 0.81, for log rank test, 1-sided p = 0.082) (data not shown).

The results of the OS data in the ITT population by randomization strata are presented in Table 20 .

Table 20 Overall Survival by randomization strata (ITT population-Study CLTR0310-301)

Randomization Strata	CPX-351		7 + 3				Hazard Ratio (95% CI)
	n	Median OS (months)	n	Median OS (months)	HR (95% CI)	P-value	
Age (years)							
60-69	96	9.63	102	6.87	0.68 (0.49, 0.95)	0.023	
70-75	57	8.87	54	5.62	0.55 (0.36, 0.84)	0.005	
AML Subtype							
CMMoL -> AML	11	9.33	12	2.28	0.37 (0.14, 0.95)	0.032	
De novo AML with MDS karyotype	41	10.09	37	7.36	0.71 (0.42, 1.20)	0.201	
MDS -> AML with prior HMA treatment	50	5.65	55	7.43	0.98 (0.64, 1.51)	0.944	
MDS -> AML without prior HMA treatment	21	15.74	19	5.13	0.46 (0.21, 0.97)	0.037	
Therapy-related AML	30	12.17	33	5.95	0.48 (0.26, 0.86)	0.012	



Secondary endpoint-Induction Response

The results of the analysis of induction responses are displayed in Table 21.

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Table 20 Proportion of subjects with a response (ITT population-Study CLTR0310-301)

Endpoint (N=CPX-351, N= 7+3)	CPX-351 n (%)	7+3 n(%)	Odds Ratio (95% CI)
CR+CRi	73 (47.7)	52 (33.3)	1.77 (1.11, 2.81) p = 0.008 (1-sided)
CR	57 (37.3)	40 (25.6)	1.69 (1.03, 2.78) p = 0.020 (1-sided)
CRi	16 (10.5)	12 (7.7)	
No response	80 (52.3)	104 (66.7)	
Age (years)			
60-69 (96, 102)			
CR	38 (39.6)	27 (26.5)	1.82 (1.00, 3.32)
CRi	10 (10.4)	10 (9.8)	
70-75 (57,54)			
CR	19 (33.3)	13 (24.1)	1.58 (0.69, 3.62)
CRi	6 (10.5)	2 (3.7)	
AML Subtype			
$_{CMMoL}$AML (11, 12)			
CR	2 (18.2)	3 (25.0)	0.67 (0.09, 4.99)
CRi	2 (18.2)	0	
$_{de\ novo}$AML (41, 37)			
CR	19 (46.3)	10 (27.0)	2.33 (0.90, 6.03)
CRi	4 (9.8)	2 (5.4)	
$_{MDS}$ AML with HMA (50, 55)			
CR	13 (26.0)	10 (18.2)	1.58 (0.62, 4.02)
CRi	5 (10.0)	8 (14.5)	
$_{MDS}$AML without HMA (21, 19)			
CR	12 (57.1)	7 (36.8)	2.29 (0.64, 8.15)
CRi	2 (9.5)	0	
t-AML (30, 33)			
CR	11 (36.7)	10 (30.3)	1.33 (0.47, 3.81)
CRi	3 (10.0)	2 (6.1)	

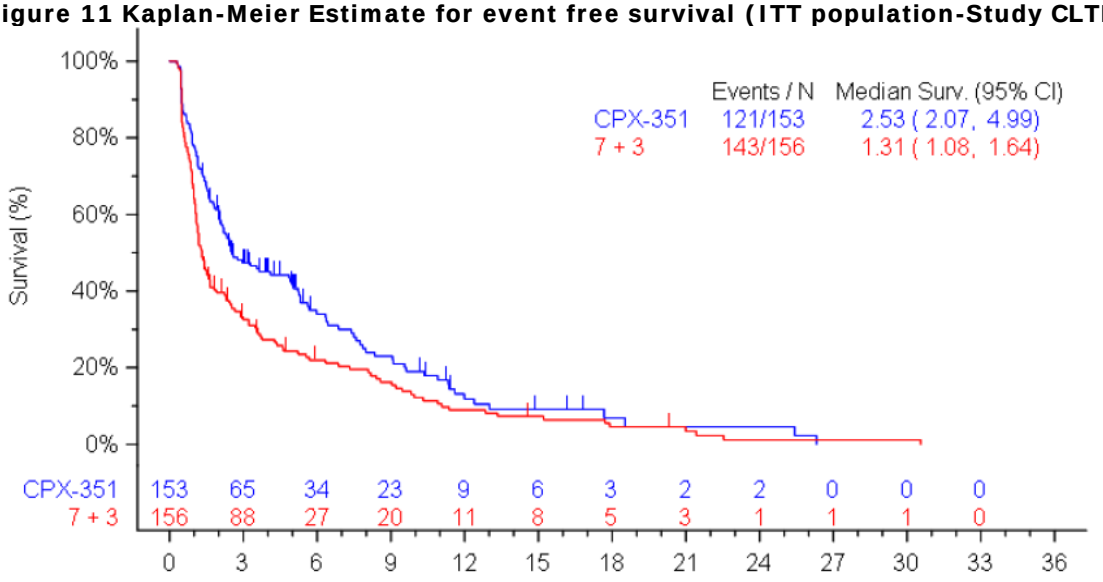
Secondary endpoint-Best Response

No subjects in either the ITT or PP populations with a CRi response were upgraded to CR. Consequently no changes occurred in the proportions of subjects with a best response compared with the induction response.

Secondary endpoint-EFS

The median EFS was 2.53 (95%CI: 2.07, 4.99) months for the Vyxeos and 1.31 (95%CI: 1.08 , 1.64) months for the 7+3 treatment arms (HR = 0.74, 1-sided, p = 0.011). A Kaplan-Meier Estimate for EFS is displayed in Figure 11.

Figure 11 Kaplan-Meier Estimate for event free survival (ITT population-Study CLTR0310-301)



Secondary endpoint- Remission Duration

Remission duration in the Vyxeos treatment arm was 6.93 months vs. 6.11 months in the 7+3 treatment arm (1-sided p = 0.147). In the per protocol population, remission duration 7.36 in the Vyxeos treatment arm compared with the 7+3 treatment arm vs. 5.95 months in the 7+3 treatment arm (1-sided p = 0.086) (data not shown).

Secondary endpoint-Morphologic leukemia-free state

The proportion of subjects achieved a MLFS is displayed in Table 22.

Table 21 Proportion of subjects achieving MLFS (ITT Population-Study CLTR0310-301)

Endpoint (N CPX-351, N 7+3)	CPX-351 N = 126 n (%)	7+3 N = 119 n(%)	Odds Ratio (95% CI)
MLFS	87 (69.0)	66 (55.5)	1.78 (1.05, 3.03) p = 0.017
Age (years)			
60-69 (82, 77)	54 (65.9)	43 (55.8)	1.52 (0.80, 2.89)
70-75 (44, 42)	33 (75.0)	23 (54.8)	2.48 (0.99, 6.18)
AML Subtype			
$_{CMMoL}$AML (9, 8)	5 (55.6)	3 (37.5)	2.08 (0.30, 14.55)
$_{de\ novo}$AML (35, 31)	23 (65.7)	14 (45.2)	2.33 (0.86, 6.29)
$_{MDS}$AML with HMA (41, 45)	28 (68.3)	26 (57.8)	1.57 (0.65, 3.81)
$_{MDS}$AML without HMA (19, 14)	14 (73.7)	11 (78.6)	0.76 (0.15, 3.92)
t-AML (22, 21)	17 (77.3)	12 (57.1)	2.55 (0.68, 9.54)

Secondary endpoint-Stem Cell Transplant

The proportion of subjects receiving stem cell transplant is displayed in Table 23.

Table 22 Proportion of Subjects Receiving Stem Cell Transplant (ITT Population-Study CLTR0310-301)

Endpoint (N CPX-351, N 7+3)	CPX-351 n (%)	7+3 n(%)	Odds Ratio (95% CI)
HSCT (153, 156)	52 (34.0)	39 (25.0)	1.54 (0.92, 2.56) p = 0.049 (1-sided)
Age (years)			
60-69 (96, 102)	36 (37.5)	33 (32.4)	1.25 (0.70, 2.25)
70-75 (57, 54)	16 (28.1)	6 (11.1)	3.12 (1.12, 8.72)
AML Subtype			
$_{CMMoL}$AML	3 (27.3)	0	Not evaluable
$_{de\ novo}$AML	17 (41.5)	11 (29.7)	1.67 (0.65, 4.28)
$_{MDS}$AML with prior HMA	14 (28.0)	14 (25.5)	1.14 (0.48, 2.71)
$_{MDS}$AML without prior HMA	7 (33.3)	5 (26.3)	1.40 (0.36, 5.49)
t-AML	11 (36.7)	9 (27.3)	1.54 (0.53, 4.49)

In the ITT population, a greater proportion of subjects in the Vyxeos treatment group received a HSCT while in CR or CRi compared with control (54.8% vs. 46.2%, respectively).

Table 23 Transplant Rate (Study CLTR0310-301)

	CPX-351 N = 153		**7 + 3 N = 156**
CR, n (%)	**57 (37.3)**		**40 (25.6)**
Received Transplant	30 (52.6)		19 (47.5)
No Transplant	27 (47.4)		21 (52.5)
CRi, n (%)	**16 (10.5)**		**12 (7.7)**
Received Transplant	10 (62.5)		5 (41.7)
No Transplant	6 (37.5)		7 (58.3)
Transplant rate	**52 (34.0)**		**39 (25.0)**
Odds ratio (95% CI)		1.54 (0.92, 2.56)	
p-value[a]		0.049[a]; 0.097[b]	

Abbreviations: CI = confidence interval.
[a] 1-sided p-value is from a comparison of rates between treatment arms and is based on the Mantel-Haenszel test stratifying by age and AML type.
[b] 2-sided p-value is from a comparison of rates between treatment arms and is based on the Mantel-Haenszel test stratifying by age and AML type.

A Kaplan-Meier analysis of the 91 subjects who received a transplant landmarked at the time of transplant showed that subjects in the Vyxeos group performed better than those in the control (HR=0.46, p = 0.009 [1-sided]). The median survival was not reached in the Vyxeos treatment group, whereas the median survival in the 7+3 treatment group was 10.25 months (data not shown).

Ancillary analyses

Efficacy results for all endpoints stratified by AML subtype are displayed in Table 25.

Table 24 Efficacy results by disease stratification factors (Study CLTR0310-301)

	AML Subtype									
	CMMoL AML		de novo AML		MDS AML with prior HMA		MDS AML without prior HMA		t-AML	
	CPX-351 N = 11	7 + 3 N = 12	CPX-351 N = 41	7 + 3 N = 37	CPX-351 N = 50	7 + 3 N = 55	CPX-351 N = 21	7 + 3 N = 19	CPX-351 N = 30	7 + 3 N = 33
Overall Survival										
Median Survival (months)	9.33	2.28	10.09	7.36	5.65	7.43	15.74	5.13	12.17	5.95
HR (95% CI)	0.37 (0.14, 0.95)		0.71 (0.42, 1.20)		0.98 (0.64, 1.51		0.46 (0.21, 0.97)		0.48 (0.26, 0.86)	
CR										
n (%)	2 (18.2)	3 (25.0)	19 (46.3)	10 (27.0)	13 (26.0)	10 (18.2)	12 (57.1)	7 (36.8)	11 (36.7)	10 (30.3)
OR (95% CI)	0.67 (0.09, 4.99		2.33 (0.90, 6.03)		1.58 (0.62, 4.02)		2.29 (0.64, 8.15)		1.33 (0.47, 3.81)	
CRi										
n (%)	2 (18.2)	0 (0)	4 (9.8)	2 (5.4)	5 (10.0)	8 (14.5)	2 (9.5)	0(0)	3 (10.0)	2 (6.1)
MLFS										
n (%)	5 (55.6)	3 (37.5)	23 (65.7)	14 (45.2)	28 (68.3)	26 (57.8)	14 (73.7)	11 (78.6)	17 (77.3)	12 (57.1)
OR (95% CI)	2.08 (0.30, 14.55)		2.33 (0.86, 6.29)		1.57 (0.65, 3.81)		0.76 (0.15, 3.92)		2.55 (0.68, 9.54)	
HSCT										
n (%)	3 (27.3)	0 (0)	17 (41.5)	11 (29.7)	14 (28.0)	14 (25.5)	7 (33.3)	5 (26.3)	11 (36.7)	9 (27.3)
OR (95% CI)	Not Evaluable		1.67 (0.65, 4.28)		1.14 (0.48, 2.71)		1.40 (0.36, 5.49)		1.54 (0.53, 4.49)	

Abbreviations: CR = complete response; CRi = complete response with incomplete neutrophil or platelet recovery; HSCT = hematopoietic stem cell transplant; MLFS = morphologic leukemia free state; OR = odds ratio

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 20. Summary of efficacy for trial CLTR0310-301

Title: A phase 3, multicenter, randomised, trial of Vyxeos(cytarabine:daunorubicin) liposome injection versus cytarabine and daunorubicin in patients 60-75 years of age with untreated high risk (secondary) AML.		
Study identifier	CLTR0310-301	
Design	Randomised, open-label, parallel-arm, standard therapy-controlled	
	Duration of main phase:	treatment phase (168-224 days)
	Duration of Run-in phase:	not applicable
	Duration of Extension phase:	Follow-up phase: 30 days after the completion of the last induction or consolidation course, and was to last until death or 5 years after randomization.
Hypothesis	Superiority	
Treatments groups	Vyxeos(cytarabine:daunorubicin) liposome injection	IV (90 min infusion) 1st Induction: 100 units/m^2/day, Days 1, 3 and 5 2nd Induction: 100 units/m^2/day, Days 1 and 3 Consolidation (up to 2 courses): 65 units/m^2/day, Days 1 and 3

	Cytarabine plus Daunorubicin (referred to as "7+3") 7 + 3 / 5 + 2, IV (control)		*1st Induction:* Cytarabine 100 mg/m^2/day, 7-day continuous infusion Daunorubicin 60 mg/m^2/day, Days 1, 2 and 3 *2nd Induction:* Cytarabine 100 mg/m^2/day, 5-day continuous infusion Daunorubicin 60 mg/m^2/day, Days 1 and 2 *Consolidation (up to 2 courses):* Cytarabine 100 mg/m2/day, 5-day continuous infusion Daunorubicin 60 mg/m^2/day, Days 1 and 2
Endpoints and definitions	Primary endpoint	Overall Survival (OS)	Duration from the date of randomization to death from any cause.
	Secondary endpoints	Event Free Survival (EFS)	Duration from study randomization to the date of induction treatment failure (persistent disease), relapse from CR or CRi or death from any cause, whichever came first.
		Response Rate	Proportion of subjects who achieved CR or CRi following induction treatment
		Remission duration	Duration from the date of achievement of a remission (CR or CRi) until the date of relapse or death from any cause.
		Morphologic Leukemia-free state MLFS)	Proportion of subjects achieved a MLFS. MLFS defined as bone marrow blasts < 5% and the absence of Auer rods and/or extramedullary disease.
		Stem Cell Transplant Rate	The number and percentage of subjects transferred for HSCT after induction treatment
Database lock	31 December 2015		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intention to treat (N= 309)		
Descriptive statistics and estimate variability	Treatment group	Vyxeos	7+3
	Number of subjects	153	156
	Median OS (months)	9.56	5.95
	95% CI	6.60 , 11.86	4.99 , 7.75
	Median EFS (months)	2.53	1.31
	95% CI	2.07 , 4.99	1.08 , 1.64
	Response Rate n (%)	73 (47.7)	52 (33.3)
	Remission duration (months)	6.93	6.11
	95%	4.60, 9.23	3.45, 8.71
	MLFS n (%)	87 (69)	66(55.5)
	Stem cell transplant rate n (%)	52 (34)	39 (25)
Effect estimate per comparison	OS	Comparison groups	Vyxeos vs 7+3
		HR	0.69
		95% CI	0.52 , 0.90
		p-value (2-sided)	0.005
	EFS	Comparison groups	Vyxeos vs 7+3
		HR	0.74
		95% CI	0.58 , 0.96
		p-value (2-sided)	0.021
	Response Rate	Comparison groups	Vyxeos vs 7+3
		Odds ratio	1.77

