16 February 2012
EMA/309145/2012
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Pixuvri

International non-proprietary name: **pixantrone**

Procedure No. **EMEA/H/C/002055**

Note

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

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Product information

Name of the medicinal product:	Pixuvri
Applicant:	CTI Life Sciences Ltd. BioPark Broadwater Road Welwyn Garden City, Herts AL73AX United Kingdom
Active substance:	pixantrone dimaleate
International Nonproprietary Name/Common Name:	pixantrone
Pharmaco-therapeutic group (ATC Code):	Anthracyclines and related substances (L01DB11)
Therapeutic indication:	Pixuvri is indicated as monotherapy for the treatment of adult patients with multiply relapsed or refractory aggressive Non Hodgkin B cell Lymphomas (NHL). The benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy.
Pharmaceutical forms:	Powder for concentrate for solution for infusion
Strength:	29 mg
Route of administration:	Intravenous use
Packaging:	vial (glass)
Package size:	1 vial

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List of abbreviations

AE	Adverse event
ALCL	Anaplastic large cell lymphoma
ANC	Absolute neutrophil count
ARA-c	Cytarabine
BBR 2778	Pixantrone
BSA	Body surface area
CHF	Congestive heart failure
CHOP	Cyclophosphamide, doxorubicin, vincristine and prednisone
CR	Complete response
CRA	Clinical research associate
CRO	Contract research organisation
CRu	Complete response unconfirmed
DLBCL	Diffuse large B-cell lymphoma
DLT	Dose limiting toxicity
DSC	differential scanning calorimetry
DVS	dynamic vapour sorption
EAE	experimental autoimmune encephalomyelitis
EOS	End of Study
EOT	End of Treatment
ESI-MS	electrospray ionization mass spectrometry
HDT	High Dose Therapy
HITT	Histologically-confirmed intent-to-treat
HPLC	high pressure liquid chromatography
IDMC	Independent Data Monitoring Committee
IAP	Independent Assessment Panel
ICH	International Conference on Harmonisation
INN	International Nonproprietary Name
IPI	International Prognostic Index
IR	infrared
IRC	Independent Radiology Committee
IWG	International Working Group
LDPE	low density polyethylene
LVEF	Left ventricular ejection fraction
Mol.Wt.	molecular weight
MTD	Maximum tolerated dose
MUGA	Multiple gated acquisition scan
NA	North America Region
NAT	N-acetyltransferases
NHL	Non-Hodgkin Lymphomas
NMR	nuclear magnetic resonance
Ph.Eur.	European Pharmacopoeia

RH	relative humidity
ROW	Rest of World
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCT	Stem cell transplant
SmPC	Summary of product characteristics
TCFU	Tumour colony forming units
TGA	thermal gravimetric analysis
UV	ultraviolet
WE	Western Europe region
XRPD	x-ray powder diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant CTI Life Sciences Ltd. submitted on 28 October 2010 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Pixuvri, through the centralised procedure falling within the Article 3(1) and point 3 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 27 July 2009.

The applicant applied for the indication in the treatment of adult patients with multiply relapsed or refractory aggressive non-Hodgkin lymphomas (NHL).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

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

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/242/2010 on the agreement of a paediatric investigation plan (PIP) including a waiver and a deferral.

At the time of submission of the application, the PIP 000713-PIP02-10 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

The application contained a critical report pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, addressing the possible similarity with authorised orphan medicinal products.

Derogation(s) of market exclusivity

Not applicable.

Conditional marketing authorisation

In accordance with Article 3 (1) of Regulation EC No 507/2006, the applicant requested the application to be considered for a Conditional Marketing Authorisation based on the following claim(s):

- The risk-benefit balance of the medicinal product, as defined in Article 1(28a) of Directive 2001/83/EC, is positive.

Based on the randomized controlled study presented in patients with multiply relapsed or refractory aggressive NHL (study PIX 301), the superiority of pixuvri was demonstrated compared to single chemotherapy agent with an increase in the Cr/CRu (20% versus 5.7%; p=0.02), an increase in median PFS (HR=0.60, 95% CI, 0.42 to 0.86, p=0.005) and a superior overall survival (median 10.2

months versus 7.6 months; HR 0.79, 95% CI 0.53, 1.18, p=0.25) The benefit risk balance of pixuvri in patients with multiply relapsed or refractory aggressive NHL is therefore considered to be positive.

From a quantitative point, of view, the benefit in the subgroup of patients previously treated with rituximab might be less as compared with what was observed in patients that had not received prior rituximab treatment. However, the efficacy of Pixuvri in patients that had received prior rituximab therapy and up to 3 prior regimens was still superior to the comparator. In Europe most patients that had multiple relapse or are refractory to treatments are expected to have received prior rituximab. Therefore there is a need to further confirm the efficacy of Pixuvri in patients previously treated with rituximab.

- It is likely that the applicant will be in a position to provide comprehensive clinical data.

The applicant claimed that it is likely to be in a position to provide the comprehensive clinical data from Phase III study PIX 306 where pixantrone in combination with rituximab is compared with gemcitabine in combination with rituximab. The study patient population includes patients with the NHL type of Diffuse Large B cell Lymphoma or Follicular grade III lymphoma who had previously been treated with at least one rituximab containing multiagent regimen. This study will support the efficacy of pixuvri in patients that had received prior rituximab of the phase III Study PIX 301. The results from study PIX 306 are expected to be available by 30 June 2015.

- Unmet medical needs to be fulfilled.

The applicant claims that there is a lack of approved and standard of care pharmacological treatment for patients with multiply relapsed or refractory aggressive NHL and that there is a need in this patient population that could be fulfilled with the proposed medicinal product.

- The benefits to public health of the immediate availability on the market of the medicinal product concerned outweighs the risk inherent in the fact that additional data are still required.

The applicant claimed that the potential risks inherent in marketing pixuvri for the specific indication, while additional, more comprehensive data will be available in the future, would be offset by the potential benefit to the patients. The RMP for pixuvri in the approved indication is considered as adequate to address any identified and unknown risks.

New active Substance status

The applicant requested the active substance pixantrone dimaleate contained in the above medicinal product to be considered as a new active substance in itself.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 18 October 2002. The Scientific Advice pertained to clinical aspects of the dossier.

Licensing status

A new application was filed in the following countries: United States of America.

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

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

Rapporteur: **Ian Hudson** Co-Rapporteur: **Kristina Dunder**

- The application was received by the EMA on 28 October 2010.
- The procedure started on 17 November 2010.
- The CHMP adopted a report on similarity of Pixuvri with Torisel on 20 January 2011.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 7 February 2011. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 4 February 2011.
- During the meeting on 14-17 March 2011, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 17 March 2011.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 26 August 2011.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 28 October 2011.
- During the CHMP meeting on 17/11/2011, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 16/12/2011.
- During the CHMP meeting on 19/01/2012, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on16/02/2012, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a conditional Marketing Authorisation to Pixuvri.

2. Scientific discussion

2.1. Introduction

Problem statement

In 2008, 73,667 men and women in the European Union were diagnosed with non-Hodgkin lymphoma (NHL), and more than 31,000 died of the disease. It occurs mainly in white populations in parts of Western Europe, North America and Australia. Diffuse large B cell lymphoma (DLBCL) is the most common aggressive NHL, constituting approximately ≥30% of all NHL. The crude incidence in the EU is 5-6/100,000/year, increasing with age from 0.3/100,000/year in 35-39 year-olds to 26.6/100,000/year in the 80 to 84 age group. Overall, > 30% of DLBCL patients will ultimately relapse. The incidence of relapsed NHL in the EU is therefore estimated to be around 1/100,000/year.

Aggressive NHLs are potentially curable malignant disorders. Anthracyclines are one of the most active drug classes in Non-Hodgkin Lymphomas (NHL), but the likelihood of cardiotoxicity rises as the cumulative dose increases.

The established first-line chemotherapy regimens typically include cyclophosphamide, anthracycline (doxorubicin), vincristine and corticosteroids (CHOP). The addition of rituximab has further improved response rates and survival in certain lymphoma entities (Coiffier et al.2002, N Eng J Med 346; Pfreundschuh et al. 2006, Lancet Oncology 7). Nevertheless, about 20 to 50% of patients either fail to respond (primary refractory disease) to front-line treatment or have relapsing disease.

In DLCBL suitable patients with adequate performance status (no major organ dysfunction, age < 65 to 70 years) a salvage regimen followed in responsive patients by high-dose treatment with stem-cell support is recommended. Patients not suitable for high-dose therapy may be treated with similar or other salvage regimens which may be combined with involved-field radiotherapy, but no salvage chemotherapy for aggressive lymphoma is considered standard. Rituximab alone induced responses in 30-35% of patients with relapsed or primary refractory DLBCL; but the CR rate was only 9%. The majority of regimens utilized beyond the front-line treatment setting do not incorporate an anthracycline or anthracenedione because of the risk for cardiac toxicity associated with an increasing cumulative lifetime anthracycline dose: by the time of first relapse, most patients have received 300 to 400 mg/m^2 of doxorubicin-equivalent cumulative dose, and thus are already near the recommended lifetime limit of 450-550 mg/m^2. The European Society of Medical Oncology makes no recommendations for therapy in patients who relapse after, or fail to respond to, second line therapy. The currently used conventional-dose programs for relapsed NHL are associated with a very low rate of response in patients with primary refractory disease, and there is no standard therapy for these patients.

In conclusion, there is no consensus regarding the best regimen for aggressive NHL beyond first relapse in patients not eligible for stem cell transplant or in disease refractory to second-line therapy, and no single agent or regimen is approved or considered standard of care in this setting.

About the product

Pixantrone is an aza-anthracenedione compound related to anthracyclines and anthracenediones such as doxorubicin and mitoxantrone, classes of drugs whose antineoplastic activity is linked to inhibition of topoisomerase II and DNA intercalation.

The proposed indication of Pixuvri is the treatment as monotherapy of adult patients with multiply relapsed or refractory aggressive NHL.

The approved indication of Pixuvri is the treatment in monotherapy of adult patients with multiply relapsed or refractory aggressive Non Hodgkin B cell Lymphomas (NHL). The benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy.

Pixuvri is supplied in vials containing 29 mg pixantrone (as dimaleate) powder for concentrate for solution for infusion. It is reconstituted with 5 ml of sodium chloride and further diluted in 250ml-500 ml sodium chloride. It is administered at 50 mg/m^2 by slow intravenous (IV) infusion over a minimum of 1 hour on Days 1, 8, and 15 of 28-day cycles for up to 6 cycles.

The development programme/Compliance with CHMP Guidance/Scientific Advice

The Applicant received EMA Scientific Advice (EMEA/CPMP/5115/02) in October 2002.

The CHMP scientific advice included the design of the AZA III-01 protocol, at that time planned as a phase III protocol proposed to support the Aggressive NHL indication. It was proposed as an open-label, randomized, Phase III comparative trial in which pixantrone substituted etoposide in the Etoposide, Methylprednisolone, Cytarabine and Cisplatin (ESHAP) chemotherapy regimen. Response rate was not well received as an endpoint and not considered a validated surrogate for the proposed design at the time. Alternate designs were suggested by the CHMP such as using a very clearly defined autologous stem cell setting, in particular similar high dose chemotherapy regimen (e.g. 'BEAM' procedure) and patient management for all patients following transplantation, and define time to progression as (co-)primary endpoint. Another suggestion was to perform a comparative study in parallel in another population of patients not eligible for ABMT/PBSCT, where duration of remission and time to progression could be observed and be related to safety issues. The CHMP emphasized the importance of follow-up data including overall survival and the occurrence of second malignancies, such as AML.

Based on the recommendations received, the Applicant modified the design of its Phase III clinical trial (PIX301 study). The latter was a randomized, active-control, multicenter, open-label study comparing single-agent treatment with pixantrone to other pre-specified single-agents (based on the physician's choice) in patients with relapsed or refractory aggressive NHL who had received two or more lines of therapy.

The main modifications were with regard to:

- The choice of the primary endpoints (CR/CRu)
- Trial performed in a non transplant setting
- Stratification (addressing national scientific advice concern)

The completed clinical development includes seven single-agent and five multiagent combination studies which have treated a total of 348 patients with pixantrone; 80% of these patients had NHL. This application is supported by a single pivotal trial (PIX-301).

The applicant also initiated a supportive phase II study (PIX-203) in patients with DLCBL comparing the response of CHOP-R (Cyclophosphamide, **D**oxorubicin, Vincristine, Prednisone plus Rituximab) versus CPOP-R (Cyclophosphamide, **P**ixantrone, Vincristine, Prednisone plus Rituximab). Enrolment was terminated in 2008 and the study report was submitted as part of the day 120 responses.

A paediatric investigation plan has been agreed for pixantrone in combination therapy for the treatment of NHL in paediatric patients aged 6 months to <18 years. A waiver has been granted for infants < 6 months. Deferral of initiation of the paediatric clinical studies until a positive risk-benefit balance in adults is confirmed by the CHMP was also granted.

Pixantrone has not been approved in any country.

2.2. Quality aspects

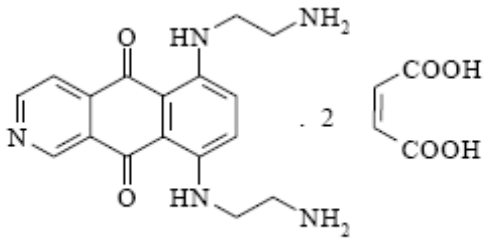
2.2.1. Introduction

Pixuvri is presented as a dark blue sterile powder for concentrate for solution for infusion containing 29 mg of the active substance pixantrone (as dimaleate) in a 20 ml glass vial. Other ingredients are defined in section 6.1 of the SmPC. Reconstitution with 5 ml 9 mg/ml sodium chloride solution for

injection yields an opaque dark blue solution containing 5.8 mg/ml pixantrone. The formulation contains no preservatives and is intended for single use.

2.2.2. Active Substance

Pixuvri contains as active substance 50 mg pixantrone dimaleate equivalent to 29mg pixantrone free base. The International Nonproprietary Name (INN) is pixantrone. The chemical name is 6, 9-bis [(2-aminoethyl) amino] benzo[g]isoquinoline-5, 10-dione dimaleate salt. The structural formula of the dimaleate is:



The molecular formula is $C_{17}H_{19}N_5O_2.2C_4H_4O_4$. The relative Mol.Wt. is 557.5. Pixantrone appears as a dark blue powder and is slightly soluble in water and propylene glycol. It is very slightly soluble in ethanol, but soluble in 0.9% NaCl and acetate buffer pH 5.2. Pixantrone dimaleate is achiral and not hygroscopic. Polymorphism has not been observed for pixantrone dimaleate.

Manufacture

At the time of the CHMP opinion, the active substance used for Pixuvri is supplied by one active substance manufacturer. Because no Ph.Eur. certificate of suitability has been issued for the active substance manufactured by the proposed supplier, detailed information about the manufacturing process, control of starting materials, reagents and solvents, control of critical steps and intermediates, process development and process validation of the active substance has been supplied by the applicant. The manufacturing process consists of three steps. All manufacturing steps are adequately described. Adequate in process controls are in place and appropriate specifications have been adopted for the starting materials, solvents and reagents. All relevant impurities, degradation products and residual solvents have been appropriately characterized. The applicant confirmed the structure of pixantrone dimaleate by UV and IR spectroscopy, NMR, ESI-MS and elemental analysis. The physico-chemical properties where characterised by solubility studies, DSC analysis, TGA analysis and dynamic vapour sorption (DVS) analysis. The absence of polymorphs was confirmed with XRPD spectra.

Specification

Pixantrone dimaleate is not described in the European Pharmacopoeia. The active substance is tested as per in-house specifications and include tests as: appearance, identification (IR), assay and related substances (HPLC), maleic acid content and identification, residual 1,4-difluorobenzene, residual fluoride content, residue on ignition (Ph.Eur.), heavy metals (Ph.Eur.), residual solvents, water content (Ph.Eur.), pH of a 0.4% w/v solution (Ph.Eur.) and bacterial endotoxins (Ph.Eur.).The specifications and tests proposed by the applicant comply with the relevant ICH guidelines and general requirements

of Ph.Eur. The specifications are adequate to control the quality of the active substance. The impurity limits are acceptable and there is no concern from the point of view of safety.

Batch analysis data have been provided on four batches. All batches were in compliance with the predefined active substance specifications and confirm consistency and uniformity of the active substance manufacture.

Stability

Pixantrone powder is stored in amber glass bottles with low density polyethylene (LDPE) stoppers with a polypropylene screw cap. The packaging complies with the Ph.Eur. requirements and the stoppers are compliant with EU directive (2002/72/EC) requirements for plastic materials in contact with food products and medicinal packaging. Specifications, testing methods, a technical drawing and certificates of analysis have been provided for the packaging.

Stability studies on the active substance have been performed at long term (25±2°C/60±5% RH) and accelerated (40±2°C/75±5% RH) conditions on four batches as per ICH Guidelines. Up to 18 months of long term stability data, and up to 6 months of accelerated stability data has been provided, confirming the stability of the active substance. The specifications tested were appearance, assay, related substances, maleic acid content, water content and bacterial endotoxins. The analytical methods used are the same as those used for the specifications. The HPLC method used for assay and related substances demonstrated to be stability indicating. The packaging used in stability trials is identical to that proposed for storage and distribution.

In conclusion, the stability data provided, support the proposed retest period at the proposed packaging and storage conditions.

2.2.3. Finished Medicinal Product

Pharmaceutical Development

Development studies demonstrated that an aqueous solution of pixantrone did not exhibit adequate stability. Hence, a lyophilized formulation was developed, which needs to be reconstituted and diluted in an infusion bag prior to administration to the patient.

Screening studies identified the excipients of choice, and the excipient concentrations have been carefully optimized in view of their function. Compatibility studies showed that pixantrone dimaleate is compatible with all excipients used in the final formulation. The selected formulation results in a lyophilized cake with acceptable appearance and reconstitution behaviour.

Pixuvri should be reconstituted with 5 ml of sodium chloride 9 mg/ml (0.9%) solution for injection and subsequently diluted in an infusion bag containing 0.9%l NaCl solution for injection. Upon reconstitution with normal saline, an opaque dark blue solution is obtained. Adequate data have been provided to demonstrate that the lyophilised powder is completely dissolved in 60 seconds with agitation.

Furthermore, data have been provided to demonstrate 24 hour compatibility at uncontrolled room temperature of Pixuvri diluted at a concentration of 0.3 mg/ml in normal saline (0.9% NaCl for injection) in commercially available infusion bags made of polyethylene or polyvinylchloride. The

diluted medicinal product was also demonstrated to be compatible with commercially available infusion set in-line filters made of polyethersulfone, cellulose acetate, and acrylic.

Pixuvri powder for concentrate for solution for infusion is filled into glass vials with rubber stoppers and aluminium crimp seals. The glass vial and butyl rubber stopper are specifically designed for lyophilization purposes and comply with the Ph.Eur. Adequate specifications and drawings have been provided for the container closure system. The vials and stoppers showed to be compatible with the drug product in both dry powder and the reconstituted state. A container closure integrity study demonstrated that the closure system is suitable for protection against microbial contamination as well as moisture transfer.

Adventitious agents

The excipients used in Pixuvri are not of animal origin with the exception of lactose monohydrate, which is derived from milk sourced from healthy cows under the same conditions as milk collected for human consumption. Hence, lactose monohydrate is compliant with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products (EMEA/410/01 Rev. 3). No excipients derived from human origin have been used.

Manufacture of the product

A detailed description of the manufacturing process and a flow scheme have been provided. Adequate in-process controls are performed and the acceptance criteria and the test methods are adequately chosen to ensure that the drug product will comply with the specification limits. The applicant has presented process validation data on three consecutive full scale validation batches. The validation results demonstrated that the manufacturing process for Pixuvri should be capable of consistently producing a finished product that meets the predefined finished product release specifications.

Product specification

The finished product release specifications include tests for appearance, appearance of container/closure system, reconstituted solution (appearance, completeness of reconstitution, visible particulates), identification (UV, HPLC), assay and related substances (HPLC), water content (Ph.Eur), uniformity of dosage units (Ph.Eur), pH (Ph.Eur.), sterility (Ph.Eur.), bacterial endotoxins (Ph.Eur.), particulate matter (Ph.Eur). The finished product specifications are standard for this type of presentation. The proposed test procedures and acceptance criteria comply with the requirements of the Ph.Eur. and ICH guidelines. All tests included in the specification have been satisfactorily described and validated. Appropriate data have been presented to justify the release specifications for each quality characteristic that is controlled. Impurities and degradation products have been evaluated and found to be acceptable from the point of view of safety. Batch analysis data are provided for three batches produced with active substance from the proposed supplier. The batches were manufactured at the proposed site, according to the proposed manufacturing process and scale. Batch analysis results comply with the predefined specifications and confirm consistency & uniformity of manufacture and indicate that the process is under control.

Stability of the product

Stability studies have been carried out under long term (5±3°C) and accelerated (25±2°C/60±5%RH) conditions on three batches according to the ICH requirements. The batches were manufactured at commercial scale using the proposed active substance and packaged in the container closure system proposed for marketing. Up to 24 months long term and up to 6 months accelerated stability data have been provided. Long term stability data (up to 42 months at 5°C) and accelerated stability data (6 months at 25 °C/60%RH) have been presented for three supporting stability batches.

The parameters tested and analytical methods used are identical to those used for the release specifications, except from identification and uniformity of dosage units which were not retested at end of shelf-life. The methods used for assay and related substances were proven to be stability indicating. The stability results demonstrated that under long term and accelerated conditions no significant change on storage was observed for any batch.

The applicant has also performed a confirmatory photostability study according to ICH Q1B. Sampled were exposed to visible and ultraviolet light in primary packing and primary packaging plus secondary packaging, placed in an inverted position to maximize light exposure. The results of the study demonstrated that the current packaging is sufficient to protect the active substance from light exposure.

Furthermore, the applicant has performed in-use stability studies (up to 24 hours data) on the reconstituted and diluted drug product, and a drug product freeze thaw study. The results of the in-use stability study indicated that Pixuvri should be used immediately after reconstitution.

As part of the stability commitment, the applicant committed that at least one batch per year for each year the finished product is produced will be placed on a long-term stability program.

In conclusion, the stability results presented were satisfactory and support the proposed shelf life for the commercially packaged product under the conditions specified in the SmPC.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The marketing authorisation application for Pixuvri, powder for concentrate for solution for infusion, contains adequate data to demonstrate the quality of the active substance and finished product. The quality of the active substance is adequately controlled and all excipients comply with the Ph.Eur. The finished product manufacturing process shows to be capable of consistently producing a finished product that meets the finished product specifications, and appropriate packaging is used to ensure the product remains stable within the agreed shelf-life.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and medicinal product has been presented in a satisfactory manner. The results of tests carried out indicate satisfactory

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 the clinic.

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

At the time of the CHMP opinion, there were no unresolved quality issues which could have an impact on the benefit/risk ratio of the medicinal product.

2.2.6. Recommendations for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

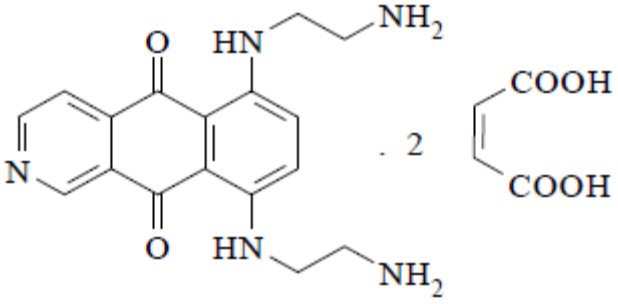
The nonclinical testing programme has evaluated the pharmacological properties of BBR 2778 (pixantrone) in both *in vitro* and *in vivo* models; the pharmacokinetics (PK) and/or toxicokinetics (TK) of BBR 2778 in mice, rats and dogs; the tissue distribution and protein binding of BBR 2778; the metabolic profile, cytochrome P450 (CYP450) inhibition, CYP450 induction, and P-glycoprotein (P-gp) inhibition of BBR 2778; excretion and mass balance of BBR 2778; as well as the toxicological profile of BBR 2778 in mice, rats and dogs; *in vitro* and *in vivo* genotoxicity studies; teratology studies in rats and rabbits; and other toxicity studies (e.g. myelotoxicity, cardiotoxicity, impurities, sudden deaths).

