EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 November 2012
EMA/CHMP/27767/2013
Committee for Medicinal Products for Human Use (CHMP)

# Assessment report

**Istodax**

International non-proprietary name: **romidepsin**

Procedure No. **EMEA/H/C/002122**

## Note

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

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
**Telep□one** +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7523 7455
**E-mail** info@ema.europa.eu **Website** www.ema.europa.eu

# Product information

| | |
|---|---|
| **Name of the medicinal product:** | Istodax |
| **Applicant:** | Celgene Europe Ltd.<br>1 Longwalk Road<br>Stockley Park<br>UB11 1DB<br>United Kingdom |
| **Active substance:** | romidepsin |
| **International Nonproprietary Name/Common Name:** | romidepsin |
| **Pharmaco-therapeutic group (ATC Code):** | Other antineoplastic agents<br>(L01XX39) |
| **Therapeutic indication:** | Treatment of adult patients with peripheral T-cell lymphoma (PTCL) that has relapsed after or become refractory to at least one prior therapy |
| **Pharmaceutical forms:** | Powder and solvent for concentrate for solution for infusion |
| **Strength:** | 5 mg/ml |
| **Route of administration:** | Intravenous use |
| **Packaging:** | powder: vial (glass); solvent: vial (glass) |
| **Package sizes:** | 1 vial + 1 vial |

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# List of abbreviations

| | |
|---|---|
| ADR | adverse drug reaction |
| AE | adverse event |
| AITL | angioimmunoblastic T cell lymphoma |
| ALC | absolute leukocyte count |
| ALCL | anaplastic large cell lymphoma |
| ALK | anaplastic lymphoma kinase |
| ALP | alkaline phosphatase |
| ALT | alanine transaminase |
| ANC | absolute neutrophil count |
| APA | action potential amplitude |
| APD90 | action potential duration at 90% repolarization |
| APTT | activated partial thromboplastin time |
| ASCT | autologous stem cell transplant |
| AST | aspartate transaminase |
| AT | as treated |
| bFGF | basic fibroblast growth factor |
| BHT | butylated hydroxytoluene |
| BSA | body surface area |
| BSEP | bile salt export pump |
| BUN | blood urea nitrogen |
| BVL | Ben Venue Laboratories |
| CHOP | cyclophosphamide, doxorubicin, vincristine and prednisone |
| CL | clearance |
| CNS | central nervous system |
| C(P)K | creatine (phospho-)kinase |
| CQA | critical Quality attribute |
| CR | complete response |
| CRu | complete response unconfirmed |
| CT | computer tomography |
| CTCL | cutaneous T-cell lymphoma |
| CYP | cytochrome P450 |
| DDI | drug-drug interaction |
| DLBCL | diffuse large B-cell lymphoma |
| DSC | differential scanning calorimetry |
| DUSP1 | dual specificity phosphatase 1 |
| DVT | deep venous thrombosis |
| EATL | Enteropathy-Associated T-cell Lymphoma |
| EBV | Epstein-Barr virus |
| ECG | electrocardiography |
| ECOG | Eastern Cooperative Oncology Group |
| FDG-PET | fluorodeoxyglucose PET |
| GC | gas chromatography |
| GOT | glutamic oxaloacetic transaminase |
| GPT | glutamic pyruvate transaminase |
| HC | histopathologically confirmed |
| HCP | Host Cell Proteins |
| HDAC | histone deacetylase |
| hERG | human Ether-à-go-go-Related Gene |
| HIF-1 | hypoxia inducible factor-1 |
| HPLC | high-performance liquid chromatography |
| HSV | herpes simplex virus |
| HZV | herpes zoster virus |
| ICE | ifosfamide, carboplatin and etoposide |
| IP | intraperitoneal(ly) |
| IRC | independent review committee |
| IV | intravenous(ly) |
| IWC | International Workshop Criteria |
| LC/MS/MS | liquid chromatography followed by tandem mass spectroscopy |
| LDH | lactate dehydrogenase |

| | |
|---|---|
| LOQ | level of quantitation |
| LSC | Liquid scintillation counting |
| LVEF | left ventricular ejection fraction |
| MCB | master cell bank |
| MCH | mean corpuscular haemoglobin |
| MCHC | mean corpuscular haemoglobin concentration |
| MCV | mean corpuscular volume |
| MRI | magnetic resonance imaging |
| MRP | multi-drug resistance associated protein |
| MST | mean survival time |
| MTD | maximal tolerated dose |
| MTT | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| MWCB | master working cell bank |
| NADPH | nicotinamide adenine dinucleotide phosphate-oxidase |
| NCE | normochromatic erythrocytes |
| NCI | National Cancer Institute |
| ND | not determined |
| NHL | non-Hodgkin's lymphoma |
| NK | natural killer |
| NMR | nuclear magnetic resonance |
| NOAEL | no observable adverse effect level |
| NOS | not otherwise specified |
| NYHA | New York Heart Association |
| OATP | organic anion-transporting polypeptide |
| ORR | overall response rate |
| OS | overall survival |
| Papp | permeability coefficient |
| PBT | persistent bioaccumulative toxic |
| PCE | polychromatic erythrocytes |
| PD | progressive disease |
| PEC | predicted environmental concentration |
| PET | positron emission tomography |
| PFS | progression free survival |
| p-gp | permeability glycoprotein |
| PI | propidium iodide |
| PO | *per os* |
| PP | per protocol |
| PPK | population pharmacokinetics |
| ppm | parts per million |
| PR | partial response |
| PSA | prostate-specific antigen |
| PTCL | peripheral T-cell lymphoma |
| PXR | pregnane X receptor |
| qRT-PCR | quantitative reverse transcriptase-polymerase chain reaction |
| RBC | red blood cells |
| RECIST | Response Evaluation Criteria in Solid Tumors |
| REAL | Revised European-American Lymphoma |
| RH | relative humidity |
| RMP | resting membrane potential |
| RRT | relative retention time |
| SAE | serious adverse event |
| SD | stable disease |
| SC | subcutaneously |
| SCID | severe combined immunodeficiency |
| TEAE | treatment-emergent adverse event |
| TBA | tert-butyl alcohol |
| TLC | thin layer chromatography |
| TTP | time to progression |
| ULN | upper limit of normal |
| VEGF | vascular endothelial growth factor |
| VTE | venous thromboembolism event |
| WBC | white blood cells |
| WFI | water for injection |

# 1. Background information on the procedure

## *1.1. Submission of the dossier*

The applicant Celgene Europe Ltd. submitted on 2 March 2011 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Istodax, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 29 June 2010.

Istodax was designated as an orphan medicinal product EU/3/05/328 on 28 October 2005. Istodax was designated as an orphan medicinal product in the following indication: treatment of peripheral T-cell lymphoma (nodal, other extranodal and leukaemic/disseminated).

The applicant applied for the following indication: treatment of adult patients with peripheral T-cell lymphoma (PTCL) that has relapsed after or become refractory to at least one prior therapy.

**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/40/2011 on the granting of a (product-specific) waiver.

### *Information relating to orphan market exclusivity*

**Similarity**

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products, because there is no authorised orphan medicinal product for a condition related to the proposed indication.

### New active Substance status

The applicant requested the active substance romidepsin 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 28 September 2005. The Scientific Advice pertained to non-clinical and clinical aspects of the dossier.

### *Licensing status*

Istodax has been given a Marketing Authorisation in the USA on 5 November 2009.

## *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: **Jens Ersbøll** Co-Rapporteur: **Pierre Demolis**

CHMP Peer reviewer: Beatriz Silva Lima

The EMA Product Team Leader: Iordanis Gravanis

- The application was received by the EMA on 2 March 2011.
- The procedure started on 23 March 2011.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 17 June 2011. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 15 June 2011.
- During the meeting on 21 July 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 21 July 2011.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 17 February 2012.
- The summary report of the GCP inspection carried out at the following sites: Memorial Sloan Kettering Cancer Center, Centre Hospitalier Lyon Sud and ICON Medical Imaging between 27-30 June 2011, 16-19 August 2011 and 22-24 August 2011, respectively, was issued on 12 October 2011.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 3 April 2012.
- During a meeting of a SAG-Oncology on 10 April 2012, experts were convened to address questions raised by the CHMP.
- During the CHMP meeting on 19 April 2012, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 21 May 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 06 June 2012.
- During the CHMP meeting on 19 June 2012, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 16-19 July 2012, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Istodax on 19 July 2012.

## *1.3. Steps taken for the re-examination procedure*

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

Rapporteur: **Pieter de Graeff** Co-Rapporteur: **Ian Hudson**

EMA Product Team Leader: Iordanis Gravanis

- The applicant submitted written notice to the EMA on 2 August 2012 to request a re-examination of Istodax CHMP opinion of 19 July 2012.
- During its meeting on 17-20 September 2012, the CHMP appointed Pieter de Graeff as Rapporteur and Ian Hudson as Co-Rapporteur.
- The applicant submitted the detailed grounds for the re-examination on 17 September 2012. The re-examination procedure started on 18 September 2012.
- The Rapporteur's Assessment Report was circulated to all CHMP members on 19 October 2012. The Co-Rapporteur's Assessment Report was circulated to all CHMP members on 18 October 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's detailed grounds for re-examination to all CHMP members on 8 November 2012.
- During the meeting on 12-15 November 2012, the CHMP, in the light of the scientific data available and the scientific discussion within the Committee, the CHMP re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the marketing authorisation.

# 2. Scientific discussion

## *2.1. Introduction*

The peripheral T-cell lymphomas are a heterogeneous group of rare disorders that result from clonal proliferation of mature post-thymic lymphocytes. According to the Revised European-American Lymphoma / World Health Organization (REAL/WHO) classification system, PTCL belongs to the subtype of mature T/natural killer (NK) cell neoplasms. PTCL is further subdivided into several subtypes. An overview of PTCL subtypes, including the relative incidence of each subtype in Europe and North America, is provided in Table 1.

**Table 1: Classification of Peripheral T-Cell Lymphomas**

| | Relative Incidence (%) | |
|---|---|---|
| **PTCL Subtype** | **Europe** | **North America** |
| PTCL, not otherwise specified (NOS) | 34.3 | 34.4 |
| Angioimmunoblastic T cell lymphoma (AITL) | 28.7 | 16.0 |
| Anaplastic large cell lymphoma (ALCL), ALK positive | 6.4 | 16.0 |
| Anaplastic large cell lymphoma (ALCL), ALK negative | 9.4 | 7.8 |
| Enteropathy-type T-cell lymphoma | 9.1 | 5.8 |
| T/NK-cell lymphoma | 4.3 | 5.1 |
| Hepatosplenic T cell lymphoma | 2.3 | 3.0 |
| Adult T cell leukaemia/lymphoma | 1.0 | 2.0 |
| Subcutaneous panniculitis-like T-cell lymphoma | 0.5 | 1.3 |

Of the approximately 65,000 new cases of non-Hodgkin's lymphoma (NHL) diagnosed in the US each year, with a similar incidence reported in the EU, PTCLs comprise between 5% and 10% of the cases (Armitage and Weisenburger, 1998; Jemal *et al*, 2007; Groves *et al*, 2000). These T-cell neoplasms account for approximately 10% to 15% of all lymphoid neoplasms (Armitage, 2006; Jaffe *et al*, 2001). The incidence is higher on the Asian continent, with approximately 15% to 20% of all lymphomas classified as PTCL or natural killer (NK)/T-cell lymphoma (Ascani *et al*, 1997; Anderson *et al*, 1998). Compounding the difficulty in the diagnosis and treatment of such an uncommon entity, the classification of PTCL is complex, with as many as 15 distinct pathologic histologies now recognised (Jaffe *et al*, 2001).