		95% CI	1.11, 2.81
		p-value (2-sided)	0.016
	Remission Duration	Comparison groups	Vyxeos vs 7+3
		HR	0.77
		95% CI	0.47 , 1.26
		p-value (2-sided)	0.294
	MLFS	Comparison groups	Vyxeos vs 7+3
		Odds ratio	1.78
		95% CI	1.05, 3.03
		p-value (1-sided)	0.017
	Stem Cell Transplant Rate	Comparison groups	Vyxeos vs 7+3
		Odds ratio	1.54
		95% CI	0.92, 2.56
		p-value (2-sided)	0.097
Notes	Stratification factors: Age (60-69 vs 70-75) and AML type t-AML ($_{MDS}$AML with documented history of MDS with prior treatment with hypomethylating agents vs $_{MDS}$AML with documented history of MDS without prior treatment with hypomethylating agents vs $_{de\ novo}$AML with karyotype characteristic of MDS vs $_{CMMoL}$AML with documented history of CMMoL)		

Clinical studies in special populations

	Age 65-74 (Older subjects number / total number)	**Age 75-84 (Older subjects number / total number)**	**Age 85+ (Older subjects number / total number)**
Controlled Trials	298/323	30/41	0
Non Controlled trials	25/323	11/41	0

Supportive studies

- **Study 204**

This was a Phase 2 multicenter, randomised, open-label, parallel-arm, fixed-dose study in subjects between 60 and 75 years with newly diagnosed AML (de novo or secondary).

Subjects were randomised 2:1 to receive either CPX-351or standard induction (7 + 3) stratified by standard or high risk category: High risk: > 70 years, prior hematologic disorder, ≥ 3 cytogenetic abnormalities at baseline and standard risk: all other subjects

The primary objective was to estimate the rate of response and confirm the safety of Vyxeos compared with 7 + 3 as first line therapy in elderly patients with standard or high risk AML. Secondary objectives were to determine OS, EFS and CR duration, the rate of aplasia after 1 or 2 inductions and effectiveness of Vyxeos on response between de novo AML and secondary AML.

The primary endpoint CR used the same assessment criteria as that for Study 301.

Patients had a treatment phase (up to 2 inductions and 2 consolidations) and a follow-up Phase (from approx. 30 days after last induction or consolidation for up to 2 years from randomization).

- Vyxeos arm: 100 units/m^2 IV over 90 minutes on Days 1, 3, and 5 for the first induction and on days 1 and 3 for second induction. For consolidation, 100 units/m^2 on Days 1 and 3.
- 7 + 3 arm: first induction with cytarabine 100 mg/m^2/day on Days 1 through 7 by continuous infusion, and daunorubicin 45 to 60 mg/m^2/day on Days 1, 2, and 3. For second induction and consolidation cytarabine was dosed on days 1 through 5 and daunorubicin on Days 1 and 2.

Subjects in the control arm were permitted to cross over to receive Vyxeos if a response was not achieved after 2 induction courses or after 1 induction if investigators determined response to a second induction would be unlikely. Efficacy data were analysed based on assigned treatment. The data after crossover were excluded from analyses. A total of 127 subjects were randomised, 86 to Vyxeos and 41 to 7 + 3. The efficacy analysis set (any subject randomised and treated with Vyxeos or 7+3) included 125 subjects (84 subjects in Vyxeos, 41 subjects in 7 + 3). After 1 or 2 inductions, 10 subjects from the 7 + 3 group crossed over to the Vyxeos group. Mean (SD) age was 67.9 (4.69) years. Baseline demographics and disease characteristics (AML type, stratification risk, cytogenetic risk, and ECOG performance status) were well balanced across treatment groups. The majority of patients were high risk (61%), intermediate cytogenetics risk (68%) and had de novo AML (65%).

Results

The efficacy results are summarised below.

Table 25 Efficacy results (Efficacy Evaluable Analysis Set -Study 204)

	Number of Subjects	
	CPX-351 N = 84	**7 + 3 N = 41**
Primary Endpoint		
Morphologic Complete Remission (CR)		
n (%)	41 (48.8)	20 (48.8)
95% CI	(37.7, 60.0)	(32.9, 64.9)
p-value[a]	0.5745	
Secondary Efficacy Endpoints		
Remission Duration (days)		
n	56	21
Median	275	235
Min, Max	40, 730	36, 703
Time to Remission (days)		
n	56	21
Median	49	40
Min, Max	32, 163	21, 89
Patient with Aplasia During the Study, n (%)	60 (71.4)	17 (41.5)
Aplasia at Day 14 (First Induction)	55 (65.5)	15 (36.6)
Number with Stem Cell Transplant	13 (15.5%)	10 (24.4%)
CR, n (%)	41 (48.84)	20 (48.8)
Received Stem Cell Transplant	10 (24.4)	4 (20.0)
Did not Receive Stem Cell Transplant	31 (75.6)	16 (80.0)
CRi, n (%)	15 (17.9)	2 (4.9)
Received Stem Cell Transplant	2 (13.3)	1 (50.0)
Did not Receive Stem Cell Transplant	13 (86.7)	1 (50.0)
< CRi, n (%)	28 (33.3)	20 (48.8)
Received Stem Cell Transplant	1 (3.6)	5 (25.0)
Did not Receive Stem Cell Transplant	27 (96.4)	15 (75.0)

EFS at 1 year (Efficacy Evaluable Set) showed a median EFS 161 days for Vyxeos and 55 days for 7 + 3 (p=0.0971), HR 0.70 (95% CI (0.5, 1.1). Improved EFS for CPX 351 was seen in both age groups.

The Kaplan-Meier estimate for median survival could not be estimated for any treatment group as the median was not reached because of insufficient follow up. The HR of OS at 1 year for the Vyxeos versus 7 + 3 was 0.89 (95% CI 0.5, 1.6). The Vyxeos and 7 + 3 treatment groups had 39/84 (46.4%) and 18/41 (43.9%) deaths at 1 year, respectively.

Study 205

This was a Phase 2 multicenter, randomised, open-label, parallel-arm, active controlled study in patients between the ages of 18 and 65 with AML in first relapse. Subjects were stratified according to the European Prognostic Index and age, and received either Vyxeos or intensive salvage therapy.

The primary objective was to estimate the efficacy of Vyxeos compared with intensive salvage therapy in patients with AML in first relapse. Primary endpoint was survival at 1-year but the study was not powered to detect a statistically significant difference. Secondary variables included CR (including CRi) rate and duration, EFS, rate of aplasia, and rate of transfer for stem cell transplant.

Subjects were permitted to receive up to 2 inductions and 2 consolidations. The study comprised a treatment phase (up to 2 inductions and up to 2 consolidations) and a follow-up phase, from the completion of the treatment phase, and lasted for 1 year from the date of randomization. Only subjects with recovery from all the therapy related nonhematologic AEs and documented CR or CRi were eligible for consolidation.

The dose of Vyxeos was 100 units/m^2 by 90 minute IV infusion, on Days 1, 3, and 5 for the first induction and on Days 1 and 3 for a second induction or consolidation. Intensive salvage therapy (was chosen by the investigator.

Results

A total of 126 subjects were randomised, Vyxeos (81 subjects) and salvage therapy (45 subjects). The efficacy evaluable analysis set included the 81 subjects in the Vyxeos treatment and 44 subjects in the salvage group who received at least 1 dose of study medication.

The demographic characteristics of the subjects were similar in the 2 treatment groups. Subjects had a mean (SD) age of 50.2 (11.57) years, ranging from 20 to 66 years; most subjects (82%) were between 18 and 60 years old, had de novo AML (89.6%) or of intermediate cytogenetics risk (60.5%).

The proportion of subjects surviving at 1 year, the primary endpoint, was higher in the Vyxeos arm (35.8%, 29/81 subjects) than in the salvage therapy arm (27.3%, 12/44 subjects), but the difference was not statistically significant (p = 0.43; Fisher' s Exact). The median survival was 259 days (8.51 months) in the Vyxeos and 191 days (6.28 months) in the salvage arm (p=0.19; based on log rank test; HR of survival by 1-year was 0.75 with 95% CI of [0.5, 1.2]).

The rates of CR (37.0% vs 31.8%, respectively), CRi (12.3% vs 9.1%), and CR or CRi (49.4% vs 40.9%) indicated clinical improvement in the Vyxeos arm compared with salvage therapy, but the differences were not significant. The median duration of remission for subjects who achieved CR or Cri was 301 days in the Vyxeos arm and 259 days in the salvage arm. Median EFS was longer in the Vyxeos arm (75 days) than in the salvage (43 days), HR of 0.67 (95% CI of [0.4, 1.0], p=0.06) for EFS by 1-year.The rates of aplasia at any time during the study and at Day 14 (first induction) were higher in the Vyxeos arm than in the salvage arm (76.5% vs 54.5%, respectively, at any time and 75.3% vs 52.3%, respectively, at Day 14). The

percentage of subjects transferred to stem cell transplant was similar in the 2 arms: 46.9% in the Vyxeos arm and 47.4% in the salvage arm.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

This application is supported by a single phase III clinical study (301) and the overall design is considered adequate.

The patient population has been adequately selected and reflects those patients with high risk AML with documented prior haematological disorder as defined in the proposed indication. The pivotal study included only patients without prior anti-AML treatment and who were above 60 years of age. The comparator was the standard 7+ 3 regimen which is appropriate and allows a clear comparison of the liposomal formulation combination of cytarabine and daunorubicin versus the two components administered separately. The choice of endpoints with OS as primary is satisfactory considering the poor prognosis in this type of AML population. All other secondary endpoints are also considered adequate.

There is sufficient follow up for data maturity and to allow comprehensive assessment of results. The conduct of the study appears adequate and there are no concerns. Overall, the study design is in line with the main CHMP anti-cancer guideline and followed previous CHMP scientific advice.

The phase II study 205 provides supportive data of Vyxeos in patients with AML (de novo or secondary) above 18 years of age and in first relapse.

The proposed dose of Vyxeos and the regimen schedule, as administered in the pivotal study, have been justified by a dose finding study (101), data from earlier phase II studies (204 and 205) and an exposure dose analysis and it is considered acceptable.

Efficacy data and additional analyses

Both arms in the pivotal study were balanced with regards to baseline demography and disease characteristics. A significant improvement of 3.6 months in OS with Vyxeos compared to the 7+3 standard treatment has been demonstrated in the pivotal study ITT population (median OS 9.56 months vs 5.95 months; HR = 0.69, 95% CI : 0.52 to 0.90, 1-sided p = 0.003). This is a clinically relevant effect as the 7+3 treatment has been the standard for over four decades and no therapy has shown to offer superior efficacy. The OS benefit was seen across all randomization strata or those with the adverse risk FLT3 mutation, except for the MDS AML with prior hypomethylating agent therapy.

The favourable result is OS is supported by the outcome in secondary endpoints. A significantly greater proportion of subjects in the Vyxeos group achieved CR or CR+ CRi compared with the 7+3 group (CR 37.3% vs 25.6%; CR+CRi 47.7% vs. 33.3%). No clinical differences were seen in remission duration between Vyxeos vs 7+3 (median 6.93 months vs. 6.11 months). Favourable results for Vyxeos versus 7+3 treatment were also documented for EFS (median 2.53 months vs 1.31 months, HR = 0.74, 1-sided p = 0.011), achieving a morphologic leukaemia free state (69.0% vs. 55.5%) or the rate of bridging to transplant (52 [34.0%] vs. 39 [25.0%]).

More patients in the Vyxeos treatment group received a HSCT in CR or CRi compared with patients in CR or CRi for the 7+3 regimen (54.8% vs. 46.2%, respectively).

An analysis landmarked at the time of SCT, showed that subjects who received a HSCT in the Vyxeos treatment group had significantly longer OS than the 7+3 group (HR=0.46, p = 0.009, 1-sided) with median survival not reached in the Vyxeos versus 10.25 months for 7+3. It is known that survival in high risk AML increases if the patient undergoes transplant, especially if achieved CR after induction.

Overall the results across randomization data favoured Vyxeos over 7+3, especially across age subgroups. Most but not all the analyses across stratification subgroups showed positive outcome for Vyxeos. For the subgroup of patients with prior MDS treated with hypomethylating agents the control arm had a longer median OS than the experimental arm (7.43 m vs 5.65 m) despite the slight favourable results for CPX 351 on CR/CRi, MLFS and patients receiving SCT. It is noted that the HR does not favour the control arm (0.98) and results in subgroups not powered for confirmatory efficacy should always be viewed with caution as some will be in the opposite direction just by chance.

Overall, the results from study 301 support the indication in untreated patients with high risk AML as defined by therapy-related or with myelodysplasia changes. Although this study was conducted in patients of 60 years up to 75 years no clinically differences in outcome were seen across both age subgroups (60-69 years/70-75 years). PK data did not show variability with regards to age covariate and free fractions of the active substances relative to the encapsulated active substances are similar in the studied population to that of adults below the age of 60 years. In addition, data from study 205 in AML patients in first relapse showed a beneficial trend with Vyxeos versus salvage therapy (consisting mainly of cytarabine and an anthracycline and another agent) in patients below 65 years, the majority below 60 years. Vyxeos is an optimised liposomal formulation of cytarabine and daunorubicin which is used to treat in AML of all ages fit for intensive chemotherapy although newly diagnosed younger patients may receive a more intensive treatment such as cytarabine + anthracycline + a third agent. On the other hand, there is no possible rationale why the results seen in study 301 cannot be extrapolated to adults of 18 years up to 60 years if they were to receive the standard 7+3. It appears there are no differences in disease biology with regards to t-AML and AML-MRC between age's subgroups in adults.

A point to mention is that Vyxeos is a more convenient therapy administered as 90 minutes' infusions on separate days instead of the 7-days or 5-days continuous infusion cytarabine (with additional daunorubicin administration) of the standard 7+3.