2.3.2. Pharmacology

Brief summary

The applicant conducted *in vitro* mechanism of action studies, along with *in vitro*/*in vivo* cytotoxic activity assays (including several experimental tumour models). The animal models used for the primary pharmacodynamic endpoint, cytotoxic activity, were considered by the applicant to be relevant and predictive of the antitumour efficacy seen with BBR 2778 in human clinical trials for aggressive NHL. Secondary pharmacodynamics were conducted based on the immunosuppressor properties of BBR 2778, and stand-alone safety pharmacology studies were completed. Formal stand-alone pharmacodynamic drug interaction studies were not conducted, but this is not considered to be a major deficiency since several primary pharmacodynamic studies were conducted in combination with other anticancer agents.

Physical chemistry
Structure of the active substance
Site of labelling (see structure).



Isomerism.	BBR 2778 is achiral and has no regioisomers
Molecular weight.	557.5
Solubility in water.	0.5% w/v
Pka.	6.0
Solubility in other solvents.	1.2% in 0.9% NaCl, 1.0% in acetate buffer ph 5.2, 0.6% in propylene glycol, 0.04% in ethanol.

Primary pharmacodynamic studies

The mechanism of action of BBR 2278 was investigated in cell-free and cell-based studies. The *in vitro* cytotoxicity of BBR 2278 was compared *in vitro* with other anti-cancer drugs. There were also *in vivo* efficacy studies in tumour models of haematopoietic origin in comparison with other anti-cancer agents and *in vitro* and *in vivo* efficacy studies in combination with other cytotoxic agents.

The mechanistic studies show that, similar to mitoxantrone, BBR 2778 binds to DNA *in vitro*. However, the potency of BBR 2778 in comparison with mitoxantrone is variable and dependent upon experimental conditions (e.g. different ionic strengths, pH conditions).

The interaction of BBR 2778 with DNA and topoisomerase II was qualitatively similar to that of mitoxantrone, but quantitatively different as shown by the lower amount of DNA single-strand breaks, double-strand breaks, and DNA-protein cross-links in L1210 leukemia cells *in vitro*. The *in vitro* cell-killing effects of BBR 2778 did not seem to be solely related to stimulation of topoisomerase II mediated DNA cleavage or to formation of DNA breaks, since these were more numerous in S180 than in L1210 leukemia cells treated with BBR 2778, yet the former were less sensitive to BBR 2778 as measured by cytotoxicity. The lack of correlation between DNA breaks and cytotoxicity suggests that other mechanisms contribute to the cytotoxicity of BBR 2778. Gene regulation studies in HS-Sultan human Non-Hodgkin Lymphomas showed that although the final effects of BBR 2778 and mitoxantrone on cell proliferation were qualitatively similar, the immediate effects of BBR 2778 were different.

The cytotoxic activity of BBR 2778 *in vitro* was compared with other anticancer agents against a variety of tumour cells. BBR 2778 showed greater cytotoxicity *in vitro* against tumour cell lines derived from haematological tumours than those derived from solid tumours. BBR 2778 was less cytotoxic than mitoxantrone and was approximately as cytotoxic as doxorubicin in haematological tumour cells, while it had a higher IC50 against solid tumour cells.

BBR 2778 showed complete cross-resistance with mitoxantrone and doxorubicin in one MDR over-expressing cell line (LoVo/DX), and was also cross-resistant with doxorubicin in the MCF7/ADR breast cancer cell line. However, it showed only partial cross-resistance with mitoxantrone in the HT29/Mitox, a cell line having a specific mechanism of resistance to mitoxantrone not attributable to P-glycoprotein or to the MDR gene.

In a tumour colony forming units (TCFUs) assay partial cross-resistance with mitoxantrone was also observed in 58 human tumours derived from primary tissue culture belonging to 10 tumour types. No resistance for BBR 2778 was observed in *in vitro* studies in resistant cell lines after a year of BBR 2778 exposure.

Although BBR 2778 was not very potent in the *in vitro* cytotoxicity assays, it demonstrated antitumour activity *in vivo* against multiple tumour models of hematopoietic and solid tumour origin. Overall, the data suggest greatest efficacy of BBR 2778 against haematological malignancies, where BBR 2778 was superior to mitoxantrone and doxorubicin.

BBR 2778 had a wide range of active doses in haematological murine tumour models. Mitoxantrone and doxorubicin, in the same experimental conditions, showed their best activity only at their

maximum tolerated dose (MTD), whereas BBR 2778 showed a high level of efficacy at doses as low as approximately one third of its MTD.

In solid tumour models, the antitumour activity of BBR 2778 was comparable with that of standard agents used as comparators. Full dose-response studies were carried out for each test compound.

In summary, BBR 2778 has a broad spectrum of antitumour activity against haematological and solid tumour models. The activity in the haematological tumours was superior to that of standard agents and was present at a wide range of well tolerated doses. The combination studies demonstrated the potential of BBR 2778 as a therapeutic agent against a broad range of malignancies. The broad range of active nontoxic doses allows combination with other anticancer drugs.

BBR 2778 is primarily metabolized by the formation of mono- and di-acetyl derivatives, all of which were much less potent than the parent drug.

Secondary pharmacodynamic studies

In an experimental autoimmune encephalomyelitis (EAE) rat model of multiple sclerosis repeated IV administration of mitoxantrone and BBR 2778, BBR 2778 caused more profound and long-lasting lymphopenia that was still evident at the end of the 60-day observation period. Histopathology showed the lower cardiotoxicity of BBR 2778.

In a study in MG Lewis rats and rat-derived T-cell lines, BBR 2778 showed strong antiproliferative activity *in vitro* in the nanomolar range and BBR 2778 administration reduced the severity of disease compared with both vehicle and mitoxantrone-treated animals.

When administered to mice according to doses and schedules that demonstrated similar antitumour efficacy, BBR 2778 was less immunosuppressive than mitoxantrone. However, after repeated treatments, lymphocytes were reduced more markedly by BBR 2778 than by mitoxantrone.

Safety pharmacology programme

Effects on cardiovascular, respiratory and central nervous systems (CNS) were evaluated in GLP-compliant studies (see table 1).

Target organs other than the cardiac and CNS, e.g. bone marrow and kidneys, were investigated both via their functionality markers (i.e. haematology and blood enzymes) and via histopathological assessment during the repeated dose toxicity studies in rodents and non rodents.

Table 1: List of Safety Pharmacology studies

Type of study	Study Report	Species	Duration of Dosing	Dosing Schedule	Recovery period	Dose range
Central Nervous System						
CNS-modified Irwin test	RR/03/2008/TG	mouse	single	once	NA	12.5-50 mg/kg
Cardiovascular and Respiratory Systems						
hERG assay	99973	HEK293 cells	NA	NA	NA	0.05µM-100µM
hERG assay	99981	HEK293 cells	NA	NA	NA	0.05µM-100µM
Cardiovascular QT + respiratory	10670	dog (anaesthetised)	single	once	NA	2.5-6.5 mg/kg
Cardiovascular QT+ respiratory respiratory	30570	dog (anaesthetised)	single	once	NA	10 mg/kg
NA: Not applicable						

Cardiovascular and Respiratory Systems

Cardiovascular (including QT assessment) and respiratory effects of BBR 2778 administered by slow i.v. infusion were tested at doses from 2.5 to 10 mg/kg in male anaesthetised dogs at up to 240mg/kg in two separate experiments. There were no treatment related changes in any of the parameters under examination. This result differed from that observed during a single dose study in dogs, in which BBR 2778 at the IV bolus dose of 10 mg/kg resulted in transient tachycardia during and after administration. A non-GLP toxicokinetic study (Study Report 10670) showed a relationship between dose level and systemic exposure: mean plasma concentrations of BBR 2778 were proportional to the administered doses.

Further GLP and ICH-compliant studies were conducted *in vitro* to investigate possible effects of BBR 2778 on hERG (human-ether-a-go-go related gene) potassium channels stably expressed in HEK293 cells. BBR 2778, at a concentration of 100 µM induced a very slight reduction (about 10% vs controls) of the tail current, whereas positive controls had a 96% reduction at 100 nM. In patients, the Cmax of BBR 2778 after a dose of 84 mg/m2 corresponded to about 3100 nM. It can therefore be concluded that at clinical doses, potassium channel inhibition is unlikely to occur.

Central Nervous System (CNS)

In a modified Irwin test (Study Report RR/03/2008/TG) performed in CD1 mice at 12.5, 25 and 50 mg/kg after single IV bolus administration, the NOAEL for behavioural, neurological and autonomic responses, and body temperature was 25 mg/kg. At the highest dose (50 mg/kg), males showed reduced body tone and passivity in handling and elicited response tests. A temporary decrease (not statistically significant) in body temperature (-7% vs controls) was observed 2 hours after treatment, and this decrease recovered 5 hours after treatment. These findings agreed with symptoms observed during the single-dose toxicity studies in the same animal species at similar dose levels of 49 or 65 mg/kg. Pharmacokinetics studies showed that the compound is not extensively distributed to the CNS (Study Reports 950232 and 940738) and limited penetration occurs after fast bolus intravenous injection. Based on these findings and the slow injection rate in patients (1-hour infusion), the applicant considered that a low potential for CNS toxicity is expected in humans.

Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies were not performed; however, drug combination studies have been conducted to evaluate the antitumour efficacy of BBR 2778 in combination with cytotoxic drugs.

2.3.3. Pharmacokinetics

The pharmacokinetics of BBR 2778 was evaluated in mice, rats, and dogs after single and repeated administration of different doses. The compound was investigated in mice at dose levels of 40 mg/kg after single administration and 16 mg/kg after repeated administration, in rats at dose levels of 63 and 96 mg/kg after single administration and 25 mg/kg after repeated administration, and in dogs at dose levels from 2.5 to 10 mg/kg after single administration and from 0.8 to 1.6 mg/kg after repeated administration.

Some pharmacokinetics and ADME studies were conducted with [14C] BBR 2278. The radiolabel was located on the two carbon atoms of each diamino chain of the molecule as depicted in Figure 2.

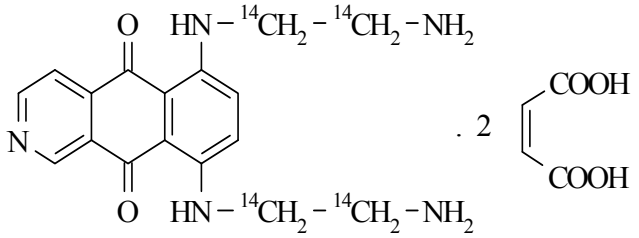


Figure 2: BBR 2778 ^{14}C labelled atoms

In order to characterize the pharmacokinetics of the drug, the concentrations of BBR 2778 in plasma and urine were determined by HPLC/UV bioanalytical methods. Several methods were developed..

The extraction methods applied during non-GLP studies were based either on solid phase extraction or liquid/liquid extraction from plasma. Urine was analyzed after dilution with buffer. The limits of quantitation (LOQ) of the HPLC/UV methods were 0.05 and 0.3 μg/mL in mouse and rat plasma, respectively, and 0.15 μg/mL for both mouse and rat urine. The LOQ in dog plasma was 0.010 μg/mL.

In the toxicological GLP studies, validated HPLC/UV analytical methods based on liquid/liquid extraction were developed with a limit of quantification of 0.005 μg/mL for plasma from rats and dogs. Linearity, accuracy and precision of the methods were within the standard acceptance criteria for a bioanalytical method (RSD <15%).

Absorption

As BBR 2278 is intended for i.v. administration, no absorption studies have been conducted and this is considered acceptable.

Following i.v. administration, the volume of distribution exceeded total body water in all species suggesting extensive distribution into tissues. In the mouse, systemic exposure increased after weekly repeated administration for 4 weeks and was 2-fold higher than that after a single dose. In the rat, drug accumulation occurred after 6 months of repeated administration. The applicant claimed that this accumulation was consistent with the generally compromised physiological condition of the animals. In the dog, there was no significant overall time dependency in Cmax and AUC as expected on the basis

of the estimated t1/2 of 1.9 to 9.0 hours. There were some gender related differences in PK parameters in rodents but not in the dog.

Distribution

Tissue distribution studies were conducted in the mouse after single and repeated administration and the rat after single administration. The route of administration was i.v. In both species, drug related radioactivity distributed very rapidly in almost all organs and tissues and it was persistent. Radioactivity was present 8 days post-treatment in most organs and mainly in the spleen, lungs, kidney (both species) and thymus (rat). There were no major gender or species related difference in tissue distribution. The distribution to the brain depended on the rate of administration. After a bolus injection radioactivity was distributed all over the brain tissue and spinal cord whereas after slow infusion it was present only in the subarachnoid space and ventricles. After repeated administration in the mouse, there was evidence of accumulation of drug related radioactivity. This indicates slow elimination from the tissues and systemic circulation after repeated dosing. There was limited binding to serum proteins. Placental transfer and milk excretion studies were not conducted. This is acceptable in view of the intended patient population.

Metabolism

The metabolism of BBR 2278 was investigated *in vitro* and *in vivo*. No *in vivo* studies were conducted in the dog, the non-rodent species used in single and repeated dose toxicity studies. Acetylation was the main metabolic pathway in rodents whilst it was stated that dogs are not capable of acetylation of this class of compound. The dog was considered to be a suitable non-rodent species because the cytotoxicity of BBR 2278 was associated with the parent compound, not the metabolites.

After microsomal incubation in all species BBR 2278 underwent oxidative biotransformation in the side chain resulting in ring closure. *In vitro*, there were no important differences between species. No additional metabolites were present in humans which were not observed in animals.

In vivo, the metabolic profile confirmed the *in vitro* findings: the compound was mainly metabolised with the formation of mono- and di-acetyl derivatives in the primary amino groups of the diaminoethyl side chain. Unchanged drug accounted for the majority of circulating and excreted radioactivity and the metabolism produced a variety of metabolites, none of which accounted for more than the parent drug.

Excretion

Excretion and mass balance were investigated following single (mice and rats) or repeated (male mouse) i.v. administration of 14C BBR 2278. In both rodent species, drug related radioactivity was eliminated in urine and faeces/bile with the majority being non-renal elimination. In the rat and mouse the renal clearance was about 400 and 4 times lower than total plasma clearance respectively. At the end of the collection period 16-47% (mouse/rat) of the administered dose was still found in the carcass, in agreement with the persistent distribution of drug related radioactivity. After repeated administration to the mouse once a week for 4 weeks, elimination of radioactivity in urine and faeces was comparable with the excretion after a single dose. Following i.v. administration, the volume of distribution exceeded total body water in all species suggesting extensive distribution into tissues.

In the mouse, systemic exposure increased after weekly repeated administration for 4 weeks and was 2-fold higher than that after a single dose. In the rat, drug accumulation occurred after 6 months of repeated administration. In the dog there was no significant overall time dependency in Cmax or AUC as expected on the basis of the estimated t1/2 of 1.9-9.0 hours. There were some gender differences in PK parameters in rodents but not in the dog.

Interactions

The compound showed a moderate inhibition capacity towards CYP1A2 activity. The Ki for BBR 2778 against CYP1A2 was determined as 5 - 10 µM and the type of inhibition was at least partly of a competitive nature. BBR 2778 was a substrate for active efflux transporters such as P-gp.

2.3.4. Toxicology

Single dose toxicity

Studies were conducted in the mouse, rat and dog. Administration was by i.v.injection, except for a study in the mouse. The studies incorporated a recovery period ranging from 8 days to 8 weeks (see table 2).

Preliminary studies showed that BBR 2778, administered intravenously as a bolus injection to CD-1 mice, induced death of the animals during or immediately after the administration of the test compound, starting at the dose level of 50 mg/kg. In the subsequent experiments, BBR 2778 was administered by slow infusion in order to avoid this toxic effect.

In the pivotal IV toxicity study in mice, BBR 2778 was administered intravenously by slow infusion at dose levels of 65 mg/kg and 49 mg/kg and acute lethal toxicity was observed in some animals, probably due to peak plasma levels reached during administration. These doses are much higher than those used in anti-tumour activity studies. Histopathology revealed precipitates in the lung vessels, which may have contributed to the observed mortality. The main target organs of toxicity in surviving animals were the hematopoietic system, kidneys and testes which could be anticipated for an anti-proliferative agent. Effects on bone marrow and kidneys were reversible by Day 29, while the effects on testes lasted longer. BBR 2778 was more toxic in male than in female mice.

Intravenous toxicity studies in rats indicated that BBR 2778 induced a dose-dependent mortality in the range of 80 to 140 mg/kg (injection rates of 0.4 mL/min); death occurred, in many cases during or immediately after dosing. The main overt signs of toxicity observed in dead and surviving animals of both sexes at all doses of BBR 2778 were: dyspnea, piloerection, reduced motility, swollen snout and bluish discolouration of the skin. These signs lasted no longer than four days, with the exclusion of bluish discolouration which lasted the whole period (15 days) of the study. Acute toxicity was similar in male and female rats. Target organs for toxicity in rats were testes, spleen, bone marrow, gastrointestinal mucosa, kidney and heart.

A single administration of 3.25 mg/kg of BBR 2778 in the rat induced slight myelotoxic effects that had complete recovery about 15 days after the treatment.

In the definitive study in the dog, BBR 2778 at 10 mg/kg (injection rate of 2 mL/min) administered as a single dose by the intravenous route , resulted in the death of animals at one week after treatment, due to pulmonary edema, which followed severe immunodepression (test compound-related) with consequent bacteraemia. The dose of 1 mg/kg IV in dogs was considered generally to be well tolerated. After an 8-week recovery period, slight testicular alterations were observed in the male dog, whereas alterations of lymphatic tissues related to immunosuppression were no longer present. In dogs, tachycardia and ECG changes also occurred immediately after treatment only in non-anaesthetised animals given the compound at a 2 mL/min injection rate. In safety pharmacology studies when the BBR2778 solution was injected at about 0.7 mL/min, these findings were not confirmed, indicating that cardiotoxicity is dependent on the rate of injection.

Table 2: Acute Toxicology Studies

Type of study	Study #	Species	Injection rate (mL/min)	Dosing Duration	Dosing Schedule	Recovery period	Dose range (mg/kg)
Acute Tox	11/TOX/93	Mouse	2	Single	NA	28-d	50, 60, 70, 80, 90
Acute Tox	02/S/1994	Mouse	0.1	Single	NA	28-d	50, 70,80, 90, 110
Acute Tox	11/S/1994	Mouse	0.1	Single	NA	28-d	49, 65
Acute Tox	03/S/1994	Mouse	NA (IP injection)	Single	NA	28-d	70 to 160
nnAcute Tox	13/S/1994	Rat	0.83	Single	NA	14-d	3.25
Acute Tox	14/S/1994	Rat	0.4	Single	NA	28-d	80, 100, 120, 140
Acute Tox	950037	Dog	2	Single	NA	8-d	10
Acute Tox	950154	Dog	2	Single	NA	8-w	1, 10

Repeat dose toxicity

The toxicological programme consisted of a series of repeat dose studies carried out in mice, rats, and dogs given IV weekly treatment for 4 weeks followed by a 4-week recovery period. An additional dog study was performed with daily IV treatment for 5 consecutive days. Most of these preliminary studies were conducted using male animals, as it was shown to be the most sensitive gender in single dose studies. Pivotal studies were carried out on both genders (see table 3).

The two pivotal studies were:

- An 18-week intravenous toxicity study in rats (18 males and 18 females), consisting of six treatment cycles of 2 doses with a 7-day interval followed by a 14-day observation period and including an 8-week recovery period after the 6th cycle (RR/08/2003/TG).
- An 24-week intravenous toxicity study in dogs (4 groups of 6 males and 6 females), consisting of six treatment cycles of 3 doses with a 7-day interval followed by a 14-day observation period and including an 8-week recovery period after the 6th cycle (RTC 26890).

Table 3. Repeated Dose Toxicology Studies

Type of study	Study #	Species	Injection rate (mL/min)	Dosing Duration	Dosing Schedule	Recovery period	Dose range (mg/kg)
Repeated Tox	01/S/1995	Mouse	0.1	4-w	q7dx4	8-w	16, 48
Repeated Tox	05/S/1995	Mouse	0.1	4-w	q7dx4	28-d	16, 48
Repeated Tox	04/S/1994	Mouse	NA (IP injection)	5-d	q1dx5	28-d	10, 15, 20, 25, 30, 35
Repeated Tox	10/S/1994	Mouse	NA (IP injection)	5-d	q1dx5	28-d	15, 18, 21, 24, 27

Repeated Tox	12/S/1994	Mouse	NA (IP injection)	5-d	q1dx5	28-d	21
Repeated Tox	02/S/1995	Rat	0.83 and 0.4	4-w	q7dx4	28-d	3.25, 32.5
Repeated (cyclic) + TK	RR/08/2003/TG	Rat	2	18-w	q7dx2 (6 cycles spaced by 14 days)	8-w	13.5, 19, 25
Repeated Tox	RE/05/2003/TG	Rabbit	2	3-d	q1dx3	14-d	5, 6, 7
Repeated Tox+ TK	16/S/1996	Dog	2	4-w 6-w	q7dx4 q7dx2 (two cycles spaced by 14 days)	28-d	1, 2.5
Repeated (cyclic) Tox	RE/05/2004/TG	Dog	2	8-w	q7dx3 (two cycles spaced by 14 days)	14-d	1.6, 2, 2.5
Repeated Tox	950029	Dog	2	5-d	q1dx5	8-w	0.2, 2.0
Repeated (cyclic) + TK	26890	Dog	2	26-w	q7dx3 (6 cycles spaced by 14 days)	8-w	0.8, 1.2, 1.6
NA = Not applicable IP = Intraperitoneal							

The pivotal, GLP 6-cycle toxicity studies conducted for BBR 2778 in rats and dogs by intravenous bolus dosing have identified the lympho-hematopoietic organs and male reproductive tract as primary target organs of toxicity in both species, with the heart and kidney also being target organs in the rat.

Lympho-Haematopoietic Organs: In the 6-cycle rat toxicity study, a dose-dependent decrease in haematocrit, haemoglobin, RBC and WBC count (mainly lymphocytes) was recorded during the treatment period, even at the lowest dose tested (13.5 mg/kg). This was also observed in the 24-week pivotal dog study, again at the lowest dose tested (0.8 mg/kg, 15.8 mg/m2).

Male Reproductive Tract: BBR 2778-related effects on the testes were observed in the 6-cycle rat toxicity study. A dose-dependent decrease in testes size and weight was observed, with only partial reversibility at the end of the recovery period. Tubular atrophy of the testes was observed in a dose-dependent manner and persisted at the completion of the recovery period. Similar effects on the male reproductive tract were observed in other rodent (mouse and rat) toxicity studies of shorter duration. In dogs, significant decreases in testes weight vs. control were seen in males at all doses after 6 cycles of treatment. Decreased testes weight was still present at the end of recovery for all animals. Marked testicular atrophy and absence of sperm in the epididymides were seen in all treated males after 6 cycles and this finding persisted for all animals at the end of recovery. Consistent with the findings in the pivotal study, tubular epithelium degeneration of the testes was observed in dogs at doses as low as 0.2 mg/kg/day for 5 consecutive days.

Heart: Histopathological changes were observed in a dose-dependent manner for the myocardium (degenerative/vacuolar changes, interstitial fibrosis and inflammation) of male and female rats in the 6-cycle toxicity study. Cardiac toxicity was observed only at doses ≥ 13.5 and 19 mg/kg in male and female rats, respectively, i.e. at doses shown to induce mortality and severe multi-organ lesions. Cardiotoxicity was not observed in dogs.

Kidneys: In the 6-cycle rat toxicity study, urine volume was generally increased in all treated groups as compared to control animals at the end of treatment period (except for low dose females) and at the end of recovery. Analysis of the urine revealed increased levels of leukocytes, proteins, and erythrocytes in treated animals. The severe renal effects observed following repeated administration of BBR 2778 to rats were always associated with lesions observed in the myocardial tissue. In general, dose-dependent increases in progressive tubular and glomerular nephropathy were observed with a greater severity in males, but this finding was still prevalent in males and females. This finding was not fully reversible at the end of the recovery period. In dogs, there were minimal adverse findings for BBR 2778 in the kidney. Following 5 consecutive days of dosing at 2 mg/kg/day, protein and blood were observed in the urine that was graded as slight to severe.