The peripheral T-cell malignancies usually affect adults and most entities are more common in men. The disease often presents with involvement of multiple sites, including lymph nodes, blood, bone marrow, spleen, liver, skin, sinonasal cavity and other organs. There is a correlation between specific clinicopathologic entities and the primary site of involvement. Most PTCL subtypes follow an aggressive clinical course with a poor prognosis and patients with this disease require prompt treatment.

Peripheral T-cell lymphoma (PTCL) is a life-threatening form of non-Hodgkin lymphoma (NHL) that exhibits an aggressive clinical behaviour. Long-term outcome in patients with this disease is extremely poor with 5-year overall survival rates of approximately 32% for patients with the 2 most common subtypes. In particular, patients relapsing following front-line therapy have an extremely poor prognosis (Vose, 2008) with current salvage therapy rarely resulting in complete responses, and when remission does occur, it is generally of short duration.

Currently there is no consensus on standard therapy for PTCL. Anthracycline-containing regimens such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) are commonly used (Vose *et al*, 2008), but this therapy has never been established prospectively as a preferred or even effective therapy for PTCL (Horwitz, 2007). In the International T-cell Lymphoma Clinical/Pathologic Project (Vose *et al*, 2008), no significant difference was observed in outcomes between patients who received anthracycline-containing regimens and those receiving regimens without an anthracycline. High-dose chemotherapy and ASCT have been used as salvage therapy for PTCL with marginal success (Rodriguez *et al*, 2001; Feyler *et al*, 2007). A review of 40 patients with relapsed PTCL preparing to proceed to ASCT as a salvage therapy highlights the lack of durable responses with current combination approaches. Most patients (70%) responded to second-line therapy with ICE (ifosfamide, carboplatin, etoposide); however, 83% of patients relapsed less than 4 months following ASCT (Horwitz *et al*, 2005). Despite these results, given the poor prognosis with currently available therapies, transplant has been employed recently as initial consolidation therapy following successful remission induction for patients with advanced PTCL (Rodriguez *et al*, 2007).

Romidepsin is an anti-neoplastic agent originally isolated from Chromobacterium violaceum strain 968 that has been identified as a novel histone deacetylase (HDAC) inhibitor. HDAC inhibitors have been shown to induce hyper-acetylation of histones and other non-histone protein species resulting in a variety of phenotypic changes, including induction or repression of genes, G1 and G2/M arrest of the cell cycle, morphological reversion of transformed cells, differentiation, cell growth inhibition, apoptotic cell death, and inhibition of angiogenesis. The target proteins include factors regulating gene expression, cell proliferation, cell migration and cell death, and play a role in angiogenesis and immune response. While not completely understood, it is clear that the mechanisms of HDAC inhibitor-induced transformed cell death may involve more than one pathway and that romidepsin exhibits both cytotoxic and cytostatic properties. With regard to histone target proteins, romidepsin preferentially interacts with zinc ions in the active site of HDAC class I enzymes and inhibits class II enzymes to a lesser extent.

The applicant applied for the following indication: Istodax is indicated for the treatment of adult patients with peripheral T-cell lymphoma (PTCL) that has relapsed after or become refractory to at least one prior therapy. In response to the CHMP List of Outstanding Issues, the proposed indication was amended as follows: Istodax is indicated for the treatment of adult patients with peripheral T-cell lymphoma (PTCL) that has relapsed after or become refractory to at least two prior therapies. The proposed dose of romidepsin was 14 mg/m2 administered intravenously on Days 1, 8 and 15 of a 28-day cycle.

The application contained an EMA Decision (P/40/2011) on the granting of a product-specific waiver for romidepsin in the treatment of peripheral T-cell lymphoma (nodal, extranodal and leukaemic/ disseminated). The waiver was granted for all subsets of the paediatric population and in accordance with article 11(1)(c) of Regulation (EC) No 1901/2006 on the grounds that the specific medicinal product does not represent a significant therapeutic benefit over existing treatments for paediatric patients. This was because the treatments used in medical practice (multi-agent chemotherapy) for ALCL would result in a 3 year overall survival of 78 % (PTCL other than ALK-positive ALCL) to 95% (ALK-positive ALCL). ALCL is the malignancy of paediatric interest within PTCL and indeed a substantial portion of adult patients with ALCL were included in studies presented in the product-specific waiver application. In contrast, taking the limited paediatric data together with non-clinical efficacy data, romidepsin did not seem particularly active. Even though no paediatric patient with ALCL or a paediatric ALCL cell line or xenograft was studied, the lack of activity in view of a non-targeted, a priori broad mechanism of action was considered to greatly reduce the priority that romidepsin may have had for further paediatric trials.

## *2.2. Quality aspects*

### 2.2.1. Introduction

The finished product is presented as powder and solvent for concentrate for solution for infusion containing 5 mg/ml of romidepsin as active substance. The powder contains apart from the active substance povidone and hydrochloric acid; the solvent is comprised by propylene glycol and anhydrous ethanol.

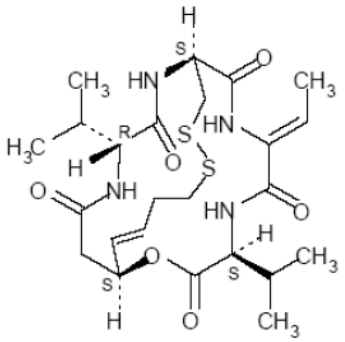
Istodax is available as a dual pack with a sterile single-use vial containing 10 mg of lyophilised romidepsin and a second sterile vial containing 2 ml of solvent.

The reconstituted solution of 2 ml contains 5 mg/ml of romidepsin and is further diluted for infusion to contain 0.1 to 0.02 mg/ml.

Due to a restricted GMP certificate which does not allow it to be used for new MAs, the originally proposed finished product manufacturing site was replaced during the evaluation by another manufacturer. At the time of the CHMP Opinion an updated GMP certificate, which includes small volume aseptically manufactured liquids, was not yet available. This is a major concern with regard to the suitability of the proposed manufacturing site.

### 2.2.2. Active Active Substance

Romidepsin is a white non-hygroscopic crystalline powder, very slightly soluble in water. The chemical name is (1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis(1-methylethyl)-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone corresponding to the molecular formula $C_{24}H_{36}N_4O_6S_2$, it has a relative molecular mass of 540.71 and the following structural formula:




The structure of romidepsin has been confirmed by the following methods: 1H NMR and 13C NMR spectroscopy, two-dimensional proton-carbon, mass spectroscopy, infrared spectroscopy, elemental analysis. Copies of relevant spectra have been provided as well as satisfactory interpretation.

Romidepsin originates from microbial biosynthesis as a single enantiomer with defined stereochemistry at four chiral centres and defined geometric isomers at the two alkene groups. The determined single-crystal structure is in agreement with the proposed stereochemical structure of romidepsin. Absolute stereochemical configuration has been determined by Single-crystal X-ray crystallography. Enantiomeric purity is controlled routinely by specific optical rotation. Polymorphism has been observed for romidepsin.

An extensive screen to identify polymorphs of romidepsin was conducted. The manufacturing process is robust to produce the desired crystalline form. Polymorphism has been sufficiently documented

considering that the crystalline form is not a CQA since romidepsin is used in solution. The polymorphic form has been characterised by X-Ray Powder Diffraction and DSC.

## *Manufacture*

Romidepsin is a bicyclic depsipeptide produced by fermentation. It is synthesised as a secondary metabolite by a strain of Chromobacterium violaceum. The fermentation is a 2-stage process with one seed stage and one production stage. Romidepsin is purified by standard chromatographic and recrystallisation processes. The manufacturing process is robust and produces consistently only one polymorphic form. The cyclic peptide contains an intramolecular disulfide bridge which makes it bicyclic. The stereochemistry of the active substance is predetermined by the biosynthetic pathway.

Suitable normal operating ranges for the process parameters for the sterilization steps, seed culture stage and production stage taking into account the batch size have been set. There are no critical process parameters.

The source, history and the generation of the production strain have been sufficiently documented. Absence of setting storage period for master working cell bank (MWCB) has been sufficiently justified.

However, a suitable retest period remains to be set for MCB. This issue remains unresolved.

Carry-over of potential impurities from fermentation process to active substance has also been discussed. Suitable statements from the suppliers have been provided regarding the absence of pesticides and toxins in raw materials of vegetable origin. Although the residual levels found for DNA are deemed acceptable, a discrepancy on the reported levels of residual DNA remains to be explained and a summary of validation results remained to be provided.

As far as the residual levels of Host Cell Proteins (HCP) found in active substance are concerned, the level of residual HCP in romidepsin does not represent a safety risk taking into account the maximal daily dose (14 mg/m² per day). Nevertheless, raw data for the tested batches showing the residual levels of HCP and confirmation that the clinical batch has been tested by the same method for residual HCP remain to be provided.

## *Specification*

The active substance specification includes tests and limits for: appearance (visual), identity (Ph.Eur., specific rotation), assay (HPLC), related substances (HPLC), residual solvents (GC), water content (Ph.Eur.), heavy metals (Ph.Eur.), and residue ignition (Ph.Eur.), Bacterial endotoxins (Ph.Eur.) and microbial count (Ph.Eur.).

Batch analysis data from a sufficient number of batches are presented. These include  batches produced at the intended commercial site and scale and 3 of which are the validation and primary stability batches. The other batches were produced for nonclinical and clinical studies and supporting stability studies. These batches represent the different manufacturing methods employed during the development.

The polymorphic form has been monitored for the first five commercial batches to demonstrate the consistency in the manufacturing process. The batch analysis results support the absence of a test for polymorphic form in the active substance specification.

All batches have been (re-)tested using the validated HPLC method for related substances. With the exception of the two early non-clinical batches the results for all the unspecified impurities are below LOQ. Overall the results are within the specifications and consistent from batch to batch.

In addition, there are some issues with regard to the proposed limits and testing methods for certain impurities that remain to be clarified. The details can be found in section 2.2.6 of this report. These issues are considered minor and are not expected to adversely affect the benefit risk balance of the product.

### *Stability*

Three commercial batches manufactured have been subjected to stability studies at accelerated (40°C/75% RH) and long-term conditions (25°C/60% RH) for 6 and 36 months respectively and photo-stability studies according to EU/ICH Guideline Q1B.