No data are available on the use of Vyxeos in children and this has been reflected in the SmPC.

2.5.4. Conclusions on the clinical efficacy

Efficacy of Vyxeos in the proposed dose/schedule in adult patients with untreated therapy-related AML or AML with myelodysplasia-related changes has been demonstrated.

2.6. Clinical safety

A total of 629 subjects were treated in the clinical studies and out of these, 403 subjects received at least one dose of Vyxeos.

Safety data are presented for the pooled safety population that included all subjects in clinical studies 101, 204, 205, 206 and 301 who received at least one dose of 100 or 101 units/m^2 of Vyxeos. The 101 units/m^2 dose is considered the same as the 100 units/m^2 dose. In Study 204, 10 subjects from the 7 + 3 group crossed over to Vyxeos and their data collected during treatment with 7 + 3 were summarised with 7 + 3, and data collected after cross over were summarised with Vyxeos.

The pooled population includes a broad range of AML patients with different AML subtypes, newly diagnosed and relapsed patients and of an age range of 18 to 80 years.

Patient exposure

The overall extend of exposure is displayed in Table 27.

Table 26 Overall Extent of exposure (Pooled safety population)

	Induction Period		Consolidation Period[a]			Treatment Period	
	CPX-351 100 units/m² N = 375	All Controls[b] N = 236	CPX-351 65 units/m² N = 52	CPX-351 100 units/m² N = 63	All Controls[b] N = 55	CPX-351 N = 375	All Controls[b] N = 236
Exposure (days)[c]							
Mean (SD)	3.5 (0.92)	8.9 (3.05)	3.0 (1.08)	2.6 (0.91)	8.0 (2.85)	4.3 (1.57)	10.8 (4.54)
Median (Min, Max)	3.0 (1, 8)	8.0 (2, 16)	2.5 (2, 6)	2.0 (2, 4)	6.0 (3, 14)	3.0 (1, 9)	8.0 (2, 23)
Inductions							
1 Induction[d]	289 (77.1)	170 (72.0)	NA	NA	NA	289 (77.1)	170 (72.0)
2 Inductions	85 (22.7)	66 (28.0)	NA	NA	NA	85 (22.7)	66 (28.0)
Consolidations							
1 Consolidation	NA	NA	27 (51.9)	45 (71.4)	29 (52.7)	72 (19.2)	29 (12.3)
2 Consolidations	NA	NA	24 (46.2)	18 (28.6)	26 (47.3)	42 (11.2)	26 (11.0)
≥ 3 Consolidations	NA	NA	1 (1.9)	0	0	1 (0.3)	0
Cumulative Number of Infusions per Subject[e]							
Mean (SD)	3.5 (0.92)	1.3 (0.45)	3.1 (1.15)	2.6 (0.91)	1.5 (0.50)	4.3 (1.59)	1.6 (0.77)
Median (Min, Max)	3.0 (1, 8)	1.0 (1, 2)	3.0 (2, 6)	2.0 (2, 4)	1.0 (1, 2)	3.0 (1, 9)	1.0 (1, 4)
Cumulative Dose of Daunorubicin (mg/m²)[f]							
Mean (SD)	152.11 (40.556)	212.98 (57.198)	85.30 (30.452)	107.61 (40.974)	162.34 (59.752)	181.79 (59.867)	243.78 (85.586)
Median (Min, Max)	132.00 (43.8, 353.7)	180.00 (43.8, 308.9)	86.98 (51.5, 165.8)	88.00 (41.8, 179.8)	120.00 (85.6, 261.5)	133.63 (43.8, 387.6)	181.82 (43.8, 531.7)
Cumulative Dose of CPX-351 (units/m²)							
Mean (SD)	345.71 (92.174)	NA	193.87 (69.209)	244.58 (93.122)	NA	413.17 (136.062)	NA
Median (Min, Max)	300.00 (99.5, 803.8)	NA	197.68 (117.1, 376.9)	200.00 (95.0, 408.7)	NA	303.70 (99.5, 880.9)	NA

Abbreviations: Max = maximum; Min = minimum.

[a] The CPX-351 65 units/m² consolidation dose was tested in Study 206 and Study 301. The CPX-351 100 units/m² consolidation dose was tested in Study 101 (101 units/m²), Study 204, and Study 205.

In the pivotal study 301 all subjects received an initial induction, approximately one-third in both arms received a second induction and more subjects in the Vyxeos group received both an initial and second consolidation than in the 7+3 (Table 28).

Table 27 Study 301: Exposure to experimental agents (safety population- CLTR0310-301)

	CPX-351 N = 153	7+3 N = 151
First induction, n (%)	153 (100)	151 (100)
Second induction, n (%)	48 (31)	51 (34)
First consolidation, n (%)	49 (32)	32 (21)
Second consolidation, n (%)	23 (15)	12 (7.9)
Cumulative dose		
CPX-351, median (units)	814	
Cytarabine, median (mg)		1743.0
Daunorubicin, median (mg)		447.0
Length of treatment exposure, median (days)[a]	19.00	10.00
Length of treatment phase, median (days)[b]	62.00	41.00

[a] Length of treatment exposure = last date of experimental agent administration – first date of experimental agent.

[b] Length of treatment phase = date of end of treatment phase – first date of experimental agent + 1.

Adverse events

In the Pooled Safety population, all subjects reported at least 1 TEAE (Table 29).

Table 28 Overall Summary of Treatment Emergent AE (Pooled Safety Population)

	Number of subjects, n (%)						
	Induction Period		Consolidation Period[a]			Treatment Period	
	CPX-351 100 units/m^2 N = 375	All Controls N = 236	CPX-351 65 units/m^2 N = 52	CPX-351 100 units/m^2 N = 63	All Controls N = 55	CPX-351 N = 375	All Controls N = 236
Any TEAEs[b]	375 (100)	236 (100)	44 (84.6)	61 (96.8)	49 (89.1)	375 (100)	236 (100)
TEAEs by Maximum NCI-CTC Grade[c]	375 (100)	236 (100)	44 (84.6)	61 (96.8)	49 (89.1)	375 (100)	236 (100)
Grade 1	2 (0.5)	4 (1.7)	12 (23.1)	6 (9.5)	5 (9.1)	2 (0.5)	4 (1.7)
Grade 2	31 (8.3)	21 (8.9)	4 (7.7)	9 (14.3)	14 (25.5)	27 (7.2)	20 (8.5)
Grade 3	251 (66.9)	158 (66.9)	20 (38.5)	36 (57.1)	17 (30.9)	239 (63.7)	150 (63.6)
Grade 4	63 (16.8)	28 (11.9)	7 (13.5)	5 (7.9)	9 (16.4)	73 (19.5)	33 (14.0)
Grade 5	28 (7.5)	25 (10.6)	1 (1.9)	5 (7.9)	4 (7.3)	34 (9.1)	29 (12.3)
Grade 3 to 5	342 (91.2)	211 (89.4)	28 (53.8)	46 (73.0)	30 (54.5)	346 (92.3)	212 (89.8)
TEAEs by Closest Relationship[d]							
Not related	13 (3.5)	16 (6.8)	8 (15.4)	12 (19.0)	10 (18.2)	12 (3.2)	16 (6.8)
Related	362 (96.5)	220 (93.2)	36 (69.2)	49 (77.8)	39 (70.9)	363 (96.8)	220 (93.2)
Serious TEAEs	145 (38.7)	76 (32.2)	25 (48.1)	33 (52.4)	24 (43.6)	189 (50.4)	91 (38.6)
TEAEs leading to discontinuation	7 (1.9)	3 (1.3)	0	0	0	7 (1.9)	3 (1.3)
TEAEs leading to death	28 (7.5)	25 (10.6)	1 (1.9)	5 (7.9)	4 (7.3)	34 (9.1)	29 (12.3)

Abbreviations: AE = adverse event; NCI-CTC = National Cancer Institute Common Terminology Criteria; SAE = serious adverse event; TEAE = treatment emergent adverse event.

[a] The CPX-351 65 units/m^2 consolidation dose was tested in Study 206 and Study 301. The CPX-351 100 units/m^2 consolidation dose was tested in Study 101 (101 units/m^2), Study 204, and Study 205.

[b] For SAEs or AEs identified as a bleeding event, a cardiac event, infection or rash, a TEAE is defined as an AE that started after the first dose of induction 1. For all other AEs, a TEAE is defined as an AE that started after the first dose of induction 1 and not more than 30 days after the last dose date.

[c] If a subject experiences an AE with more than one NCI-CTC grade, the subject is counted only once in maximum grade category.

[d] If a subject experiences an AE with more than one relationship, the subject is counted only once in closest relationship category. Related category includes possibly, probably, and definitely related.

Note: The 'All Controls' group included subjects from Study 204, Study 205 and Study 301 treated as follows: during the Induction Period, 'All Controls' included subjects treated with 7 + 3 and Salvage therapy; during the Consolidation Period, 'All Controls' included subjects treated with 5 + 2 and Salvage / Other therapy; during the Treatment Period, 'All Controls' included subjects treated with 7 + 3 and Salvage therapy.

An overall summary of AEs for the safety population is displayed in Table 30.

Table 29 Study 301 Overall summary AE (safety analysis population)

	CPX-351 (N=153) n (%)	7 + 3 (N=151) n (%)	All Patients (N=304) n (%)
Patients with AEs (any grade)	153 (100)	151 (100)	304 (100)
Patients discontinued due to an AE	3 (2.0)	2 (1.3)	5 (1.6)
Patients with serious AEs	90 (59)	65 (43)	155 (51)
Patients with Grade 3 AEs	85 (56)	92 (61)	177 (58)
Patients with Grade 4 AEs	25 (16)	16 (11)	41 (13)
Patients with Grade 3 or 4 AEs	110 (72)	108 (72)	218 (72)
Patients with Grade 5 AEs	31 (20)	29 (19)	60 (20)
Patients with Grade 5 AEs during Treatment Period	31 (20)	29 (19)	60 (20)
Patients with Grade 5 AEs during Follow-up	19 (12)	11 (7.3)	30 (9.9)
Patients with related AEs	146 (95)	143 (95)	289 (95)
Patients with Grade 5 Related AEs	27 (18)	27 (18)	54 (18)
Patients with Grade 3, 4 or 5 AEs	141 (92)	137 (91)	278 (91)

- *Common AEs*

For Vyxeos the most frequently reported TEAEs by PT were febrile neutropenia (63.2%), nausea (51.2%), diarrhoea (45.6%), constipation (42.7%) and oedema peripheral (41.3%). In the All Controls group, the most frequently reported TEAEs by PT were diarrhoea (65.7%), febrile neutropenia (59.7%), nausea (53.4%), oedema peripheral (43.2%) and constipation (39.4%).

Table 30 TEAEs reported in ≥ 10% of subjects in any treatment group, by system organ class and preferred term – treatment period (pooled safety population)

System Organ Class Preferred Term	CPX-351 N = 375	Controls 7 + 3 N = 192	Controls Salvage N = 44	Controls All Controls N = 236
Any TEAEs[a]	375 (100)	192 (100)	44 (100)	236 (100)
Blood and Lymphatic System Disorders	**258 (68.8)**	**132 (68.8)**	**19 (43.2)**	**151 (64.0)**
Febrile neutropenia	237 (63.2)	126 (65.6)	15 (34.1)	141 (59.7)
Cardiac Disorders	**154 (41.1)**	**72 (37.5)**	**11 (25.0)**	**83 (35.2)**
Tachycardia	57 (15.2)	23 (12.0)	7 (15.9)	30 (12.7)
Atrial fibrillation	26 (6.9)	20 (10.4)	1 (2.3)	21 (8.9)
Gastrointestinal Disorders	**338 (90.1)**	**185 (96.4)**	**39 (88.6)**	**224 (94.9)**
Nausea	192 (51.2)	103 (53.6)	23 (52.3)	126 (53.4)
Diarrhoea	171 (45.6)	129 (67.2)	26 (59.1)	155 (65.7)
Constipation	160 (42.7)	82 (42.7)	11 (25.0)	93 (39.4)
Vomiting	103 (27.5)	43 (22.4)	16 (36.4)	59 (25.0)
Abdominal pain	72 (19.2)	34 (17.7)	9 (20.5)	43 (18.2)
Stomatitis	57 (15.2)	25 (13.0)	6 (13.6)	31 (13.1)
Abdominal distention	43 (11.5)	22 (11.5)	2 (4.5)	24 (10.2)
Dyspepsia	32 (8.5)	14 (7.3)	5 (11.4)	19 (8.1)
General Disorders and Administration Site Conditions	**338 (90.1)**	**171 (89.1)**	**38 (86.4)**	**209 (88.6)**
Oedema peripheral	155 (41.3)	91 (47.4)	11 (25.0)	102 (43.2)
Fatigue	143 (38.1)	65 (33.9)	16 (36.4)	81 (34.3)
Chills	117 (31.2)	54 (28.1)	10 (22.7)	64 (27.1)
Pyrexia	109 (29.1)	39 (20.3)	13 (29.5)	52 (22.0)
Mucosal inflammation	54 (14.4)	32 (16.7)	9 (20.5)	41 (17.4)
Asthenia	38 (10.1)	22 (11.5)	9 (20.5)	31 (13.1)
Infections and Infestations	**289 (77.1)**	**130 (67.7)**	**33 (75.0)**	**163 (69.1)**
Pneumonia	74 (19.7)	39 (20.3)	4 (9.1)	43 (18.2)
Bacteraemia	40 (10.7)	6 (3.1)	4 (9.1)	10 (4.2)
Injury, Poisoning and Procedural Complications	**120 (32.0)**	**56 (29.2)**	**11 (25.0)**	**67 (28.4)**
Transfusion reaction	29 (7.7)	16 (8.3)	6 (13.6)	22 (9.3)
Metabolism and Nutrition Disorders	**204 (54.4)**	**101 (52.6)**	**27 (61.4)**	**128 (54.2)**
Decreased appetite	127 (33.9)	76 (39.6)	14 (31.8)	90 (38.1)
Hypokalaemia	47 (12.5)	14 (7.3)	5 (11.4)	19 (8.1)
Fluid overload	23 (6.1)	21 (10.9)	2 (4.5)	23 (9.7)
Musculoskeletal and Connective Tissue Disorders	**181 (48.3)**	**85 (44.3)**	**17 (38.6)**	**102 (43.2)**
Back pain	54 (14.4)	24 (12.5)	6 (13.6)	30 (12.7)
Arthralgia	50 (13.3)	9 (4.7)	3 (6.8)	12 (5.1)
Pain in extremity	44 (11.7)	17 (8.9)	6 (13.6)	23 (9.7)
Nervous System Disorders	**239 (63.7)**	**103 (53.6)**	**27 (61.4)**	**130 (55.1)**
Headache	120 (32.0)	45 (23.4)	15 (34.1)	60 (25.4)
Dizziness	74 (19.7)	43 (22.4)	5 (11.4)	48 (20.3)
Dysgeusia	23 (6.1)	13 (6.8)	5 (11.4)	18 (7.6)
Psychiatric Disorders	**195 (52.0)**	**94 (49.0)**	**14 (31.8)**	**108 (45.8)**
Insomnia	85 (22.7)	43 (22.4)	10 (22.7)	53 (22.5)
Anxiety	65 (17.3)	25 (13.0)	6 (13.6)	31 (13.1)
Confusional state	44 (11.7)	26 (13.5)	1 (2.3)	27 (11.4)
Respiratory, Thoracic and Mediastinal Disorders	**292 (77.9)**	**141 (73.4)**	**25 (56.8)**	**166 (70.3)**
Cough	118 (31.5)	37 (19.3)	10 (22.7)	47 (19.9)
Epistaxis	111 (29.6)	35 (18.2)	12 (27.3)	47 (19.9)
Dyspnoea	86 (22.9)	37 (19.3)	4 (9.1)	41 (17.4)
Hypoxia	60 (16.0)	35 (18.2)	1 (2.3)	36 (15.3)
Oropharyngeal pain	60 (16.0)	22 (11.5)	6 (13.6)	28 (11.9)
Pleural effusion	52 (13.9)	32 (16.7)	2 (4.5)	34 (14.4)
Skin and Subcutaneous Tissue Disorders	**315 (84.0)**	**124 (64.6)**	**26 (59.1)**	**150 (63.6)**
Rash	147 (39.2)	48 (25.0)	10 (22.7)	58 (24.6)
Petechiae	71 (18.9)	23 (12.0)	6 (13.6)	29 (12.3)
Pruritus	65 (17.3)	19 (9.9)	4 (9.1)	23 (9.7)
Hyperhidrosis	38 (10.1)	13 (6.8)	2 (4.5)	15 (6.4)
Alopecia	12 (3.2)	24 (12.5)	4 (9.1)	28 (11.9)
Vascular Disorders	**172 (45.9)**	**87 (45.3)**	**13 (29.5)**	**100 (42.4)**
Hypotension	76 (20.3)	40 (20.8)	5 (11.4)	45 (19.1)
Hypertension	57 (15.2)	29 (15.1)	6 (13.6)	35 (14.8)

Abbreviations: AE = adverse event; MedDRA = medical dictionary for regulatory activities; SAE = serious adverse event; TEAE = treatment emergent adverse event.