NOAELs were not identified in the pivotal rat and dog toxicity studies.

Genotoxicity

All the Ames tests were conducted using Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and TA102. The methods included both plate incorporation and pre-incubation. BBR 2778 was positive in stain TA98 in study R09010 in which in both experiments there was a slight statistically significant increase in the number of revertant colonies at the highest non-toxic dose tested (50µg/plate) for strain TA98 either with or without metabolic activation. In study 9648-001EXT there was a concentration related increase in revertant numbers with strain TA1537 in the presence of S9 metabolic activation using the pre-incubation method. These strains are considered to detect changes at guanine-cytosine sites within target histidine genes. BBR 2778 was clastogenic both *in vitro* and *in vivo*.

Overall, the evidence indicates that BBR 2778 should be considered genotoxic.

Carcinogenicity

No carcinogenicity study has been submitted.

Reproduction Toxicity

Fertility and early embryonic development

Cytotoxic anticancer compounds such as BBR 2778 are assumed to cause embryotoxicity and foetal toxicity as indicated in the CPMP Note for Guidance for the pre-clinical evaluation of anticancer medicinal products (CPMP/SWP/997/96). Neither fertility study nor early embryonic development study has been submitted with pixantrone dimaleate.

Embryo-fœtal development

Segment II studies in rats and rabbits were carried out to elucidate the potential for reproductive toxicity in the case of inadvertent administration to a pregnant woman. BBR 2778 was administered for 3 consecutive days during the major period of organogenesis (Days 9 to 11).

In rats, doses from 3.25 to 13.0 mg/kg/day (gestation days: 9-11) induced maternal and foetal toxicity consisting of significant reduction of dams' body weight, increase in post-implantation loss and reduction in number of viable pups and mean fetal weight. The highest dose of 24.0 mg/kg/day provoked total resorptions. As malformed fetuses were found at the lowest dose, no NOAEL could be established .

In rabbits, doses of 3 to 6mg/kg also at gestational age 9-11 days induced maternal toxicity and fetal growth retardation, including delayed ossification.

Prenatal and postnatal development, including maternal function

No pre-post natal development study has been submitted with pixantrone dimaleate in line with the CPMP Note for Guidance for the pre-clinical evaluation of anticancer medicinal products (CPMP/SWP/997/96).

Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

No study in juvenile animals was conducted with pixantrone, as it is intended for the treatment of adult patients with multiple relapsed or refractory aggressive NHL as monotherapy.

Toxicokinetic data

Studies were conducted in the mouse, rat, and dog. The route of administration was i.v. except for 3/5 studies in the mouse, where the route was i.p. All the studies incorporated a recovery period to check for reversibility of treatment related effects. Most of the preliminary studies were conducted in male animals only, as it was shown that this was the most sensitive gender in single dose studies (see table 4).

The dog was the non-rodent species used in single and repeated dose toxicity studies. Acetylation was the main metabolic pathway in rodents whilst it was stated that dogs are not capable of acetylation of this class of compound. Nevertheless, the applicant considered that the dog was a suitable non-rodent species because the cytotoxicity of BBR 2278 was associated with the parent compound, not the metabolites. The applicant also stated that dogs are recognized as the best *in vivo* model for the assessment of cardiovascular toxicity, which is one of the major side effects produced by anthracycline -like and anthracenedione-like cytotoxic agents.

The main findings were myelotoxicity, nephrotoxicity and testes damage, haematological toxicity that tended to recover, and kidney damage in rats and testicular histopathological changes in rats and dogs that were non-reversible.

Table 4 : Overview of Toxicokinetic Studies

Type of Study	Test system	Method of Administration	Doses (mg/kg/day)	GLP Compliance	Study Number
Repeated dose toxicity (18-week/6 cycles)	Rat	Intravenous	13.5, 19, 25 Cycle: 2 treatments at 7 day intervals followed by 2 week observation period	yes	RR/08/ 2003/TG
Cardiovascular and Respiratory Function (single)	Dog	Intravenous	2.5, 4.5, 6.5	yes	RTC 10670[1]
Cardiovascular and Respiratory Function (single)	Dog	Intravenous	10	yes	RTC 30570[1]
Repeated dose toxicity (4-week)	Dog	Intravenous	1, 2.5 weekly for 2 weeks followed by a 2 week observation period	yes	03/PKB/ 96[2]

Repeated dose toxicity (24-week/6 cycles)	Dog	Intravenous	0.8, 1.2, 1.6 Cycle: 3 treatments at 7 day intervals followed by 2 week observation period	yes	26890
1 Study Nos. RTC 10670 and RTC 30570 are safety pharmacology studies. 2 TK study related to toxicology study report 16/S/96					

Local Tolerance

Local tolerance was evaluated as part of the intravenous toxicity studies in rats and dogs. These studies were stated to have been conducted using the same formulation (BBR 2778 in 0.9% saline) as intended to be marketed. The dosing frequency in the rat and dog toxicity studies was equivalent to or higher than the frequency applied in the intended clinical use. In addition, stand-alone local tolerance studies were conducted in mice with bulking agents intended to be used in the clinical formulation (mannitol, polyvinylpyrrolidone). Further development in formulation led to selection of lactose, and further studies were not carried out as it is not expected to worsen local tolerability, in view of the well-documented safety of lactose in pharmaceutical preparations for intravenous injections. As the final formulation chosen did not include lactose, no further studies of use of lactose as a bulking agent were carried out.

Since the findings in the rat were not observed in dogs, it is likely that they were mainly due to needle trauma to the small vessels of rodents.

Other toxicity studies

Immunotoxicity

BBR 2778 and mitoxantrone were studied (Study Report RE/10/2008/PH) for their ability to interfere with T-cell mediated immune reactivities, such as blastogenic response to Concanavalin A (ConA), delayed type hypersensitivity (DTH) reaction and generation of cytotoxic lymphocytes in CD2F1 mice (Vecchi 1993). Drugs were employed at doses and treatment regimen (mitoxantrone at 3 mg/kg and BBR 2778 at 18 mg/kg given intravenously on Days 1, 4 and 7), which displayed comparable antitumor activity when applied in murine tumour models.

One day after treatment, BBR 2778 and mitoxantrone decreased the blastogenic response to ConA to the same level, while 8 days after treatment BBR 2778 was less suppressive than mitoxantrone. Generation of cytotoxic lymphocytes was significantly reduced by both compounds at the same level at both times tested (1 and 8 days after treatment). DTH reactivity was not significantly modified by BBR 2778, while mitoxantrone completely inhibited this parameter. Overall, BBR 2778 was less immunosuppressive than mitoxantrone.

Studies on impurities

Mouse acute toxicity of two different batches of BBR 2778 was assessed in order to see if the impurity RT 0.61 found in batch number 8083 could induce toxicity. The two batches exerted a similar acute toxicity. A second mouse toxicity study was conducted to determine whether a degradation product (NPH006759) in batch 210282-1 could induce toxicity as compared to a batch (02E607) that did not contain the degradant. The two batches were equitoxic. BBR 3558, an impurity of BBR 2778, induced reverse mutations in the Ames test (see table 5).

Table 5. Studies on impurities

Type of study	Study #	Species	Injection rate (mL/min)	Duration of Dosing	Dosing Schedule	Recovery period	Dose range (mg/kg)
Acute Tox:impurities	01/2002/B	Mouse	0.1	Single	NA	28-d	65
Acute Tox: impurities	RR/07/2008/TG	Mouse	0.1	Single	NA	28-d	65
Ames test: impurities	9648-004EXT	*in vitro*	NA	NA	NA	NA	0.5 to 918 ug/plate

Cardiotoxicity

Doxorubicin and mixantrone are anthracycline-based compounds that are known to cause cardiotoxicity. As BBR 2778 is a novel anthracenedione on the basis of its biochemical and biological properties, the potential cardiotoxicity of BBR 2778 was investigated in comparison with doxorubicin and mitoxantrone. Potential exacerbation of cardiotoxicity by BBR 2778 was investigated in doxorubicin-induced animal models of cardiac toxicity, and comparative studies were performed with the individual compounds in healthy animals.

Occasional cardiovascular findings in the toxicology studies in rats and dogs prompted further investigation. Cardiac alterations were recorded at the high dose in one study in mice and rats, possibly related, at least in rats, to the chronic renal disease status. Although in the single dose toxicity study in dogs some changes in cardiac function were observed after an IV bolus injection, in a safety pharmacology study in dogs, no respiratory or cardiac parameters were altered, including QT interval, after a slower administration (i.e. IV infusion) of BBR 2778 at up to 10 mg/kg (200 mg/m2).

BBR 2778 was compared to equiactive doses of doxorubicin and mitoxantrone in treatment-naïve and in doxorubicin-pretreated mice using a schedule of treatment reported in the literature as optimal for the evaluation of cardiotoxicity of these agents. BBR 2778, administered twice a week for 4 weeks at up to 27 mg/kg, did not induce any cardiotoxic effects, whereas mitoxantrone was cardiotoxic at all the doses tested (1.1, 1.8 and 2.5 mg/kg). In addition, doxorubicin-induced cardiotoxicity was enhanced to a lesser extent by BBR 2778 than by mitoxantrone or doxorubicin alone. Thus, the cardiotoxic potential of BBR 2778 was lower than that of the reference compounds doxorubicin and mitoxantrone.

Sudden death

In the BBR 2778 toxicity studies, mortality was observed during or immediately after treatment. A series of studies have been conducted to investigate the possible contributions of dose rate and length of infusion and precipitation, as well as the thrombogenic and arrhythmogenic potential of BBR 2778.

Higher injection rates resulted in unexpected, early mortality and several studies were performed to investigate the sudden death phenomenon. BBR 2778 thrombogenic activity was evaluated in male mice pretreated with antithrombotic agents (acetylsalicylic acid or hydrocortisone sodium succinate) at doses which inhibited the sudden death due to pulmonary thrombosis and pulmonary artery constriction induced by sodium arachidonate. Pretreatment with both antithrombotic agents failed to prevent the sudden death phenomenon induced by BBR 2778. Heparin pretreatment markedly reduced the incidence of sudden deaths in both mice and rats, but this result was ambiguously associated with a reduction in the delayed mortality and in the intensity of the blue colouring induced by BBR 2778. Moreover, the addition to plasma or serum samples of heparin and BBR 2778 caused the formation of a blue precipitate.

The evaluation of the main hemocoagulative parameters as PTT (partial thromboplastin time), PT (prothrombin time), T-C (thrombin-coagulase time), fibrinogen and D-D (DDimer) after BBR 2778

administration showed a reduction in fibrinogen concentration and an increase in T-C. No changes in D-D, PT and PTT values were observed.

The onset of arrhythmias, related or not related with a cardiac electrolytic imbalance, could explain the rapidity with which the sudden death phenomenon developed.

Two studies performed in the mouse and the rat at severely toxic doses (LD60-80) showed marked alterations of electrocardiographic tracings characterized by atrium-ventricular blocks and anomalies of the QRS complex. Since these arrhythmic phenomena appeared towards the end of the infusion, at the moment of onset of animal agony, it cannot be determined whether these alterations were caused by a direct arrhythmogenic activity of BBR 2778 or by a preagonic ischemic condition. For this reason, a third study was performed to evaluate the possible arrhythmogenic activity of BBR 2778 at sublethal doses. In this study, the reading of electrocardiographic tracings identified the presence of slight morphological alterations of some electrocardiographic waves. These changes could indicate minimal alterations in the conduction of cardiac stimulus (depolarization/repolarisation) caused by an electrolytic imbalance.

Studies to investigate a possible systemic electrolytic imbalance caused by BBR 2778 were performed both in mice and rats at lethal and sublethal doses. In one of the studies, animals were placed under forced respiration conditions to eliminate possible interference of respiratory acidosis on the hematic concentration of the electrolytes measured. In all the studies, a marked dose-dependent increase in potassium values was observed, which was toxicologically relevant.

Myelotoxicity

Myelotoxic effects were expected for BBR 2778 and were evident from toxicity studies. Specific studies were carried out to compare the myelotoxicity of BBR 2778 to that of other marketed anticancer drugs.

The pivotal study used a method (Colony Forming Units – Spleen) originally set up for the evaluation of the myelotoxic effect exerted by ionizing radiation (Till 1961). It was shown that myelotoxicity of BBR 2778 is similar to that of mitoxantrone at equitoxic doses in the male mouse (BBR 2778 at 65 mg/kg and mitoxantrone at 10.4 mg/kg corresponding to the LD10). This study was not performed according to GLP; nevertheless, the reporting is of adequate standard and it was conducted at the highest quality standards in force at the time of the experiments.

2.3.5. Ecotoxicity/environmental risk assessment

The applicant has presented an experimentally determined logK_{ow} which is below the threshold value of 4.5. Thus, pixantrone is not considered a PBT drug. By refining Fpen for the prevalence of Non Hodgkin's lymphoma the $PEC_{surface\ water}$ is set to 0.00636 μg.l-1, which is below the trigger value of 0.01μg.l-1.

Table 6: Summary of main study results

Substance (INN/Invented Name): Pixantrone/Pixuvri			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	1.1067	Potential PBT (N)
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.00636	μg/L	> 0.01 threshold (N)

Other concerns (e.g. chemical class)			(N)

No environmental risk has been identified as a consequence of the use of pixantrone.

2.3.6. Discussion on non-clinical aspects

A general effect of BBR 2778 that was observed in all *in vivo* experiments was bluish discolouration diffusely observed in all organs. The greatest occurrence was in animals that died immediately after dosing which was attributed to rapid distribution of administered dark-bluish solutions of the drug.

In rodents, the sudden death of animals during or immediately after i.v. bolus administration appeared to be primarily attributable to the injection rate and dose volume. The clinical relevance of this finding is that it indicates the importance of administration of pixantrone as a slow infusion.

The pivotal, GLP 6-cycle toxicity studies conducted for BBR 2778 in rats and dogs by intravenous bolus dosing have identified the lympho-hematopoietic organs and male reproductive tract as primary target organs of toxicity in both species, with the heart and kidney also target organs in the rat. NOAELs were not identified in the pivotal rat and dog toxicity studies.

The hematotoxicity and myelotoxicity of BBR 2778 were directly compared to mitoxantrone. Studies in mice demonstrated that these compounds from the same therapeutic class displayed a similar myelotoxicity at equitoxic doses.

Because of the chemical similarity of BBR 2778 with other cardiotoxic drugs, cardiotoxicity studies were of particular relevance.

The use of anthracyclines and anthracenediones is associated with irreversible and cumulative cardiotoxicity. Histopathological changes were observed in a dose-dependent manner for the myocardium (degenerative/vacuolar changes, interstitial fibrosis and inflammation) of male and female rats in the 6-cycle toxicity study. Cardiotoxicity was not observed in a safety pharmacology study in dogs, no respiratory or cardiac parameters were altered, including QT interval, after i.v. infusion of BBR 2778 at up to 10 mg/kg (200 mg/m2).

In vitro, a slight alteration of potassium channel function was seen only at the high concentration of 100 µM, which is much higher than 3100 nM, the maximum Cmax of BBR 2778 in patients given 84 mg/m2 as a single agent.

The cardiotoxic potential of BBR 2778 appears to be lower than that of the reference compounds doxorubicin and mitoxantrone determined using equiactive dose levels in terms of efficacy in the same experimental conditions/models in mice.

As anticipated based on the cytotoxic mechanisms of action of the compound, experimental data indicated that BBR 2778 is genotoxic.

Due to the proposed clinical indication, the carcinogenic potential of BBR 2778 has not been studied. According to current guidelines, when the life-expectancy in the indicated population is short, no long-term carcinogenicity studies are required. In pregnant rats, BBR 2778 was embryotoxic, visceral malformations were observed (e.g. absence of thymus, hydronephrosis, enlarged ureters, increased incidence of ectopic or pelvic kidney), and incomplete ossification of the skeleton (primarily attributed to lower fetal weights). BBR 2778 was not embryotoxic in rabbits, with smaller fetuses observed for treated animals but no corresponding toxicological differences upon comparisons of fixed fetus heads. Minor changes in ossification were attributed to maternal toxicity or low fetal weight.

BBR 2778-related effects on the testes were observed in the 6-cycle rat toxicity study. In dogs, significant decreases in testes weight vs. control were seen in males at all doses after 6 cycles of treatment.

Assessment of paediatric data on non-clinical aspects

The Applicant submitted a Paediatric Investigational Plan (EMEA-000713-PIP02-10) to the Paediatric Committee (PDCO) for which assessment began on 12 August 2010. The preclinical program consists of a juvenile mouse cardiotoxicity study (Study No. 1); a study of toxicokinetics of sudden death in mice (Study 2); and, a study to evaluate pixantrone efficacy using *in vivo* xenograft models of paediatric solid tumours (study 3). These studies are described below.

Study 1, in 10-day old mice, will evaluate the toxicokinetics, clinical signs and symptoms (including markers of nephro- and cardiotoxicity) in several tissues including kidneys, heart, hematopoietic tissue. Mice will be administered (intravenously or intraperitoneally) 27 mg/kg of pixantrone twice weekly up to 35 days. The study will also include a reversal period. This study will be completed prior to any child under the age of 12 may receive pixantrone.

Study 2 will evaluate the toxicokinetics of various doses of pixantrone and the potential to cause sudden death. This study will be completed prior to any child under the age of 12 may receive pixantrone.

Study 3 will evaluate pixantrone in mouse xenograft models against a broad range of paediatric solid tumors to support general paediatric development using similar methodology as the Paediatric Preclinical Testing Program at NCI (adult mice for kidney/rhabdoid tumors, Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, neuroblastoma, and non-glioblastoma brain tumors. Results from Study 3 are needed to support potential enrolling paediatric patients with solid tumors in to the proposed clinical trials.

2.3.7. Conclusion on the non-clinical aspects

The CHMP considers the following measures necessary to address the non clinical issues:

Further investigation of the phototoxic potential of pixantrone is considered necessary (see section 2.4 clinical aspects). A non-clinical in vivo phototoxicity study should be conducted, and has been included in the RMP.

2.4. Clinical aspects

2.4.1. Introduction

At the time of submission, the completed clinical development (see Figure 3) includes seven single-agent and five multiagent combination studies which have treated a total of 348 patients with pixantrone; 80% of these patients had NHL and extensive prior anthracycline exposure.

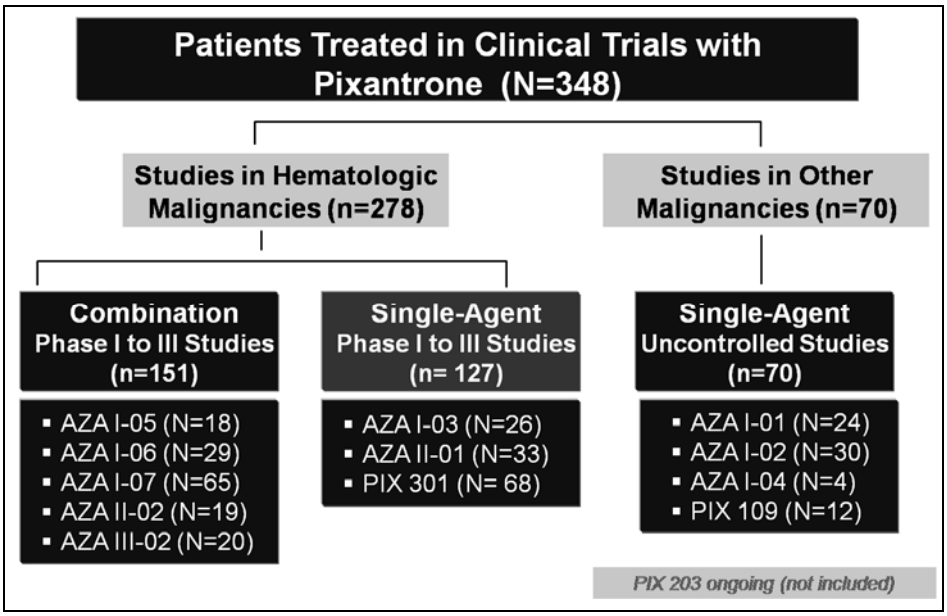


Figure 3: Clinical development of pixantrone

An additional study PIX 203 was submitted during the procedure in which 61 patients received pixantrone as part of a combination regimen (see Table 7).

The indication applied for initially was the following:
Pixuvri is indicated as monotherapy for the treatment of adult patients with multiply relapsed or refractory aggressive Non Hodgkin Lymphomas (NHL).

The final proposed indication is as follows:
Pixuvri is indicated as monotherapy for the treatment of adult patients with multiply relapsed or refractory aggressive Non Hodgkin B cell Lymphomas (NHL). The benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy.

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: Pixantrone Completed Clinical Development.

Study ID	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	# of Subjects	Healthy Subjects or Diagnosis of Patients	Treatment Duration	Study Status; Type of Report
PIX301	Primary: Complete response rate Secondary: Overall survival, progression-free survival, time to response, duration of response, dose intensity, cardiac function, safety, PK	Open-label Randomized Controlled Comparative Two arm (BBR 2778 or physician's choice single comparator agent) Sponsor blinded to treatment	BBR 2778 85 mg/m^2 IV (1 hour infusion)	140 (70/arm)	Relapsed aggressive NHL	Days 1, 8 and 15 of a 4 week cycle, up to 6 cycles	Complete; Full
AZA-I-01	Primary: MTD Secondary: Toxicity profile, DLT, PK, Phase II dose, Anti-tumor activity	Open-label Dose-escalation Uncontrolled	BBR 2778 20 – 240 mg/m^2 IV (1 hour infusion)	24	Advanced solid tumors	Every 3 weeks, up to 10 cycles.	Complete; Full
AZA-I-02	Primary: MTD Secondary: Toxicity profile, DLT, PK, Phase II dose, Anti-tumor activity	Open-label Dose-escalation Uncontrolled	BBR 2778 5.0 – 150.0 mg/m^2 IV (1 hour infusion)	30	Progressive malignant disease	Days 0, 7 and 14 of a 28-day course	Complete; Full
AZA-I-03	Primary: MTD Secondary: Toxicity profile, DLT, PK, Phase II dose, Anti-tumor activity	Open-label Dose-escalation Uncontrolled	BBR 2778 5.0 – 84.0 mg/m^2 IV (1 hour infusion)	26	NHL/CLL	Days 0, 7 and 14 of a 28-day course	Complete; Full
AZA-I-04	Primary: MTD Secondary: Toxicity profile, DLT, PK, Phase II dose, Anti-tumor activity	Open-label Uncontrolled Dose-escalation	BBR 2778 180 - 270 mg/m^2 IV (1 hour infusion)	4	Advanced solid tumors	Day 0 of a 21-day course	Complete; Full
AZA-I-05 AZA-I-05 PK	Primary: MTD, efficacy dose Secondary: Safety profile, DLT, PK, toxicity vs. systemic exposure, Efficacy	Open-label Uncontrolled Non-randomized	BBR 2778 80 mg/m^2 in combination with cytarabine, methyl-prednisolone and cisplatin IV (1 hour infusion)	19	Relapsed aggressive NHL	Every 3 weeks, up to 6 cycles	Complete; Full

Study ID	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	# of Subjects	Healthy Subjects or Diagnosis of Patients	Treatment Duration	Study Status; Type of Report
AZA-I-06 AZA-I-06 PK	Primary: MTD, efficacy dose Secondary: Safety profile, DLT, PK, toxicity vs. systemic exposure, Efficacy	Open-label Uncontrolled Non-randomized	BBR 2778 80 - 120 mg/m^2 in combination with fludarabine, dexamethasone and rituximab IV (1 hour infusion)	29	Relapsed or refractory indolent NHL	Every 4 weeks, up to 8 cycles	Complete; Full
AZA-I-07	Primary: MTD Secondary: DLT, PK, recommended dose, safety, efficacy	Open-label Dose-escalation Uncontrolled Non-randomized	BBR 2778 80 - 180 mg/m^2 in combination with cyclo-phosphamide, vincristine and prednisone IV (1 hour infusion)	65	Relapsed aggressive NHL	Every 3 weeks, up to 6 cycles	Complete; Full
PIX109	Primary: MTD, Activity in population	Open-label Dose-escalation Randomized	BBR 2778 80 - 110 mg/m^2 IV (1 hour infusion)	12	AML	Days 1, 2 and 3 of a 21-day cycle, up to 2 cycles.	Complete; Full
AZA-II-01	Primary: Response rate Secondary: response duration, progression free survival, overall survival, safety	Open-label Non-randomized	BBR 2778 85 mg/m^2 IV (1 hour infusion)	33	Relapsed aggressive NHL	Days 1, 8 and 15 of a 4 week cycle, up to 6 cycles	Complete; Full
AZA-II-02	Primary: Anti-tumor activity Secondary: Safety	Open-label Non-randomized	BBR 2778 80 mg/m^2 in combination with cytarabine, methyl-prednisolone and cisplatin IV (1 hour infusion)	19	Relapsed aggressive NHL	Every 3 weeks, up to 6 cycles	Complete; Full
AZA-III-02*	Primary: Time to progression Secondary: Response rates, time to response, duration of response, overall survival, safety	Open-label Randomized Comparative Controlled Two arm (BBR 2778 plus rituximab or rituximab alone)	BBR 2778 90 mg/m^2 IV (1 hour infusion)	38 (20 experimental, 18 control)	Relapsed indolent NHL	Cycle 1: Days 2 and 8 of a 21-day cycle. Cycle 2 to 6: Days 1 and 8 of a 21-day cycle	Complete; Full

Study ID	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	# of Subjects	Healthy Subjects or Diagnosis of Patients	Treatment Duration	Study Status; Type of Report
PIX203	Primary: Response rate Secondary: Overall survival, PFS, safety, tolerability, duration of response, overall response rate and time to treatment failure	Open label Randomized Control comparative Two arms	CPOP-R (Day 1: Cyclophosphamide 750 mg/m2 IV, Pixantrone 150 mg/m2 IV, Vincristine 1.4 mg/m2 IV and Rituximab 375 mg/m2 IV: Days 1-5 Prednisolone 100 mg daily orally) CHOP-R (Day 1: Cyclophosphamide 750 mg/m2 IV, Doxorrubicin 50 mg/m2 IV, Vincristine 1.4 mg/m2 IV and Rituximab 375 mg/m2 IV: Days 1-5 Prednisolone 100 mg daily orally)	124 patients (61 in CPOP-R arm/ 63 in CHOP-R arm)	Untreated CD 20-positive diffuse large B cell NHL stage ≥ II	Every 3 weeks up to 8 cycles	Complete (submitted during procedure as part of Day 120 response. Report dated 15/4/2011)
AML = acute myelogenous leukemia; CLL = chronic lymphocytic leukemia; DLT = dose-limiting toxicity; IV – intravenous; MTD = maximum tolerated dose; NHL = non-Hodgkin Lymphoma; PK = pharmacokinetic; NHL = non-Hodgkin Lymphoma *AZA III-02 was the only controlled study utilizing a combination regimen and had 18 patients in the control group. These patients are not included in the integrated analysis of the combination therapy studies.							