Based on the inherent physicochemical stability of romidepsin and lack of trends, no formal statistical analysis was performed. The investigated parameters were appearance, assay, water content and related substances. At the long-term storage condition, all specifications were met, no degradation products were observed, and no trends were noted. At the accelerated conditions all acceptance criteria were met throughout this 6 month study. A very slight increase in total impurities was observed at 6 months under the accelerated storage condition and an increase in water content was observed, but the results were well within the acceptance limits. No increases in total impurities have been observed at the 25°C/60% RH storage condition through 36 months. However, the stability indicating character of HPLC method for purity should be better demonstrated.

Photostability study

An ICH confirmatory photo-stability study was performed on one batch. In the directly exposed samples 3 unidentified impurities were detected by HPLC. None of these impurities were present in the dark control sample. The directly exposed sample had reduced assay compared the dark control sample. It can be concluded though from the stability results obtained to date that the packaging controls combined with the recommended storage condition provide an effective and proven protection to the drug product from photo-degradation thus rendering a separate testing method for such degradants unnecessary.

## 2.2.3. Finished Medicinal Product

### *Pharmaceutical Development*

Istodax is a lyophilised powder for solution for injection. It is manufactured as a lyophilised, sterile finished product containing 10 mg/vial romidepsin. It is supplied in a dual-pack configuration with a diluent vial for use in reconstitution.

Because of the poor water solubility of romidepsin, a tert-butyl alcohol non-aqueous co-solvent system was developed.

Insoluble particles have been observed in trace amounts in romidepsin solution for lyophilisation. Appropriate measures to minimise or essentially eliminate their formation in full-scale production have been put in place. Details on the method applied to demonstrate the absence of insoluble particles are awaited as well as clarification as to how levels are adequately controlled in the active substance.

In addition polymerisation of the active substance product is an impurity present in the active substance but is not formed during manufacture and storage of the finished product; consequently this impurity should be adequately controlled in the active substance.

There is no evidence of incompatibility of the active substance with the excipients. Accelerated and formal, long-term stability studies of the finished product have demonstrated that povidone and trace

quantities of HCl have no effect on romidepsin assay, purity, or any other measurable quality or performance attributes. The composition of the drug product has remained unchanged throughout clinical development and the formulation of the batches used in clinical development is the same as the commercial ones.

For both the powder and the diluent vials, a container-closure system integrity test has been performed to prove the adequacy of the proposed container/process to assure a suitable barrier to microbial contamination. All test vials passed the test. Moreover, container-closure integrity had been demonstrated on 46 month-old retain stability vials, which showed no evidence of dye intrusion. Finally all stability samples to date have passed sterility testing, providing further assurance of the integrity and suitability of the proposed container-closure system to maintain sterility.

The ability of the container-closure system to control moisture in the lyophilizsed drug product has also been demonstrated. The risk of interaction of the drug product with the glass vial or elastomer seal is considered to be minimal because the drug product is a dry form.

With regard to the diluent, as there is a potential for components of the container closure system to leach impurities into the organic diluent, a thorough study was performed to detect and quantify potential container closure leachables.

The potential leachable components were identified and presented. Evaluation of diluent container-closure leachables was also being performed as part of ongoing stability studies. Analytical methods were developed and validated to detect and quantify these potential leachables at appropriate levels. Potential leachables are either not detected or are measured at trace levels.

### *Adventitious agents*

Neither the active substance nor the excipients used in the manufacture of Istodax are of human or animal origin.

### *Manufacture of the product*

The manufacturing process of the powder consists of the following main steps: compounding of the raw materials, prefiltration of the bulk solution, sterilisation of the bulk solution by filtration, aseptic filling into sterile vials, lyophilisation, backfilling with nitrogen, stoppering and sealing of the vials.

The manufacturing process of the diluent consists of compounding of the raw materials, sterilisation of the bulk solution, aseptic filling into sterile vials and stoppering. There are no intermediates in the manufacturing process.

The suitability of the sterilising filters has been demonstrated. The aseptic processing is regularly validated by process simulation studies. Two media fill tests were described that cover a large range of vials sizes.

The media fill program at the proposed manufacturer covers all container/closure combinations on the respective filling line. Media fills are performed using the ‘worst-case’ scenario. Results from media fills identical to those presented for the lyophilised powder are included.

The manufacturing process has been satisfactorily validated and the batch analysis results for the validation batches demonstrate compliance with the specifications.

A study was provided to support the proposed holding time of the bulk solution.

Retrospective validation data for three consecutive production batches have been submitted. The process validation data and stability data supporting the new proposed manufacturer have been provided.

The manufacturing process has been validated by a number of studies for the major steps of the manufacturing process and has been demonstrated to be capable and to be able to reproducibly produce finished product of the intended quality. The in process controls are adequate for this pharmaceutical form.

The batch analysis data on two development batches and on three consecutive commercial production batches shows that the product can be manufactured reproducibly according to the agreed finished product specification, which is suitable for control of this intravenous pharmaceutical form.

## *Product specification*

The finished product release specifications include appropriate tests for appearance (visual), Clarity, colour of reconstituted solution (visual), identification (HPLC, UV), drug substance assay and impurities (HPLC), moisture content (Ph.Eur.), uniformity of dosage units (Ph.Eur.), reconstitution time (visual), residual tert-butyl alcohol (GC), particulate matter (Ph.Eur.), bacterial endotoxins (Ph.Eur.) and sterility (Ph.Eur.).

Analytical results are provided for Istodax powder batches manufactured for the clinical studies and for registration including results for 11 batches manufactured at the commercial scale and for 6 smaller scale development batches. The results presented are within the specifications.

Analytical results are provided for Istodax diluent batches manufactured for the clinical studies and for registration including results for 9 batches manufactured at the commercial scale. The results presented are within the specifications. The presented batch analysis results confirm consistency and uniformity of manufacture and indicate that the process is capable and under control.

The proposed specification limits for assay and impurities are in principle acceptable however they should be tightened to reflect the observed levels from batch analysis and stability studies. It should be further clarified if an unknown degradant which appears in samples stored at accelerated conditions in the active substance is also detected and quantified by the method applied for testing of related substances in the finished product.

## *Stability of the product*

Stability data of three validation and one engineering powder batches manufactured by the new proposed manufacturer and stored under long term conditions for up to 6 months (powder) at 25ºC/60%RH and for up to three months under accelerate conditions at 40ºC/75%RH were presented. Although these are limited data from the proposed manufacturer, additional, stability data from batches manufactured at the originally proposed site with the same formulation and a process that is essentially the same as the commercial process and packed in the primary packaging proposed for marketing of three commercial scale batches were provided. Those batches were stored under long term conditions for 36 months (powder) and for 48 (diluent) at 25ºC/60%RH and for up to six months under accelerate conditions at 40ºC/75%RH according to ICH guidelines were provided.

Istodax powder samples were tested for appearance, colour of solution, clarity of solution, completeness of solution, reconstitution time, assay, impurities, particulate matter, moisture, sterility, and bacterial endotoxins. For the diluent, the tests included appearance, assay, sterility, bacterial endotoxins and leachables. The analytical procedures used were stability indicating, however it remains

to be confirmed also for the impurities method in relation to the the unknown degradant (RRT 1.89) which appears in samples stored at accelerated conditions.

Regarding the powder, all the data recorded whatever the storage conditions and duration were well within the specifications. There was no apparent decreasing trend for the assay. No increasing trend of degradation product formation has been observed throughout the study. A difference in the moisture content has been observed between the batches manufactured at proposed and the original site. Although the moisture content as such raises no concerns, the difference in the results between sites should be further discussed.

Regarding the diluent, all the data recorded whatever the storage conditions and duration were well within the specifications. Vials stored at 25ºC/60% RH in the inverted position were also tested for leachables. Potential leachables met requirements.

Photostability Studies

A photostability study was performed as per ICH Q1B Option 2. Regarding the powder, the assay had a tendency to decrease and the impurities to increase for the exposed vial but there was an apparent mass imbalance. The reduced assay level and the observed mass imbalance of the exposed vials proved to be due to the formation of polymeric species.

Regarding the diluent, the appearance of the solution of the exposed vials did not evolved and the assay remained unchanged.

Based on available stability data, the proposed shelf-life and storage conditions are acceptable.

## 2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The active substance Istodax is a bicyclic depsipeptide originating from microbial biosynthesis and is produced by fermentation. The finished product is a sterile powder and a diluent both produced by aseptic process. Information on development, manufacture and control of the active substance and finished product has been generally presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance.

During the evaluation, the originally proposed finished product manufacturing site has been given a restricted GMP certificate that would not allow it to be used to support new MAs. This was a major concern and consequently an alternative manufacturer has been proposed. The necessary supportive data from this manufacturer have been provided but an updated GMP certificate is still awaited. At the time of the CHMP opinion, there were a number of minor unresolved quality issues that should be satisfactorily addressed prior to any positive recommendation.

## 2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

At the time of the Opinion there were no major quality issues unresolved with the exception of the GMP certificate to support the suitability of the proposed manufacturing facility. The GMP certificate due to an oversight did not include small volume aseptically manufactured liquids. An updated certificate is expected but was not yet available at the time of the Opinion.

However there were still some other minor quality issues that do not adversely affect the benefit risk balance of the product. It is expected that they would have been addressed at the last stage of the evaluation prior to a positive Opinion. In the context of the current procedure they remain unresolved and are raised as recommendations to be taken into account for the future development of the product (see section 2.2.6).

### 2.2.6. Recommendations for future quality development

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

1. A suitable retest period should be set for MCB.
2. The discrepancy on the reported levels of residual DNA should be explained and a summary of validation results should be provided.
3. As far as the residual levels of Host Cell Proteins (HCP) found in active substance are concerned, the raw data should be submitted for the tested batches showing the residual levels of HCP and it should be confirmed that the clinical batch has been tested by the same method for residual HCP.
4. The proposed limit for a specified impurity is not justified by stability results and should correspond to the levels observed from batch analysis.
5. The unknown degradant formed during storage at 40°C/75% RH should be identified, characterised and should be qualified. On the other hand information regarding three process related impurities should be updated accordingly.
6. The choice of the standard used in the validation of the method for the determination of residual HCP in romidepsin, should be justified and the HCP loading amounts should be clarified. It is acceptable to not submit a full method validation according to ICHQ2, however, at least LOQ and accuracy at LOQ should be determined. Furthermore the specifications for romidepsin should be updated by including a test for residual HCP with a suitable limit.
7. The polymerisation products of romidepsin should be adequately controlled in the active substance. Non-routine testing of polymers might be justified when experience will have been gained by analysis of further batches with a validated quantitative method depending on the results.
8. The stability indicating character of HPLC method for purity of the active substance should be demonstrated.
9. Details on the method applied to demonstrate the absence of insoluble particles in the active substance should be provided and clarification should be provided how levels are adequately controlled in the active substance.
10. The specification limits for assay and impurities for the finished product should be tightened to reflect the observed levels from batch analysis and stability studies.
11. It should be clarified if an unknown degradant, which appears in the active substance, is also detected and quantified by the method applied for testing of related substances in the finished product. It should also be confirmed that the impurities method in relation to this unknown degradant is stability indicating.
12. A discussion on the moisture content observed during storage in the batches from the finally proposed manufacturer should be presented.