[a] For SAEs or AEs identified as a bleeding event, a cardiac event, infection or rash, a TEAE is defined as an AE that started after the first dose of induction 1. For all other AEs, a TEAE is defined as an AE that started after the first dose of induction 1 and not more than 30 days after the last dose date.

Note: The 'All Controls' treatment group includes subjects from Study 204, Study 205, and Study 301 who were treated with 7 + 3 and / or Salvage therapy.

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- *TEAEs ≥ Grade 3*

A summary of the TEAEs of Grade ≥ 3 reported in at least 3% of subjects in any treatment group is displayed in Table 32.

Table 31 Treatment Emergent Adverse Events of Grade ≥ 3 Reported in at Least 3% of Subjects in any Treatment Group, by SOC and PT- Treatment Period (Pooled Safety Population)

System Organ Class Preferred Term	CPX-351 N = 375	Controls		
		7 + 3 N = 192	Salvage N = 44	All Controls N = 236
Any TEAEs Grade ≥ 3 [a, b]	346 (92.3)	172 (89.6)	40 (90.9)	212 (89.8)
Blood and Lymphatic System Disorders	**245 (65.3)**	**131 (68.2)**	**19 (43.2)**	**150 (63.6)**
Febrile neutropenia	233 (62.1)	125 (65.1)	15 (34.1)	140 (59.3)
Neutropenia	13 (3.5)	6 (3.1)	2 (4.5)	8 (3.4)
Pancytopenia	4 (1.1)	1 (0.5)	2 (4.5)	3 (1.3)
Thrombocytopenia	14 (3.7)	5 (2.6)	3 (6.8)	8 (3.4)
Cardiac Disorders	**30 (8.0)**	**25 (13.0)**	**2 (4.5)**	**27 (11.4)**
Atrial fibrillation	10 (2.7)	6 (3.1)	0	6 (2.5)
Gastrointestinal Disorders	**59 (15.7)**	**31 (16.1)**	**3 (6.8)**	**34 (14.4)**
Diarrhoea	11 (2.9)	11 (5.7)	1 (2.3)	12 (5.1)
General Disorders and Administration Site Conditions	**71 (18.9)**	**36 (18.8)**	**8 (18.2)**	**44 (18.6)**
Aesthenia	3 (0.8)	1 (0.5)	2 (4.5)	3 (1.3)
Fatigue	31 (8.3)	10 (5.2)	1 (2.3)	11 (4.7)
Mucosal inflammation	4 (1.1)	5 (2.6)	2 (4.5)	7 (3.0)
Infections and Infestations	**217 (57.9)**	**95 (49.5)**	**27 (61.4)**	**122 (51.7)**
Bacteraemia	36 (9.6)	4 (2.1)	4 (9.1)	8 (3.4)
Bacterial sepsis	7 (1.9)	1 (0.5)	3 (6.8)	4 (1.7)
Cellulitis	13 (3.5)	3 (1.6)	2 (4.5)	5 (2.1)
Clostridium difficile colitis	5 (1.3)	6 (3.1)	0	6 (2.5)
Enterobacter sepsis	2 (0.5)	0	2 (4.5)	2 (0.8)
Enterococcal bacteraemia	14 (3.7)	10 (5.2)	3 (6.8)	13 (5.5)
Escherichia bacteraemia	7 (1.9)	6 (3.1)	2 (4.5)	8 (3.4)
Fungal sepsis	1 (0.3)	0	2 (4.5)	2 (0.8)
Klebsiella bacteraemia	4 (1.1)	7 (3.6)	0	7 (3.0)
Klebsiella sepsis	2 (0.5)	1 (0.5)	3 (6.8)	4 (1.7)
Pneumonia	60 (16.0)	27 (14.1)	3 (6.8)	30 (12.7)
Pneumonia fungal	12 (3.2)	4 (2.1)	0	4 (1.7)
Sepsis	22 (5.9)	12 (6.3)	0	12 (5.1)
Staphylococcal bacteraemia	27 (7.2)	7 (3.6)	3 (6.8)	10 (4.2)
Staphylococcal sepsis	4 (1.1)	1 (0.5)	2 (4.5)	3 (1.3)
Streptococcal bacteraemia	11 (2.9)	2 (1.0)	2 (4.5)	4 (1.7)
Streptococcal sepsis	9 (2.4)	2 (1.0)	2 (4.5)	4 (1.7)
Urinary tract infection bacterial	2 (0.5)	4 (2.1)	2 (4.5)	6 (2.5)
Urinary tract infection enterococcal	6 (1.6)	1 (0.5)	2 (4.5)	3 (1.3)
Investigations	**35 (9.3)**	**22 (11.5)**	**3 (6.8)**	**25 (10.6)**
Ejection fraction decreased	8 (2.1)	7 (3.6)	0	7 (3.0)

Metabolism and Nutrition Disorders	**42 (11.2)**	**19 (9.9)**	**9 (20.5)**	**28 (11.9)**
Decreased appetite	6 (1.6)	9 (4.7)	0	9 (3.8)
Hypokalaemia	21 (5.6)	5 (2.6)	3 (6.8)	8 (3.4)
Hyponatraemia	6 (1.6)	0	3 (6.8)	3 (1.3)
Neoplasms Benign, Malignant and Unspecified (including cysts and polyps)	**6 (1.6)**	**2 (1.0)**	**2 (4.5)**	**4 (1.7)**
Acute myeloid leukaemia	1 (0.3)	1 (0.5)	2 (4.5)	3 (1.3)
Nervous System Disorders	**36 (9.6)**	**15 (7.8)**	**4 (9.1)**	**19 (8.1)**
Syncope	10 (2.7)	4 (2.1)	3 (6.8)	7 (3.0)
Psychiatric Disorders	**16 (4.3)**	**17 (8.9)**	**1 (2.3)**	**18 (7.6)**
Delirium	3 (0.8)	8 (4.2)	0	8 (3.4)
Renal and Urinary Disorders	**28 (7.5)**	**14 (7.3)**	**3 (6.8)**	**17 (7.2)**
Renal failure	5 (1.3)	6 (3.1)	1 (2.3)	7 (3.0)
Renal failure acute	17 (4.5)	8 (4.2)	2 (4.5)	10 (4.2)
Respiratory, Thoracic and Mediastinal Disorders	**76 (20.3)**	**45 (23.4)**	**2 (4.5)**	**47 (19.9)**
Dyspnoea	17 (4.5)	5 (2.6)	0	5 (2.1)
Hypoxia	36 (9.6)	24 (12.5)	1 (2.3)	25 (10.6)
Pulmonary oedema	5 (1.3)	9 (4.7)	0	9 (3.8)
Respiratory failure	14 (3.7)	9 (4.7)	0	9 (3.8)
Skin and Subcutaneous Tissue Disorders	**43 (11.5)**	**10 (5.2)**	**1 (2.3)**	**11 (4.7)**
Rash	16 (4.3)	1 (0.5)	0	1 (0.4)
Vascular Disorders	**44 (11.7)**	**19 (9.9)**	**3 (6.8)**	**22 (9.3)**
Hypertension	23 (6.1)	10 (5.2)	3 (6.8)	13 (5.5)
Hypotension	15 (4.0)	3 (1.6)	0	3 (1.3)

Abbreviations: AE = adverse event; NCI-CTC = National Cancer Institute Common Terminology Criteria; SAE = serious adverse event; TEAE = treatment emergent adverse event.

[a] For SAEs or AEs identified as a bleeding event, a cardiac event, infection or rash, a TEAE is defined as an AE that started after the first dose of induction 1. For all other AEs, a TEAE is defined as an AE that started after the first dose of induction 1 and not more than 30 days after the last dose date.

[b] If a subject experiences an AE with more than one NCI-CTC grade, the subject is counted only once in maximum grade category.

Note: The 'All Controls' treatment group includes subjects from Study 204, Study 205, and Study 301 who were treated with 7 + 3 and / or Salvage therapy.

In Study 301, similar percentages of subjects in the Vyxeos group (92%) and the 7 + 3 (91%) reported AEs Grade ≥ 3. The most frequently reported in both groups were febrile neutropenia (68% and 71%, in the Vyxeos and 7 + 3, respectively), pneumonia (20% and 15%), and hypoxia (13% and 15%).

- *Related TEAEs*

The most frequently related TEAEs reported in Vyxeosgroup were febrile neutropenia (48.3%), nausea (41.1%), diarrhoea (34.4%), rash (32.3%) and fatigue (28.0%), and in the all controls group were diarrhoea (48.7%), febrile neutropenia (46.2%), nausea (44.9%), decreased appetite (33.1%) and fatigue (23.3%).

In study 301, AE related to study drug by the investigator were reported by 95% of subjects in each treatment group and the most frequent was febrile neutropenia (60% each arm). A summary of the related teae reported in at least 5% of subjects in any treatment group is displayed in Table 33.

Table 32 Related TEAE Reported in at Least 5% of Subjects in Any Treatment Group, by System Organ Class and Preferred Term - (Pooled Safety Population)