2.4.2. Pharmacokinetics

Absorption

After reconstitution, Pixuvri is an aqueous solution intended for administration via intravenous infusion.

Following intravenous administration, plasma concentrations of pixantrone base reached the maximal concentration at the end of infusion and then declined poly-exponentially. The pharmacokinetics of Pixuvri was dose-independent in the 3 mg/m2 to105 mg/m2 dose range and no substantial differences were observed when the medicinal product was given as a single agent or in combination studies. Average exposures as single agent accounted for:

Pixuvri Dose (mg/m^2)	Number of patients	AUC (0-24h) (ng.hr/ml)
33	3	982 ± 115
49	6	1727 ± 474
88	2	3811

From an analysis of population PK data, for a target recorded dose of 50 mg/m2 of pixantrone base the median 28-day cycle exposure was 6320 ng.hr/ml (90% CI, 5990-6800 ng.hr/ml), for 3 doses / 4 week cycle.

Distribution

After 1-hr intravenous infusion, maximum plasma concentrations of pixantrone were reached at end of infusion. Thereafter, plasma concentrations declined poly-exponentially. There was no apparent dose dependency. Pharmacokinetic data after repeated doses is basically non-existent, but this is accepted given the intermittent dosing schedule. Distribution was extensive, with volume of distribution (Vss) estimations from single-agent studies varying between 94 to 972 L/m^2 in different studies and at different doses. Also the terminal half-life of pixantrone varied considerably between doses and studies and ranged between 6 to 36 hr. CL values ranged between 20-59 L/hr/m^2.

The protein binding in human plasma was weak, 57%, and independent of pixantrone concentration. A similar degree of binding was observed in plasma from different animal species.

Elimination

There is no human mass-balance study but it is considered that in view of the very long term collection period needed it would be very difficult to conduct such study in cancer patients. In a non-clinical mass-balance study in the rat, a relatively large part of the radioactivity (47%) persisted in the carcass after 192 hours (8 days), in agreement with a large body of distribution and a slow tissue release. In pharmacokinetic studies in humans, plasma sampling was only performed for 24 or 48 hours. Thus, it cannot be completely excluded that a long terminal elimination phase has been missed. However, there is no trend in the clinical safety data indicating that pixantrone concentrations accumulate markedly over time at 3 weekly administrations per 28-day period.

In animal studies, drug-related material was predominantly excreted in faeces, and urinary excretion data from humans may indicate a low renal excretion of pixantrone and its metabolites. However, as urinary sampling was only made for 24 hours, final conclusions on the importance of renal clearance cannot be drawn. Nevertheless, elimination in humans is likely to be primarily via hepatic routes. The degree of metabolism in human material *in vitro* was low, and the primary metabolic pathway was conjugation (acetylation) via N-acetyltranferase (NAT). Metabolites were only present in small quantities in urine and were found to be inactive. An *in vitro* study has confirmed there is no reversible metabolism to active pixantrone. It is not known which NAT isoform is involved in the metabolism of pixantrone but there is very limited transformation of pixantrone into acetylated metabolites and therefore a significant consequence of genetic polymorphism is not expected.

Pixantrone has a moderate to high total plasma clearance of 72.7 l/hr and a low renal excretion accounting for less than 10% of the administered dose in 0-24 hours. The terminal half-life ranged from 14.5 to 44.8 hr with a mean of 23.3 ± 8.0 (n=14, CV=34%) and a median of 21.2 hr. Due to the limited contribution of renal clearance, plasma clearance is mainly non-renal. Pixuvri may be metabolised in the liver and/or excreted in the bile. As metabolism appears to be limited, biliary excretion of unchanged pixantrone may be the major elimination pathway. Hepatic clearance approximates the hepatic plasma flow, suggesting a high hepatic extraction ratio and, therefore, efficient parent active substance elimination. Hepatic uptake of pixantrone is possibly mediated by OCT1 active transporters and biliary excretion by P-gp and BCRP.

Pixantrone had only a weak or no capability to inhibit P-gp, BCRP, and BSEP transport mechanism *in vitro*.

Pixantrone did inhibit OCT1-mediated metformin transport in vitro, but is not expected to inhibit OTC1 *in vivo* at clinically relevant concentrations. Pixantrone was a poor inhibitor of OATP1B1 and OATP1B3 uptake transporters in vitro.

Considering the *in vitro* metabolism, human urinary excretion and animal data together, the most important route of elimination for pixantrone in humans appears to be biliary excretion of unchanged compound.

Polymorphism in NATs or hepatic transport proteins may increase pharmacokinetic variability, but at present this cannot be handled in clinical practice in other ways than by individual dose adjustments based on haematological response.

Dose proportionality and time dependencies

- **Dose proportionality**

After the administration of pixantrone as a single agent or in combination with other drugs, systemic exposure (AUC0-24h) increased with dose, without a marked deviation from dose-linearity from 5 to 180 mg/m2 as shown in Figure 4 below.

In the single agent studies AUC0-24h accounted for more than 80% of the AUC.

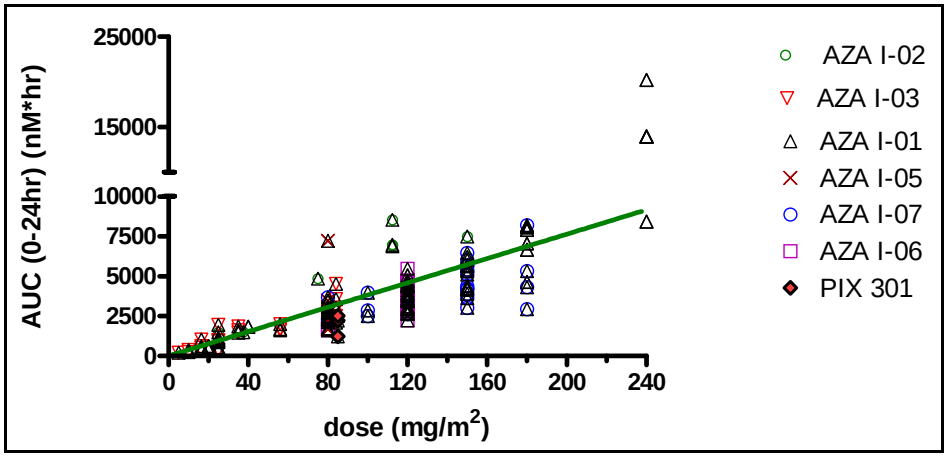


Figure 4: Relationship between AUC and Dose After Administration of Pixantrone as a Single Agent and in Combination Studies

- **Time dependency**

Time dependence was assessed in study PIX301. Although no definitive conclusions could be drawn due to the limited number of subjects, the PK appears time independent. The systemic exposures calculated after single and repeated administrations are comparable, as expected given its terminal half-life and the dosage interval. This is consistent with the lack of cumulative neutropaenia observed in the study.

Special populations

Intra- and inter-individual variability

In the phase I studies, inter-individual variability (CV%) was generally around 25-50%. The different dose groups were, however, small. As there is basically no data on repeated administration, intra-individual variability has not been determined.

Renal and hepatic impairment

There are no specific studies in renal or hepatic impairment.

Because the renal excretion appears not to be an important route of elimination caution should be exercised when administering pixuvri in patients with renal impairment (see section 4.2 of the SmPC).

Pixantrone is likely mainly eliminated via hepatic routes, and hepatic impairment may therefore be expected to increase pixantrone exposure. As biliary excretion may be the major route of elimination, pixantrone treatment should not be administered to patients with severe liver impairment (see section 4.3 of the SmPC). A reduced starting dose is not considered appropriate, as the effect of hepatic impairment is unknown and underexposure should be strictly avoided. Subsequent doses will be individualised based on e.g. haematological toxicity.

Acknowledging the difficulties in performing a specific pharmacokinetic study in patients with NHL and hepatic impairment, the CHMP accepted the absence of such study. The limited data on NHL patients with mild degree of liver impairment is reassuring with no significant worsening of liver function and with a safety profile similar to the rest of the safety population. The SmPC wording is considered appropriate to compensate for the lack of data in patients with hepatic impairment. Caution should be exercised in patients with mild or moderate liver impairment (see section 4.2 of the SmPC).

Gender

The gender effect on the pharmacokinetics of pixantrone was investigated within the retrospective pop-PK analysis performed with data from Phase I single-agent studies. No significant effect of gender was observed.

Elderly

The retrospective pop-PK analysis performed with data from Phase I single-agent trials did not highlight any influence of age on systemic clearance and volume of distribution. In the population analysis, there were only a few subjects above 65 years and no subjects above 70 years. The age range was somewhat wider among the patients included in the statistical analysis.

Race

There was no apparent difference in pharmacokinetic between Caucasian and Hispanics. Although most PK population were Caucasian the elimination of pixantrone is mainly hepatic with a limited degree of metabolism via acetylation by NAT. Therefore, no significant PK differences are expected in patients of Asian or African background.

Weight and Body surface area (BSA)

Clearance appears to be, to some extent, dependent on body size measures and dosing based on BSA is acceptable. However, data in obese patients is limited and it is advised to exercise caution when administering pixuvri in obese patients (see SmPC section 4.2).

Children

Pixantrone is at present not indicated for children, and no paediatric data are yet available.

Pharmacokinetic interaction studies

The risk for pharmacokinetic drug-drug interactions with pixantrone has mainly been evaluated *in vitro*.

Pixantrone metabolism appears to be mediated via N-acetyltransferases (NATs). The overall importance of metabolism for pixantrone elimination cannot be fully determined but may be small. In addition, interactions involving inhibition of NATs are not recognised as a problem to the same extent as interactions involving CYPs. Thus, the risk for clinically relevant interactions on the metabolic level affecting pixantrone concentrations is expected to be low. Based on *in vitro* studies, pixantrone was found to be a substrate for the membrane transport proteins P-gp/BRCP and OCT1 and agents which inhibit these transporters have the potential to decrease hepatic uptake and excretion efficiency of pixantrone. Blood counts should be closely monitored when co-administered with agents that inhibit such transporters such as cyclosporine A or tacrolimus, commonly used to control chronic graft-versus-host disease, and the anti-HIV agents: ritonavir, saquinavir or nelfinavir.

In addition, caution should be taken when pixantrone is continuously co-administered with efflux transport inducers such as rifampicin, carbamazepin and glucocorticoids, as pixantrone excretion might be increased with a consequent decrease of systemic exposure.

In vitro, pixantrone inhibited CYP1A2 at clinically relevant concentrations. The interaction has not been confirmed *in vivo*, however warnings in the SmPC for CYP1A2 substrates with narrow therapeutic index such as theophylline have been included (see SmPC section 4.5). The potential for pixantrone to reversibly inhibit CYP2B6 and CYP2C8 at clinically relevant concentration is low. Definite conclusions on the risk for inhibition of CYP2C8 activity cannot be drawn from the data submitted. Although the *in vitro* signal is relatively weak, given that an inhibitory potential cannot be completely excluded based on *in vitro* data, a warning against some relevant CYP2C8 substrates such as *repaglinide*, *rosiglitazone*, *or paclitaxel* has been included in section 4.5 of the SmPC.

2.4.3. Pharmacodynamics

Mechanism of action

No clinical pharmacodynamics studies were conducted.

Primary and Secondary pharmacology

In humans haematological toxicity, mainly neutropaenia and leucopaenia is the most common adverse event and determined the dose limiting toxicity (DLT). There is a direct relationship between exposure and neutropenia. During the pivotal study after an initial decline from baseline to cycle 2, mean neutrophil nadirs remain stable through subsequent cycles.

Data pooled from four single agent studies (AZA-I-01, 02, 03 and PIX 301) using different doses of pixantrone and in different cancer populations have shown a relationship between plasma exposure (AUC) and pharmacodynamic effect (PFS and neutropaenia).

No specific studies on pharmacodynamic interactions with other medicinal products or substances and genetic differences in PD response were submitted but none are required.

2.4.4. Discussion on clinical pharmacology

Dosing based on BSA as proposed for pixantrone is acceptable

The proposed contraindication in patients with severe liver impairment and the caution recommended when administering pixuvri in patients with renal impairment and in patients with mild or moderate liver impairment is acceptable.

2.4.5. Conclusions on clinical pharmacology

Overall, based on the data submitted, the clinical pharmacology of pixantrone has been adequately investigated

2.5. Clinical efficacy

2.5.1. Dose response study(ies)

Three phase 1 dose-escalation single agent studies explored two different treatment regimens: pixantrone every 3 weeks and pixantrone weekly for 3 consecutive weeks with 1 week rest. The MTD and recommended dose was assessed in each study and the dose-limiting toxicity was reversible grade 4 neutropenia of more than 4 days' duration (see table 8).

Table 8: MTD and RD in the phase I dose escalation studies

Study No	Disease	Schedule	MTD	RD
AZA I-01	Solid tumour	q3w	240 mg/m^2	180 mg/m^2
AZA I-02	Solid tumour	q1wx3/q4w	150 mg/m^2	75 mg/m^2
AZA I-03	NHL/CLL	q1wx3/q4w	84 mg/m^2	84 mg/m^2

MTD: maximum tolerated dose; RD: recommended dose
q3w: once every 3 weeks/ 3 week cycle
q1wx3/q4w: once a week for 3 weeks/4 week cycle

The final schedule selected was once a week for 3 weeks on a 4 week cycle with a recommended dose of between 75-84 mg/m^2.

AZA I-03 enrolled 26 patients with relapsed heavily pretreated NHL or CLL. There were 5 responses, 3 CRs and 2 PRs. All CRs occurred in the 84 mg/m^2 dose cohort and included a patient with relapsed DLBCL, a patient with primary refractory follicular NHL with bone marrow involvement and pleural effusions, and a patient with relapsed advanced aggressive B-NHL.

Based on this data the dose of 85 mg/m^2 on days 1,8,15 of a 28 day cycle was chosen for further phase II development (AZA II-01).

2.5.2. Main study

PIX301: international, multicentre, randomised, active controlled, open-label Phase III study designed to compare the efficacy and safety of pixantrone to that of physician's choice of protocol-specified single-agent therapies in patients with relapsed or refractory aggressive NHL who had received at least two prior NHL regimens.

Methods

Study Participants

Inclusion Criteria

1. Histologically confirmed aggressive [de novo or transformed] NHL according to REAL/WHO classification. Lymphoma types permitted were:

- follicular lymphoma – grade III
- transformed indolent lymphoma (areas of follicularity allowed)
- diffuse large B-cell lymphoma
- mediastinal large B-cell lymphoma
- primary effusion lymphoma (includes previously called immunoblastic lymphoma)
- peripheral T-cell lymphoma not otherwise characterized (encompasses diffuse mixed cell lymphoma)
- anaplastic large cell lymphoma and T/null cell, primary systemic type

2. Patients must have received rituximab in prior regimens in those countries where it was the standard of care and available at the patient's institution and when neoplastic cells expressed CD20.
3. At least one objectively measurable lesion as demonstrated by CT, spiral CT, or MRI that could be followed for response as a target lesion. Patients with skin lesions, palpable lymph nodes, spleen or bone marrow as the only site of disease were NOT eligible.
4. Relapse (with evidence of disease progression) after 2 or more prior regimens of chemotherapy, including: first-line treatment with a standard anthracycline-containing regimen such as CHOP or equivalent, at least 1 additional combination chemotherapy regimen. High dose chemotherapy or chemoradiotherapy with autologous stem cell support counted as 1 prior regimen. Allogenic transplant counted as 1 prior regimen.
5. Patients must have been sensitive to the last anthracycline/anthracenedione containing regimen. Sensitive was defined as a response (confirmed or unconfirmed PR or CR) to an anthracycline/anthracenedione with relapse after a response duration ≥ 6 months.
6. Age ≥ 18 years.
7. ECOG performance status of $\leq$ 2.
8. Hb ≥ 8g/dL, neutrophils ≥ 1.5 x 109/L and platelets ≥ 50 x109/L; if there was bone marrow involvement, neutrophils > 0.5 x 109/L, platelets >10 x 109/L and the ability to provide platelet transfusion were acceptable.
9. Serum bilirubin $\leq$ 1.5 x the institution's upper limit normal (ULN) and creatinine $\leq$ 1.5 ULN and alkaline phosphatase $\leq$ 2.0 x the institution's ULN and AST or ALT $\leq$ 2.0 x the institution's ULN. If hepatic involvement by lymphoma was present, AST or ALT could be $\leq$ 5.0 x the institution's ULN.
10. Patients previously treated with one of the comparative agents had to be sensitive to that agent, if it was to be used in this trial. Sensitive was defined as previous response to that agent with relapse after a response duration ≥ 6 months.
11. LVEF ≥ 50% determined by MUGA scan.

Exclusion criteria

1. Prior treatment with a cumulative dose of doxorubicin or equivalent exceeding 450 mg/m² according to the calculation index X/450 + Y/160 > 1 where X was the doxorubicin dose in mg/m² and Y the mitoxantrone dose in mg/m².
2. Histological diagnosis of Burkitt lymphoma, lymphoblastic lymphoma, or mantle cell lymphoma.
3. Active CNS lymphoma involvement based on clinical evaluation

4. HIV-related lymphoma.
5. Any chemotherapy, radiotherapy, or other anticancer treatment (including corticosteroids ≥ 10 mg/day of prednisone or equivalent) within the 2 weeks before randomization. For radioimmunoconjugate therapy, there was to be 8 weeks since last dose or platelet recovery to ≥ 50 x 109/L prior to randomization.
6. Clinically significant cardiovascular abnormalities (equal to NYHA grade III- IV), myocardialinfarction within the prior 6 months, severe arrhythmia, uncontrolled hypertension, or uncontrolled angina.
7. History of, or clinical symptoms suggesting, HIV infection.

Treatments

Patients were randomly assigned in a 1:1 ratio by IVRS to one of two treatment groups: pixantrone or comparator.

Experimental arm

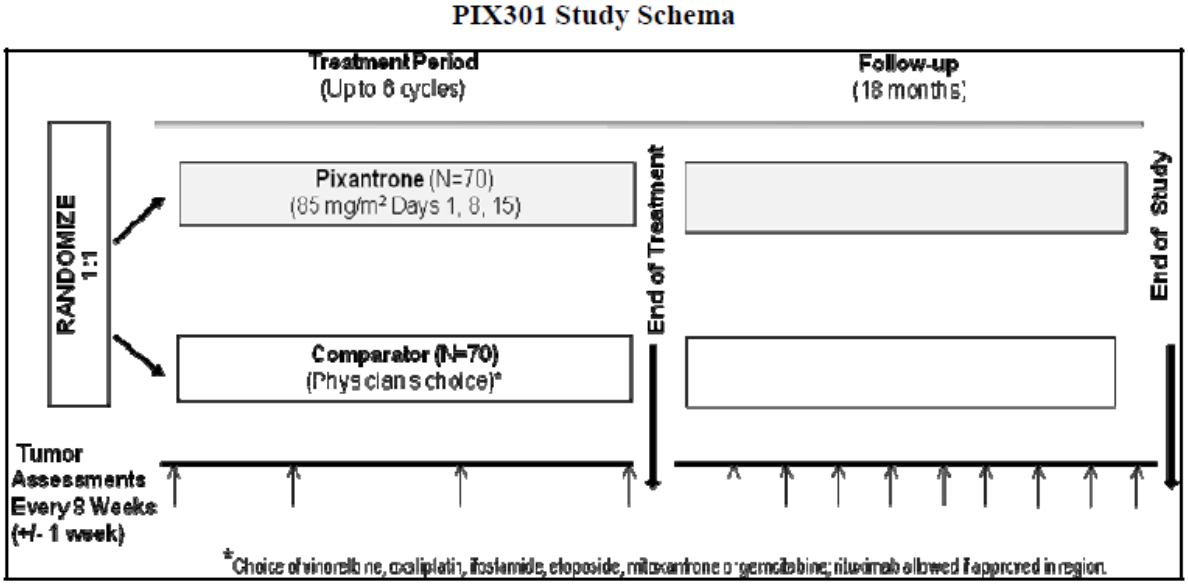
Pixantrone 85 mg/m^2 by IV infusion on Days 1, 8, and 15 of each 4-week cycle for up to 6 cycles

Comparator arm

Physician's choice of 1 out of 6 specified single agents that are approved for cancer indications other than aggressive NHL but with demonstrated activity in aggressive NHL. Vinorelbine, oxaliplatin, ifosfamide, etoposide, mitoxantrone, gemcitabine (US) for up to 6 cycles or rituximab (US) were administered using the protocol-specified doses and schedules.

After completion of treatments (up to 6 cycles) there was an 18 month follow up.

The study scheme is shown below.



Objectives

Primary objective

To compare the efficacy of BBR 2778 (pixantrone) to a selection of single agents in terms of the complete response (CR) and unconfirmed complete response (CRu) rate.

Outcomes/endpoints

Primary endpoint

CR or CRu rate (ITT) assessed by the Independent Assessment Panel (IAP) based on the Report of the International Workshop to Standardize Response Criteria. These criteria are also known as the International Working Group (IWG) criteria (Cheson 1999).