## *2.3. Non-clinical aspects*

### 2.3.1. Introduction

*In vivo* pharmacology studies were conducted in mice. *In vivo* safety pharmacology studies were conducted in rats and dogs and they were GLP compliant, as were some of the *in vitro* safety pharmacology studies. The pharmacokinetics of romidepsin was investigated in rat and dog using the clinical product and route of administration in single-dose PK and GLP-compliant repeat-dose toxicokinetic studies. Single dose toxicity studies were conducted in rats and dogs and they were GLP compliant. A large number of repeat-dose toxicity studies were submitted: these were conducted in mice, rats and dogs and the pivotal among them were GLP compliant.

The Applicant received Scientific Advice from the CHMP. The non-clinical advice related particularly to the cardiac safety, drug-drug interactions, and the need for long-term toxicity testing in a non-rodent species.

### 2.3.2. Pharmacology

#### *Primary pharmacodynamic studies*

The inhibitory activity of romidepsin on HDAC function was evaluated in vitro using recombinant HDACs (Furumai *et al*, 2002; Miller *et al*, 2003; Bradner *et al*, 2010).

**Table 2: *In vitro* inhibition activities of romidepsin on recombinant HDAC**

| **$IC_{50}$ values for romidepsin (nM)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| HDAC enzyme<br>Class | **1**<br>**I** | **2**<br>**I** | **3**<br>**I** | 4<br>IIa | 5<br>IIa | 6<br>IIb | 7<br>IIa | **8**<br>**I** | 9<br>IIa |
| Furumai et al., 2002 & Miller et al., 2003 | 36 | 47 | | 510 | | 14,000 | | | |
| Bradner et al., 2010[a] | 0.002 | 0.004 | 0.150 | 21 | 550 | 9.5 | 1250 | 0.150 | 1100 |

The primary pharmacological studies focused on HDAC inhibition-induced gene expression and effects on cell cycle, cell proliferation, and apoptosis. These are summarised in the following Table 3.

**Table 3: Summary of studies investigating the mechanism of action of romidepsin**

| **Test system, concentrations, endpoints** | **Main findings** |
|---|---|
| **Study no.CRR910025 – *in vitro*** | |
| Endpoints, cell lines<br>- Cytotoxicity for Various human tumour cell lines (see list on right column)<br>- Effect on DNA, RNA and protein synthesis in murine leukaemia L1210 cells ($^{14}$C-thymidine, $^{14}$C-uridine, $^{14}$C-leucine incorporation)<br>- Cell cycle and gene expression (c-myc and Ha-ras mRNAs, northern dot hybridisation):Ras 1 cells | Cytotoxicity: the following $IC_{50}$ values were determined<br>- Lung tumours (A549, PC-9, PC-1, PC-10, ADH, LX-1): 0.3 to 2.4 ng/mL<br>- Stomach tumours (MKN28, MKN74): 0.9 ng/mL<br>- Breast tumours (MCF-7, ZR-75-1): 0.6 to 0.7 ng/mL<br>- Colon tumours (Colo-201, SW 480): 0.3 to 1.0 ng/mL<br>- Human normal fibroblasts: >1000 ng/mL<br>- Human normal endothelium: 7 ng/mL<br>- Murine embryonic cells (BALB/3T3, NHI/3T3): 3.2 to 9.2 ng/mL<br><br>Macromolar synthesis: concentration-dependent ↓ RNA synthesis from 5.4 to 54 ng/mL (-22% to -85%) – more potent than effect on DNA or protein synthesis<br><br>Cell cycle and expression of c-myc and Ha-ras mRNAs: G0/G1 arrest and potent ↓ c-myc mRNA expression at 2.5 ng/mL – reversible upon drug removal from culture medium |

| Test system, concentrations, endpoints | Main findings |
|---|---|
| **Study no.CRE040013 – *in vitro*** | |
| Endpoints, cell lines<br>- Cytotoxicity: human leukaemia cell lines U-937, K562, and CCRF-CEM<br>- apoptosis: U-937 cells<br>- cell cycle: U-937 cells<br>- differentiation: human leukaemia U-937 cells<br>- gene expression ($p21^{WAF1/Cip1}$ and gelsolin mRNAs, qRT-PCR): U-937 cells<br>- chromatin immuno-precipitation: U-937 cells<br><br>Romidepsin concentration: 0.5, 5 ng/mL | Cytotoxicity: the following $IC_{50}$ values were determined for growth inhibition: U-937: 5.92 nM / K562: 8.36 nM / CCRF-CEM: 6.95 nM<br><br>Apoptosis: ↑ apoptosis in treated vs. control cells, beginning at 20 h incubation and with time-dependent ↑ up to 72 h. At the latter time-point, 53% of treated cells were annexin V- and PI-positive (at 5 ng/mL; 0.5 ng/mL not investigated)<br><br>Cell-cycle: at 5 ng/mL, ↓ G1 fraction and G2/M arrest at12 h exposure, ↑ sub-G1 fraction at 24 h exposure. G1 arrest observed in cell treated at 0.5 ng/mL for 48 h and then cultured in drug-free medium for 24 h<br><br>Differentiation: induction of differentiation at 0.5 and 5 ng/mL (based on expression of Cd11b and CD14)<br><br>$p21^{WAF1/Cip1}$ and gelsolin mRNAs levels: time-dependent ↑ expression level of both mRNAs from 1 to 24 hour incubation ($p21^{WAF1/Cip1}$: 9- to 654-fold, gelsolin: 2- to 152-fold) (at 5 ng/mL; 0.5 ng/mL not investigated)<br><br>Chromatin immuno-precipitation: ↑ acetylation of histone H3 (P1 and P2 regions of $p21^{WAF1/Cip1}$ promoter) and histone H4 (in all regions of $p21^{WAF1/Cip1}$ promoter and exon 2) (at 5 ng/mL; 0.5 ng/mL not investigated) |
| **Study no.CRE020081 – *in vitro*** | |
| Cell line: PSA-secretory human prostate carcinoma (LNCaP)<br><br>Endpoints:<br>- Cytotoxicity<br>- Gene expression (PSA mRNA, qRT-PCR)<br>- PSA levels<br><br>Duration: 48-h exposure | Cytotoxicity: concentration-dependent inhibition of cell growth; $IC_{50}$ = 3.36 ng/mL<br><br>PSA mRNA levels: biphasic modulation<br>- slight ↑ PSA mRNA levels at 0.1 and 0.32 ng/mL (111% and 131.3% of control levels)<br>- marked ↓ PSA mRNA levels at 1 and 3.2 ng/mL: (36.9% and 1.9% of control levels)<br><br>PSA secretion: biphasic modulation of PSA levels<br>- slight concentration-dependent ↑ PSA levels from 0.0488 to 0.391 ng/mL (122.9% to 157.2% of control levels)<br>- marked concentration-dependent ↓ PSA levels at ≥0.781 ng/mL (22.6% of control levels at 3.13 ng/mL which is close to the IC50 / up to 18.6% of control levels at 50 ng/mL) |
| **Study no.CRE010158 – *in vitro and in vivo*** | |
| Endpoints, cell lines<br>- cell cycle and apoptosis: prostate cancer (PC-3)<br>- gene expression ($p21^{WAF1/Cip1}$ mRNA– qRT-PCR): prostate cancer (PC-3, DU-145), renal cancer (ACHN)<br><br>Romidepsin concentration: 5 ng/mL | Cell cycle and apoptosis: cell cycle arrest at G2/M rather than G1 after 12 h exposure, induction of apoptosis (14.8% cells annexin V-positive/PI-negative after 48 h exposure)<br><br>$p21^{WAF1/Cip1}$ mRNA levels: time-dependent ↑ mRNA levels of $p21^{WAF1/Cip1}$ in PC-3 and DU-145 cells from 1 to 24 hours (up to 36-fold and 9-fold in respective cell lines) |
| Test system: nude mice (20M/group) implanted SC with human PC-3 or ACHN tumour xenografts<br><br>Doses, route: 3.2 mg/kg, administered IV 3 weeks post-implantation<br><br>Endpoint: gene expression ($p21^{WAF1/Cip1}$ and c-myc mRNA levels determined by qRT-PCR, in tumours isolated prior and 1, 3, 6, and 24 h post-dose) | PC-3 cells:<br>- ↑ $p21^{WAF1/Cip1}$ mRNA levels for up to 24 h post-dose, peak at 3h post-dose<br>- ↓ c-myc mRNA levels over the 24-h post-dose period<br><br>ACHN cells:<br>- no change in $p21^{WAF1/Cip1}$ mRNA levels<br>- ↑ c-myc mRNA levels at 24 h post-dose<br><br>(note: basal levels of $p21^{WAF1/Cip1}$ and c-myc mRNA were lower and higher, respectively, in PC-3 than in ACHN cells) |