	Number of subjects, n (%)						
	Induction Period		Consolidation Period[a]			Treatment Period	
	CPX-351 100 units/m² N = 375	All Controls N = 236	CPX-351 65 units/m² N = 52	CPX-351 100 units/m² N = 63	All Controls N = 55	CPX-351 N = 375	All Controls N = 236
Any Related TEAEs	362 (96.5)	220 (93.2)	36 (69.2)	49 (77.8)	39 (70.9)	363 (96.8)	220 (93.2)
Blood and Lymphatic System Disorders	**187 (49.9)**	**114 (48.3)**	**14 (26.9)**	**17 (27.0)**	**15 (27.3)**	**197 (52.5)**	**116 (49.2)**
Febrile neutropenia	173 (46.1)	106 (44.9)	14 (26.9)	13 (20.6)	11 (20.0)	181 (48.3)	109 (46.2)
Cardiac Disorders	**68 (18.1)**	**36 (15.3)**	**3 (5.8)**	**5 (7.9)**	**7 (12.7)**	**76 (20.3)**	**40 (16.9)**
Atrial fibrillation	16 (4.3)	7 (3.0)	0	0	3 (5.5)	16 (4.3)	9 (3.8)
Tachycardia	20 (5.3)	9 (3.8)	1 (1.9)	2 (3.2)	1 (1.8)	23 (6.1)	10 (4.2)
Gastrointestinal Disorders	**271 (72.3)**	**192 (81.4)**	**18 (34.6)**	**29 (46.0)**	**26 (47.3)**	**274 (73.1)**	**194 (82.2)**
Abdominal distension	8 (2.1)	11 (4.7)	1 (1.9)	1 (1.6)	2 (3.6)	10 (2.7)	13 (5.5)
Abdominal pain	32 (8.5)	26 (11.0)	2 (3.8)	2 (3.2)	3 (5.5)	36 (9.6)	28 (11.9)
Constipation	74 (19.7)	34 (14.4)	1 (1.9)	4 (6.3)	6 (10.9)	77 (20.5)	37 (15.7)
Diarrhoea	120 (32.0)	110 (46.6)	4 (7.7)	8 (12.7)	10 (18.2)	129 (34.4)	115 (48.7)
Dyspepsia	18 (4.8)	11 (4.7)	1 (1.9)	0	3 (5.5)	19 (5.1)	14 (5.9)
Mouth haemorrhage	16 (4.3)	5 (2.1)	2 (3.8)	2 (3.2)	1 (1.8)	19 (5.1)	6 (2.5)
Nausea	149 (39.7)	101 (42.8)	5 (9.6)	16 (25.4)	8 (14.5)	154 (41.1)	106 (44.9)
Stomatitis	46 (12.3)	25 (10.6)	0	8 (12.7)	2 (3.6)	51 (13.6)	26 (11.0)
Vomiting	70 (18.7)	42 (17.8)	3 (5.8)	8 (12.7)	4 (7.3)	76 (20.3)	44 (18.6)
General Disorders and Administration Site Conditions	**218 (58.1)**	**130 (55.1)**	**18 (34.6)**	**22 (34.9)**	**20 (36.4)**	**228 (60.8)**	**133 (56.4)**
Asthenia	21 (5.6)	19 (8.1)	1 (1.9)	0	1 (1.8)	22 (5.9)	19 (8.1)
Chills	46 (12.3)	19 (8.1)	3 (5.8)	4 (6.3)	2 (3.6)	51 (13.6)	21 (8.9)
Fatigue	97 (25.9)	48 (20.3)	8 (15.4)	10 (15.9)	10 (18.2)	105 (28.0)	55 (23.3)
Mucosal inflammation	38 (10.1)	31 (13.1)	1 (1.9)	3 (4.8)	2 (3.6)	41 (10.9)	32 (13.6)
Oedema peripheral	58 (15.5)	40 (16.9)	4 (7.7)	2 (3.2)	3 (5.5)	61 (16.3)	43 (18.2)
Pyrexia	46 (12.3)	20 (8.5)	5 (9.6)	8 (12.7)	5 (9.1)	56 (14.9)	25 (10.6)
Infections and Infestations	**141 (37.6)**	**82 (34.7)**	**13 (25.0)**	**18 (28.6)**	**9 (16.4)**	**158 (42.1)**	**88 (37.3)**
Pneumonia	29 (7.7)	20 (8.5)	3 (5.8)	2 (3.2)	3 (5.5)	33 (8.8)	23 (9.7)
Metabolism and Nutrition Disorders	**112 (29.9)**	**85 (36.0)**	**5 (9.6)**	**9 (14.3)**	**11 (20.0)**	**120 (32.0)**	**95 (40.3)**
Decreased appetite	86 (22.9)	73 (30.9)	4 (7.7)	8 (12.7)	6 (10.9)	93 (24.8)	78 (33.1)
Hypokalaemia	13 (3.5)	3 (1.3)	0	0	3 (5.5)	13 (3.5)	6 (2.5)
Musculoskeletal and Connective Tissue Disorders	**47 (12.5)**	**26 (11.0)**	**3 (5.8)**	**5 (7.9)**	**5 (9.1)**	**53 (14.1)**	**30 (12.7)**
Myalgia	1 (0.3)	3 (1.3)	3 (5.8)	1 (1.6)	0	5 (1.3)	3 (1.3)
Nervous System Disorders	**104 (27.7)**	**62 (26.3)**	**8 (15.4)**	**7 (11.1)**	**8 (14.5)**	**113 (30.1)**	**67 (28.4)**
Dizziness	25 (6.7)	13 (5.5)	1 (1.9)	1 (1.6)	5 (9.1)	27 (7.2)	18 (7.6)
Dysgeusia	14 (3.7)	17 (7.2)	2 (3.8)	1 (1.6)	1 (1.8)	17 (4.5)	18 (7.6)
Headache	45 (12.0)	24 (10.2)	2 (3.8)	3 (4.8)	2 (3.6)	49 (13.1)	25 (10.6)
Psychiatric Disorders	**51 (13.6)**	**27 (11.4)**	**3 (5.8)**	**3 (4.8)**	**6 (10.9)**	**55 (14.7)**	**32 (13.6)**
Confusional state	14 (3.7)	10 (4.2)	1 (1.9)	0	2 (3.6)	15 (4.0)	12 (5.1)
Insomnia	21 (5.6)	10 (4.2)	2 (3.8)	1 (1.6)	2 (3.6)	23 (6.1)	12 (5.1)
Respiratory, Thoracic and Mediastinal Disorders	**144 (38.4)**	**77 (32.6)**	**8 (15.4)**	**10 (15.9)**	**13 (23.6)**	**151 (40.3)**	**83 (35.2)**
Cough	32 (8.5)	16 (6.8)	2 (3.8)	1 (1.6)	2 (3.6)	33 (8.8)	18 (7.6)
Dyspnoea	29 (7.7)	18 (7.6)	1 (1.9)	1 (1.6)	3 (5.5)	31 (8.3)	20 (8.5)
Epistaxis	53 (14.1)	18 (7.6)	2 (3.8)	3 (4.8)	4 (7.3)	57 (15.2)	22 (9.3)
Hypoxia	27 (7.2)	18 (7.6)	1 (1.9)	1 (1.6)	1 (1.8)	29 (7.7)	19 (8.1)
Oropharyngeal pain	18 (4.8)	14 (5.9)	1 (1.9)	3 (4.8)	2 (3.6)	22 (5.9)	16 (6.8)
Pleural effusion	24 (6.4)	13 (5.5)	1 (1.9)	0	1 (1.8)	25 (6.7)	14 (5.9)
Skin and Subcutaneous Tissue Disorders	**230 (61.3)**	**95 (40.3)**	**11 (21.2)**	**29 (46.0)**	**14 (25.5)**	**240 (64.0)**	**100 (42.4)**
Alopecia	9 (2.4)	22 (9.3)	1 (1.9)	1 (1.6)	2 (3.6)	11 (2.9)	24 (10.2)
Petechiae	24 (6.4)	14 (5.9)	4 (7.7)	4 (6.3)	2 (3.6)	32 (8.5)	15 (6.4)
Pruritus	38 (10.1)	14 (5.9)	1 (1.9)	0	0	39 (10.4)	14 (5.9)
Rash	111 (29.6)	36 (15.3)	4 (7.7)	19 (30.2)	4 (7.3)	121 (32.3)	39 (16.5)
Rash maculo-papular	26 (6.9)	10 (4.2)	0	0	1 (1.8)	26 (6.9)	11 (4.7)
Rash erythematous	14 (3.7)	5 (2.1)	0	2 (3.2)	3 (5.5)	16 (4.3)	7 (3.0)
Vascular Disorders	**54 (14.4)**	**34 (14.4)**	**5 (9.6)**	**6 (9.5)**	**4 (7.3)**	**64 (17.1)**	**36 (15.3)**
Hypotension	25 (6.7)	16 (6.8)	4 (7.7)	2 (3.2)	2 (3.6)	30 (8.0)	18 (7.6)
Hypertension	15 (4.0)	12 (5.1)	0	0	1 (1.8)	15 (4.0)	12 (5.1)

Abbreviations: NCI-CTC = National Cancer Institute Common Terminology Criteria; TEAE = treatment emergent adverse event.

[a] The CPX-351 65 units/m² consolidation dose was tested in Study 206 and Study 301. The CPX-351 100 units/m² consolidation dose was tested in Study 101 (101 units/m²), Study 204, and Study 205.

Note: If a subject experiences an AE with more than one relationship, the subject is counted only once in closest relationship category. Related category includes possibly, probably, and definitely related.

Note: The 'All Controls' group included subjects from Study 204, Study 205 and Study 301 treated as follows: during the Induction Period, 'All Controls' included subjects treated with 7 + 3 and Salvage therapy; during the Consolidation Period, 'All Controls' included subjects treated with 5 + 2 and Salvage / Other therapy; during the Treatment Period, 'All Controls' included subjects treated with 7 + 3 and Salvage

- *AEs of special interest*

Infections

The incidence of infection was 94.1% in the Vyxeos group compared with 1.9% in all controls regardless of treatment period. Most subjects experienced Grade 3 Infection AEs in both groups. The most frequently reported infection AE in both groups was febrile neutropenia (63.2% vs 59.7%, respectively). The incidence of other infection AEs was similar between Vyxeos vs all controls, with imbalances for pyrexia (29.1% vs 22.0%), bacteraemia (10.7% vs 4.2%), and clostridium difficile infection (5.3% vs 1.7%).

Pneumonia, sepsis and bacteriaemia were the most frequently seen serious infection ADRs in the clinical trial population. The incidence of infection events was 78.1%; the incidence of non-serious events of infections was 73.1%, the incidence of serious events of infections was 28.5%; the incidence of infections which led to discontinuation is 0.5%. The incidence of fatal infections was 6.9%. The fatal infections experienced were sepsis and pneumonia (SmPC, section 4.8).

Bacterial and viral infection occurred with similar frequency between Vyxeos and All Controls (87.5% vs 84.7%, and 13.1% vs 10.2%, respectively). Fungal Infection occurred more frequently in subjects receiving Vyxeos (18.9%) than in control (11.9%). No viral or fungal infections led to discontinuation of study or death in any treatment group.

The most frequently reported bacterial Infection in Vyxeos and control was Febrile neutropenia (63.2% and 59.7% of subjects, respectively). The most common viral infection reported with Vyxeos was Herpes simplex (1.9%) and for All Controls it was oral herpes (3.0%). The most commonly reported fungal Infection was pneumonia fungal (3.5% in Vyxeos versus 1.7% all controls).

Almost all subjects in study 301 in both treatment groups had at least 1 infection AE (92.8% vs. 92.7% in the Vyxeos and 7+3 arms respectively) and most were Grade 3 to 5 (84% vs 86%). The most frequently reported were febrile neutropenia, chills, pneumonia, pyrexia, sepsis, cellulitis, and bacteraemia. The most notable difference between the arms was for bacteraemia (9.8% vs. 2.6%), especially for grade 5 infections (7.2% vs. 2.6%). No grade 4 or 5 viral infections and no grade 5 fungal infections were reported. There were no notable differences between arms in viral or fungal infections.

Bleeding

A summary of bleeding AE by NCI-CTC Grade is displayed in Table 34.

Table 33 Summary of bleeding AE by NCI-CTC Grade – Treatment Period (Pooled Safety Population)

System Organ Class Preferred Term	Number of subjects, n (%)	
	CPX-351 N = 375	All Controls N = 236
Subjects with Bleeding AEs, n (%)	**259 (69.1)**	**139 (58.9)**
Bleeding AEs by NCI-CTC Grade		
Grade 1	143 (38.1)	86 (36.4)
Grade 2	68 (18.1)	39 (16.5)
Grade 3	36 (9.6)	10 (4.2)
Grade 4	4 (1.1)	1 (0.4)
Grade 5	8 (2.1)	3 (1.3)
Grade 3-5	48 (12.8)	14 (5.9)
Serious Bleeding AEs	21 (5.6)	9 (3.8)
Bleeding AEs leading to discontinuation	1 (0.3)	0
Bleeding AEs leading to death	8 (2.1)	3 (1.3)

The most common haemorrhagic event was epistaxis, and the majority of these were considered not serious (29.1%). The incidence of haemorrhage events is 69.1%; the incidence of non-serious events of haemorrhage was 67.2 %; the incidence of serious events of haemorrhage is 5.6%; the incidence of haemorrhage which led to discontinuation is 0. The incidence of fatal haemorrhage was 2.1% .Serious or fatal haemorrhagic events, including fatal central nervous system (CNS) haemorrhages, associated with severe thrombocytopenia were seen in patients treated with Vyxeos (SmPC, section 4.8).

In study 301 more subjects in Vyxeos than in the 7+3 group had a bleeding-related AE (74.5% vs. 59.6%, respectively), most were grade 1 to 2, and grade 3 to 5 occurred in 12% vs 8.6%, respectively. Four (2.6%) subjects in each arm had a grade 5 bleeding-related AE. The most frequent bleeding-related AEs were epistaxis, petechiae, mouth Haemorrhage and ecchymosis. The most notable difference between the Vyxeos and 7+3 groups was for epistaxis (35.9% vs. 17.9%, respectively), mouth haemorrhage (10.5% vs. 5.3%, respectively), and blood blister (9.2% vs. 3.3%, respectively).

Cardiac

During treatment period, cardiac AEs were more frequent in the Vyxeos group than in the All Controls but the difference is driven by Grade 1 AEs while similar incidences were reported for Grade 3-5 AEs.

Table 23 Summary of Cardiac AE by NCI-CTC Grade - Treatment Period (Pooled Safety Population)

System Organ Class Preferred Term	Number of subjects, n (%)	
	CPX-351 N = 375	All Controls N = 236
Subjects with Cardiac AEs, n (%)	**188 (50.1)**	**99 (41.9)**
Cardiac AEs by NCI-CTC Grade		
Grade 1	103 (27.5)	36 (15.3)
Grade 2	45 (12.0)	31 (13.1)
Grade 3	29 (7.7)	14 (5.9)
Grade 4	9 (2.4)	12 (5.1)
Grade 5	2 (0.5)	6 (2.5)
Grade 3 to 5	40 (10.7)	32 (13.6)
Serious Cardiac AEs	26 (6.9)	26 (11.0)
Cardiac AEs leading to discontinuation	3 (0.8)	2 (0.8)
Cardiac AEs leading to death	2 (0.5)	6 (2.5)

Abbreviations: AE = adverse event; MedDRA = medical dictionary for regulatory affairs; NCI-CTC = national cancer institute common terminology criteria; SAE = serious adverse event; TEAE = treatment emergent adverse event.

Note: For SAEs or AEs identified as a bleeding event, a cardiac event, infection or rash, a TEAE is defined as an AE that started after the first dose of induction 1. For all other AEs, a TEAE is defined as an AE that started after the first dose of induction 1 and not more than 30 days after the last dose date.

Note: If a subject experiences an AE with more than 1 NCI-CTC grade for a category, the subject is counted only once in maximum grade under that category.

Note: The 'All Controls' treatment group includes subjects from Study 204, Study 205, and Study 301 who were treated with 7 + 3 and / or Salvage therapy.

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The most frequently reported serious ADRs were decreased ejections fraction and congestive cardiac failure. The incidence of all cardiotoxicity events was 72.0%; the incidence of non-serious events of cardiotoxicity was 68.5 %; the incidence of serious events of cardiotoxicity was 9.1%; the incidence of cardiotoxicity which led to discontinuation is 0. 5%. Incidence of fatal cardiotoxicity events is 0.5%. Cardiac arrest was reported

as a fatal event; the patient experienced thrombocytopenia and neutropenia which contributed to cardiac arrest (SmPC, section 4.8).

The most frequently reported Cardiac AE (Vyxeos vs all controls) were tachycardia (15.2% vs 12.7%, respectively), followed by chest pain (7.2% vs 5.9%) and atrial fibrillation (6.9% vs 8.9%) and there were small imbalances in pericardial effusion (5.1% vs 2.5%) and cardiac murmur (4.3% vs 0.8%).

Grade 5 AEs were reported for 2 subjects in Vyxeos and included cardio-respiratory arrest (n = 1) and sudden cardiac death (n = 1).

The most frequently reported Serious Cardiac AEs was ejection fraction decreased (2.4% for Vyxeos vs 3.4% all controls).

The incidence of normal and abnormal ECGs was similar between the Vyxeos group and All Controls.

Although an imbalance of ~10% incidence in Cardiac AEs was observed between Vyxeos and control groups during treatment (50.1% vs 41.9%), the incidence was similar during induction (45.3% vs 40.7%) while it was lower during consolidation for subjects receiving Vyxeos 65 units/m^2 versus All Controls versus Vyxeos 100 unit/m^2 (15.4% vs 21.8% vs 31.7%) driven primarily by a reduced incidence of atrial fibrillation, tachycardia and pericardial effusion.

Only 5 patients (3 in Vyxeos and 2 in all controls) had high prior use of anthracycline ($\geq$ 500 mg/m^2). Within the subgroups with low (n=94) and medium (n=65) prior anthracycline use, subjects in the Vyxeos group experienced a higher incidence of Cardiac AEs compared with control (41.8% vs 25.9%, and 40.4% vs 16.7%, respectively) which was driven by the higher incidence of tachycardia in the Vyxeos (17.9% in low prior use and 21.3% in medium prior use).

In study 301 nearly all subjects (96.7%) were anthracycline naïve and there were no differences between groups having a cardiac AE (49.0% vs. 47.7%, respectively) and most were grade 1 to 2 (grade 3 to 5 reported for 16% vs 18%). The most frequently reported AEs for Vyxeos and 7+3 groups were tachycardia (15% vs 11.3%), atrial fibrillation (6.5% vs 10.6%), chest pain (7.8% vs 9.3%), ejection fraction decreased (5.9% vs 6.6%) and chest discomfort (5.9% vs 4%).