Secondary endpoint

- Progression-free survival (PFS)
- Response rate lasting at least 4 months
- Overall survival (OS)

Other pre-specified endpoints:

- Overall response rate (ORR): The total proportion of patients with a CR/CRu/PR as assessed by the IAP.
- Time to response
- Time to CR(CR or CRu)
- Duration of response

Efficacy was assessed on Days 50, 106, and at any time a patient discontinued study treatment (End Of Treatment or EOT), then every 8 weeks ± 1 week during the follow-up period.

An independent assessment was conducted centrally by an Independent Radiology Committee (IRC) comprised of a radiologist and by an Independent Assessment Panel (IAP) comprised of a radiologist, oncologist, and pathologist. An independent central pathology assessment was also planned.

The IRC and IAP assessments were organized by an independent CRO and included physicians not affiliated with the Sponsor and blinded to treatment assignment.

The IRC based its assessment on the radiologic response. The IAP subsequently provided the final assessment of response. The investigator response was also collected and used as a sensitivity analysis to assess concordance of investigator and IAP responses.

The IRC and IAP remained blinded during the follow-up period and response assessments in follow-up were included in the End of Study (EOS) analysis.

Sample size

The planned enrolment was 320 patients based on the CR seen on AZA-II-01 study of 15%. There were limited literature data on outcomes in the third-line setting and it was believed that the CR/CRu rate with comparators was ≤ 5%. In order to detect a 10% difference on CR/CRu rate between the arms a total of 320 patients (160 per arm) was required to achieve 80% power.

An interim efficacy analysis that was to occur after 50% of the planned 320 patients had been accrued was cancelled. At the time enrolment was discontinued, the IAP remained blinded to patient treatment assignment, and a majority of the patients had not yet had independent assessment of response. With the enrolment of 140 patients, the study was considered sufficiently powered (about 80%) to detect a 15% difference in the CR/CRu rate, assuming a ≥ 18% CR/CRu rate in the pixantrone arm.

Randomisation

Randomisation was 1:1 ratio.

Stratification was by 3 factors:

- Region (North America [NA] vs. Western Europe [WE] vs. Rest of World [ROW])

- International Prognostic Index (IPI) Score- internationally accepted prognostic index for patients with aggressive NHL.

- Prior SCT

Blinding (masking)

This was an open-label study. Treatment assignment was known to patients, investigators, clinical research associates (CRAs) and pharmacovigilance but the IRC and IAP were blinded to treatment assignments and to investigator assessments of response.

Statistical methods

Statistical Analysis Plan (SAP):

- The first and primary analysis, the End of Treatment (EOT), used a database cutoff after the last patient completed the end-of-treatment visit.
- An End of Study (EOS) analysis was performed on data from the treatment and follow-up periods.

There were 2 predefined study populations

- ITT (primary analysis)
- HITT (secondary analysis). This was a histologically-confirmed aggressive NHL population by retrospective independent central pathology assessment

Patients who had neither progressed nor died were censored for PFS at the date last assessed and found to be progression-free. Patients receiving a new treatment for aggressive NHL in the absence of progression were considered as progressing at the time of retreatment. Since the study was designed to follow patients through 18 months after 6 cycles of treatment, patients alive after 24 months were censored at 24 months.

Results

After 4 years the study was closed to enrolment (140 patients randomized) due to extremely slow accrual.

Baseline disease and demographics were similar between the two groups with no significant differences. DLBCL followed by transformed indolent lymphoma were the most common NHL types. Very few patients had T cell NHL. All patients had received at least two prior chemotherapy treatments and above 50% had received at least three regimens. Previous median anthracycline dose received was approx.300 mg/m^2. The majority of patients were refractory to their last therapy (57% refractory both groups) and a mean time from last chemotherapy to randomisation was longer than 13 months. Of note no black patients were recruited.

Region was one of the stratification factors and notably only 38/140 patients were recruited in "Western Europe".

Participant flow

PIX301 Patient Disposition, n (%)	Pixantrone (N=70)	Comparator (N=70)
Study Completion		
Completion of Protocol Treatment (6 Cycles)	20 (28.6%)	16 (22.9%)
Discontinued Treatment	50 (71.4%)	54 (77.1%)
Reasons for Treatment Discontinuation		
Progressive/Relapsed Disease	28 (40.0%)	39 (55.7%)
AEs	15 (21.4%)	9 (12.9%)
Follow-up Period		
Entered follow-up	52 (74.3%)	43 (61.4%)
Completed 18 Months of Follow-up	**15 (28.8%)**	**11 (25.6%)**
Died During Follow-up	30 (57.7%)	26 (60.5%)

Only 36 patients completed protocol-defined treatment (28.6% of the pixantrone group and 22.9% of the comparator group). The most common reason for discontinuation was progressive/relapsed disease (40% of the pixantrone group, 56% of the comparator group).

52 patients in the pixantrone group and 43 patients in the comparator entered the follow-up period and constitute the follow-up population.

Recruitment

Study period: First patient randomized 12 October 2004; last patient last treatment visit 28 August 2008; database cut-off for End-of-Treatment (EOT) analysis 30 September 2008; last patient last follow-up 16 February 2010.

Conduct of the study

Study 301 was conducted in 66 sites in 17 countries: Argentina, Bulgaria, Colombia, Ecuador, France, Germany, Hungary, India, Italy, Peru, Poland, Romania, Russia, Ukraine, United Kingdom, the United States and Uruguay.

Enrolment was not distributed evenly among the geographically defined strata. Eight patients were enrolled from North America (all from the US), 38 patients from Western Europe, and 94 patients from the Rest of World.

Changes in the Conduct of the Study or Planned Analyses
A country-specific protocol for Spain was generated with the amendment to the original protocol that showed only etoposide and mitoxantrone as options in the comparator group.

Major amendments are listed below:

Amendment 1 (14 October 2004):

- Inclusion criteria modified to state patients must be sensitive to their last anthracycline/anthracendione regimen.

Amendment 2 (01 March 2005):

A country-specific protocol for the US was generated with the amendment to the protocol (non-Spain) that added gemcitabine and rituximab to the existing list of permitted drugs for the comparator group.

- Gemcitabine and rituximab (CD 20+ patients only) were added as options for comparator group drugs and dosage specifications for oxaliplatin were removed.
- Follicular lymphoma grade III was removed from eligible disease types.

Amendment 3 (08 February 2006):

- "With evidence of disease progression" added to inclusion criteria requiring relapse after 2 or more prior regimens and "(confirmed or unconfirmed PR or CR)" added to inclusion criteria requiring prior response to anthracycline/anthracenedione.
- Expected accrual time changed from 12 to 36 months to reflect slower than originally expected enrollment.
- Geographic region for stratification previously defined as "Eastern Europe" changed to "Rest of World," and text added to state that stratification variables will be investigated as covariates for the primary and secondary analyses.

Amendment 4 (07 December 2006):

- The secondary endpoint time to progression (TTP) was changed to progression-free survival (PFS).

Amendment 4 (20 June 2007):

- Follicular lymphoma (grade III) was added to inclusion criterion #1.

Baseline data

For demographics refer to table 9 for baseline histology see table 10, for baseline disease characteristics see table 11 and for prior NHL treatment table 12.

Table 9: PIX301 Demographic Characteristics (ITT Population)

	Pixantrone (N=70)	Comparator (N=70)
Age at Randomization (years)		
Mean (SD)	58.2 (13.5)	56.2 (12.9)
Median (range)	60.0 (18-80)	58.0 (26-82)
Age Category at Randomization, n (%)		
18 to < 30	5 (7.1%)	2 (2.9%)
30 to < 40	2 (2.9%)	9 (12.9%)
40 to < 50	9 (12.9%)	7 (10.0%)
50 to < 60	18 (25.7%)	21 (30.0%)
60 to < 70	20 (28.6%)	21 (30.0%)
70 to < 80	15 (21.4%)	9 (12.9%)
≥ 80	1 (1.4%)	1 (1.4%)
Sex, n (%)		
Male	46 (65.7%)	40 (57.1%)
Female	24 (34.3%)	30 (42.9%)
Race, n (%)		
Caucasian	46 (65.7%)	44 (62.9%)
Black	0	0
Asian	10 (14.3%)	13 (18.6%)
Hispanic	7 (10.0%)	6 (8.6%)
Native American	1 (1.4%)	1 (1.4%)
Other	6 (8.6%)	6 (8.6%)
Baseline ECOG Performance Status, n (%)		
0	26 (37.1%)	23 (32.9%)
1	30 (42.9%)	32 (45.7%)
2	14 (20.0%)	14 (20%)
3	0	1 (1.4%)
Geographic Region, n (%)		
North America	4 (5.7%)	4 (5.7%)
Western Europe	19 (27.1%)	19 (27.1%)
Rest of World	47 (67.1%)	47 (67.1%)
Weight (kg)		
Mean (SD)	70.9 (15.8)	68.7 (15.3)
Median (range)	70.0 (45-117)	67.5 (37-115)

SD=standard deviation
Fisher exact test was used to compare proportions between the group and a two-sided student's t-test was used in the comparison of means between treatment groups.
Source: 14.1.3 and 14.1.3.4 (PIX301 CSR)

Table 10: PIX301 Baseline Histology

	Pixantrone (N=70)	Comparator (N=70)

Diffuse large B-cell lymphoma	53 (75.7%)	51 (72.9%)
Transformed indolent lymphoma	10 (14.3%)	9 (12.9%)
Follicular lymphoma grade III	1 (1.4%)	2 (2.9%)
Peripheral T-cell lymphoma NOC	3 (4.3%)	7 (10.0%)
Anaplastic large cell lymphoma/null cell/primary systemic	3 (4.3%)	1 (1.4%)

Table 11: PIX301 Baseline Disease Characteristics

	Pixantrone (N=70)	**Comparator** (N=70)
Duration of NHL (months)		
Mean (SD)	43.6 (35.6)	46.6 (51.7)
Median (range)	32.0 (7-160)	31.6 (0-333)
Ann Arbor Stage of NHL, n (%)		
I/II	19 (27.1%)	14 (20.0%)
III/IV	51 (72.9%)	56 (80.0%)
International Prognostic Index, n (%)		
0, 1	21 (30.0%)	17 (24.3%)
≥ 2	49 (70%)	52 (74.3%)
Missing	0	1 (1.4%)
Number of Extranodal Sites, n (%)		
0	35 (50%)	35 (50%)
≥ 1	34 (48.6%)	33 (47.1%)
Missing	1 (1.4%)	2 (2.9%)
Time from Last Chemotherapy to Randomization (months)		
Mean (SD)	13.6 (15.7)	13.2 (23.5)
Median (range)	9.0 (1-86)	8.0 (1-190)

SD=standard deviation
Fisher exact test was used to compare proportions between the groups, and a two-sided student's t test was used in the comparison of means between treatment groups. P-values are for reference purposes only.
Source: 14.1.5 (PIX301 CSR)

Table 12: PIX301 Prior NHL Treatment

	Pixantrone (N=70)	**Comparator** (N=70)
Chemotherapy Regimens		
Mean (SD)	2.9 (1.2)	3.1 (1.2)
Median (range)	3.0 (2.0-9.0)	3.0 (2.0 -9.0)
Number of Chemotherapy Regimens		
2	**32 (45.7%)**	**24 (34.3%)**
3-5	**35 (50%)**	**42 (60%)**
≥ 6	3 (4.3%)	4 (5.7%)
Category of Prior Chemotherapy		

	Pixantrone (N=70)	**Comparator** (N=70)
Biologics (anti-CD20 mAB)	38 (54.3%)	39 (55.7%)
Anthracyclines/anthracenediones	70 (100.0%)	70 (100.0%)
Other Topoisomerase Inhibitors[a]	53 (75.7%)	55 (78.6%)
Platinum-based agents	36 (51.4%)	35 (50.0%)
Antimetabolites	42 (60.0%)	44 (62.9%)
Alkylating agents	70 (100.0%)	70 (100.0%)
SPs/MIs (spindle poison/mitotic inhibitors)	70 (100.0%)	69 (98.6%)
Corticosteroids	66 (94.3%)	65 (92.9%)
Other[b]	**21 (30.0%)**	**30 (42.9%)**
Disease Response Category		
Refractory	**40 (57.1%)**	**40 (57.1%)**
Relapsed	28 (40.0%)	30 (42.9%)
Missing	2 (2.9%)	0
Patients who had Radiotherapy, n (%)		
	34 (48.6%)	**30 (42.9%)**
Received SCT, n (%)		
	11 (15.7%)	10 (14.3%)
Anthracycline Dose Equivalent (mg/m$^{2)b}$		
Mean (SD)	284.8 (98.1)	321.9 (119.0)
Median (range)	**292.9 (51-472)**	**315.5 (15-681)**

a Other topoisomerase inhibitors were etoposide and teniposide.
b Other includes targeted therapies, nonclassified anticancer therapies and supportive therapies.
Fisher exact test was used to compare proportions between groups and a two-sided student's t test was used to compare means between treatment groups. P-values are for reference purposes only
* P-value ≤ 0.05.

Numbers analysed

Table 13- PIX301 Analysis Populations

	Pixantrone	**Comparator**
Intent-to-Treat Population	70 (100%)	70 (100%)
Histologically Intent-to-Treat Population	54 (77.1%)	50 (71.4%)

The number of patients in the ITT as well as HITT populations was similar in both arms.

Thirty six (36) patients of the ITT population were not in the HITT population and they were not centralised to one particular region. Retrospective data on these 36 patients showed

- 25 patients had confirmed aggressive NHL
- 3 patients had low-grade NHL
- For 6 patients there was insufficient information
- 2 patients had no pathology reports

Outcomes and estimation

Table 14- Efficacy results ITT population by IAP (primary and secondary endpoints)

Endpoint		Pixantrone (n=70)	Comparator (n=70)	
Primary	CR/CRu at EOT by IAP	14 (20%) 95% CI (11.4%, 31.3%)	4 (5.7%) 95% CI (1.6%, 14.0%)	p=0.021
Primary, supportive	CR/CRu at EOS by IAP	17 (24.3%) 95% CI (14.8%, 36.0%)	5 (7.1%) 95% CI (2.4%, 15.9%)	p=0.009
Primary, supportive	CR/CRu at EOT by investigator	12 (17.1%) 95% CI (9.2%, 28.0%)	4 (5.7%) 95% CI (1.6%, 14.0%)	p=0.060
Primary, supportive	CR/CRu at EOS by investigator	15 (21.4%) 95% CI (12.5%, 32.9%)	6 (8.6%) 95% CI (3.2%, 17.7%)	p=0.056
Primary, supportive	CR/CRu at EOT, HITT	9 (16.7%) 95% CI (7.9%, 29.3%)	3 (6.0%) 95% CI (1.3%, 16.5%)	p=0.126
Primary, supportive	CR/CRu at EOS, HITT	10 (18.5%) 95% CI (9.3%, 31.4%)	4 (8.0%) 95% CI (2.2%, 19.2%)	p=0.154
Secondary	PFS by IAP	Median 5.3 m 95% CI (2.3, 6.2)	Median 2.6 m 95% CI (1.9, 3.5)	HR (95% CI)=0.60 (0.42, 0.86), p=0.005
Secondary, supportive	PFS investigator	Median 4.2 m 95% CI (2.4, 6.9)	Median 2.6 m 95% CI (1.9, 3.5)	HR (95% CI)=0.64 (0.45, 0.92), p=0.015
Secondary, supportive	PFS, HITT	Median 5.0 m 95% CI (2.3, 6.1)	Median 2.6 m 95% CI (1.9, 3.4)	HR (95% CI)=0.54 (0.36, 0.82), p=0.003
Secondary	OS	Median 10.2 m 95% CI (6.4, 15.7)	Median 7.6 m 95% CI (5.4, 9.3)	HR (95% CI)=0.79 (0.53, 1.18), p=0.251
Secondary	RR ≥4 months	12 (17.1%)	6 (8.6%)	p=0.206
Pre-specified	CR/CRu/PR rate by IAP at EOS	28 (40%)	10 (14.3%)	p=0.001

For the primary endpoint at EOT by IAP, there were 8 (11.4%) CRs in the Pixantrone arm vs 0 CR in the comparator arm and 6 (8.6%) CRu in the Pixantrone arm vs 4 (5.7%) CRu in the comparator arm.

The results also favoured pixantrone for duration of all responses, duration of CR/CRu and time to CR although no statistical significant differences were observed. Time to response was the same for both arms (median 1.9 months).

Ancillary analyses

1. Investigator Assessment

Three cases of CR were recorded in the comparator arm (4.3%) that were not seen in the IAP assessment and led to a non statistical difference in CR/Cru rate at the EOT. Twelve (12) of 70 patients (17.1%; 95% CI 9.2%, 28.0%) in the pixantrone treatment group had achieved a CR/CRu at the time of the EOT analysis, compared with 4 of 70 patients (5.7%; 95% CI 1.6%, 14.0%) in the comparator

group (*P=0.060*). PFS showed statistical significant difference in favour of pixantrone (4.2 months vs. 2.6 months, HR=0.64 (0.45, 0.92), *P=0.015*).

2. HITT analysis

The HITT population consisted of 104 patients (54 pixantrone vs 50 comparator). 36 patients of the ITT population were not in the HITT (25 patients had confirmed aggressive NHL, 3 had low-grade NHL, 6 had insufficient information and 2 patients had no pathology reports).

In this analysis all the endpoints favoured pixantrone but only PFS (5.0 months vs. 2.6 months, HR=0.54 (0.36, 0.82), p = 0.003) and ORR at EOT (CR/Cru/PR 33.3% vs. 16.0%, p = 0.04) showed a statistically significant difference.

Subgroup analyses

Table 15: Number of patients in subgroups

Subgroup	Pixantrone (N=70)	Comparator (N=70)
Prior SCT	11	10
Western Europe	19	19
Prior Rituximab	38	39
Source: PIX301 CSR Tables 14.1.3, 14.1.5, and 14.1.6		

i. The Effect of Rituximab on Efficacy of Pixantrone

Thirty eight (54.3%) patients randomized to the pixantrone arm and 39 (55.7%) patients randomized to the comparator arm, received rituximab therapy prior to study entry.

Table 16 presents the overall response rates (ORRs) for pixantrone and comparator patients who did or did not receive prior rituximab.

Table 16: Overall response rate by prior rituximab experience and prior line of therapy

	Prior lines of therapy	Received Prior Rituximab		No Prior Rituximab	
		Pixantrone	Comparator	Pixantrone	Comparator
ORR, n (%)	Overall	12/38 (31.6%)	7/39 (17.9%)	14/32 (43.8%)	3/31 (9.7%)
	2	5/10 (50.0%)	0/9	11/22 (50.0%)	2/15 (13.3%)
	3	6/15 (40.0%)	3/16 (18.8%)	4/9 (44.4%)	1/16 (6.3%)
	4 or more	1/13 (7.7%)	4/14 (28.6%)	1/1 (100.0%)	0/0
Source: Table 2.7.4.7.2.15, PIX301 CSR Table 14.2.11.4					

The effect of prior rituximab therapy on progression-free survival (PFS) was also evaluated by the number of prior regimens. The median time to progression and the hazard ratio are presented in Table 17.

Table 17: Progression-free survival by prior rituximab experience and prior line of therapy

	Prior lines of therapy	Received Prior Rituximab		No Prior Rituximab	
		Pixantrone	Comparator	Pixantrone	Comparator
PFS, median (months) (95% CI); Hazard Ratio (95% CI)	Overall	3.3 (2.3, 5.7)	2.5 (1.9, 3.4)	6.1 (2.2, 10.3)	3.4 (1.7, 4.1)
		0.83 (0.51, 1.34)		0.40 (0.23, 0.69)	
	2	5.7 (1.1, 14.6)	2.8 (0.7, 4.3)	5.7 (2.0, 9.0)	1.9 (0.8, 4.9)
		0.28 (0.08, 0.94)		0.49 (0.24, 0.99)	
	3	3.3 (1.1, 6.7)	2.8 (1.4, 7.8)	6.5 (1.9, NA)	3.4 (1.3, 4.1)
		1.44 (0.66, 3.15)		0.29 (0.10, 0.82)	
Source: Tables 2.7.4.7.2.9, 2.7.4.7.2.10, 2.7.4.7.2.11, 2.7.4.7.2.12, 2.7.4.7.2.13, and 2.7.4.7.2.14.					

ii. Efficacy of Pixantrone in Patients with Prior Stem Cell Transplantation

Twenty-one patients with prior autologous stem cell transplants were enrolled in PIX301. Patients with prior stem cell transplants had a median of 3.0 and a mean of 4.1 prior regimens including conditioning regimens for stem cell transplantation. All stem cell transplant patients, with the exception of 1 in the comparator arm, received prior rituximab therapy.

Of the 11 pixantrone patients with prior stem cell transplants, 2 were responders (18%), 1 CR and 1 partial response (PR). In the control group there were 3 responders (33%), 2 CRu and 1 PR. Given the small number of patients and the high number of prior regimens, it is not possible to draw firm conclusions regarding the efficacy of pixantrone in patients following stem cell transplant.

iii. Efficacy in European Patients

Table 18, provides a comparison of the demographic and baseline disease characteristics between patients recruited from WE versus ROW.

There were a total of 38 WE patients enrolled in PIX301, 19 per study arm. Of the 19 patients enrolled in Europe, there were 3 responders (16%); 2 with PR and 1 with CRu.

Most patients from Europe were heavily pre-treated with multiple combinations regimens including rituximab (Table 8), had a short interval from their last regimen, and nearly half of the patients had rapidly advancing disease. Compared to other patients enrolled in the study, patients entered in Europe were later stage patients with highly aggressive disease.

Table 8: Baseline demographic and disease characteristics of western Europe compared to rest of world

	Western Europe		Rest of World	
	Pixantrone (N=19)	**Comparator (N=19)**	**Pixantrone (N=47)**	**Comparator (N=47)**
Gender				
Male	14 (73.7%)	6 (31.6%)	30 (63.8%)	30 (63.8%)
Female	5 (26.3%)	13 (68.4%)	17 (36.2%)	17 (36.2%)
Race				
Caucasian	18 (94.7%)	18 (94.7%)	24 (51.1%)	22 (46.8%)
Non-Caucasian	1 (5.3%)	1 (5.3%)	23 (48.9%)	25 (53.2%)
Age				
≤ 60 Years	8 (42.1%)	8 (42.1%)	29 (61.7%)	33 (70.2%)
> 60 Years	11 (57.9%)	11 (57.9%)	18 (38.3%)	14 (29.8%)
Duration of NHL (Median no. of Months)	32.4	31.3	29.7	33.0
Prior Stem Cell Transplant				
Yes	5 (26.3%)	5 (26.3%)	4 (8.5%)	4 (8.5%)
No	14 (73.7%)	14 (73.7%)	43 (91.5%)	43 (91.5%)
Number of Previous Chemotherapy Regimens				
2	6 (31.6%)	3 (15.8%)	25 (53.2%)	20 (42.6%)
3	5 (26.3%)	9 (47.4%)	18 (38.3%)	22 (46.8%)
4	6 (31.6%)	3 (15.8%)	1 (2.1%)	3 (6.4%)
5	1 (5.3%)	3 (15.8%)	3 (6.4%)	1 (2.1%)
6+	1 (5.3%)	1 (5.3%)	0	1 (2.1%)
Prior Anti-CD20 mAB				
Yes	17 (89.5%)	17 (89.5%)	17 (36.2%)	18 (38.3%)
No	2 (10.5%)	2 (10.5%)	30 (63.8%)	29 (61.7%)
Time from Start of Most Recent Chemotherapy to Randomization (Months)				
Median	6.0	8.0	9.0	8.0
Time from End of Most Recent Chemotherapy to Randomization (Months)				
Median	3.0	6.0	5.0	6.0
Source: Tables 2.7.4.7.1.5, 2.7.4.7.1.7, 2.7.4.7.1.9, and 2.7.4.7.1.14; PIX301 CSR Tables 14.1.3.2, 14.1.5.2, and 14.1.6.2				

Summary of main study(ies)

The following table summarises 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 19: Summary of Efficacy for trial PIX301

Title: Pixantrone (BBR 2778) versus Other Chemotherapeutic Agents for Third-line Single Agent Treatment of Patients with Relapsed Aggressive Non-Hodgkin's Lymphoma: A Randomized, Controlled, Phase III Comparative Trial

Study identifier	PIX301		
Design	Multicenter, multinational, open-label, randomised, comparative study		
	Duration of treatment: Duration of Run-in phase: Duration of Extension phase:		Up to 28 weeks not applicable not applicable
Hypothesis	Superiority		
Treatments groups	Pixantrone		85 mg/m^2 on days 1, 8, and 15 of 28-day (four-week) cycles, 70 patients randomised
	Most appropriate comparator drug selected from a provided list		Reference for maximum dose to administer was provided in the protocol
Endpoints and definitions	Primary endpoint	Complete response (CR) and complete response unconfirmed (CRu rate)	It was defined as the proportion of patients with CR or CRu as assessed by the Independent Assessment Panel (IAP)
	Secondary endpoint	Overall Survival (OS)	It was defined as the time between the date of randomisation and the date of death due to any cause. If a patient was not known to have died, survival was censored at the time of last contact/last date patient was seen alive. Patients still alive at the end of the study were censored at that time.
	Secondary endpoint	Response Rate Lasting at Least 4 months:	It was defined as the total proportion of patients with CR, CRu, or partial response (PR) with a difference from the first documented objective response to disease progression or death of at least 4 months. Patients who had a response and later underwent engraftment were censored at the start of the induction treatment.
	Secondary endpoint	Progression-Free Survival (PFS)	It was defined as the time between the date of randomisation and the date of the initial documentation of progressive/relapsed disease or death due to any cause. Patients who received subsequent therapy were considered as progressing at the start of that therapy. PFS for patients who were alive without disease progression at their date of last tumor assessment was censored at the date of last tumor assessment.
	Other endpoint	Overall Response Rate (ORR)	It was defined as the total proportion of patients with CR, CRu, or PR as assessed by the IAP.
	Other endpoint	Time to Response	It was defined as the time between the date of randomisation and the date of the initial response independent of the duration.
	Other endpoint	Time to Complete Response:	It was defined as the time between the date of randomisation and the date of the initial CR or CRu.