| Test system, concentrations, endpoints | Main findings |
|---|---|
| **Study no.CRE040012 – *in vitro and in vivo*** | |
| Endpoints, cell lines<br>- gene expression (VEGF and bFGF mRNAs, 24h incubation, qRT-PCR): PC-3 and ACHN cells<br>- gene expression (VEGF and HIF-α mRNAs in hypoxic conditions, up to 16h incubation, qRT-PCR): PC-3 cells<br>- chromatin immuno-precipitation: PC-3 cells (2h incubation under hypoxia)<br><br>Romidepsin concentration: 5 ng/mL | VEGF and bFGF mRNAs levels:<br>- PC-3 cells ↓ VEGF and bFGF mRNAs levels (-51% and -94% at 12 h)<br>- ACHN cells: ↓ bFGF mRNA levels (-81% at 12 h), no impact on VEGF mRNA<br><br>VEGF and HIF--α mRNAs under hypoxia<br>↓ VEGF mRNA levels, no impact on HIF--α mRNA levels (*note: transcription of VEGF gene is induced by hypoxia, and tumour angiogenesis extends under conditions of hypoxia – HIF-1 is the transcription factor of VEGF gene under hypoxia and its activity is primarily determined by hypoxia-induced stabilisation of HIF--α which is a component subunit of HIF-1*)<br><br>Chromatin immuno-precipitation: ↑ acetylation of histone H3 and H4 associated with the VEGF promoter, especially in the P2 region which contains HIF binding site |
| Test system: nude mice (M, no.unknown) implanted SC with human PC-3 or ACHN tumour xenografts<br><br>Doses, route: 3.2 mg/kg, administered IV once tumours reached 100-300 mg<br><br>Endpoint: gene expression (VEGF and bFGF mRNA levels determined by qRT-PCR, in tumours isolated prior and 1, 3, 6, and 24 h post-dose) | PC-3 cells:<br>- ↓ VEGF mRNA levels from 3h post-dose (-68% at 24h)<br>- ↓ bFGF mRNA levels from 1h post-dose (-88% at 24h)<br><br>ACHN cells:<br>- no change in VEGF abd bFGF mRNA levels<br><br>(note: basal level of bFGF mRNA was lower in PC-3 than in ACHN cells, no great variation regarding basal VEGF mRNA levels) |
| **Study no.CRE040010 – *in vitro and in vivo*** | |
| Endpoints, cell lines<br>- gene expression profile (Geneship analysis using Affimetrix GeneChips): U-397, PC-3, and ACHN cells<br><br>Romidepsin concentration: 5 ng/mL<br><br>Duration: 0.1, 3, 12 and 24-h exposure | 205 of 7070 genes profiled affected by romidepsin:<br>- 105 genes were up-regulated in response to romidepsin including $p21^{WAF1/Cip1}$, IL-8, and caspase 9<br>- 100 genes were down-regulated in response to romidepsin, with the most common examples being MAPK and cyclin A2 |
| Test system: nude mice (6M/group) implanted SC with human PC-3, SC-6, A498, or ACHN tumour xenografts<br><br>Doses, route: 1.8 and 3.2 mg/kg Q4Dx3 – administered IV once tumours reached 100-300 mg<br><br>Endpoint:<br>- Tumour weight changes (10 days following the final injection)<br>- gene expression profile (Geneship analysis using Affimetrix GeneChips – confirmation by measuring the expression of DUSP-1 and caspase 9 mRNA by qRT-PCR) | Antitumour activity<br>- PC-3 and SC-6 tumours: romidepsin-sensitive (98% and 84% ↓ in tumour growth at 3.2 mg/kg)<br>- A498 and ACHN tumours: romidepsin-insensitive (29% and 20% ↓ in tumour growth at 3.2 mg/kg)<br><br>Gene expression profile: comparison of results obtained in romidepsin-sensitive vs. romidepsin-insensitive tumours<br>- 27 genes had high expression in romidepsin-sensitive tumours, and low expression in romidepsin-insensitive tumours; 49 genes had an opposite expression profile<br>- e.g.: the expression of caspase 9 and DUSP1 (regulator of cell proliferation and apopotis) were 3-fold higher in romidepsin-sensitive tumours than in romidepsin-insensitive tumours |

The efficacy of romidepsin has been examined in vitro for its cytotoxic effects across multiple human tumour cell lines, and in vivo in numerous studies performed in mice implanted with human tumour xenografts (both solid and haematologically-derived tumours) or bearing murine tumours. These studies are summarised in the following Table 4, although some *in vitro* cytotoxicity data were summarised in Table 3.

**Table 4: Summary of pharmacology studies characterising the anti-tumour effects of romidepsin**

<table>
<tr><th>Test system, concentrations, endpoints</th><th>Main findings</th></tr>
<tr><td colspan="2">Study no.CRE010130 – in vitro</td></tr>
<tr><td>Cell lines:<br>Various human tumour cell lines (see list on right column)<br><br>Endpoints<br>Cytotoxicity</td><td>Cytotoxicity: the following $IC_{50}$ values were determined – shown as mean (range)<br>- Lymphoma (CCRF-CEM, U-937, THP-1, ML-3, HL-60, JOSK-1, K562, JOK-1): 3.23 ng/mL (0.60-4.52)<br>- Renal tumours (OUR10, ACHN, A-498): 4.97 ng/mL (4.25-5.98)<br>- Prostate tumours (PC-3, DU-145): 1.61 (1.21-2.01)<br>- Colon tumours (SW-480, Colo201, HT-29): 1.61 ng/mL (1.11-2.23)<br>- Lung tumours (PC-10, NCI-H69): 3.83 ng/mL (3.12-4.53)</td></tr>
<tr><td colspan="2">Study no.CRE040013 – in vivo</td></tr>
<tr><td>Test system: SCID mice (6M/group + 12 M control) inoculated IP with U-937 cells<br><br>Doses, route: 0, 0.1, 0.18, 0.32, 0.56, 1 mg/kg given IP, either once or twice a week beginning 24h post-inoculation<br><br>Endpoints: survival evaluated up to 60 days post-dose (mean survival time, MST)</td><td>
<table>
<tr><td></td><td>Control</td><td>Once a week</td><td>Twice a week</td></tr>
<tr><td>MST (days)</td><td>20</td><td>↑ (22.5-30.5)</td><td>↑ (25-33)</td></tr>
<tr><td>First death on</td><td>Day 18</td><td colspan="2"><Day 18 at 1 mg/kg (toxicity of romidepsin at this dose)</td></tr>
<tr><td>No alive on Day 24</td><td>1/12</td><td>0.18 mg/kg: 6/6<br>0.56 mg/kg: 6/6</td><td>0.32 mg/kg: 6/6<br>0.56 mg/kg: 6/6</td></tr>
<tr><td>No alive on Day 60</td><td>0/12</td><td>0.56 mg/kg: 1/6</td><td>0.56 mg/kg: 1/6</td></tr>
</table>
</td></tr>
<tr><td colspan="2">Study no.CRE000198 – in vivo</td></tr>
<tr><td>Test system: athymic nude mice (10 F/group) implanted with LOX IMVI melanoma xenografts<br><br>Route: IV or IP<br><br>Doses: variable according to the route, and treatment schedule (from daily to intermittent treatments)<br><br>Endpoints: tumour regression, relative tumour growth and growth delay, no. tumour-free on Day 37, median time to doubling, net log cell kill</td><td>Intravenous route of administration was more efficacious and better tolerated than the intreaperitoneal route of administration; an increase in the number of animals with tumor regression and an increase in the growth delay and log net cell kill values were observed with intravenous administration.<br><br>With intravenous administration, a regimen consisting of high-dose, intermittent administration was superior to that of lower-dose, daily administration.</td></tr>
<tr><td>Test system: athymic nude mice (10 F/group) implanted with LOX IMVI melanoma xenografts<br><br>Route: IV<br><br>Doses:<br>- 0, 3.60, 5.30, 8 mg/kg Q4D x 3 (1 cycle, beginning on day 6 after implantation)<br>- 1.60, 2.40 mg/kg Q4D x 3 (2 cycles beginning on Days 6 and 23 after implantation)<br><br>Endpoints: see above (no. tumour-free on Day 75)</td><td>1.6 mg/kg (2 cycles): 1/10 death, body weight ↓ 11.9%, 1/10 with complete regression, growth delay of 502%, net cell kill of -0.5 log<br><br>2.4 mg/kg (2 cycles): 1/10 death, body weight ↓ 12.9%, 3/10 with complete regression, growth delay of 720%, net cell kill of 2.0 log<br><br>3.60 mg/kg (1 cycle): 0/10 death, body weight ↓ 11.2%, 6/10 with complete regression, growth delay of 493%, net cell kill of 3.7 log<br><br>5.3 mg/kg (1 cycle): 0/10 death, body weight ↓ 15.1%, 8/10 with complete regression, growth delay of 524%, net cell kill of 4.0 log, 1/10 tumour free on Day 75<br><br>8 mg/kg (1 cycle): 0/10 death, body weight ↓ 19.9%, 8/10 with complete regression, growth delay of 870%, net cell kill of 8.0 log, 1/10 tumour free on Day 75<br><br>All regimens were tolerated and effective against LOX melanomas. The MTD was 8 mg/kg based on the 20% decrease in body weight, and the 8 mg/kg Q4Dx3 regimens appears to be superior based on survival, tumour regression, and net cell kill</td></tr>
</table>

| Test system, concentrations, endpoints | Main findings |
|---|---|
| Test system: athymic nude mice (10 F/group) implanted with NCI-H522 small cell lung tumour xenografts<br><br>Route: IV<br><br>Doses: 0, 1.6, 2.4, 3.6, 5.3, 8 mg/kg Q4Dx3 (treatment began on Day 13 post-implantation)<br><br>Endpoints: see above (no. tumour-free on Day 46) | 1.6 mg/kg: 1/10 death, body weight ↓ 10.5%, 0/10 with either partial or complete regression, growth delay of 40%, net cell kill of -0.4 log<br><br>2.4 mg/kg: 1/10 death, body weight ↓ 11.8%, 0/10 with either partial or complete regression, growth delay of 69%, net cell kill of -0.1 log<br><br>3.60 mg/kg: 3/10 deaths, body weight ↓ 20.4%, 0/10 with either partial or complete regression, growth delay of 106%, net cell kill of 0.3 log<br><br>5.3 mg/kg: 2/10 deaths, body weight ↓ 22.7%, 0/10 with either partial or complete regression, growth delay of 95%, net cell kill of 0.2 log<br><br>8 mg/kg: 7/10 deaths, body weight ↓ 25.7%, 0/10 with either partial or complete regression, growth delay of 90%, net cell kill of 0.1 log<br><br>**Romidepsin has limited activity against NCI-H522 tumour xenografts, and was less tolerated than in previous experiments (1/10 to 7/10 deaths)** |
| Test system: athymic nude mice (10 F/group) implanted with UACC-62 melanoma xenografts<br><br>Route: IV<br><br>Doses: 0, 1.6, 2.4, 3.6, 5.3, 8 mg/kg Q4Dx3 (treatment began on Day 19 post-implantation)<br>Endpoints: see above (no. tumour-free on Day 48) | 1.6 mg/kg: 0/10 death, body weight ↓ 9.9%, 0/10 with either partial or complete regression, growth delay of 85%, net cell kill of 0<br><br>2.4 mg/kg: 0/10 death, body weight ↓ 16.3%, 0/10 with either partial or complete regression, growth delay of 107%, net cell kill of 0.2 log<br><br>3.60 mg/kg: 3/10 deaths, body weight ↓ 15.5%, 0/10 with either partial or complete regression, growth delay of 145%, net cell kill of 0.6 log<br><br>5.3 mg/kg: 0/10 death, body weight ↓ 19.1%, 0/10 with completed regression and 1/10 with partial regression, 1/10 tumour-free on Day 48, growth delay of 97%, net cell kill of 0.1 log<br><br>8 mg/kg: 4/10 deaths, body weight ↓ 20.6%, 0/10 with either partial or complete regression, growth delay of 95%, net cell kill of 0.1 log<br><br>**Romidepsin has limited activity against UACC-62 tumour xenografts** |
| Test system: athymic nude mice (10 F/group) implanted with MX-1 mammary carcinomas<br><br>Route: IV or IP<br><br>Doses: variable according to the treatment regimen (start Day 11 postimplantation)<br>- 0.64 to 2.16 mg/kg/day for 5 days<br>- 2.40 to 8 mg/kg Q4D x3<br>- 1.07 to 3.60 mg/kg Q4D x 6<br>- 2.40 to 8 mg/kg Q7Dx4<br><br>Endpoints: see above (no. tumour-free on Day 45) | **Romidepsin had moderate anti-tumour activity against MX-1 tumour xenografts**. At the doses and regimens tested in this study, the best anti-tumour effects were observed, and higher total doses were tolerated, with the 2 intermittent dosing regimens (dosing every 4 days for a total of 3 or 6 doses). At 8 mg/kg Q4D x 3 and 3.60 Q4Dx6, there were however 9/10 and 7/10 deaths, respectively. |