Hypersensitivity

Hypersensitivity reactions were very common ADRs in Vyxeos clinical trials. The most frequently reported hypersensitivity ADRs was rash and the majority of these were not serious (38.9%). The incidence of all hypersensitivity events was 66.9%; the incidence of non-serious events of hypersensitivity events was 66.4 % of which 38.9 % were rash; the incidence of serious events of hypersensitivity is 1.1%; the frequency of hypersensitivity which led to discontinuation is 0. The frequency of fatal hypersensitivity events was 0 (SmPC, section 4.8).

Rash AEs were more common in the Vyxeos than in the All Controls group (58.4% vs 36.9%) during the treatment Period, mainly PT rash (39.2% vs 24.6%), but all AEs were $\leq$ Grade 3, no subjects discontinued due to rash and only 3 subjects (Vyxeos group) had a SAE rash.

The incidence of rash was higher during induction for both Vyxeos and controls groups (56.5% vs 36.4%) and during consolidation the 65 units/m2 dose demonstrated a lower incidence (11.5%) compared with the 100 units/m2 (38.1%) and was similar to the All Control (16.4%).

Serious adverse event / deaths / other significant events

Serious adverse event

More SAEs were reported in the Vyxeos group than the all controls (50.4% vs 38.6%).

Table 34. Serious TEAE in at least 2 % of subjects in any treatment group, by SOC and PT- treatment period (pooled safety population)

System Organ Class Preferred Term	CPX-351 N = 375	Controls		
		7 + 3 N = 192	Salvage N = 44	All Controls N = 236
Any Serious TEAEs[a]	**189 (50.4)**	**72 (37.5)**	**19 (43.2)**	**91 (38.6)**
Blood and lymphatic system disorders	**50 (13.3)**	**13 (6.8)**	**4 (9.1)**	**17 (7.2)**
Febrile neutropenia	38 (10.1)	10 (5.2)	2 (4.5)	12 (5.1)
Neutropenia	2 (0.5)	0	1 (2.3)	1 (0.4)
Pancytopenia	1 (0.3)	0	1 (2.3)	1 (0.4)
Cardiac disorders	**16 (4.3)**	**17 (8.9)**	**2 (4.5)**	**19 (8.1)**
Arrhythmia supraventricular	0	0	1 (2.3)	1 (0.4)
Cardiac failure	2 (0.5)	1 (0.5)	1 (2.3)	2 (0.8)
Sinus bradycardia	0	0	1 (2.3)	1 (0.4)
General disorders and administration site conditions	**21 (5.6)**	**5 (2.6)**	**1 (2.3)**	**6 (2.5)**
Death	0	0	1 (2.3)	1 (0.4)
Pyrexia	11 (2.9)	0	0	0
Infections and infestations	**92 (24.5)**	**30 (15.6)**	**10 (22.7)**	**40 (16.9)**
Aspergillosis	0	0	1 (2.3)	1 (0.4)
Bacteraemia	8 (2.1)	0	1 (2.3)	1 (0.4)
Bacterial sepsis	4 (1.1)	0	2 (4.5)	2 (0.8)
Catheter site infection	1 (0.3)	0	1 (2.3)	1 (0.4)
Cellulitis	6 (1.6)	1 (0.5)	1 (2.3)	2 (0.8)
Enterobacter sepsis	1 (0.3)	0	1 (2.3)	1 (0.4)
Fungal sepsis	0	0	1 (2.3)	1 (0.4)
Klebsiella sepsis	0	1 (0.5)	1 (2.3)	2 (0.8)
Pneumonia	20 (5.3)	6 (3.1)	2 (4.5)	8 (3.4)
Pseudomonas infection	0	0	1 (2.3)	1 (0.4)
Respiratory syncytial virus infection	0	0	1 (2.3)	1 (0.4)
Sepsis	16 (4.3)	6 (3.1)	0	6 (2.5)
Staphylococcal bacteraemia	4 (1.1)	2 (1.0)	2 (4.5)	4 (1.7)
Staphylococcal sepsis	1 (0.3)	0	1 (2.3)	1 (0.4)
Streptococcal bacteraemia	2 (0.5)	0	2 (4.5)	2 (0.8)
Investigations	**15 (4.0)**	**9 (4.7)**	**0**	**9 (3.8)**
Ejection fraction decreased	9 (2.4)	8 (4.2)	0	8 (3.4)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**5 (1.3)**	**2 (1.0)**	**2 (4.5)**	**4 (1.7)**
Acute myeloid leukaemia	1 (0.3)	1 (0.5)	2 (4.5)	3 (1.3)
Nervous system disorders	**16 (4.3)**	**2 (1.0)**	**2 (4.5)**	**4 (1.7)**
Convulsion	2 (0.5)	0	1 (2.3)	1 (0.4)
Syncope	5 (1.3)	0	1 (2.3)	1 (0.4)
Renal and urinary disorders	**6 (1.6)**	**3 (1.6)**	**1 (2.3)**	**4 (1.7)**
Renal failure acute	6 (1.6)	2 (1.0)	1 (2.3)	3 (1.3)
Respiratory, thoracic and mediastinal disorders	**36 (9.6)**	**23 (12.0)**	**0**	**23 (9.7)**
Acute respiratory failure	9 (2.4)	3 (1.6)	0	3 (1.3)
Pulmonary oedema	1 (0.3)	4 (2.1)	0	4 (1.7)
Respiratory failure	11 (2.9)	7 (3.6)	0	7 (3.0)
Skin and subcutaneous tissue disorders	**5 (1.3)**	**0**	**1 (2.3)**	**1 (0.4)**
Blister	0	0	1 (2.3)	1 (0.4)

Abbreviations: AE = adverse event; MedDRA = medical dictionary for regulatory activities; SAE = serious adverse event; TEAE = treatment emergent adverse event.

[a] For SAEs or AEs identified as a bleeding event, a cardiac event, infection or rash, a TEAE is defined as an AE that started after the first dose of induction 1. For all other AEs, a TEAE is defined as an AE that started after the first dose of induction 1 and not more than 30 days after the last dose date.

Note: The 'All Controls' treatment group includes subjects from Study 204, Study 205, and Study 301 who were treated with 7 + 3 and / or Salvage therapy.

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In study 301 more subjects in the Vyxeos group had an SAE than in the 7+3 (59% vs. 43%, respectively) and the most commonly reported SAEs for CPX 351 were febrile neutropenia (7.8%), sepsis (7.8%) and respiratory failure (7.2%).

Table 35 Study 301 Number of Subjects with TEAE by PT in ≥ 3% (Safety Population)

Preferred Term	CPX-351 N = 153 n (%)	7+3 N = 151 n (%)	All Subjects N = 304 n (%)
Any serious adverse event	90 (59)	65 (43)	155 (51)
Febrile Neutropenia	12 (7.8)	8 (5.3)	20 (6.6)
Respiratory Failure	11 (7.2)	8 (5.3)	19 (6.3)
Ejection Fraction Decreased	9 (5.9)	9 (6.0)	18 (5.9)
Sepsis	12 (7.8)	5 (3.3)	17 (5.6)
Pneumonia	10 (6.5)	6 (4.0)	16 (5.3)
Disease Progression	6 (3.9)	6 (4.0)	12 (3.9)
Acute Respiratory Failure	6 (3.9)	3 (2.0)	9 (3.0)

Deaths

During the Study Period (Treatment + Follow-up), fewer deaths were reported with Vyxeos compared with 7 + 3 (59.7% vs.74%, respectively) (Table 37).

Table 36 Deaths by Period (Pooled safety population)

	Induction Period		Consolidation Period[a]			Study Period[a]	
	CPX-351 100 units/m^2 N = 375	7 + 3 N = 192	CPX-351 65 units/m^2 N = 52	CPX-351 100 units/m^2 N = 63	5 + 2 N = 32	CPX-351 N = 375	7 + 3 N = 192
Deaths	29 (7.7)	18 (9.4)	1 (1.9)	8 (12.7)	3 (9.4)	224 (59.7)	142 (74.0)
Deaths due to TEAEs	24 (6.4)	18 (9.4)	1 (1.9)	4 (6.3)	3 (9.4)	70 (18.7)	36 (18.8)
≤ 7 days							
Deaths	0	0	0	0	0	0	0
Deaths due to TEAEs	0	0	0	0	0	0	0
8 to 30 days							
Deaths	14 (3.7)	15 (7.8)	1 (1.9)	2 (3.2)	2 (6.3)	21 (5.6)	19 (9.9)
Deaths due to TEAEs	14 (3.7)	15 (7.8)	1 (1.9)	2 (3.2)	2 (6.3)	21 (5.6)	19 (9.9)
≤ 30 days							
Deaths	14 (3.7)	15 (7.8)	1 (1.9)	2 (3.2)	2 (6.3)	21 (5.6)	19 (9.9)
Deaths due to TEAEs	14 (3.7)	15 (7.8)	1 (1.9)	2 (3.2)	2 (6.3)	21 (5.6)	19 (9.9)
31 to 60 days							
Deaths	9 (2.4)	2 (1.0)	0	0	0	26 (6.9)	19 (9.9)
Deaths due to TEAEs	9 (2.4)	2 (1.0)	0	0	0	23 (6.1)	9 (4.7)
≤ 60 days							
Deaths	23 (6.1)	17 (8.9)	1 (1.9)	2 (3.2)	2 (6.3)	47 (12.5)	38 (19.8)
Deaths due to TEAEs	23 (6.1)	17 (8.9)	1 (1.9)	2 (3.2)	2 (6.3)	44 (11.7)	28 (14.6)
61 to 90 days							
Deaths	1 (0.3)	1 (0.5)	0	2 (3.2)	1 (3.1)	18 (4.8)	12 (6.3)
Deaths due to TEAEs	1 (0.3)	1 (0.5)	0	2 (3.2)	1 (3.1)	12 (3.2)	4 (2.1)
> 90 days							
Deaths	5 (1.3)	0	0	4 (6.3)	0	159 (42.4)	92 (47.9)
Deaths due to TEAEs	0	0	0	0	0	14 (3.7)	4 (2.1)

Abbreviations: TEAE = treatment emergent adverse event.
[a] The Study Period includes the Induction Period, the Consolidation Period, and the Follow-up Period.
Note: The 'All Controls' group included subjects from Study 204, Study 205 and Study 301 treated as follows: during the Induction Period, 'All Controls' included subjects treated with 7 + 3 and Salvage therapy; during the Consolidation Period, 'All Controls' included subjects treated with 5 + 2 and Salvage / Other therapy; during the Treatment Period, 'All Controls' included subjects treated with 7 + 3 and Salvage
Note: The number of days was calculated relative to the first dose of the induction or consolidation period.

In study 301 fewer deaths were reported in the Vyxeos group (69.3%) compared with the 7 + 3 (84.8%).

Table 37. Study 301: Deaths by period (safety analysis population)

Period	CPX-351 N = 153	7 + 3 N = 151
Number of subjects who died	**106 (69.3)**	**128 (84.8)**
≤ 7 days	0	0
8 to 30 days	9 (5.9)	16 (10.6)
31 to 60 days	12 (7.8)	16 (10.6)
≤ 60 days	21 (13.7)	32 (21.2)
Treatment Phase	12 (7.8)	17 (11.3)
Follow-up Phase[a]	93 (60.8)	109 (72.2)

[a] The Follow-up Phase began approximately 30 days after completion of all therapy.
Note: Percentages are based on the number of subjects in each treatment group of the Safety Analysis Population.
Note: Periods start on first day of treatment.
Note: An additional 3 deaths were observed after the end of the Follow-up Phase: 1 in the CPX-351 treatment group and 2 in the 7 + 3 treatment group.

Laboratory findings

Haematology

Treatment with either Vyxeos or 7+3 led to neutropenia and thrombocytopenia within 5 to 7 days of the first induction course. The time to recovery in absolute neutrophils count (ANC) was delayed for a median of 7 days (i.e. recovery longer than 28 days) for subjects receiving Vyxeos compared with 7 + 3. During consolidation treatment, time to ANC recovery was similar for subjects receiving 65 and 100 units/m^2 Vyxeos.

Platelet nadir was observed around day 14 regardless of treatment, but time to recovery is delayed for Vyxeos compared with 7 + 3.

For subjects in Vyxeos arm who received only 1 induction, the median time from the start of induction to recovery from neutropenia (ANC value ≥ 1000/µL) was 37 days (vs. 29 days in control) and recovery from thrombocytopenia (≥ 100,000/µL) was 40 days (vs. 30 days in control). Similarly, the median time to recovery for all subjects in the ITT population was longer in the Vyxeos group than in the control (44 vs. 35 days for ANC and 49 vs. 44 days for platelets, respectively).

Although subjects treated with 2 inductions spend longer periods of time with neutropenia and thrombocytopenia no clinically meaningful differences were observed in the time to recovery from neutropenia and thrombocytopenia compared to those who received one induction.

There were no clinically relevant changes in hemoglobin for Vyxeos compared with control treatment.

In study 301 all subjects had a haematological toxicity of any grade during study and no notable differences were seen between Vyxeos and 7+3. Most subjects had grade 4 event, regardless of the grade they had prior to treatment, mainly neutropaenia (99% each arm) and thrombocytopaenia (98% vs 99%).

Chemistry

No clinically relevant changes in serum creatinine or serum bilirubin were observed. There was no indication of a difference in transaminase elevations during Study 301 for either study treatment.

Serum Copper

Since Vyxeos contains copper encapsulated in the liposome, which acts as a chelating agent to maintain a slow release rate for daunorubicin, subjects were monitored for serum copper concentrations during each of the clinical studies in the Vyxeos program.

In the Pooled Safety Population, upon administration of Vyxeos, a rise in serum copper levels during induction and consolidation periods was evident compared with control but, by the last observation post baseline median levels had begun to return to normal ranges.

Study 206 included specific PK analysis of serum copper following administration of 3 doses of 100 units/m^2 Vyxeos every other day that resulted in increases in serum copper concentrations which returned to baseline levels within 10 to 11 days after the last dose.

Safety in special populations

- *Age*

The overall Summary of TEAE by geriatric status is displayed in Table 39.

Table 38 Overall Summary of TEAE by geriatric status - Study Period (Pooled Safety Population)

	CPX-351		All Controls	
	Age < 65 Years N = 163	Age ≥ 65 Years N = 212	Age < 65 Years N = 93	Age ≥ 65 Years N = 143
Any TEAEs[a]	**163 (100)**	**212 (100)**	**93 (100)**	**143 (100)**
TEAEs by Maximum NCI-CTC Grade[b]	163 (100)	212 (100)	93 (100)	143 (100)
Grade 1	1 (0.6)	1 (0.5)	2 (2.2)	1 (0.7)
Grade 2	11 (6.7)	13 (6.1)	5 (5.4)	14 (9.8)
Grade 3	93 (57.1)	123 (58.0)	63 (67.7)	77 (53.8)
Grade 4	26 (16.0)	37 (17.5)	9 (9.7)	20 (14.0)
Grade 5	32 (19.6)	38 (17.9)	14 (15.1)	31 (21.7)
Grade 3 to 5	151 (92.6)	198 (93.4)	86 (92.5)	128 (89.5)
TEAEs by Closest Relationship[c]				
Not related	6 (3.7)	6 (2.8)	10 (10.8)	6 (4.2)
Related	157 (96.3)	206 (97.2)	83 (89.2)	137 (95.8)
Serious TEAEs	92 (56.4)	126 (59.4)	37 (39.8)	66 (46.2)
TEAEs leading to discontinuation	3 (1.8)	5 (2.4)	1 (1.1)	2 (1.4)
TEAEs leading to death	32 (19.6)	38 (17.9)	14 (15.1)	31 (21.7)

Abbreviations: AE = adverse event; MedDRA = medical dictionary for regulatory activities; NCI-CTC = National Cancer Institute Common Terminology Criteria; SAE = serious adverse event; TEAE = treatment emergent adverse event.