	Other endpoint	Duration of Response	It was defined as the time from the first documented objective response to disease progression/relapse or death. Patients who received subsequent therapy were considered as progressing at the start of that therapy. Patients who were still responding at the date of their last tumor assessment were censored at the date of last tumor assessment.
Database lock	Cut-off date (End of treatment[EOT]): 30 September 2008 Follow-up period: 18 months (End of study [EOS])		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat EOT and/or EOS		
Descriptive statistics and estimate variability And Effect estimate per comparison	Treatment group	Pixantrone	Comparator agents
	Number of subject	70	70
	CR/CRu rate (EOT), n (%)	14 (20.0)	4 (5.7)
	95% CI	11.4% – 31.3%	1.6% – 14.0%
	P-value (Fisher exact test)	0.021	
	Difference (95% CI)	14.3% (3.5% - 25.1%)	
	OS (EOS) No of events (%)	47 (67)	52 (74)
	Median survival (months) (95% CI)	10.2 (6.4 – 15.7)	7.6 (5.4 – 9.3)
	P-value (Log-rank test)	0.251	
	Hazard Ratio (95% CI)	0.79 (0.53-1.18)	
	Responses Lasting ≥ 4 Months (EOS) No of patients (%)	12 (17.1)	6 (8.6)
	95% CI	9.2% - 28.0%	3.2% - 17.7%
	P-value (Fisher exact test)	0.206	
	Difference (95% CI)	8.6% (-2.4% - 19.6%)	
	PFS (EOS) No of events (%)	58 (83)	64 (91)
	Median PFS (months) (95% CI)	5.3 (2.3 – 6.2)	2.6 (1.9 – 3.5)
	P-value (Log-rank test)	0.005	

	Hazard Ratio (95% CI)	0.60 (0.42-0.86)	
	ORR (EOT) No of patients (%)	26 (37.1)	10 (14.3)
	95% CI	25.9% – 49.5%	7.1% – 24.7%
	P-value (Fisher exact test)	0.003	
	Difference (95% CI)	22.9% (8.9% - 36.8%)	
	Time to complete response (CR/CRu rate) (EOS) No of complete responders (%)	17 (24%)	5 (7.1%)
	Median time to response (months) (95% CI)	2.0 (1.7 – 3.7)	3.6 (2.3 – 19.0)
	P-value (Log-rank test)	0.237	
	Hazard Ratio (95% CI)	1.92 (0.64-5.77)	
	Time to response (CR/CRu/PR) (EOS) No of responders (%)	28 (40%)	10 (14.3%)
	Median time to response (months) (95% CI)	1.9 (1.8 – 2.3)	1.9 (1.6 – 2.3)
	P-value (Log-rank test)	0.304	
	Hazard Ratio (95% CI)	0.68 (0.32-1.43)	
	Duration of complete response (CR/CRu rate) (EOS) No of complete responders (%)	17 (24%)	5 (7.1%)
	Median duration of response (months) (95% CI)	9.6 (4.0 – 20.8)	4.0 (1.0 – 5.1)
	P-value (Log-rank test)	0.081	
	Hazard Ratio (95% CI)	0.32 (0.09-1.23)	
	Duration of all responses (CR/CRu/PR) (EOS) No of responders (%)	28 (40%)	10 (14.3%)
	Median duration of response (months) (95% CI)	7.0 (3.8 – 11.6)	4.5 (0.0 – 6.0)
	P-value (Log-rank test)	0.226	
	Hazard Ratio (95% CI)	0.62 (0.28-1.36)	

Analysis performed across trials (pooled analyses and meta-analysis)

No analyses performed across trials were submitted.

Clinical studies in special populations

Because patients with clinically significant impaired hepatic and renal function were excluded in general from AZA II-01 and PIX301 studies, drug exposure and efficacy in these populations has not been formally evaluated. Further, the efficacy of pixantrone has not been formally evaluated in this indication in patients resistant to anthracyclines, as patients in AZA II-01 and PIX301 were to have been sensitive to prior anthracyclines. However, it has been reported that in DLBCL the vast majority of patients are sensitive to first line anthracycline therapy (Coiffier 2002). Because in the pivotal study the majority of patients (around 75%) had been diagnosed with DLBCL the study population is representative of the majority of the target population. The data have been reflected in the SmPC (section 5.1).

Supportive study

Study AZA-II-01:

This was an uncontrolled open-label Phase II study evaluating pixantrone 85 mg/m^2 on Days 1, 8, and 15 in 28-day cycles as treatment for adult patients with relapsed aggressive NHL.

Efficacy endpoints

- Primary: ORR (CR and PR) in ITT population according to investigator assessment
- Secondary: PFS and OS

Tumour assessment was performed at the end of the second cycle and every two cycles thereafter. Patients could receive up to 6 cycles in the absence of disease progression.

Patient population

Eligible patients with aggressive NHL could have received 0 to 3 prior regimens of chemotherapy containing an anthracycline/anthracenedione and must have been potentially sensitive to them, i.e., either previously responsive to but relapsed after a disease progression-free interval ≥ 6 months or never received an anthracycline/anthracenedione.

The median age of patients was 65 years and 54% were male. The majority of patients had DLBCL (73%), low-high IPI, advanced stages of the disease and had received at least two prior chemotherapy regimens with a median anthracycline cumulative dose of 300 mg/m^2. Of note 21% of patients had mantle cell lymphoma.

Results

33 patients were enrolled and treated with at least one dose of pixantrone. Only 6 patients completed the study (6 cycles) and 23 discontinued because of disease progression (n=19) or toxicity (n=4). The results are summarized below:

Table 20: AZA-II-01 Summary of Efficacy Response Parameters ITT population (N=33)

Confirmed responders (CR+PR)	**9 (27.3%)**
	(95% CI 13.3-45.5)
CR	5 (15.2%)
PR	4 (12.1%)
Unconfirmed PR	**5 (15.2%)**
SD	3 (9.1%)
PD	13 (39.4%)
Not Evaluable	3 (9.1%)
Median PFS	**106 days**
	(95% CI 51-199 days)
Median Survival	**229 days**
	(lower 95% CI 160 days)
CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease; PFS=progression-free survival; CI=confidence interval	

All but one of the confirmed responses were in patients with DLBCL or other high-grade B-cell lymphoma. The efficacy results in the ITT population (33) were similar to the PP (31) analysis.

Study PIX-203

This was an open label, randomised, multicenter, comparative Phase II study. The primary objective was to compare the response rate of CPOP-R (cyclophosphamide, pixantrone, vincristine and prednisone + rituximab) regimen against the standard CHOP-R (cyclophosphamide, doxorrubicin, vincristine and prednisone + rituximab) and to show the response rate for CPOP-R was not inferior to CHOP-R in patients with Diffuse Large B Cell Lymphoma (DLBCL).

Patient population

Patients aged ≥ 18 years with untreated and histologically confirmed CD20 positive DLBCL according to the REAL/WHO classification and of stage II, III or IV . Adequate organ function and ECOG performance status ≤ 2 were inclusion criteria required.

124 patients were randomised (1:1): 61 in CPOP-R arm and 63 in CHOP-R arm

The total planned sample had originally been 280 patients but the study was terminated earlier for business reasons.

Treatments

All patients were due to be administered 4 cycles of CPOP-R or CHOP-R (21 day cycle) followed by 4 additional cycles if following cycle 4 a partial response was recorded or 2 further cycles if a complete response had been recorded.

- CPOP-R arm

  Day 1: Cyclophosphamide 750 mg/m^2 IV, pixantrone 150 mg/m^2 IV, vincristine 1.4 mg/m^2 IV and rituximab 375 mg/m^2 IV

  Prednisone 100 mg daily days 1 to 5

- CHOP-R arm

Day 1: Cyclophosphamide 750 mg/m^2 IV, doxorubicin 50 mg/m^2 IV, vincristine 1.4 mg/m^2 IV and rituximab 375 mg/m^2 IV

Prednisone 100 mg daily days 1 to 5

Tumours were assessed with CT, spiral CT or MRI scans at baseline, after cycle 4, at the end of treatment (EOT), every 3 months for 1 year after EOT, every 6 months for 24 months thereafter and at any time disease progression was suspected. The disease response was determined by an independent assessment panel (IAP).

Patients who discontinued treatment were followed up for 36 months after EOT.

Endpoints

Primary: CR/Cru rate by IAP

Secondary: OS, PFS, ORR, duration of response and TTF (time to treatment failure)

As only 124 patients were enrolled in the study instead of the planned 280, this study was not powered to detect statistical significance. The primary analysis was based on ITT population.

Results

Demographic and baseline characteristics were balanced across both treatment arms.

- CR/CRu

The CR/CRu rate for the CPOP-R arm was 72% (24 patients [39%] with CR and 20 [33%] with CRu) compared to 79% for the CHOP-R arm (28 patients [44%] with CR and 22 [35%] with CRu).

- ORR

In the CPOP-R arm the ORR was 82% (24 patients [39%] with CR, 20 [33%] with CRu, and 6 [10%] with PR) compared to 87% for the CHOP-R arm (28 patients [44%] with CR, 22 [35%] with CRu, and 5 [8%] with PR).

- PFS

PFS results were HR 1.03 (95% CI 0.55, 1.91) and p value 0.93.

- OS was better for patients in CHOP-R arm compared to CPOP-R arm with a HR of 2.34 (95% CI 1.05, 5.22; p= 0.032.)
- Duration of response was not reached for either arm
- TTF was not reached for CPOP-R arm and was 15.1 months for CHOP-R arm.

The study was terminated early, so did not have power to detect noninferiority using the original assumptions of the primary endpoint analysis failed its primary objective. From the efficacy point of view no further conclusions can be drawn for the purpose of this application as pixantrone was administered with a different dose and time schedule, in a different target population of untreated patients and in combination with other medicinal products against the first line standard treatment of CHOP-R regimen

2.5.3. Discussion on clinical efficacy

The pivotal study was designed to compare pixantrone as single agent for six cycles versus physician's choice of protocol specified single agent therapies in aggressive NHL with at least two prior therapies. The enrolled patients are representative of the target population and overall both groups were balanced.

The choice of single arm comparator from a pre-specified list was considered acceptable.

The choice of CR as primary endpoint is not considered acceptable for a single phase III trial and PFS or overall survival would have been more appropriate. Looking at the positive results of pixantrone in the heavily pretreated population on study, this point is not of major concern. The primary analysis was met for the primary endpoint at the end of treatment phase and also at the end of the follow up but not in other analysis by investigator assessment or histological confirmed population. However, PFS showed consistent statistical significance favouring pixantrone across all analysis and other variables that did not reach statistical significance also favoured pixantrone except for time to response which was equal in both groups.

The study was stopped to enrolment by the sponsor in March 2008 due to slow recruitment, nearly 9 months prior to the analysis being conducted in November 2008. Database cut-off for the primary analyses occurred on 30 September 2008, after the last patient completed the end of treatment (EOT) visit. During that time, there were no plans to continue the study. All activities were focused on completion of data collection, data cleaning, and preparing for final analysis. Based upon the above information, the efficacy analysis conducted met the definition of a final analysis and not an interim analysis.

It has been confirmed that the sponsor remained blinded when these decisions were being taken and it is appropriate to accept the analyses provided as final with no need to adjust for the type I error.

Modern treatment strategies of aggressive lymphoma generally include rituximab upfront and the consideration of high dose chemotherapy followed by a stem cell transplant in eligible patients. This means that almost all patients suffering from a second or later relapse in Europe have been exposed to rituximab, and eligible patients also to a stem cell transplant. In the PIX301 study, only approximately 55% of patients were previously treated with rituximab and 15% had undergone transplantation, with a marked geographically skewed distribution as the corresponding fractions in the Western Europe/US and Rest of world regions are 91% *vs* 37% and 28% *vs* 8%, respectively.

Response rates to pixantrone were superior to comparator irrespective of prior rituximab use (32% vs. 18%, 50% vs. 10% pixantrone vs. comparator, with and without prior rituximab respectively).

An evaluation of these data by the number of regimens patients had received prior to study entry showed small impact of prior rituximab therapy on the treatment effect in patients who received 2 prior lines of therapy (50% vs. 0%, 50% vs. 13.3%, pixantrone vs. comparator with and without prior rituximab use). Similar results were observed with patients who received 3 prior lines of therapy (40% vs. 18.8%, 44.4% vs. 6.3%, pixantrone vs. comparator, with or without prior rituximab use).

With increasing numbers of prior regimens there was a decrease in the response rate to pixantrone. This is most apparent in patients who received 4 or more prior regimens (i.e. use of pixantrone as 5th line therapy). Nearly all patients (27/28) who received 4 or more prior regimens had also received prior rituximab. As all but one pixantrone patient with 4 or more prior regimens had received prior rituximab, it is not possible to evaluate the effect of prior treatment with rituximab versus the expected diminished response rates seen in patients treated after 4 or more prior regimens. An imbalance in the prior rituximab population with 4 or more prior lines of therapy received was seen in

favour of the comparator (11/13 pixantrone patients were refractory versus 9/14 refractory patients in the comparator).

Although the outcome was not as favourable as in the patients without prior rituximab the PFS was still more favourable in the pixantrone arm versus the comparator arm regardless of prior rituximab use, particularly in patients with only two prior regimens. These data support the efficacy of pixantrone in patients that have received prior rituximab and up to 3 prior treatment regimens.

The subgroup analysis data show that the advantage of pixantrone over comparator detected in the ITT population is lower in the group of patients pre-treated with rituximab and diminishes further with increasing number of prior regimens. The results seen for Western European patients are explained partly by more heavily pre-treated patients and, importantly, by a higher prevalence of prior rituximab therapy. Still, in the group of pre-treated patients with rituximab, pixantrone shows a numerically better effect than the comparator in patients pre-treated with up to 3 regimens. This benefit needs to be further confirmed by a phase III study. The indication has been reworded with the statement "benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy". This proposed indication reflects the available data and is considered acceptable.

Additional efficacy data needed in the context of a conditional MA

Response rates to pixantrone were superior to comparator irrespective of prior rituximab use, however the benefit of pixuvri in patients that had received prior treatment with rituximab was not as favourable as in the patients without prior rituximab. Therefore additional efficacy data is needed in the context of a conditional MA to further confirm the benefit of pixuvri in the subgroup of pre-treated patients with rituximab.

The applicant shall provide the comprehensive clinical data from the Phase III study PIX 306 where pixantrone in combination with rituximab is compared with gemcitabine in combination with rituximab. The study patient population includes patients with the NHL type of Diffuse Large B cell Lymphoma or Follicular grade III Lymphoma who had previously been treated with at least one rituximab containing multiagent regimen. This study will support the efficacy of pixuvri in patients that had already received prior rituximab. The results from study PIX 306 are expected to be available in Q.2 2015.

2.5.4. Conclusions on the clinical efficacy

Efficacy of Pixuvri has been shown in the approved indication of treatment in monotherapy of adult patients with multiply relapsed or refractory aggressive Non Hodgkin B cell Lymphomas (NHL). The benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy.

In view of the smaller benefit in patients previously treated with rituximab, the CHMP considers the following measure necessary to address the missing efficacy data in the context of a conditional MA:

- The results of study PIX 306 should be submitted to further confirm the benefit of Pixuvri in patients that had received prior treatment with rituximab.

2.6. Clinical safety

Patient exposure

Twelve clinical studies were completed and safety was evaluated in all of them providing a safety population of 407 cancer patients, including 345 patients with NHL.

In total 348 patients received pixantrone: 129 in uncontrolled single agent studies, 68 in the controlled single study (PIX301) and 151 in combination therapy studies.

In the pixantrone arm of the pivotal study, the mean and median number of cycles received was higher (≥ 3 cycles) than in the uncontrolled single-agent studies and the dose showed less variability than in any other studies (mean 82.4 mg/m^2). Dose reductions were infrequent in both treatment groups (18% pixantrone vs 15% comparator). More patients in the pixantrone group required a dose delay (40% vs 22%), but the majority of delays affected one dose only. Only one patient missed a dose (pixantrone arm).

Additional safety data come from study PIX 203 with 124 patients with NHL of whom 61 received pixantrone as part of a combination chemotherapy (CPOP-Rituximab) at a dose of 150 mg/m^2 IV on day 1 of a 21 day cycle.

Adverse events

AE by System Organ Class

Practically all patients that received pixantrone experienced AE. The most frequent AE were seen in the blood (mainly neutropaenia), gastrointestinal and respiratory systems as well as general disorders (see table 21).

Table 21: Patients with Adverse Events by System Organ Class

System Organ Class	Uncontrolled Single Agent Therapy		Controlled Single Agent Therapy (PIX301)		Combination Therapy
	NHL (n=59)	**Other Malignancies** (n=70)	**Pixantrone Group** (n=68)	**Comparator Group** (n=67)	**All Studies** (n=151)
Patients with any adverse event	55 (93.2%)	70 (100.0%)	66 (97.1%)	61 (91.0%)	151 (100.0%)
Infections and infestations	22 (37.3%)	33 (47.1%)	29 (42.6%)	19 (28.4%)	85 (56.3%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	7 (11.9%)	28 (40.0%)	7 (10.3%)	13 (19.4%)	6 (4.0%)
Blood and lymphatic system disorders	50 (84.7%)	50 (71.4%)	52 (76.5%)	34 (50.7%)	140 (92.7%)
Immune system disorders	2 (3.4%)	2 (2.9%)	1 (1.5%)	1 (1.5%)	4 (2.6%)
Metabolism and nutrition disorders	5 (8.5%)	32 (45.7%)	21 (30.9%)	14 (20.9%)	66 (43.7%)
Psychiatric disorders	5 (8.5%)	15 (21.4%)	9 (13.2%)	5 (7.5%)	47 (31.1%)
Nervous system disorders	8 (13.6%)	27 (38.6%)	10 (14.7%)	14 (20.9%)	98 (64.9%)
Eye disorders	4 (6.8%)	7 (10.0%)	2 (2.9%)	4 (6.0%)	28 (18.5%)
Ear and Labyrinth Disorders	1 (1.7%)	2 (2.9%)	3 (4.4%)		12 (7.9%)
Cardiac disorders	8 (13.6%)	11 (15.7%)	14 (20.6%)	9 (13.4%)	24 (15.9%)
Vascular disorders	6 (10.2%)	22 (31.4%)	7 (10.3%)	8 (11.9%)	45 (29.8%)
Respiratory, thoracic and mediastinal disorders	12 (20.3%)	18 (25.7%)	29 (42.6%)	15 (22.4%)	73 (48.3%)

System Organ Class	Uncontrolled Single Agent Therapy		Controlled Single Agent Therapy (PIX301)		Combination Therapy
	NHL (n=59)	**Other Malignancies** (n=70)	**Pixantrone Group** (n=68)	**Comparator Group** (n=67)	**All Studies** (n=151)
Gastrointestinal disorders	22 (37.3%)	53 (75.7%)	34 (50.0%)	27 (40.3%)	128 (84.8%)
Hepatobiliary disorders	2 (3.4%)	2 (2.9%)	5 (7.4%)	1 (1.5%)	6 (4.0%)
Skin and subcutaneous tissue disorders	14 (23.7%)	45 (64.3%)	20 (29.4%)	14 (20.9%)	119 (78.8%)
Musculoskeletal and connective tissue disorders	8 (13.6%)	21 (30.0%)	13 (19.1%)	9 (13.4%)	65 (43.0%)
Renal and urinary disorders	7 (11.9%)	37 (52.9%)	10 (14.7%)	5 (7.5%)	69 (45.7%)
Reproductive system and breast disorders		3 (4.3%)	1 (1.5%)		10 (6.6%)
General disorders and administration site conditions	25 (42.4%)	52 (74.3%)	42 (61.8%)	31 (46.3%)	130 (86.1%)
Investigations	9 (15.3%)	34 (48.6%)	22 (32.4%)	19 (28.4%)	80 (53.0%)
Injury, poisoning and procedural complications	2 (3.4%)	7 (10.0%)	3 (4.4%)	2 (3.0%)	15 (9.9%)
NHL = non-Hodgkin Lymphoma Source: Table 2.7.4.7.3.2.1.1					

Common AE (pivotal study)

The AE seen in the pixantrone arm of the pivotal study are in general in line with the AE seen in uncontrolled single agent studies in NHL except for a more frequent neutropaenia and cough and less incidence of lymphopaenia in the pivotal study.