| Test system, concentrations, endpoints | Main findings |
|---|---|
| **Study no.CRR910025 – *in vivo*** | |
| Test system, doses, route: mouse (no. unknown) implanted:<br>- IP with murine ascitic tumours (*P388 and L1210 leukaemia, B16 melanoma*): 0.032 to 1.8 mg/kg/day for 5 days (P388) or 9 days, IP route<br>- SC or ID with murine solid tumours (*colon38 carcinoma, B16 melanoma, M5076 reticular cell sarcoma, Lewis lung carcinoma, colon 26 adenocarcinoma, MethA sarcoma*): 0.32 to 10 mg/kg Q3D x 4, IV route<br><br>Endpoints: survival in mice with ascitic tumours, tumour size change in mice with solid tumours | Murine ascitic tumours: ↑ survival vs. controls, maximal at 0.56 mg/kg for each tumour. At the two highest dose levels (1 and 1.8 mg/kg, survival was not improved due mostly to romidepsin-related toxicity)<br><br>Murine solid tumours: ↓ tumour size vs controls in some tumours; the effect depended also on the day of treatment initiation<br>- colon38 carcinoma and M5076 reticular cell sarcoma: inhibition of tumour growth when romidepsin was administered from either Day 1, or Day 4 or Day 7 post-implantation<br>- B16 melanoma: inhibition of tumour growth when romidepsin was administered from Day 1 post-implantation<br>- MethA sarcoma: inhibition of tumour growth when romidepsin was administered from either Day 4 or Day 7 post-implantation<br>- Lewis lung carcinoma and colon 26 adenocarcinoma: no effect |
| Test system, doses, route: mouse (no. unknown) implanted IP with murine ascitic tumours (P388 leukaemia) resistant to MMC, or 5FU, or VCR, or CPM, or doxorubicin: 0.18 to 1 mg/kg/day for 4 days, IP route<br><br>Endpoints: mean survival time (MST) | Mice implanted with MMC, 5FU, VCR and CPM-resistant P388 tumours and treated with romidepsin had an increased MST than mice implanted with P388 sensitive to these drugs.<br><br>No effect on the survival of mice implanted with doxorubicin-resistant tumours. |
| Test system: nude mice (no.unknown) implanted (SC) with human lung carcinomas (LX-1, LU-65, A549, LC-6, PC-9), stomach carcinoma (SC-6), colon adenocarcinoma (colo201), or mammary carcinomas (MX-1, MCF-7)<br><br>Doses, route: 1.8 to 10 mg/kg Q4D x 3, IV route<br><br>Endpoint: tumour size change | ≥ 80% tumour growth inhibition in mice implanted with LU-65, LC-6, SC-6, and MX-1 tumour xenografts.<br><br>The dose of 10 mg/kg Q4D x 3 was found to be toxic to mice (survival rate <65%). |
| **Study no.CRE010131 – *in vivo*** | |
| Test system: athymic nude mice (6/sex/group) implanted with renal carcinoma (ACHN, RXR-631L), prostate cancer (PC-3, DU-145) or colon cancer (HT-29, HCC2998, HCT-15)<br><br>Doses, route: 0, 1.8, 3.2 mg/kg Q4D x 3). Positive control was paclitaxel (24 mg/kg/day for 5 days) – IV route<br><br>Endpoints: body weight, antitumour activity evaluated based on tumour size change (final/initial) in treated animals vs controls | No mortality in any group. Body weight changes were equivalent in groups treated with romidepsin at 3.2 mg/kg and those treated with paclitaxel at 25 mg/kg (up to -19%).<br><br>**Romidepsin showed anti-tumour activity against RXF-631L and PC-3 tumour xenografts. This activity was comparable to that of paclitaxel.** There was no clear relation to the dose in RXF-631L-implanted animals.<br><br>In addition, paclitaxel showed anti-tumour activity against DU-145, HT-29 and HCC2998 tumour xenografts. |

## *Secondary pharmacodynamic studies*

No specific secondary pharmacodynamic studies were submitted. However, the effect of romidepsin on various receptors was studied in the course of the safety pharmacology programme (please refer to miscellaneous studies in Table 5 below).

## *Safety pharmacology programme*

The safety pharmacology studies submitted are summarised in the following Table 5.

**Table 5: Summary of safety pharmacology studies**

| Type of study (ref) | Test system, study design | Results |
|---|---|---|
| ***Cardiovascular system: in vitro studies*** | | |
| hERG assay<br>(GLR030533)<br>GLP | HEK293 cells transfected with hERG gene<br>Concentrations tested: vehicle, 0.3, 1, 10 µg/mL | ↓ hERG channel current at 1 and 10 µg/mL (-18.0% and -37.3%, respectively) |
| Effect on action potentials<br>(GLR030460)<br>GLP | Papillary muscle from guinea pig right ventricle<br>Concentrations: vehicle, 0.3, 1, 10 µg/mL | 10 µg/mL: ↓ APD90 and APA<br>No effect on RMP and maximum rate of depolarisation (dV/dt max) |
| Potassium channel binding assay with reduced romidepsin<br>(DVRS-003)<br>Non-GLP | Channel type and test system<br>$K_{ATP}$ channel (hamster pancreatic HIT-T15 β cells), $SK_{CA}$ (Wistar rat brain tissue), hERG (human recombinant HEK-293 cells)<br><br>Test-article and concentrations<br>Dithiothreitol-reduced romidepsin: 10 µM | No significant binding to $K_{ATP}$, $SK_{CA}$ and hERG channels |
| ***In vivo studies*** | | |
| Effect on the CNS<br>(GLR030774)<br>GLP | Rat (6M/group)<br>Route: IV infusion over 4 hours<br>Doses: 0, 0.1, 0.3, 1.0 mg/kg<br>Endpoints: symptomatology and behaviour according to Irwin's method[b] – prior administration up to 24 hours following initiation of infusion | 0.3 mg/kg: ↓ activity, ↓ muscle tone<br><br>1 mg/kg: mortality (3/6 between 8-24 hr); ↓ activity; ataxic and abnormal gait; twitches, convulsions; ↓ pain response, ↓ startle reflex; ↓ muscle tone; incomplete eyelid closure, ↑ heart rate, ↑ respiration rate (at 24 hr) |
| Effect on the CNS, cardiovascular, respiratory and blood systems<br>(GLR030775)<br>GLP | Dog (4M/group*)<br>Route: IV infusion over 4 hours<br>Doses: 0, 0.1, 0.3, 1.0 mg/kg (positive control: astemizole, 20 mg/kg administered orally)<br>Endpoints: symptomatology, spontaneous motor activity, body temperature, respiration rate, haemoglobin oxygen saturation, blood pressure, heart rate, ECG, haematology | CNS: ↑ spontaneous motor activity (at 0.1 and 0.3 mg/kg, and at up to 24 hr post-dose), ↑ body temperature (≥ 0.3 mg/kg, at up to 24 hr post-dose)<br><br>Respiratory: ↑ respiratory rate (≥ 0.1 mg/kg)<br><br>Cardiovascular:<br>- ↓ systolic, diastolic and mean blood pressure at ≥ 0.1 mg/kg<br>- ↑ heart rate at ≥ 0.1 mg/kg; maximum values were +10%, +52% (4.5 and 10-18 hr post-dose), and +34% (at 4.5-10 hr post-dose) the pre-administration values at 0.1, 0.3 and 1.0 mg/kg, respectively<br>- ↓ RR interval at ≥ 0.1 mg/kg; maximum values were -8%, -34% (10-18 hr post-dose), and -26% (at 5-10 hr post-dose) the pre-administration values at 0.1, 0.3 and 1.0 mg/kg, respectively<br>- ↑ QTc Bazett (+8%) and Fridericia (+5%) at 1 mg/kg and 6 hours post-dose<br><br>Blood: ↑ MCHC, ↓ lymphocytes, ↓ MCV at ≥ 0.3 mg/kg (24-hr post dose); ↑ RBC and haemoglobin at 1 mg/kg (24-hr post dose) |
| ***Miscellaneous*** | | |
| Binding to several receptors and channels<br>(GLR030404)<br>Non-GLP | Binding assays involving several receptors[c] and channels[d]<br>Concentrations tested: 0.3, 1, 10 µg/mL | - Inhibition of β-estradiol binding to estrogen receptors by 26.6% at 1 µg/mL and by 97.8% at 10 µg/mL<br>- Inhibition of neurokininA binding to $neurokinin_2$ receptor by 71.4% at 10 µg/mL<br><br>No binding affinity for cardio-related (or associated) adrenergic and muscarinic receptors or calcium, sodium, or potassium ion channels |

APD: action potential duration at 90% repolarisation; APA: action potential amplitude; RMP: resting membrane potential

[a] the same animals were used for each administration group. They received the vehicle first, then the test-article in ascending order and the positive control at last; the drug withdrawal interval between each administration was 6 days (1 administration / week);
[b] locomotor activity, bizarre behaviour, motor affective response, equilibrium and gait, CNS excitation, sensoro-motor responses, muscle tone, autonomic response, death;
[c] adenosine (A1, A2, A3), adrenergic ($\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, $\beta_3$, $\beta$ non-selective), angiotensin II (type 1&2), bradykinin B2, cholecytokinin$_A$, dopamine (1, 2), dopamine transporter, estrogen, endothelin (A, B), GABA (A, B), galanin, glutamate (AMPA, kainite, NMDA agonist site, NMDA glycine site), glycine, Histamine (1, 2, 3), Leukotriene ($B_4$, $D_4$), muscarinic (1, 2, 3, non-selective), neurokinin (1, 2, 3), NE transporter, nicotinic, opiate (non-selective), oxytocin, PAF, serotonin (non-selective), serotonin transporter, sigma (non-selective), testosterone, $TXA_2$, vasopressin $V_1$, VIP;

[d] Ca channel (L, N) and Na channel$_2$ from rat brain and guinea pig heart, K channel (KA, $K_{ATP}$, KV, $SK_{CA}$),

### *Pharmacodynamic drug interactions*

In vitro combination effect of romidepsin with various antitumour agents (CRE040062, non-GLP): The anticancer effect of romidepsin (0.14 and 0.28 mg/kg) in combination with various antitumour agents (adriamycin, cisplatin, 5-fluorouracil, taxol and etoposide) was investigated *in vitro* using human prostate cancer DU-145 cells. Cells were simultaneously exposed to romidepsin and the antitumour agent for 24 hours or sequentially exposed to romidepsin for 24 hours followed by the antitumour agent for 24 hours or vice versa. Cell growth inhibition after 5 days was determined by an MTT assay.

Romidepsin showed a synergistic antitumour effect with adriamycin, cisplatin or etoposide without any schedule dependency. Sequential exposure to taxol followed by romidepsin produced a synergistic effect in cells. Sequential exposure to romidepsin followed by 5-fluorouracil showed synergistic effects in cells. These findings suggest that there is a schedule dependency on synergistic antitumour effect of romidepsin with 5-fluorouracil or taxol.