Grade 3 AEs occurred with similar frequency between age groups for most SOC categories. For Vyxeos, a higher incidence was observed in subjects > 65 years for respiratory, thoracic and mediastinal disorders (17.2% of subjects < 65, and 25.0% of subjects > 65), and vascular disorders (9.2% of subjects < 65, and 14.2% of subjects > 65) driven primarily by a higher incidence in ≥ 65 years of atrial fibrillation (0.6% vs 4.2%), hypoxia (5.5% vs 12.7%) and hypertension (4.3% vs 8.0%).

Fewer deaths were reported for subjects treated with Vyxeos compared with All Controls, regardless of age group. Up to 60 days following treatment those < 65 years had comparable death rates between the 2

treatment groups (both had death rate of 12.9%) but subjects ≥ 65 years, the Vyxeos group experienced a reduced death rate compared with All Controls (12.3% vs 23.1%).

No major differences were observed for related AEs and AEs leading to discontinuation by age category.

Table 39 TEAE of Special Interest by Geriatric Status and Sponsor-defined Class – Study Period (Pooled Safety Population)

	CPX-351		All Controls	
	Age < 65 Years N = 163	Age ≥ 65 Years N = 212	Age < 65 Years N = 93	Age ≥ 65 Years N = 143
Infection Events	156 (95.7)	198 (93.4)	87 (93.5)	132 (92.3)
Bacterial Infection Events	147 (90.2)	182 (85.8)	79 (84.9)	124 (86.7)
Viral Infection Events	19 (11.7)	30 (14.2)	10 (10.8)	14 (9.8)
Fungal Infection Events	24 (14.7)	48 (22.6)	14 (15.1)	16 (11.2)
Bleeding Events	96 (58.9)	164 (77.4)	54 (58.1)	86 (60.1)
Cardiac Events	84 (51.5)	107 (50.5)	38 (40.9)	66 (46.2)
Rash Events	108 (66.3)	111 (52.4)	32 (34.4)	56 (39.2)

Abbreviations: MedDRA = medical dictionary for regulatory activities.
Note: The 'All Controls' treatment group includes subjects from Study 204, Study 205, and Study 301 who were treated with 7 + 3 and / or Salvage therapy.
MedDRA version 16.0.

- *Race*

Within the pooled population, related AEs and SAEs analysed by race category showed no major differences.

- *Gender*

In the Vyxeos group, TEAEs leading to death were more frequent for female (23.3%) than male (15.3%) subjects; the opposite was true for the all controls (22.5% males vs 14.3% females). No major differences were observed for related AEs and SAEs.

- *Renal function*

In the pooled safety population Vyxeos group, 181 subjects (48.3%) had normal renal function (CrCL ≥ 90 mL/min), 139 (37.1%) had mild renal impairment (CrCL from 60 to 89 mL/min), 54 (14.4%) had moderate renal impairment (CrCL from 30 to 59 mL/min), and 1 (0.2%) had severe renal impairment (CrCL from 15 to 29 mL/min). Overall there were insufficient numbers of subjects to evaluate possible relationships between renal function category and TEAEs leading to discontinuation or death. The incidence of SAEs was higher for the Vyxeos treatment group than the All Controls treatment group, regardless of renal function category. No notable differences were observed among renal categories in the incidence of any TEAEs Grade 3 to 5 or SAEs for either the Vyxeos or all controls (data not shown).

- *Hepatic impairment*

In the pooled safety population, the majority of subjects who received Vyxeos had bilirubin less than 1.2 mg/dL (332 subjects; 88.5%) and the remainder had bilirubin within the range of 1.2 to 3 mg/dL (42 subjects; 11.2%). There were no patients with end stage disease or bilirubin higher than 3 mg/dL. Consequently, results of this subgroup analysis should be interpreted with caution.

A higher incidence of SAEs was reported for Vyxeos group compared with all controls, regardless of hepatic function category. Few differences in the AE profile were observed for the Vyxeos treatment or when comparing the Vyxeos treatment group with all controls by hepatic function category.

Safety related to drug-drug interactions and other interactions

There were no adverse events attributable to drug-drug interactions during the Vyxeos clinical development program.

Discontinuation due to adverse events

In the Pooled Safety population, less than 3% discontinuations due to TEAEs were reported and all occurred during induction (Table 41).

Table 40 TEAE leading to discontinuation, by SOC and PT-treatment period (pooled safety population)

System Organ Class Preferred Term	CPX-351 N = 375	Controls		
		7 + 3 N = 192	Salvage N = 44	All Controls N = 236
Any TEAEs Leading to Discontinuation	**7 (1.9)**	**3 (1.6)**	**0**	**3 (1.3)**
Blood and lymphatic system disorders	**1 (0.3)**	**0**	**0**	**0**
Thrombocytopenia	1 (0.3)	0	0	0
Cardiac Disorders	**3 (0.8)**	**0**	**0**	**0**
Cardiac failure	1 (0.3)	0	0	0
Cardiomyopathy	1 (0.3)	0	0	0
Pericardial effusion	1 (0.3)	0	0	0
General disorders and administration site conditions	**2 (0.5)**	**0**	**0**	**0**
Mucosal inflammation	1 (0.3)	0	0	0
Pyrexia	1 (0.3)	0	0	0
Infections and infestations	**2 (0.5)**	**0**	**0**	**0**
Pneumonia	1 (0.3)	0	0	0
Staphylococcal bacteraemia	1 (0.3)	0	0	0
Investigations	**1 (0.3)**	**2 (1.0)**	0	2 (0.8)
Alanine aminotransferase increased	1 (0.3)	0	0	0
Aspartate aminotransferase increased	1 (0.3)	0	0	0
Ejection fraction decreased	0	2 (1.0)	0	2 (0.8)
Psychiatric disorders	**0**	**1 (0.5)**	**0**	**1 (0.4)**
Psychotic disorder	0	1 (0.5)	0	1 (0.4)
Renal and urinary disorders	**2 (0.5)**	**0**	**0**	**0**
Renal failure acute	2 (0.5)	0	0	0
Respiratory, thoracic and mediastinal disorders	**1 (0.3)**	**0**	**0**	**0**
Respiratory failure	1 (0.3)	0	0	0

Abbreviations: AE = adverse event; MedDRA = medical dictionary for regulatory affairs; SAE = serious adverse event; TEAE = treatment emergent adverse event.

[a] For SAEs or AEs identified as a bleeding event, a cardiac event, infection or rash, a TEAE is defined as an AE that started after the first dose of induction 1. For all other AEs, a TEAE is defined as an AE that started after the first dose of induction 1 and not more than 30 days after the last dose date.

Note: The 'All Controls' treatment group includes subjects from Study 204, Study 205, and Study 301 who were treated with 7 + 3 and / or Salvage therapy.

MedDRA version 16.0

In study 301 five subjects discontinued the study due to AEs, 3 subjects in the CPX-301 group (2%) due to cardiac failure, cardiomyopathy and acute renal failure (1 subject each), and 2 subjects (1.3%) in the 7 + 3 group due to SAE of ejection fraction decreased.

2.6.1. Discussion on clinical safety

At least 403 subjects received one dose of Vyxeos and included a broad population of AML patients, both newly diagnosed and relapsed, and of an age range of 18 years up to 80 years. The proposed target population and dose regimen is well represented from 153 subjects treated with Vyxeos in the pivotal study 301. The safety database in the proposed target population is considered adequate for assessment.

Patients receiving Vyxeos in the proposed regimen and doses were exposed to a reduced total dose of cytarabine and daunorubicin compared to the standard 7+3 regimen despite having received a longer duration of treatment (study 301 median duration treatment with Vyxeos 62 days versus 41 days with 7+3). This would imply an advantage of Vyxeos therapy, especially with regards to cumulative dose exposure to anthracyclines, and is considered as a clinical benefit.

The safety profile of Vyxeos appears in accordance with that expected from non-clinical data and is similar to the administration of the two components as 7+3.

The incidence of AE was similar between treatment groups except for a moderate higher incidence of serious AE with Vyxeos. However, the reported AE leading to discontinuation was low in all treatment groups (< 2%). It is reassuring to observe that despite the higher exposure and cumulative dose with Vyxeos the reported frequency of AE is not worse than 7+3.

Overall, the AE profile seen with Vyxeos was similar to all controls or 7+3 regimen. The most common AE reported with Vyxeos were febrile neutropenia, nausea, diarrhoea and constipation, and there are no differences across age subgroups.

The most frequently occurring adverse reactions (ADRs) were hypersensitivity including rash, febrile neutropenia, oedema, diarrhoea/colitis, mucositis, fatigue, musculoskeletal pain, abdominal pain, decreased appetite, headache, cough, chills, arrhythmia, pyrexia, sleep disorders, and hypotension (SmPC, section 4.8).

Vyxeos is associated with a more prolonged myelosuppression than 7+3, due to its PK properties of releasing a “full” cargo of daunorubicin and cytarabine to the bone marrow. Subsequently, it is not surprising that the recovery of neutropaenia and thrombocytopaenia takes longer for Vyxeos, around 7-10 days extra. Although bleeding AE are reported with a higher incidence with Vyxeos than 7+3 most events are of low grade. In addition, infections were reported with similar incidence in both arms (around 92%-93%) but the difference between Vyxeos and 7+3 was seen in serious infections (32% vs 21%), especially bacteriaemia. The risk of serious infections appears to be managed with supportive care as it did not translate into a higher discontinuation of study treatment or overall AE related mortality (see section 4.8).

Severe myelosuppression (including fatal infections and haemorrhagic events) has been reported in patients after administration of a therapeutic dose of Vyxeos. Serious or fatal haemorrhagic events, including fatal central nervous system (CNS) haemorrhages, associated with severe thrombocytopenia, have occurred in patients treated with Vyxeos. Baseline assessment of blood counts should be obtained, and patients should be carefully monitored during treatment with Vyxeos for possible clinical complications due to myelosuppression. Due to the long plasma half-life of Vyxeos, time to recovery of ANC and platelets may be prolonged and require additional monitoring (SmPC section 4.4 and 4.8).

Prophylactic anti-infectives may be administered during the period of profound neutropenia until ANC returns to 500/µL or greater. If myelosuppressive complications occur, appropriate supportive measures should be used, e.g., anti-infectives, colony-stimulating factors, transfusions. Blood counts should be regularly monitored until recovery (SmPC section 4.4).

Serious hypersensitivity reactions, including anaphylactic reactions, have been reported with daunorubicin and cytarabine. For moderate hypersensitivity symptoms (e.g., moderate rash, flushing, mild dyspnoea, chest discomfort) the treatment should be stopped. Intravenous diphenhydramine (20-25 mg or equivalent) and intravenous dexamethasone (10 mg) should be given. The infusion should not be restarted. When the patient is retreated, Vyxeos should be given at the same dose and rate and with premedication (see section 4.8).

For severe/life-threatening hypersensitivity symptoms (e.g., hypotension requiring vasopressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalised urticaria), the treatment should be stopped. Intravenous diphenhydramine (20-25 mg) and dexamethasone (10 mg) should be given, and epinephrine (adrenaline) or bronchodilators should be added if indicated. Infusion should not be reinitiated, and no retreat is recommended. Treatment with Vyxeos should be permanently discontinued. Patients should be monitored until symptoms resolve (SmPC sections 4.2 and 4.4). Of note rash, a known AE of cytarabine infusions was also reported at a higher incidence with Vyxeos but none of the events were of Grade 4/5, and it is easily managed with slowing the infusion or pre-medication.

A dedicated study (206) showed no evidence of an effect of Vyxeos on cardiac ventricular repolarization. Whilst in the pooled safety population, that included some patients with prior anthracycline use, there was an increase in cardiac AE in the Vyxeos versus controls (50% vs 42%) the majority were of Grade 1 or 2 and serious AE were lower with Vyxeos (7% vs 11%). Tachycardia was the most common AE reported in all treatment groups. It seems there is no difference for prior anthracycline exposed patients versus anthracycline naïve, although only 5 patients in the data had received high dose of anthracyclines (above 500 mg/m2) and 159 patients had low or medium prior exposure. Nearly all patients were anthracycline naïve in the pivotal study 301 and there were no clinically significant differences observed between Vyxeos and 7+3 with most cardiac events of Grade1-2 and in line with the pooled safety population analysis. Long term cardiotoxicity effects are unknown (see section 4.8).

Cardiotoxicity is a known risk of anthracycline treatment. Prior therapy with anthracyclines (including patients who have previously received the recommended maximum cumulative doses of doxorubicin or daunorubicin hydrochloride), pre-existing cardiac disease (including impaired cardiac function), previous radiotherapy of the mediastinum, or concomitant use of cardiotoxic products may increase the risk of daunorubicin-induced cardiac toxicity. Total cumulative doses of non-liposomal daunorubicin greater than 550 mg/m^2 have been associated with an increased incidence of treatment-induced congestive heart failure. This limit appears lower (400 mg/m^2) in patients who received radiation therapy to the mediastinum. The relationship between cumulative Vyxeos dose and the risk of cardiac toxicity has not been determined (SmPC section 4.4).

A baseline cardiac evaluation with an electrocardiogram (ECG) and a multi-gated radionuclide angiography (MUGA) scan or an echocardiography (ECHO) is recommended, especially in patients with risk factors for increased cardiac toxicity. Cardiac function should be closely monitored.Treatment with Vyxeos should be discontinued in patients with impaired cardiac function unless the benefit of initiating or continuing treatment outweighs the risk (SmPC section 4.4).

A comparison of Vyxeos with 7+3 in both, pooled analysis population or pivotal study 301 showed there were fewer deaths reported for the experimental treatment across the treatment and follow up phases. The primary cause of death during the first 30 or 60 days of the study 301 was progressive leukaemia and AE. By the end of study 301 similar proportions of patients died due to AE (~ 20%); although more grade 5 infections occurred with Vyxeos vs 7+3 (10 subjects/6.5% vs 4 subjects/2.6%) most of these deaths were not considered related to study treatment.

The incidence of serious AE was higher for Vyxeos than 7+3 in pooled analysis population and study 301 (59% vs 43%), and the most common AE reported in study 301 were febrile neutropenia and sepsis due to the prolonged myelosuppression. The most serious and frequently occurring ADRs were infection, haemorrhage, and cardiotoxicity (SmPC, section 4.8).