Table 22: Common Adverse Events in PIX301

Preferred Term	**Pixantrone** (n=68)	**Comparator** (n=67)
Any adverse event	66 (97.1%)	61 (91.0%)
Blood and lymphatic disorders	52 (76.5%)	34 (50.7%)
Anemia	21 (30.9%)	22 (32.8%)
Neutropenia	34 (50.0%)	16 (23.9%)
Leukopenia	17 (25.0%)	7 (10.4%)
Thrombocytopenia	14 (20.6%)	13 (19.4%)
Febrile Neutropenia	6 (8.8%)	2 (3.0%)
Lymphadenopathy	2 (2.9%)	5 (7.5%)
Cardiac disorders	14 (20.6%)	9 (13.4%)
Eye disorders	2 (2.9%)	4 (6.0%)
Gastrointestinal disorders	34 (50.0%)	27 (40.3%)
Nausea	12 (17.6%)	11 (16.4%)
Abdominal Pain	11 (16.2%)	7 (10.4%)
Constipation	8 (11.8%)	3 (4.5%)
Vomiting	5 (7.4%)	10 (14.9%)
Diarrhea	3 (4.4%)	12 (17.9%)
General disorders and administrative site conditions	42 (61.8%)	31 (46.3%)

Asthenia	16 (23.5%)	9 (13.4%)
Pyrexia	16 (23.5%)	17 (25.4%)
Edema peripheral	10 (14.7%)	4 (6.0%)
Fatigue	9 (13.2%)	9 (13.4%)
Mucosal inflammation	8 (11.8%)	2 (3.0%)
Hepatobiliary disorders	5 (7.4%)	1 (1.5%)
Infections and infestations	29 (42.6%)	19 (28.4%)
Pneumonia	5 (7.4%)	4 (6.0%)
Bronchitis	4 (5.9%)	0
Cellulitis	4 (5.9%)	2 (3.0%)
Investigations	22 (32.4%)	19 (28.4%)
Ejection fraction decreased	13 (19.1%)	7 (10.4%)
Weight decreased	5 (7.4%)	5 (7.5%)
Platelet count decreased	4 (5.9%)	2 (3.0%)
Metabolism and nutrition disorders	21 (30.9%)	14 (20.9%)
Anorexia	8 (11.8%)	4 (6.0%)
Dehydration	5 (7.4%)	2 (3.0%)
Musculoskeletal and connective tissue disorders	13 (19.1%)	9 (13.4%)
Pain in extremity	5 (7.4%)	2 (3.0%)
Back pain	6 (8.8%)	2 (3.0%)
Neoplasms, benign, malignant and unspecified	7 (10.3%)	13 (19.4%)
Malignant neoplasm progression	1 (1.5%)	9 (13.4%)
Nervous system disorders	10 (14.7%)	14 (20.9%)
Psychiatric disorders	9 (13.2%)	5 (7.5%)
Renal and urinary disorders	10 (14.7%)	5 (7.5%)
Chromaturia	4 (5.9%)	0
Renal failure	0	5 (7.5%)
Respiratory, thoracic and mediastinal disorders	29 (42.6%)	15 (22.4%)
Cough	15 (22.1%)	3 (4.5%)
Dyspnea	9 (13.2%)	9 (13.4%)
Rhinorrhea	4 (5.9%)	0
Pleural effusion	3 (4.4%)	4 (6.0%)
Skin and subcutaneous tissue disorders	20 (29.4%)	14 (20.9%)
Alopecia	9 (13.2%)	3 (4.5%)
Skin discoloration	7 (10.3%)	0
Vascular disorders	7 (10.3%)	8 (11.9%)
Hypotension	5 (7.4%)	3 (4.5%)

Source: Table 2.7.4.7.3.2.1.1

The incidence of AE grade 3 or 4 was lower in the comparator arm of the pivotal study compared to pixantrone. Again, in line with the overall profile neutropaenia and leucopaenia were the most common grade 3/4 AE reported (41 % and 23% respectively in the pixantrone arm).

The main differences in common AE between pixantrone and comparator were:

Neutropaenia (50% vs 23%)

- Leucopaenia (25% vs 10.4%)
- Asthaenia (23.5% vs 13.4%)
- Infections (42.6% vs 28.4%)
- Ejection fraction decreased (19.1% vs 10.4%)
- Anorexia (11.8% vs 6%)
- Neoplasm progression (1.5% vs 13.4%)
- Cough (22.1% vs 4.5%)
- Skin discolouration (10.3% vs 0%)

Skin discolouration disappears over few days to weeks as the drug is cleared.

The higher incidence of respiratory AEs, mostly grade 1-2 cough and dyspnoea, may have been associated with pixantrone itself or administration of the drug in 500 mL of saline over 1 hour. It is recommended the total volume of saline in the pixantrone infusion should be 250 mL administered IV over 1 hour.

Treatment related AE

Consistent with the overall AE profile, the most common treatment-related AEs across single agent studies were neutropenia, leucopoenia and anaemia.

Treatment-related AEs were reported by a greater proportion of patients in the pixantrone arm (81%) than the comparator arm (57%) of PIX301. It is important to note that blood counts were performed on days 1, 8, and 15 per protocol in the pixantrone patients, but only on day 1 in 52% of patients treated in the comparator arm, possibly resulting in under-reporting of haematopoietic AEs in comparator patients.

The main differences between pixantrone and comparator in the pivotal study were in line with the overall AE reported:

- Neutropaenia (48.5% vs 22.4%)
- Leucopaenia (25% vs 10.4%)
- Ejection fraction decreased (19.1% vs 4.5%)
- Skin discolouration (10.3% vs 0%)

Table 23: Treatment Emergent AE Related to Study Drug

DISORDERS	**Uncontrolled Single Agent Studies**	**Controlled Single Agent Study** (PIX301)	**Combination Therapy**

	NHL (n=59)	**Other Malignancies** (n=70)	**Pixantrone Arm** (n=68)	**Comparator Arm** (n=67)	**All Studies** (n=151)
Patients with any AE	42 (71.2%)	55 (78.6%)	55 (80.9%)	38 (56.7%)	150 (99.3%)
INFECTIONS & INFESTATIONS	6 (10.2%)	2 (2.9%)	9 (13.2%)	7 (10.4%)	46 (30.5%)
NEOPLASMS		1 (1.4%)		1 (1.5%)	2 (1.3%)
BLOOD AND LYMPHATIC*	41 (69.5%)	34 (48.6%)	46 (67.6%)	24 (35.8%)	139 (92.1%)
Neutropaenia	27 (45.8%)	23 (32.9%)	33 (48.5%)	15 (22.4%)	127 (84.1%)
Leucopaenia	29 (49.2%)	21 (30.0%)	17 (25.0%)	7 (10.4%)	120 (79.5%)
Anaemia	16 (27.1%)	18 (25.7%)	13 (19.1%)	13 (19.4%)	65 (43.0%)
Lymphopaenia	28 (47.5%)	18 (25.7%)	3 (4.4%)		72 (47.7%)
Thrombocytopaenia	9 (15.3%)	6 (8.6%)	12 (17.6%)	10 (14.9%)	56 (37.1%)
Febrile neutropaenia	1 (1.7%)	2 (2.9%)	6 (8.8%)	2 (3.0%)	22 (14.6%)
Pancytopaenia					3 (2.0%)
Other	2 (3.4%)		3(4.5%)	1 (1.5%)	
IMMUNE SYSTEM			1 (1.5%)	1 (1.5%)	
METABOLISM & NUTRITION *	1 (1.7%)	2 (2.9%)	7 (10.3%)	5 (7.5%)	50 (33.1%)
Anorexia	1 (1.7%)	2 (2.9%)	5 (7.4%)	2 (3.0%)	15 (9.9%)
PSYCHIATRIC	1 (1.7%)		2 (2.9%)	1 (1.5%)	17 (11.3%)
NERVOUS SYSTEM	6 (10.2%)	8 (11.4%)	3 (4.4%)	8 (11.9%)	74 (49.0%)
EYE	3 (5.1%)	2 (2.9%)	1 (1.5%)	1 (1.5%)	12 (7.9%)
EAR AND LABYRINTH			1 (1.5%)		6 (4.0%)
CARDIAC	5 (8.5%)	1 (1.4%)	6 (8.8%)	1 (1.5%)	9 (6.0%)
Tachycardia			2 (2.9%)		4 (2.6%)
Cardiac disorder	4 (6.8%)				
Congestive Cardiac Failure	1 (1.7%)		2 (2.9%)		
Left ventricular dysfunction		1 (1.4%)	2 (2.9%)		
Arrythmia	1 (1.7%)				1 (0.7%)
Cardiac Failure					2 (1.3%)
Angina Pectoris					1 (0.7%)
Atrial Fibrillation					1 (0.7%)
Bundle Branch Block	1 (1.7%)		1 (1.5%)		
Congestive cardiomyopathy			1 (1.5%)		
Other				1 (1.5%)	1 (0.7%)
VASCULAR	2 (3.4%)	8 (11.4%)	1 (1.5%)	3 (4.5%)	22 (14.6%)

DISORDERS	Uncontrolled Single Agent Studies		Controlled Single Agent Study (PIX301)		Combination Therapy
	NHL (n=59)	Other Malignancies (n=70)	Pixantrone Arm (n=68)	Comparator Arm (n=67)	All Studies (n=151)
RESPIRATORY, THORACIC AND MEDIASTINAL*	2 (3.4%)		6 (8.8%)	1 (1.5%)	32 (21.2%)
Dyspnoea	2 (3.4%)		4 (5.9%)		6 (4.0%)
Cough	1 (1.7%)		1 (1.5%)		8 (5.3%)
GASTROINTESTINAL	19 (32.2%)	34 (48.6%)	14 (20.6%)	17 (25.4%)	116 (76.8%)
HEPATOBILIARY			1 (1.5%)		1 (0.7%)
SKIN & SUBCUTANEOUS TISSUE	9 (15.3%)	33 (47.1%)	16 (23.5%)	7 (10.4%)	106 (70.2%)
Skin discolouration	5 (8.5%)	27 (38.6%)	7 (10.3%)	0	36 (23.8%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE	5 (8.5%)	1 (1.4%)	1 (1.5%)	2 (3.0%)	27 (17.9%)
RENAL AND URINARY		29 (41.4%)	5 (7.4%)	2 (3.0%)	57 (37.7%)
REPRODUCTIVE SYSTEM AND BREAST		1 (1.4%)			
GENERAL AND ADMINISTRATION SITECONDITIONS*	13 (22.0%)	25 (35.7%)	21 (30.9%)	18 (26.9%)	106 (70.2%)
Fatigue	3 (5.1%)	10 (14.3%)	5 (7.4%)	6 (9.0%)	58 (38.4%)
Asthenia	8 (13.6%)	12 (17.1%)	8 (11.8%)	7 (10.4%)	38 (25.2%)
Pyrexia	3 (5.1%)	1 (1.4%)	5 (7.4%)	5 (7.5%)	23 (15.2%)
Mucosal Inflammation		3 (4.3%)	8 (11.8%)	1 (1.5%)	8 (5.3%)
Chills		1 (1.4%)		2 (3.0%)	14 (9.3%)
Oedema peripheral	1 (1.7%)				8 (5.3%)
INVESTIGATIONS**	4 (6.8%)	21 (30.0%)	16 (23.5%)	7 (10.4%)	50 (33.1%)
Ejection Fraction decreased	1 (1.7%)		13 (19.1%)	3 (4.5%)	33 (21.9%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1 (1.7%)	1 (1.4%)		2 (3.0%)	3 (2.0%)

* Mentioned in table only most relevant AE with higher incidence

**Laboratory haematology/chemistry, urine and cardiac tests

Cardiac AE (pivotal study PIX 301)

Cardiac toxicity was closely monitored in the pivotal study.

Cardiac assessments of LVEF (MUGA scan), echocardiography and serum troponin T at baseline, on cycles 2 and 4, and end of treatment were conducted together with regular ECG. LVEF and serum troponin were also measured during follow up.

All patients had received previous anthracyclines/anthrazediones at equivalent doses. The only difference was that patients in pixantrone arm had higher incidence of prior congestive heart failure and cardiomyopathy whilst those in the comparator had a higher incidence of arrythmias.

At baseline 55% patients had LVEF grade 1 abnormalities, 3% had grade 2 and none were grade 3.

The mean cumulative prior anthracycline doses for patients in the pixantrone arm were 285 mg/m^2 and the mean normalized pixantrone doses administered during the trial were 822 mg/m^2 (242 mg/m^2 doxorubicin equivalent).

A summary of cardiac AE is shown below. Most events were of grade toxicity 1 or 2.

Table 24: Number of Patients (%) With Cardiac Adverse Events of Interest

Preferred Term	**Pixantrone** (n=68)	**Comparator** (n=67)
Any cardiac adverse event of interest	24 (35.3%)	14 (20.9%)
Ejection fraction decreased	13 (19.1%)*	7 (10.4%)*
Sinus tachycardia	0	3 (4.5%)
Tachycardia	3 (4.4%)	2 (3.0%)
Arrhythmia	0	1 (1.5%)
Atrioventricular block second degree	0	1 (1.5%)
Bradycardia	0	1 (1.5%)
Cardiac failure	3 (4.4%)	1 (1.5%)
Cardiac failure congestive	3 (4.4%)	0
Left ventricular dysfunction	2 (2.9%)	0
Bundle branch block (right)	1 (1.5%)	0
Cardiac arrest	1 (1.5%)	0
Source: PIX301 CSR Tables 14.3.1.8.1 and 14.3.1.8.2 * All toxicity grade 1/ 2 except 2 patients in pixantrone group of grade 3		

Looking at treatment- related cardiac events there were 9 cardiac events related to pixantrone (13%) and all were asymptomatic decreases in ejection fraction. Including all events considered likely or possibly related to pixantrone therapy, there were 14 cardiac events reported by 13/68 patients (19%), including 2 possible cases of congestive heart failure (CHF) associated with pixantrone therapy. Only one patient in the comparator arm (1.5%) had a treatment related cardiac event (LVEF decrease).

There was no demonstrable relationship between cumulative pixantrone dose to symptomatic declines in LVEF or CHF, nor was a relationship seen with prior doxorubicin equivalent cumulative exposure.

According to the independent review, data from the literature show that cardiac events in patients treated with doxorubicin at a similar cumulative total dose included a clear higher incidence of CHF.

At baseline troponin levels were higher in pixantrone arm than comparator and during study more pixantrone patients developed asymptomatic increases. However, these abnormalities were not predictive of clinically manifest cardiac events. All troponin abnormalities resolved by the 6 month follow-up except in one patient in the pixantrone arm.

Additional safety data from Study PIX 203 showed the overall safety profile between both treatment arms was similar. However, it was shown that when pixantrone is substituted for doxorubicin as part of a CHOP-Rituximab like regimen, it is associated with fewer cases of CHF, LVEF decreases of at least 20% and troponin T elevations.

No other relevant events were seen in all other safety studies.

Serious adverse event/deaths/other significant events

SAEs

Approximately half of patients receiving single agent pixantrone experienced SAEs. In the PIX301 study, SAEs more common in the pixantrone arm included infections and infestations (21% vs 17%), septic shock (3% vs 0%), and cardiac disorders (9% vs 4.5%). Acute respiratory distress syndrome (n=1) and pneumonitis (n=2) were reported in the pixantrone group only and the applicant will be requested to comment on the possible relation to the study drug. SAEs more common in the comparator group included malignant neoplasm progression (2% vs 13%), thrombocytopenia (1.5% vs 9%), and gastrointestinal disorders (6% vs 10%). SAEs due to neutropenia were reported slightly more in the pixantrone arm (13% vs 9%) as was febrile neutropenia (6% vs 3%). Two patients with neutropenic fever were reported in the uncontrolled studies. As could be expected, a substantially higher fraction of patients in the combination therapy studies had SAEs due to neutropenia and neutropenic fever (34% and 13%, respectively).

Deaths

Table 25: Number (%) of Patients with Treatment-emergent Adverse Events Resulting in Death in ≥ 1 Patient

	Uncontrolled Single Agent Therapy		**Controlled Single Agent Therapy (PIX301)**		**Combination Therapy**
System Organ Class/ Preferred Term	**NHL** (N=59)	**Other Malignancies** (N=70)	**BBR-2778 Group** (N=68)	**Comparator Group** (N=67)	**All Studies** (N=151)
Patients with any adverse event	**4 (6.8%)**	**11 (15.7%)**	**14 (20.6%)**	**14 (20.9%)**	4 (2.6%)
Infections and infestations	1 (1.7%)	1 (1.4%)	2 (2.9%)	2 (3.0%)	2 (1.3%)
Sepsis	0	1 (1.4%)	1 (1.5%)	1 (1.5%)	0
Septic shock	0	0	1 (1.5%)	0	2 (1.3%)
Pneumonia	0	0	1 (1.5%)	1 (1.5%)	0
Neoplasms benign, malignant and unspecified (including cysts and polyps)	1 (1.7%)	8 (11.4%)	**3 (4.4%)**	**8 (11.9%)**	1 (0.7%)
Malignant neoplasm progression	1 (1.7%)	4 (5.7%)	1 (1.5%)	8 (11.9%)	1 (0.7%)
Metastases to abdominal cavity	0	0	1 (1.5%)	0	0
Non-Hodgkin's lymphoma	0	0	1 (1.5%)	0	0
Cardiac disorders	0	1 (1.4%)	**4 (5.9%)**	**1 (1.5%)**	0
Cardiac failure	0	1 (1.4%)	2 (2.9%)	1 (1.5%)	0
Cardiac arrest	0	0	1 (1.5%)	0	0
Cardiac failure congestive	0	0	1 (1.5%)	0	0
Vascular disorders	0	0	2 (2.9%)	0	0
Circulatory collapse	0	0	1 (1.5%)	0	0
Hypotension	0	0	1 (1.5%)	0	0
Respiratory, thoracic and mediastinal disorders	0	0	**5 (7.4%)**	2 (3.0%)	0
Respiratory failure	0	0	2 (2.9%)	1 (1.5%)	0
Obstructive airways disorder	0	0	1 (1.5%)	1 (1.5%)	0

	Uncontrolled Single Agent Therapy		Controlled Single Agent Therapy (PIX301)		Combination Therapy
System Organ Class/ Preferred Term	**NHL** (N=59)	**Other Malignancies** (N=70)	**BBR-2778 Group** (N=68)	**Comparator Group** (N=67)	**All Studies** (N=151)
Acute respiratory distress syndrome	0	0	1 (1.5%)	0	0
Pleural effusion	0	0	0	1 (1.5%)	0
Pulmonary venous thrombosis	0	0	1 (1.5%)	0	0
Renal and urinary disorders	0	1 (1.4%)	0	**2 (3.0%)**	0
Renal failure	0	0	0	2 (3.0%)	0
General disorders and administration site conditions	2 (3.4%)	1 (1.4%)	1 (1.5%)	0	1 (0.7%)
Multi-organ failure	0	0	1 (1.5%)	0	0
Note: Cut off >= 1 Patient in either arm of PIX301.					

By system organ class, more patients in the pixantrone arm were reported with cardiac, vascular, and respiratory/thoracic disorders leading to death, and more patients in the comparator arm with neoplasms and renal disorders leading to death. In the uncontrolled single agent studies, 4/59 (7%) and 11/70 (16%) patients were reported to experience treatment-emergent AEs resulting in death in the NHL and other malignancies studies, respectively.

Table 26: Summary of Deaths—PIX301

	Pixantrone (n=68)	**Comparator** (n=67))
Patients who died	**49** (72.1%)	**52** (77.6%)
Patients who died ≤ 30 days of last dose	10 (14.7%)	12 (17.9%)
Patients who died > 30 days after last dose	39 (57.4%)	40 (59.7%)

The majority of deaths within 30 days of last study treatment were stated to be related to the patient's underlying NHL. One death in the pixantrone group was considered related to treatment (a 29 years old female, who died of septic shock on study day 8). None of the deaths within 30 days of last study treatment in the comparator group were considered related to treatment.

Three deaths that occurred more than 30 days after the last study treatment were considered related to treatment; one patient died from acute congestive heart failure and one from MDS in the pixantrone arm, and one patient died from renal failure in the comparator arm.

Laboratory findings

Haematology

Reversible neutropaenia and leukopaenia are the predominant manifestations of pixantrone hematologic toxicity. Thrombocytopenia and anaemia were also observed but at lower frequency and severity.

In PIX 301 complete blood counts were conducted during treatment with pixantrone every week whilst in more than half of the comparator it was only done every 3 weeks. Mean duration of treatment was one month longer for the pixantrone arm. The most relevant haematological events were as follows (pixantrone vs comparator):

- Neutropaenia (50% vs 23.9%) [grade 3-4: 41.2% vs 19.4%]

- Anaemia (30.9% vs 32.8%) [grade 3-4: 5.9% vs 13.4%]
- Thrombocytopaenia (20.6% vs 19.4%) [grade 3-4: 11.8% vs 10.4%]
- Febrile neutropaenia (8.8% vs 3%) [grade 3-4: 7.4% vs 3%]

More patients in the pixantrone group received growth factor support (51.5% vs 26.9%) and no relevant differences were seen in blood or platelet transfusions. Growth factor support was not routinely given as it was left up to investigator's judgement.

Neutropaenia reaches nadir on days 15-20 of each cycle and recovery normally occurs by day 28.

Most grade 4 neutropaenias were observed after cycles 1 and 2 (10% and 15%) respectively and frequency declined with subsequent cycles (9% after cycle 3, 8% after cycle 4). Observation after the last two cycles and EOT or beyond revealed less than a 5% frequency. Complications of severe neutropenia including febrile neutropaenia were uncommon in both study arms.

Infections were common across all study groups although the incidence of systemic sepsis and opportunistic systemic infections was low. In the pivotal study the pixantrone arm reported more infections than the comparator (42.6% vs 28.4% - grade 3/ 4 =18% vs 13%) and most of them were respiratory tract infections.

In all pixantrone studies, urinary tract infection, oral candidiasis, upper respiratory tract infections including nasopharyngitis, bronchitis, pharyngitis and pneumonia were the most frequently observed AEs.

Chemistry

Abnormalities were in general similar across studies and between the two arms of the pivotal trial and grade 3 or 4 abnormalities were rare.

The most common abnormalities in the pixantrone arm of the pivotal study were:

- Hyperglycaemia (55.2%)
- Hypomagnesaemia (40.8%)
- Albumin changes (45.8%)

Safety in special populations

Paediatric population:
Patients < age 18 were excluded from clinical trials. The safety and efficacy of Pixuvri in children aged < 18 years has therefore not been established and a statement has been included in section 4.2 of the SmPC.

Hepatic insufficiency:
Patients with significant hepatic impairment as evidenced by a baseline bilirubin ≥1.5 X ULN were excluded from clinical trials and severe abnormal hepatic function is a contraindication to the use of pixantrone.

Renal insufficiency:
Patients with significant renal impairment (creatinine ≥1.5 X ULN) were also excluded from clinical trials. Thus, pixantrone should be used with caution in patients with renal impairment.

Elderly patients:

Febrile neutropenia, psychiatric disorders, vascular disorders and asthenia were more common in pixantrone-treated patients 65 years or older, as was the incidence of cardiac treatment-emergent AEs (but not of grade 3-4 or ejection fraction decline). Old patients suffered less from nausea. No specific dose adjustment is required in elderly patients (aged ≥ 65 years).

Gender

Compared to men, women treated with pixantrone had higher incidences of thrombocytopenia, neutropenia, febrile neutropenia, and treatment-emergent grade 3-4 disorders of metabolism.

Fertility, pregnancy and lactation:
There are no data from the use of pixantrone in pregnant women. Studies in animals have shown reproductive toxicity.

Women of childbearing potential and their partners should be advised to avoid pregnancies.

Women and men must therefore use effective contraception during and up to 24 weeks after treatment. Pixuvri is not recommended during pregnancy and in women of childbearing potential not using contraception as indication in section 4.6 of the SmPC.

It is unknown whether Pixuvri/metabolites are excreted in human milk. A risk to the newborn/infants cannot therefore be excluded and breast-feeding should be discontinued during treatment with Pixuvri.

After repeated administrations of Pixuvri at doses as low as 0.1 mg/kg/day, a dose-dependent testicular atrophy was detected in the dogs. This effect has not been evaluated in humans. As with other agents in the general class of deoxyribonucleic acid (DNA) damaging agents, Pixuvri may be associated with fertility impairment. Whilst the effect on fertility has not been ascertained a precaution will be to advise male patients to use contraceptive methods (preferably barrier) during treatment and for a period of 6 months post-treatment to allow new sperm to mature. To avoid the risk of long term infertility sperm banking should be considered.

Patients with poor performance status

There is currently no information on the safety and efficacy of patients with poor performance status (ECOG > 2). Caution should be exercised when treating such patients.

Safety related to drug-drug interactions and other interactions

No drug-drug interaction studies have been submitted and no drug interactions have been reported in human subjects.

Discontinuation due to adverse events

Table 27: Adverse Events Leading to Withdrawal from PIX301 (extirpt)

Preferred Term	**Pixantrone** (n=68)	**Comparator** (n=67)
Any adverse event leading to withdrawal	**29 (42.6%)**	**25 (37.3%)**
Neutropenia	**7 (10.3%)**	**1 (1.5%)**
Thrombocytopenia	-	**3 (4.5%)**
Febrile neutropenia	2 (2.9%)	-

Preferred Term	Pixantrone (n=68)	Comparator (n=67)
Anemia	-	**2 (3.0%)**
Cardiac disorders	**5 (7.4%)**	**1 (1.5%)**
Cardiac failure	2 (2.9%)	1 (1.5%)
Asthenia	**5 (7.4%)**	-
Hepatobiliary disorders	**2 (2.9%)**	-
Infections and infestations	3 (4.4%)	4 (6.0%)
Ejection fraction decreased	**2 (2.9%)**	-
Neoplasms, benign, malignant and unspecified	**2 (2.9%)**	**6 (9.0%)**
Renal failure	-	**2 (3.0%)**
Respiratory, thoracic and mediastinal disorders	4 (5.9%)	7 (10.4%)

Slightly more patients were withdrawn due to AEs in the pixantrone arm (43% vs 37%). Most common AEs leading to withdrawal in the pixantrone arm were neutropenia (10%), cardiac disorders and asthenia (both 7%); two patients were withdrawn due to hepatobiliary disorders, none for renal failure. In the comparator arm, 9% of patients were withdrawn because of malignant neoplasm progression, 2 patients for renal failure, and none for hepatobiliary disorders; withdrawal due to cardiac disorder or neutropenia was uncommon (one patient in each category).