The mechanism of the combination effect of romidepsin and 5-fluorouracil was further investigated examining the expression of thymidylate synthase (a target enzyme of 5-fluorouracil) and p21WAF1/Cip1 mRNA in romidepsin-treated cells. Romidepsin induced p21WAF1/Cip1 mRNA expression. Romidepsin suppressed the expression of thymidylate synthase thereby enhancing the cytotoxicity of 5-fluorouracil.*In vivo* combination effect of romidepsin with various antitumour agents (CRE040011, non-GLP): The anticancer effect of romidepsin (0.14 and 0.28 mg/kg) in combination with various antitumour agents (irinotecan HCl, carboplatin, pacritaxel, mitomycin C or adriamycin) was investigated in mice inoculated IP with murine L1210 leukemia tumour cells. Drugs were administrated once daily for 4 consecutive days after tumour inoculation. Romidepsin showed synergistic effects when given in combination with adriamycin (only at the low dose of 1 mg/kg) but not when given in combination with the other anticancer agents.

In vivo evaluation of romidepsin in combination with gemcitabine (VVGLOU0501v2, non-GLP): The antitumour activity of romidepsin (2.5 and 5 mg/kg) was evaluated both as a single agent and in combination with gemcitabine (40 and 80 mg/kg) in the wildtype-ras Bx-PC-3 and ras-transformed PANC-1 human pancreas tumour xenograft models. As a single agent, romidepsin demonstrated some activity towards the ras-transformed PANC-1 tumour model (tumour growth inhibition of 26%) but was inactive in the wildtype-ras Bx-PC-3 line (no effect on tumour growth). In combination with gemcitabine, romidepsin demonstrated a statistically significant activity towards PANC-1 tumours (tumour growth inhibition >78%) but not towards BxPC-3 tumours (tumour growth inhibition 21-44%), suggesting agent specificity for the ras-transformed line.

## 2.3.3. Pharmacokinetics

The pharmacokinetics of romidepsin was investigated in rat and dog using the clinical product and route of administration in single-dose PK and GLP-compliant repeat-dose toxicokinetic studies.

**Table 6: PK parameters after a single dose and repeated doses**

| Species (study no.) | Analyte (Assay) Matrix | Route | Sex | Dose (mg/kg) | $T_{max}$ (h) | $C_{max}$ (ng/mL) | $AUC_{0-\infty}$ (ng.h/ml) | $t_{1/2}$ (h) |
|---|---|---|---|---|---|---|---|---|
| **Single dose studies** | | | | | | | | |
| Rat (10/sex/group) CRD040009 | $^{14}$C (LSC) - Plasma | IV | M | 0.3 | 0.083 | 277 | 484 | 13.2 |
| | $^{14}$C (LSC) - Blood | IV | M | 0.3 | 0.083 | 66.9 | 2550 | 19.6 |
| Rat (10/sex/group) 501650 | Romidepsin (LC/MS/MS) Plasma | IV (bolus) | M | 0.1 | 0 | 9.50 | 1.76[a,b] | ND[a] |
| | | | | 0.33 | 0 | 53.0 | 12.4 | 0.72 |
| | | | | 0.67 | 0 | 60.7 | 17.2 | 0.21 |
| | | | F | 0.1 | 0 | 17.9 | 2.49[a,b] | ND[a] |
| | | | | 0.33 | 0 | 37.1 | 8.21 | 0.87 |
| | | | | 0.67 | 0 | 93.0 | 19.0 | 0.48 |
| Dog (3/sex/group) GLR040231 | Romidepsin (LC/MS/MS) Plasma | IV (4-h infusion) | M | 0.3 | 1 | 48.8 | 163.4 | ND |
| | | | | 1 | 1 | 218 | 670.9 | ND |
| | | | F | 0.3 | 1.7 | 43.3 | 143.2 | ND |
| | | | | 1 | 1.3 | 146 | 454.4 | ND |
| **Repeat dose studies** | | | | | | | | |
| Rat (10/sex/group) 501650 | Romidepsin (LC/MS/MS) Plasma | IV (bolus) | M | 0.1 | 0 | 19.0 | 5.33 | 0.21 |
| | | | | 0.33 | 0 | 129 | 29.4 | 1.11 |
| | | | | 1 | 0 | 377 | 88.7 | 1.95 |
| | | | F | 0.1 | 0 | 19.2 | 4.41 | 0.23 |
| | | | | 0.33 | 0 | 92.3 | 21.3 | 0.68 |
| | | | | 1 | 0 | 362 | 74.4 | 1.18 |

M: male; F: female; IV: intravenous; ND: not determined

[a] It was not possible to estimate the kel with an acceptable degree of confidence due to an inability to characterise the terminal phase; consequently, all parameters derived from this (t½el, AUC0-inf. and % extrapolation AUC0-inf. were not estimated); [b] $AUC_{0-0.5h}$

The PK and tissue distribution of romidepsin has been investigated in rats using [14C]-romidepsin (data not shown). The general plasma disposition profile of [14C]-romidepsin derived radioactivity was characterised by a rapid initial or alpha distribution, followed by a slower terminal systemic elimination, as has been observed in studies using non-radiolabelled material. The concentration of radioactivity was higher in blood than in plasma at all time points. At similar intravenous doses, plasma Cmax was much higher than that measured with non-radiolabelled romidepsin. Total radioactivity then declined in a multi-exponential manner in both plasma and blood, although more rapidly in plasma with a t1/2 of 13.2 hours in plasma and 66.9 hours in blood for total radioactivity.

Protein binding for radiolabelled romidepsin was investigated in rat, dog and human serum, as well as in human plasma at concentrations ranging from 50 to 5000 ng/ml (data not shown). Between 50 and 5000 ng/mL, romidepsin was highly bound to plasma protein in vitro in humans (82-95%) and dogs (73-88%), while binding was lower in rats (38-41%). Specific binding to human serum albumin and α1 acid-glycoprotein was 19.91% and 93.51%, respectively, suggesting that the principal binding protein in human serum was α1 acid-glycoprotein. Plasma protein binding was found not to be concentration dependent.

The ratios of blood/plasma concentrations of [14C]-romidepsin in rat, dog and human samples were assessed in vitro over the concentration range 50 - 5000 ng/mL. The ratios were comparable across species (0.68-0.75, 0.58-0.65 and 0.59-0.60 in rats, dogs and humans, respectively). Blood/plasma ratios were almost constant in rats and humans in this concentration range. The ratio in dogs was 0.58-0.59 in the range 50-500 ng/mL; however, it increased to 0.65 at 5000 ng/mL.

The metabolism of romidepsin was investigated in vitro (liver microsomes) and in vivo (rat only). Thirty one metabolites were identified in vivo in the rat while 22 metabolites were identified in vitro in human, dog and rat.

The in vivo metabolism of romidepsin was investigated in plasma, urine, bile and faeces of rats following a single IV injection. Extensive metabolism was observed in rats with no single metabolite accounting for more than 5% of the total radioactivity.

For metabolism and pharmacokinetic interaction studies using human biomaterials, please refer to the clinical Pharmacokinetics section.

No significant qualitative and quantitative differences in romidepsin metabolism in liver microsomes were found between rats, dogs and humans.

Excretion of romidepsin into urine, faeces and bile was studied in rats and bile duct cannulated rats. The results are summarised in Table 7 below.

**Table 7: Cumulative excretion of radioactivity in bile, urine and faeces of rats**

| Species / N /sex | Analyte / Route / Dose (mg/kg) | Time (h) | Urine (% dose) | Faeces (% dose) | Bile (% dose) | Carcass (% dose) | Recovery (% dose) |
|---|---|---|---|---|---|---|---|
| Bile duct cannulated Rats / 3♂ | [$^{14}$C]-Romidepsin/ IV / 0.3 | 0-48 | 20 | 5 | 66 | 8 | 100 |
| Rats / 3♂ | | 0-168 | 17 | 79 | NA | 2 | 98 |

Less than 5% of the radioactivity excreted in the urine was identified as the unchanged parent drug.

For interactions with cytochrome P450 isoforms please refer to the clinical Pharmacokinetics section, as relevant studies were conducted using human biomaterials (liver microsomes).

The interaction of romidepsin with P-gp (permeability glycoprotein) was characterised by evaluating the bidirectional movement of romidepsin using monolayers of Caco-2 cells (Xiao *et al*, 2005). In this study, the transport of romidepsin across the Caco-2 cell monolayer in the absence and presence of P-gp and MRP inhibitors was characterised. The study showed that romidepsin had nearly unidirectional flux across Caco-2 cell monolayer, with a basolateral to apical apparent permeability coefficient (Papp) 32 times that of the apical to basolateral without any apparent saturation. Inhibition of P-gp by cyclosporine A and verapamil resulted in a decrease in the basolateral to apical Papp and an increase in the apical to basolateral Papp. In human red blood cells, the study demonstrated that there was a concentration dependent uptake and saturable efflux of romidepsin and that up-regulation of P-gp, and not changes in multidrug resistance-associated proteins, histone acetyltransferases or histone deacetylase, was responsible for the reduction in cytotoxicity by romidepsin.

## 2.3.4. Toxicology

### *Single dose toxicity*

Single dose toxicity studies conducted in rats and dogs at doses ranging from 0.7 to 5.1 mg/kg and from 0.01 to 1 mg/kg, respectively, were submitted. They are summarised in Table 8.

**Table 8: Summary of single-dose toxicity studies**

| Study ID / GLP | Species / Number / Sex Group | Dose (mg/kg) / Route | Approx. lethal dose / observed max non-lethal dose |
|---|---|---|---|
| GLR910291 / GLP | Rats/ 5/sex/group | 0.7, 1.0, 1.4, 1.9, 2.6, 3.6, 5.1 / IV bolus | **3.6 / ND** |
| **Major findings**<br>**3.6: Mortality:** 5/10. **Gross pathology**: Diffuse petechiae in glandular stomach 1♂.<br>**≥3.6: Gross pathology:** Clouding of thymic parenchyma; Sporadic or many dark red foci in thymus; Dark red coloration of lungs.<br>**5.1: Mortality:** 9/10. | | | |
| GLR910294 / GLP | Dogs/ 1/sex/group | 0.01, 0.1, 1.0 / IV bolus | **ND / 1.0** |
| **Major findings**<br>**≥0.1:** Body weight ↓, Food consumption ↓. **Organ weight:** ↓Thymus. **Clinical signs**: ↓Spontaneous motility; ↑ Body temp. **Gross pathology**: Atrophy of thymus (↓cortical lymphocytes).<br>**0.1: Clinical signs**: Wetness of eyeballs; Dryness of nasal tip. **Haematology**: ↓Lymphocytes. **Clinical chemistry**: ↓Phospholipids.<br>**1.0: Clinical signs**: Irregular heart rate; Tremor; Cold to touch, Congestion of eye mucosa; Irregular respiration; Cough; Salivation. **Haematology**: ↓ WBC. **Clinical chemistry**: ↑GOT; ↑glucose; ↓Ca; ↓K; ↓BUN; ↑Total cholesterol; ↑ phospholipids. | | | |
| IRN25566 / GLP | Rats/ 1-2♀/group | 17.5, 55, 175, 550 / Oral gavage | **55 / 17.5** |
| **Major findings**<br>**≥55: Mortality** 1/2. **Gross pathology**: Dark liver; Thin appearance.<br>**≥175: Mortality** 2/2. **Gross pathology**: Dark pancreas; Small spleen;<br>**550: Mortality** 1/1. **Gross pathology**: Dark heart, lungs, pituitary and adrenal glands; Reddened brain. | | | |

ND: not determined; BUN: blood urea nitrogen; Ca: calcium; ECG: electrocardiography; GOT: glutamic oxaloacetic transaminase; K: potassium; Temp: temperature; WBC: white blood cells.