Daunorubicin has been associated with local tissue necrosis at the site of medicinal product extravasation. In clinical studies with Vyxeos, one event of extravasation occurred, but no necrosis was observed. Care should be taken to ensure that there is no extravasation of medicinal product when Vyxeos is administered. Vyxeos should be administered intravenously only. Vyxeos should not be administered via an intramuscular, intrathecal, or subcutaneous route (SmPC, sections 4.2 and 4.4).

There is no evidence of clinically significant abnormal serum copper levels due to the copper encapsulated in the liposome. In clinical trials the copper levels remained below 530 microg/mL (4 times above the ULN). Each vial contains 100 mg of copper gluconate, which corresponds to 14 mg of elemental copper. Vyxeos should only be used in patients with a history of Wilson's disease or other copper-related disorder if the benefits outweigh the risks (SmPC section 4.4).

Administration of live or live-attenuated vaccines in patients that are immunocompromised by chemotherapeutic agents may result in serious or fatal infections. Vaccination with a live vaccine should be avoided in patients receiving Vyxeos. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished (SmPC section 4.4).

It should be taken into consideration that the absorption of oral accompanying medicinal products may be considerably influenced by gastrointestinal mucositis and/or diarrhoea frequently occurring in association with intensive chemotherapy (SmPC section 4.4).

No relevant changes were noted for chemistry laboratory investigations or urine analysis. Vyxeos treatment did not affect s-Crea or s-Bil implying no harmful renal or hepatic effects. There was no indication of a difference in transaminase elevations during Study 301 for either study treatment.

Vyxeos may induce hyperuricemia secondary to rapid lysis of leukaemic cells. Blood uric acid levels should be monitored and appropriate therapy initiated in the event that hyperuricemia develops (SmPC section 4.4).

Hepatic or renal impairment may increase the risk of toxicity associated with daunorubicin and cytarabine. Evaluation of hepatic and renal function using conventional clinical laboratory tests is recommended prior to administration of Vyxeos and periodically during treatment. There is no experience with Vyxeos in patients with baseline serum bilirubin greater than 50 µmol/L, severe renal impairment (creatinine clearance less than 30 mL/min), or end stage renal disease. Vyxeos should only be used in patients with a severe hepatic and/or renal impairment if the benefits outweigh the risks (SmPC, section 4.4).

Although there was a higher incidence of bleeding events in patients above 65 years, the overall safety profile of Vyxeos is considered similar across age subgroups (< or > 65 years) which is important given the intensity of the proposed chemotherapy regimen.

There were no adverse events attributable to drug-drug interactions during the Vyxeos clinical development program.

Throughout all analyses the consolidation dose of 65 mg/m^2 showed an improved safety profile compared to 100 mg/m^2 providing a strong justification for the proposed consolidation dosage.

For the purpose of this application in AML patients with poor survival prognosis, the lack of long term safety data is not a concern.

Vyxeos must not be substituted or interchanged with other daunorubicin and/or cytarabine containing products. Due to substantial differences in the pharmacokinetic parameters, the dose and schedule recommendations for Vyxeos are different from those for daunorubicin hydrochloride injection, cytarabine injection, daunorubicin citrate liposome injection, and cytarabine liposome injection. The medicinal product name and dose should be verified prior to administration to avoid dosing errors. (SmPC section 4.4).

Women of childbearing potential should avoid becoming pregnant while receiving Vyxeos. Women of childbearing potential should use effective contraception while they or their male partner undergo treatment. Women of childbearing potential should not receive treatment until pregnancy is excluded. Women of childbearing potential should undergo pregnancy testing before initiation of Vyxeos. Men with sexual partners of reproductive potential and women should use effective contraception during treatment and for 6 months following the last dose of Vyxeos (SmPC section 4.6).

There are no data on the use of Vyxeos in pregnant women. Based on results from animal studies and its mechanism of action, Vyxeos should not be used during pregnancy, unless the clinical condition of the woman requires treatment and justifies the potential risk to the foetus (SmPC section 4.6).

If the medicinal product is used during pregnancy, or if the patient becomes pregnant while receiving Vyxeos, the woman should be informed of the potential hazard to the foetus. In any case, cardiologic examination and a blood count are recommended in foetuses and newborns born to mothers who received treatment during pregnancy SmPC section 4.6).

It is not known whether Vyxeos is excreted in human milk. Because many medicinal products are excreted in human milk and because of the potential for serious adverse reactions in nursingbreast feeding infants from Vyxeos, mothers should be advised to discontinue nursingbreast feeding during Vyxeos therapy (SmPC section 4.6).

Vyxeos has minor influence on the ability to drive and use machines. Fatigue and dizziness have been reported with the use of Vyxeos. Therefore, caution is recommended when driving or operating machines (SmPC section 4.7).

If overdose occurs, exacerbation of adverse reactions associated with Vyxeos is expected and supportive treatment (incuding anti-infectives, blood and platelet transfusions, colony-stimulating factors, and intensive care as needed) should be provided until the patient recovers. Patients should be observed carefully over time for signs of cardiotoxicity and provide appropriate supportive therapy as clinically indicated (SmPC section 4.9).

It is contraindicated in patients with history of serious hypersensitivity to the active substances or to any of the excipients listed in section 6.1

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

Overall, the safety profile of Vyxeos is similar to standard 7+3 and is considered acceptable. The most common AEs reported with Vyxeos are febrile neutropenia, nausea, diarrhoea and constipation. Vyxeos produces a prolonged myelosuppression and a higher incidence of low grade bleeding events and serious infections that can be managed with appropriate supportive care.

2.7. Risk Management Plan

Safety concerns

No important safety concerns were identified. As this drug combination (cytarabine and daunorubicin) has been in use for several decades the risks are well known, and they are also clearly presented in the PI. No critical difference between Vyxeos and the therapy with conventional daunorubicin/cytarabine combination is expected in this respect.

Pharmacovigilance plan

N/A

Risk minimisation measures

No additional risk minimisation measures are applicable.

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.2 is acceptable.

2.8. 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.

Periodic Safety Update Reports submission requirements

Based on different PUSR frequencies for daunorubicin and cytarabine, the CHMP is of the opinion that a separate entry in the EURD list for Vyxeos (cytarabine:daunorubicin) liposome for injection is needed, as it cannot follow the already existing entry for daunorubicin / cytarabine. The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request the alignment of the new PSUR cycle with the international birth date (IBD). The IBD is 3.08.2017. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. 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

3.1. Therapeutic Context

3.1.1. Disease or condition

Vyxeos (daunorubicin and cytarabine) is proposed for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukaemia (t-AML) or AMLwith myelodysplasia-related changes (AML-MRC).

3.1.2. Available therapies and unmet medical need

In the EU (European Union), approved agents include decitabine (Dacogen) which is authorised for the treatment of adult patients with newly diagnosed de novo or secondary AML, according to the WHO classification, who are not candidates for standard induction chemotherapy. Azacitidine (Vidaza) is also authorised for the treatment of adult patients who are not eligible for HSCT with AML with 20-30 % blasts and multi-lineage dysplasia, according to WHO classification and AML with >30% marrow blasts according to the WHO classification. In addition, histamine dihydrochloride (Ceplene) is authorised for adult patients with AML in first remission concomitantly treated with interleukin-2 (IL-2). Midostaurin (Rydapt) is authorised in combination with standard daunorubicin and cytarabine induction and high dose cytarabine consolidation chemotherapy followed by midostaurin single agent maintenance therapy for adult patients with newly diagnosed AML who are FLT3 mutation positive. Finally, Mylotarg is authorised in combination therapy with daunorubicin (DNR) and cytarabine (AraC) for the treatment of patients age 15 years and above with previously untreated, de novo CD33 positive AML, except acute promyelocytic leukaemia (APL).

AML has a poor prognosis with survival of 37.9% after one year and 17.1% after 5 years from diagnosis that decreases steeply with age, from 49.6% (patients aged 15-44 years) to 3.8% (patients aged ≥ 75 years). Amongst all AML types, high-risk AML as defined by therapy-related AML or AML with myelodysplasia-related changes carries a very poor prognosis and represents an unmet medical need.

3.1.3. Main clinical studies

The main clinical study was study CLTR0310-301 a phase III, multicenter, randomised, trial of Vyxeos (cytarabine: daunorubicin) liposome injection versus cytarabine and daunorubicin in patients 60 -75 years of age with untreated high risk (secondary) AML study.

3.2. Favourable effects

- Improved median OS by 3.6 months (ITT population median OS 9.56 months CPX 351 versus 5.95 months 7+3; HR 0.69, 95% CI: 0.52 to 0.90; 2-sided p = 0.005).
- Improved median EFS by 1.2 months (ITT population: median EFS 2.53 months CPX 351 versus 2.31 months7+3; HR 0.74, 95% CI: 0.58 to 0.96; 2-sided p = 0.021).
- Improved MLFS (ITT population: 69% Vyxeos vs. 55.5%7+3; OR 1.78, 95% CI: 1.05 to 3.03, 1-sided p = 0.017)
- Improved rate of HSCT (ITT population: 34% Vyxeos vs. 25% 7+3; OR 1.54, 95% CI: 0.92 to 2.56; 2-sided p = 0.097).

• Easier dosing schedule with Vyxeos of three (or two) 90-minutes infusions every other day compared to 7-days (or 5-days) continuous infusion of cytarabine along with subsequent administration of daunorubicin at induction. Patients can receive Vyxeos consolidation in the out-patient setting.

3.3. Uncertainties and limitations about favourable effects

There are no important uncertainties or limitations about the favourable effects.

3.4. Unfavourable effects

Due to the neutropenia experienced with Vyxeos, infections of various types were very common ADRs. Pneumonia, sepsis and bacteriaemia were the most frequently seen serious infection ADRs in the clinical studies population. The incidence of infection events was 78.1%; the incidence of non-serious events of infections was 73.1%, the incidence of serious events of infections was 28.5%; the incidence of infections which led to discontinuation was 0.5%.

A variety of haemorrhagic events were seen in clinical studies. The most common haemorrhagic event was epistaxis, and the majority of these were considered not serious (29.1%). The incidence of haemorrhage events was 69.1%; the incidence of non-serious events of haemorrhage was 67.2 %; the incidence of serious events of haemorrhage was 5.6%; the incidence of haemorrhage which led to discontinuation was 0. The incidence of fatal haemorrhage was 2.1%.

Hypersensitivity reactions were very common ADRs in Vyxeos clinical studies. The most frequently reported hypersensitivity ADRs was rash and the majority of these were not serious (38.9%). The incidence of all hypersensitivity events was 66.9%; the incidence of non-serious events of hypersensitivity was 66.4 %, of which 38.9 % were rash; the incidence of serious events of hypersensitivity was 1.1%. There were no hypersensitivity events which led to discontinuation and no fatal hypersensitivity events were recorded (see section 4.4).

3.5. Uncertainties and limitations about unfavourable effects

There are no important uncertainties and limitations about the unfavourable effects.

3.6. Effects Table

Table 41 Effects Table for Vyxeos for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukaemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC)-study CLTR0310-301(data cut-off: 31 December 2015).

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
Overall survival (OS)	Time form randomization to death from any cause	Months	9.56 (6.60- 11.86)	5.95 (4.99-7.75)	HR 0.69(0.52-0.90), 2-sided p=0.005	
Event free survival	Time from randomizatio	Months	2.53 (2.07-4.99)	1.31 (1.08-1.64)	HR 0.74 (0.58-0.96),	

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
(EFS)	n to the date of induction treatment failure, relapse from CR or CRi or death from any cause, whichever came first.				2-sided p=0.021	
Unfavourable Effects (1)						
Infection	Grade 3-5	%	58.7	50.5		
Haemorrhage	Grade 3-5	%	13.1	6.8		
Rash	Grade 3-5	%	4	0.5		
Febrile Neutropenia	Grade ≥ 3	%	62.1	65.1		
Pneumonia	Grade ≥ 3	%	16	14.1		
Hypoxia	Grade ≥ 3	%	9.6	12.5		

Notes: (1) The frequencies of the unfavourable effects were derived from the frequency of all adverse events in the pooled safety population.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Vyxeos has demonstrated a clinically significant improvement in overall survival compared to the standard of care 7+3 in the proposed population of patients with newly diagnosed AML with MRC and therapy related AML. This is remarkable given the very poor prognosis of these patients and their unmet medical need. Secondary endpoints support the primary outcome, in particular an increased rate of HSCT which is potentially the only curative treatment in AML.

The safety profile appears similar to the known profile of 7+3 except for a more prolonged myelosuppression due to the liposomal formulation and an increase in serious AE, in particular infections. These appear to be managed with appropriate supportive care as the rate of discontinuations is low. There is no evidence of increased cardiac toxicity and in fact, despite longer exposure to treatment the cumulative doses of daunorubicin are lower than with 7+3.

3.7.2. Balance of benefits and risks

In view of the effect in terms of OS and the known ADRs for such a combination (cytarabine and daunorubicin), the benefit-risk balance in the proposed indication is considered positive.

3.7.3. Additional considerations on the benefit-risk balance

The pivotal study showed no clinically relevant differences in outcome across age subgroups (60-69 years/70-75 years) although it excluded patients below 60 years of age. Vyxeos is an optimised liposomal formulation of cytarabine and daunorubicin which is used as standard therapy in patients of all ages. Most patients that receive the standard treatment need to be fit for intensive chemotherapy, and therefore, whether the patient's performance status and their suitability to receive the treatment is based on clinical judgement and the physician's decision. In some younger patients with newly diagnosed AML, other treatments, like 7+3 plus a third agent, are sometimes used. However, for patients below 60 years scheduled to receive the standard 7+3, Vyxeos seems an optimised formulation which is very likely to be superior to 7+3 given the results in > 60 years from the pivotal study. It appears there are no differences in disease biology for t-AML or AML-MRC between adult ages subgroups. Prognosis is very poor, and these subtypes of AML represent an unmet medical need irrespective of age. The exposure response analysis conducted in adult patients, included few patients below age of 60 and it indicated the proposed dose is the most appropriate. PK data did not show variability with regards to age covariate and results from studies 101 and 206 showed that free fractions of daunorubicin and cytarabine in patients below 60 years are similar to those above 60 years. It is debated in the literature whether a higher cytarabine dose should be administered to younger patients with the aim to improve efficacy. However, there are discrepant results across published studies which have led to different dosages used across institutions. As the Vyxeos liposomal formulation delivers a higher "cargo" of daunorubicin and cytarabine to the bone marrow the dose of cytarabine seems acceptable for younger adults. Overall, extrapolation of data from the pivotal study can reasonably be made to adults below the age of 60 years.

3.8. Conclusions

The overall B/R of Vyxeos is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Vyxeos is not similar to Vidaza, Ceplene, Dacogen, Rydapt and Mylotarg, 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, the CHMP considers by consensus that the risk-benefit balance of Vyxeos is favourable in the following indication:

Vyxeos is indicated for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukaemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC).

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

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product

Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

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

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

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

Risk Management Plan (RMP)

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

An updated RMP should be submitted:

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

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

Not applicable.

Appendix

1. CHMP AR on similarity dated 31 May 2018

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