Post marketing experience

N/A

2.6.1. Discussion on clinical safety

Safety data from 12 clinical studies are available and a total of 348 patients received pixantrone, including 68 patients in the controlled pivotal study and 129 patients in uncontrolled single agent trials. The majority of patients in the pivotal trial received at least 4 cycles of treatment with the recommended dose.

Practically all patients that received pixantrone experienced AEs. The type and incidence of AE correlated in general across all single agent studies and the most common AEs seen in the pivotal study were neutropaenia (50%), leucopaenia (25%), anaemia (31%), thrombocytopaenia (21%), asthenia (23%), pyrexia (23%), cough (22%), decreased ejection fraction (19%) and nausea (18%). Characteristic of pixantrone is a reversible skin discoloration.

Analysis of treatment related AEs still showed neutropaenia as the most common AE (49%) and it was the main AE leading to discontinuation from the pivotal study (10%). Haematological side effects were also the most common associated with grade 3 or 4 toxicity. With the recommended dose and schedule, neutropenia is usually transient, reaching its nadir on days 15-22 following administration on days 1, 8, and 15 with recovery usually occurring by day 28.

No cases of overdose have been reported with Pixuvri. Single doses of pixantrone base up to 158 mg/m2 have been given in dose-escalation clinical trials without evidence of dose-related toxicity.

Thrombocytopenia and anaemia were of lower frequency and severity than neutropaenia and there were no differences in blood or platelet transfusions between treatment groups. Most grade 4

neutropenias were observed after cycles 1 and 2 and frequency declined with subsequent cycles. Of note complications of severe neutropenia including febrile neutropenia were uncommon and growth factor support was left at the discretion of the investigator.

Careful monitoring of blood counts is required, including leukocyte, red blood cells, platelet and absolute neutrophil counts. Recombinant hematopoietic growth factors may be used according to institutional or European Society for Medical Oncology (ESMO) guidelines and dose modifications should be considered. The use of Pixantrone dimaleate is contraindicated in the case of profound bone marrow suppression.

Cardiac toxicity was closely monitored in the pivotal study and a higher incidence of cardiac events was seen in the pixantrone group (35% vs 21%).Only 9 cases of cardiac events were considered related to pixantrone (13%) and all were asymptomatic decreases of ejection fraction. Overall events observed were relatively mild and asymptomatic and there were no clear cases of pixantrone-associated CHF as typically described in the literature for other anthracyclines. There was no demonstrable relationship between cumulative pixantrone dose to symptomatic declines in LVEF or CHF, nor was a relationship seen with prior doxorubicin equivalent cumulative exposure.

Changes in cardiac function including decreased LVEF or fatal congestive heart failure (CHF) may occur during or after treatment with Pixuvri.

Additional data from study PIX-203 has shown that when pixantrone is substituted for doxorubicin as part of a CHOP-Rituximab like regimen, it is associated with fewer cases of CHF, LVEF decreases of at least 20% and troponin T elevations.

Active or dormant cardiovascular disease, prior therapy with anthracyclines or anthracenediones, prior or concurrent radiotherapy to the mediastinal area or concurrent use of other cardiotoxic medicinal products may increase the risk of cardiac toxicity. Cardiac toxicity with Pixuvri may occur whether or not cardiac risk factors are present.

Patients with cardiac disease or risk factors such as a baseline LVEF value of < 45% by multigated radionuclide (MUGA) scan, clinically significant cardiovascular abnormalities (equal to New York Heart Association (NYHA) grade III or IV), myocardial infarction within the last 6 months, severe arrhythmia, uncontrolled hypertension, uncontrolled angina, or prior cumulative doses of doxorubicin or equivalent exceeding 450 mg/m2 should receive careful risk versus benefit consideration before receiving treatment with Pixuvri.

Cardiac function should be monitored before initiation of treatment with Pixuvri and periodically thereafter. If cardiac toxicity is demonstrated during treatment, the risk versus benefit of continued therapy with Pixuvri must be evaluated.

The occurrence of secondary acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) is a well described complication of chemotherapy regimens containing anthracyclines and other topoisomerase II inhibitors.

Infections, including pneumonia, cellulitis, bronchitis and sepsis have been reported during clinical trials. Infections have been associated with hospitalisation, septic shock and death. Patients with neutropenia are more susceptible to infections, although in the clinical studies there was no increased incidence of atypical, difficult to treat infections, such as systemic mycotic infections or infections with opportunistic organisms such as Pneumocystis jiroveci.

Pixuvri should not be administered to patients with an active, severe infection or in patients with a history of recurring or chronic infections or with underlying conditions which may further predispose them to serious infection.

Pixantrone may induce hyperuricaemia as a consequence of the extensive purine catabolism that accompanies drug-induced rapid lysis of neoplastic cells (tumour-lysis syndrome) and can lead to electrolyte imbalances which can result in kidney damage. Blood uric acid levels, potassium, calcium phosphate and creatinine should be evaluated after treatment in patients at high risk for tumour lysis (elevated LDH, high tumour volume, high baseline uric acid or serum phosphate levels). Hydration, urine alkalinisation, and prophylaxis with allopurinol or other agents to prevent hyperuricaemia may minimise potential complications of tumour lysis syndrome.

Immunisation may be ineffective when given during Pixuvri therapy. Immunisation with live virus vaccines is contraindicated due to the immunosuppression associated with Pixuvri therapy.

If extravasation occurs the administration should be stopped immediately and restarted in another vein. The non-vesicant properties of Pixuvri minimise the risk of local reaction following extravasation.

Photosensitivity is a theoretical risk based on *in vitro* data and no confirmed cases have been reported in the clinical trial program. As a precaution, patients should be advised to follow sun protection strategies, including wearing sun protective clothing and using sunscreen. Since most medicinal product-induced photosensitivity reactions are caused by wavelengths within the UV-A range, sunscreen that strongly absorbs UV-A is recommended.

The applicant has confirmed that an in vivo phototoxicity study will be conducted to clarify this point.

It is not known whether pixantrone has an effect on the ability to drive a car or use machines.

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

Additional safety data needed in the context of a conditional MA

None required

Conclusions on the clinical safety

The clinical safety profile of pixuvri in the proposed indication is acceptable.

The CHMP considers the following measure necessary to address issues related to safety:

- The applicant should conduct a non clinical *in vivo* phototoxicity study to address the theoretical risk of photosensitivity.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

The applicant must ensure that the system of pharmacovigilance is in place and functioning before the product is placed on the market.

Risk Management Plan

The applicant submitted a risk management plan identifying relevant important identified/potential risks and important missing information.

Summary of the risk management plan

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Identified risks		
Cardiac failure	• Routine pharmacovigilance	• Instructions with regard to monitoring and identification section 4.4 of SmPC under the heading of "Cardiotoxicity" • Further information is given in Section 4.8 of the SmPC under the heading of "Cardiac toxicity". • Instructions include pre-dose evaluation of cardiac function and periodic monitoring also in Sections 4.4 and 4.8 of the SmPC • Pixantrone will only be used on units with experience in prescribing intravenous chemotherapy (SmPC Section 4.2)
Myelotoxicity	• Routine pharmacovigilance	• Special precautions as per Section 4.4 of the SmPC under the heading of "Myelosuppression" • Additional information is provided in Section 4.8 of the SmPC under the heading of "Haematologic toxicities and complications of neutropenia" • Pixantrone will only be used on units with the facilities for regular monitoring of clinical, haematological and biochemical parameters during and after treatment as described in SmPC Section 4.2 • There is a contraindication with regard to treating patients with profound myelosuppression in Section 4.3
Serious infections	• Routine pharmacovigilance	• Advice in Section 4.4 of the SmPC under the heading of "Infection" particularly with regard to the warning not to administer to patients with active severe infection or recurrent infections
Tumour lysis syndrome	• Routine Pharmacovigilance	• Section 4.4 of the SmPC has information with regard to the potential risk of tumour lysis syndrome
Potential risks		
Therapy related AML/MDS	• Routine pharmacovigilance including literature reviews to detect reports of haematological malignancies involving anthracycline treatment that may include pixantrone	• Advice section 4.4 of SmPC is given with regard to the potential risk of "Secondary Malignancy"

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Reproductive toxicity	• Reporting of pregnancy directly to the company • Reports of male infertility as part of routine pharmacovigilance	• Specific advice SmPC Section 4.6 is given under the heading of "Pregnancy" • SmPC Section 4.6 provides a warning regarding avoiding pregnancy • SmPC Section 5.3 provides information with regard to preclinical evidence • For breastfeeding advice is provided in Section 4.6 of the SmPC under the heading of "Lactation" • Advice is also provided in Fertility in Section 4.6 of the SmPC under the heading of "Fertility"
Photosensitivity	• Routine pharmacovigilance • An *in vivo* phototoxicity study in rodents will be performed using pixantrone dimaleate at relevant clinical doses.	• A paragraph in Section 4.4 of the SmPC provides guidance with regard to the theoretical risk of "Photosensitivity" and measures that can be taken
CYP1A2 and CYP2C8	• Routine pharmacovigilance	• Advice in Section 4.5 of the SmPC on potential interactions specifically through CYP1A2 is provided with reference to the fact that no drug-drug interaction studies have been performed but information is provided about possible interactions based on CYP1A2 metabolism. Similar warning is provided for potential effect on CYP2C8.
Missing information		
Use in children	• Three studies: PIX 111 to be completed by February 2015, PIX 211 to be completed by August 2018 and PIX 311to be completed by November 2021 and a preclinical juvenile toxicity study to determine the safety of pixantrone in children initially from 5-18 years of age and also of >6 months after initial safety in older children established	• Advice on the lack of data is provided in Section 4.2 of the SmPC
Safety in people with significant hepatic and renal impairment	• Routine pharmacovigilance • Additional safety information from approximately 350 patients will become available from the completion of PIX306 study in June 2015	• Advice on the lack of information and need to be cautious in renal failure and be used with caution in patients with mild or moderate liver impairment is provided in SmPC section 4.2. • Pixuvri is controindicated in patients with severe hepatic impairment is provided in SmPC Section 4.3.
Safety in patients with severely abnormal cardiac function	• Routine pharmacovigilance • Additional safety information from approximately 350 patients will become available from the completion of PIX306 study in June 2015	• Specific precautions in Section 4.4 in the SmPC as described for cardiotoxicity • Also a contraindication for patients with severely abnormal cardiac function in Section 4.3 • Requirement to have pre-dose cardiac function (e.g. MUGA or ECHO)

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Safety in patients with poor bone marrow reserve	• Routine pharmacovigilance	• SmPC Section 4.3 contraindicates patients with profound bone marrow suppression • Advice in SmPC section 4.4 under the heading of "Myelosuppression" provides advice on the fact that recombinant haematopoietic growth factors may be used according to institutional or European Society for Medical Oncology (ESMO) guidelines
Off label use	• Routine pharmacovigilance • AEs/SAE data collection, the Pharmacovigilance department will ensure the 'use-indication' for all spontaneous reported SAEs , including literature articles/manuscripts are also obtained and databased.	• Section 4.1 of the SmPC shows the indication for treatment with pixantrone • Section 4.3 of the SmPC indicates that the following patients the use of pixantrone is contraindicated ▪ Hypersensitivity to pixantrone dimaleate, or to any of the excipients ▪ Profound bone marrow suppression ▪ Severely abnormal cardiac function
Safety in Elderly patient > 75 years of age	• Routine Pharmacovigilance (also see cardiotoxicity, renal and hepatic impairment)	• Apart from the instructions with regard to comorbidities no other risk minimisation activities are planned and the recommendation for elderly patients is for no dose adjustment
Safety in non-Caucasians	• Routine pharmacovigilance (also see cardiotoxicity)	• Apart from instructions with regard to the risk of cardiotoxicity no other risk minimisation activities are planned
Safety in patient with poor performance status	• Routine pharmacoviliance	• Information about the lack of information has been added to Section 4.2 of the SmPC under the heading of " Patients with poor performance status"
Safety in patient with prior mediastinal radiotherapy	• Routine Pharmacovigilance (also see cardiotoxicity)	• Information with regard to the risk of cardiotoxicity is provided in Section of 4.4 the SmPC

The CHMP, having considered the data submitted, was of the opinion that the below pharmacovigilance activities in addition to the use of routine pharmacovigilance are needed to investigate further some of the safety concerns:

Description	Due date
In vivo phototoxicity study	Q4 2012
Phase III randomised study comparing pixantrone plus rituximab with gemcitabine plus rituximab (**PIX 306**)	Q2 2015

No additional risk minimisation activities were required beyond those included in the product information.

2.8. 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

Benefits

Beneficial effects

There is currently no approved treatment or standard of care for patients with aggressive NHL that had relapsed two or more times after other therapies. Cure is normally not expected in this patient population and the probability of responding to therapy with a durable effect is very small. Pixantrone has been developed to address this medical need.

The pivotal study was designed to compare pixantrone as single agent for six cycles versus physician's choice of protocol specified single agent therapies in aggressive NHL with at least two prior therapies. The enrolled patients are representative of the target population.

The primary analysis was met for the primary endpoint at the end of treatment and also at the end of the follow up. PFS (secondary endpoint) showed consistent statistical significance favouring pixantrone across all analysis. Overall survival (secondary endpoint) was prolonged with pixantrone treatment although no statistical significant difference was observed.

Uncertainty in the knowledge about the beneficial effects.

The advantage of pixantrone over comparator detected in the ITT population is lower in the group of patients pre-treated with rituximab and diminishes further with increasing number of prior regimens. Pixantrone showed to be more active than the comparator in the group of patients pretreated with up to 3 regimens, including rituximab. However, the benefit in this subset needs to be further confirmed in view of the low number of patients.

There is a lack of data on black patients but from the PK/PD point of view no relevant clinical difference is expected. The lack of data in this group of patients has been addressed in the RMP.

Risks

Unfavourable effects

Overall the data are considered sufficient for the assessment of the safety profile of Pixuvri in the proposed indication.

Bone marrow suppression is the most frequent and severe toxicity associated with pixantrone treatment. Neutropaenia is the predominant manifestation whilst thrombocytopenia and anaemia occurred at less frequency and severity. More patients in pixantrone arm received growth factor support compared to comparator but blood or platelet transfusions were similar to comparator. Neutropaenia reaches nadir on days 15-20 of each cycle and recovery normally occurs by day 28. Most

grade 4 neutropaenias were observed after cycles 1 and 2 and frequency declined with subsequent cycles. Complications of severe neutropenia including febrile neutropaenia seem uncommon. Neutropaenia is the main cause for discontinuation of treatment.

Infections were common but the incidence of systemic sepsis and opportunistic systemic infections was low.

Cardiac toxicity was closely monitored in the pivotal study and a higher incidence of cardiac events was seen in the pixantrone group. However, only 9 cases of cardiac events were considered related to pixantrone (13%) and all were asymptomatic decreases of ejection fraction. Overall events observed were relatively mild and asymptomatic and there were no clear cases of pixantrone-associated CHF as typically described in the literature for other anthracyclines.

There was no demonstrable relationship between cumulative pixantrone dose to symptomatic declines in LVEF or CHF, nor was a relationship seen with prior doxorubicin equivalent cumulative exposure.

Uncertainty in the knowledge about the unfavourable effects

Patients with significant hepatic or renal impairment were excluded from the clinical trials. This lack of data is reflected by appropriate wording in the SmPC and the proposed RMP.

The clinical implication of a positive non-clinical phototoxicity assay is undetermined. Although no clear clinically significant photosensitisation was observed in the safety database, phototoxicity may be a rare condition and it is concluded that a non-clinical *in vivo* study should be performed post approval.

Benefit-risk balance

Importance of favourable and unfavourable effects

Given the lack of standard of care and the poor prognosis for patients with multiple relapses/refractory aggressive NHL the improvement seen in CR/CRu, supported by the results of secondary endpoints of PFS and OS in the pivotal study is considered meaningful and of clinical relevance.

Although haematological toxicity was the main manifestation of pixantrone it is reversible. Cardiac toxicity is seen at lower frequency and with an apparent less severity than that reported with other anthracyclines.

Benefit-risk balance

The favourable effect seen in terms of CR/CRu, supported by the results of secondary endpoints of PFS and OS in the full study population outweighs the risks associated with pixantrone therapy.

Additional efficacy data are needed to confirm the benefit of pixuvri in patients that had received prior treatment with rituximab. The applicant shall provide the comprehensive clinical data from the Phase III study PIX 306 where pixantrone in combination with rituximab is compared with gemcitabine in combination with rituximab.

Discussion on the benefit-risk balance

Benefit/risk is considered favourable but there is limited data in the group of patients pretreated with rituximab. The available data has shown a better efficacy outcome of pixantrone over comparator in patients pretreated with rituximab and who have received up to 3 prior treatments. However, it is

acknowledged that data in this subgroup of patients is limited. Therefore, a conditional approval is recommended pending the results from Study PIX 306 to support the efficacy of Pixuvri in patients that had received prior rituximab therapy.

Following consultation with the applicant, the CHMP considered the granting of a conditional marketing authorisation pixantrone. Pixantrone aims at the treatment of seriously debilitating diseases or life-threatening diseases and falls within the scope of Commission Regulation 507/2006 on the conditional marketing authorisation. The Committee found that although comprehensive clinical data referring to the efficacy of the medicinal product had not been supplied, all of the following requirements were met:

- The risk-benefit balance of the medicinal product, as defined in Article 1(28a) of Directive 2001/83/EC, is positive.

Based on the randomized controlled study presented in patients with multiply relapsed or refractory aggressive NHL (study PIX 301), the favourable effect seen in terms of CR/CRu, supported by the results of secondary endpoints of PFS and OS in the full study population outweighs the risks associated with pixantrone therapy.

- It is likely that the applicant will be in a position to provide comprehensive clinical data.

From a quantitative point, of view, the benefit in the subgroup of patients previously treated with rituximab might be less as compared with what was observed in patients that had not received prior rituximab treatment. However, the efficacy of Pixuvri in patients that had received prior rituximab therapy and up to 3 prior regimens was still superior to the comparator. In Europe most patients that had multiple relapse or are refractory to treatments are expected to have received prior rituximab. Therefore there is a need to further confirm the efficacy of Pixuvri in patients previously treated with rituximab.

Comprehensive clinical data will be provided through a Phase III study PIX 306 where pixantrone in combination with rituximab is compared with gemcitabine in combination with rituximab. The study patient population includes patients with the NHL type of Diffuse Large B cell Lymphoma or Follicular grade III lymphoma who had previously been treated with at least one rituximab containing multiagent regimen. This study will support the efficacy of pixuvri in patients that had received prior rituximab of the phase III Study PIX 301. The results from study PIX 306 are likely to be available in Q2 2015.

- Unmet medical needs to be fulfilled.

There is a lack of approved and standard of care pharmacological treatment for patients with multiply relapsed or refractory aggressive NHL and there is a need in this patient population that could be fulfilled with the proposed medicinal product. The CHMP concluded that the product fulfils an unmet medical need due to the lack of available alternative treatments in this population.

- The benefits to public health of the immediate availability on the market of the medicinal product concerned outweighs the risk inherent in the fact that additional data are still required.

The CHMP considered that the potential risks inherent in marketing pixuvri for the specific indication, while additional, more comprehensive data will be available in the future, would be offset by the potential benefit to the patients. The CHMP agreed that the RMP for pixuvri in the approved indication was adequate to address any identified and unknown risks.

The CHMP concluded that all the requirements for the granting of a conditional marketing authorisation had been met.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus decision is of the opinion that Pixuvri is not similar to Torisel® within the meaning of Article 3 of Commission Regulation (EC) No. 847/200.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the risk-benefit balance of Pixuvri in "the treatment of adult patients with multiply relapsed or refractory aggressive Non-Hodgkin B-cell Lymphomas (NHL). The benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy."

is favourable and therefore recommends the granting of the conditional 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)

Conditions and requirements of the Marketing Authorisation

Risk Management System and PSUR cycle

The MAH must ensure that the system of pharmacovigilance, presented in Module 1.8.1 of the marketing authorisation, is in place and functioning before and whilst the product is on the market.

The MAH shall perform the pharmacovigilance activities detailed in the Pharmacovigilance Plan, as agreed in version 4 of the Risk Management Plan (RMP) presented in Module 1.8.2 of the marketing authorisation and any subsequent updates of the RMP agreed by the CHMP.

As per the CHMP Guideline on Risk Management Systems for medicinal products for human use, the updated RMP should be submitted at the same time as the next Periodic Safety Update Report (PSUR).

In addition, an updated RMP should be submitted:

- When new information is received that may impact on the current Safety Specification, Pharmacovigilance Plan or risk minimisation activities
- Within 60 days of an important (pharmacovigilance or risk minimisation) milestone being reached
- at the request of the EMA

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

Not applicable

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14(7) of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
To conduct a randomised controlled Phase 3 study (PIX306) of pixantrone-rituximab vs gemcitabine-rituximab in patients with aggressive B-cell NHL, who failed front line CHOP-R who are not eligible for autologous stem cell transplant (ASCT) (2^{nd} line) or failed ASCT (3^{rd} or 4^{th} line). A clinical study report should be submitted.	30 June 2015

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

Not applicable.

Divergent position(s) to the majority recommendation are appended to this report.

New Active Substance Status

Based on the CHMP review of data on the quality, non-clinical and clinical properties of the active substance, the CHMP considers that pixantrone (as dimaleate) is to be qualified as a new active substance.

Divergent Positions

DIVERGENT POSITION EXPRESSED BY CHMP MEMBERS

The benefit in terms of CR and PFS is driven by patients treated in "rest of the world".

No benefit has been demonstrated for target population relevant for the clinical practice in Western Europe: No clear benefit for pixantrone over comparator is demonstrated for patients with previous treatment with anti-CD20 or stem cell transplant and most importantly, patients in treated in North America or Western Europe.

The study results observed in patients treated in "rest of the world" cannot be extrapolated to the Western European population because the population differed clearly in baseline characteristics, e.g. age, performance status, histology, relevant prior treatments, including rituximab use and stem cell transplantation, and refractoriness to prior treatments.

The safety profile – based on the very limited data base is unfavourable compared to the reference treatment options.

In terms of efficacy no benefit for pixantrone over comparator is demonstrated for patients <65 years, male gender, previous treatment with anti-CD20 or stem cell transplant or ≥3 chemo regimens, and most importantly, patients in "North America" or "Western Europe". Therefore, based on the limited data presented, the benefit-risk ratio is deemed to be negative.

Considerations on conditional approval

Albeit it is likely that the applicant will be able to provide comprehensive data and it is not theoretically excluded that pixantrone may have the potential to fulfil an unmet medical need in patients with multiply relapsed or refractory aggressive NHL, two prerequisites of article 2 as of CD 507/2006 are not met:

1. The risk-benefit balance of the product is not positive but negative (see above).
2. The benefits to public health of the immediate availability do not outweigh the risks inherent in the fact that additional data are still required. Rather, immediate availability of the medicinal product on the European market will hamper the clarification of relevant scientific questions such as the benefit in a (Western) European population.

Overall conclusion

Based on the data submitted the benefit-risk ratio of Pixivuri in "the treatment of adult patients with multiply relapsed or refractory aggressive Non-Hodgkin B-cell Lymphomas (NHL). The benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy." is negative. Neither a full nor a conditional approval is an option. The MAA has to be rejected.

Pierre Demolis	Andrea Laslop	Jan Mueller-Berghaus
Harald Enzmann	Hubert Leufkens	Barbara van Zwieten-Boot
Ingunn Hagen Westgaard	Jan Mazag	