## *Repeat dose toxicity*

Repeat-dose toxicity studies were conducted in mice, rats and dogs using different treatment schedules. A summary of the repeat-dose toxicity studies is presented in Table 9 below.

**Table 9: Repeat-dose toxicity studies**

| Study ID / GLP | Species / Number / Sex / Group | Dose / Route / Regimen | NOAEL (mg/kg/day) |
|---|---|---|---|
| SRI-Chm-91-1145-6464-LXXX / Non-GLP | NCr nude Mouse / 5♀ / group | 2.4, 3.6, 5.3, 8.0 / IV bolus / Q4Dx3<br>or<br>0.64, 0.96, 1.44, 2.16 / IV Bolus / QDx5 | ND<br><br>ND |
| **Q4Dx3:**<br>**≥ 2.4: Haematology:** ↓ RBC; ↓WBC; ↓ Platelets; ↓ Leukocytes. **Clinical chemistry:** ↑ LDH. **Bone marrow:** ↓ Femoral counts; ↑M/E ratios; ↓Lymphocytes in bone marrow. **Histopathology:** ↑Haematopoietic cells in spleen and bone marrow; Chronic focal inflammation of the heart consisting of neutrophilic cellular infiltration and extravascular foci of cellular exudate rich in granulocytes with few fibroblasts.<br>**≥ 5.3: Organ weight:** ↓ Rel. heart.<br><br>**QDx5:**<br>**2.16: Mortality:** 1/5. **Organ weight:** ↓ Abs. & rel. heart. **Haematology:** ↓ RBC; ↓ Platelets; ↓Reticulocytes. | | | |
| SRI-Chm-91-1145-6464-LXXX / Non-GLP | NCr nude Mouse / 5♀ / group | 1.6, 2.4, 3.6, 5.3 / IP bolus / Q4Dx3<br>or<br>0.2, 0.3, 0.45, 0.66/ IP bolus / Q3H Q4Dx3 | ND<br><br>ND |
| **Q4Dx3:**<br>**≥ 1.6: Haematology:** Lymphopenia. **Clinical chemistry**: ↑ LDH; ↑M/E ratios. **Bone marrow:** ↓Lymphocytes in bone marrow. **Histopathology**: ↑Haematopoietic cells in spleen and bone marrow; Chronic focal inflammation of the heart consisting of neutrophilic cellular infiltration and<br>extravascular foci of cellular exudate rich in granulocytes with few fibroblasts.<br><br>**Every three hours, Q4Dx3:**<br>**0.2 and 0.3: Haematology:** ↓ RBC; ↓Leukocytes; ↓Reticulocytes. **0.3: Haematology:** ↓Platelets.<br>**≥ 0.45: Mortality:** 10/10. | | | |

| Study ID / GLP | Species / Number / Sex / Group | Dose / Route / Regimen | NOAEL (mg/kg/day) |
|---|---|---|---|
| SRI Chm-92-653-6464-LXXXIX / GLP | CD2F1 Mouse / 10♂ / group | 3.6, 5.3, 8.0 / IV bolus / Q7Dx4 or 2Wx4<br><br>Recovery: 22 days (all groups) | ND |
| **≥ 3.6:** Body weight ↑. **Clinical signs**: Local reactions at injection site (Sores; Swollen; Scaly; Necrotic); Sloughing skin around base of tail/anal area. **Haematology**: ↓ RBC; ↓ HGB; ↓ HCT; ↑MCV; ↑MCH; ↑Platelets; ↑Reticulocytes; ↑Nucleated RBC; ↑WBC. 2W only: ↓MCHC; ↑WBC; ↑Neutrophils. **Clinical chemistry**: ↑AST; ↓Total protein. **Gross pathology**: Lesions of tail skin; Enlarged and/or pale spleen; Pale femoral bone marrow; Small and/or pale, mottled testes<br>Q7D only: Pale carcass.<br>**5.3: Clinical signs**: Hunched and/or thin animals.<br>**8.0: Mortality**: Q7D: 4/10. 2W: 2/10. **Clinical signs**: Hunched and/or thin animals. **Haematology**: 2W only: ↑ Platelets; Mild thrombocytosis. **Histopathology**: Inflammation of skin of tail; myeloid and erythroid cell proliferation in spleen; Erythroid cell necrosis in spleen; Myeloid hyperplasia of bone marrow; Bone marrow depletion (unspecified); Haematopoietic foci in liver; Testicular degeneration; Atrophy of thymus. Q7D only: Atrophy of splenic red pulp; Fatty degeneration in the liver.<br><br>**After recovery**<br>**≥ 3.6: Histopathology:** Testicular degeneration.<br>**8.0: Haematology**: Mild thrombocytosis. | | | |
| GLR910292 / non-GLP | SD Rat / 5 / sex / group | 0.1, 0.32, 1.0 / IV bolus / Q1Dx14 | ND |
| **≥ 0.1: Haematology**: ↓ Erythrocytic parameters; ↓Platelets; ↓Lymphocytes. **Clinical chemistry**: ↓ Albumin; ↑Cholesterol; ↑ GOT; ↑γ-GTP. **Histopathology**: Spleen: extramedullary haematopoiesis and lymphocyte degeneration. Thymus: lymphocyte degeneration and necrosis, atrophy and interstitial inflammatory cell infiltration. | | | |
| GLR910293 / GLP | SD Rat / 12-18 / sex / group | 0.0032, 0.010, 0.032, 0.10 / IV bolus / Q1Dx28<br><br>Recovery: 4 weeks (0.032 and 0.10 groups) | ND |
| **≥ 0.01: Organ weight:** ↓Adrenal glands; ↓Salivary glands; ↓Thymus; ↓Liver; ↓Seminal vesicle; ↓Heart; ↓Lungs; ↓Epididymis; ↓Prostate; ↓Ovary; ↑ Spleen. **Haematology**: ↓ WBC; ↓Lymphocytes; ↑Segmented neutrophiles; ↓HCT; ↓HGB; ↓MCH; ↓MCV; ↓Platelets. **Clinical chemistry**: ↑PT; ↓Albumin; ↑AST; ↑LDH; ↑γGTP; ↑CPK; ↑Total cholesterol; ↓Triglyceride; ↓Phospholipids; ↓K; ↑Bilirubin; ↓A/G; ↓Glucose; ↓Total protein.<br>**≥ 0.032:** Body weight ↓; Food consumption↓.<br>**After recovery**<br>**≥ 0.01: Organ weight:** ↓Ovary. | | | |
| SRI-LIF-96-183-8000-XCI / GLP | Fischer 344 Rat / 10♂ / group | 0.33, 1.67, 3.3 / IV 4 hour infusion / Q4Dx3<br><br>Recovery: 22 days (0.33 and 3.3 groups) | ND |
| **≥ 0.33: Haematology**: ↓WBC; ↓ Lymphocytes; ↓Segmented neutrophiles; ↓Eosinophiles; ↓Monocytes; ↓RBC; ↓HCT; ↓ HGB; ↓PLT; ↓ Reticulocytes values; ↑ Nucleated RBCs; RBC basophilic stippling.<br>**Clinical chemistry**: ↑ ALP; ↑ AST; ↑ ALT; ↑Fibrinogen; ↑ BUN. **Gross pathology**: Small and/or gelatinous thymus; Gelatinous salivary gland or skin (subcutaneous tissue); Fluid in thoracic cavity; Thickness, dark colour or a mass in the catheterised blood vessel. **Histopathology**: Lesions in lymph nodes, spleen, thymus, subcutaneous skin tissues and near injection site blood vessel (Depletion of lymphoid cells; Necrosis in lymph nodes, spleen and thymus; Thymic oedema; Inflammation of SC skin tissue at injection site).<br>**≥ 1.67: Mortality**: 1.67: 3/10; 3.3: 10/10. Body weight ↓. **Clinical signs**: Poor grooming; Emaciation; Hunched posture; Hypoactivity; Laboured breathing; Sunken and/or squinting eyes; Eye discharge; Cool or warm to touch; Salivation; Urogenital discharge; Prostration (maybe attributed to moribund / deteriorating condition); Swelling in areas adjacent to catheter placement. **Histopathology**: Depletion of haematopoietic cells in bone marrow.<br><br>**After recovery**<br>**0.33: Haematology:** ↓ Lymphocytes. **Histopathology**: Necrosis of injection site blood vessel. | | | |

| Study ID / GLP | Species / Number / Sex / Group | Dose / Route / Regimen | NOAEL (mg/kg/day) |
|---|---|---|---|
| SRI-LIF-96-184-8000-XCII / GLP | Fischer 344 Rat / 10♂ / group | 0.1, 1.0, 1.67 / IV 4 hour infusion / Q4Dx3<br><br>Recovery: 22 days (all groups) | ND |
| **≥ 0.1: Haematology**: ↓ WBC; ↓ Lymphocytes; ↓ Monocytes; ↓ Platelets. **Gross pathology**: Small and/or gelatinous thymus; Small spleen; Fluid in thoracic cavity; Thick catheterised blood vessel; Gelatinous or thick SC skin; Thin carcass. **Histopathology**: Depletion of lymphoid cells and necrosis in lymph nodes and spleen; Necrosis of catheterised blood vessels.<br>**≥ 1.0: Clinical signs**: Squinting eyes; Poor grooming; Pallor; Hunched bodies; Cool to touch; Emaciation. **Haematology**: ↓ Segmented neutrophiles; ↓Eosinophiles; ↓ RBC; ↓ HCT; ↓ HGB; ↓ Reticulocytes; ↑ Nucleated RBC; ↓PLT; RBC basophilic stippling. **Clinical chemistry**: ↑ ALP; ↑ AST; ↑ Fibrinogen. **Histopathology**: Necrosis in thymus; Depletion of haematopoietic cells in bone marrow; Lymphoid cell depletion in thymus.<br>**1.67: Mortality:** 2/10. Body weight ↓. **Clinical signs**: Swelling of forelimb, neck, face, and/or head. **Clinical chemistry**: ↑ ALT. **Histopathology**: inflammation and edema of injection site SC skin tissues; Thymic oedema. **Gross pathology**: Liver adhesion; Gelatinous skin.<br>**After recovery**<br>**≥ 1.0: Haematology**: ↓ Lymphocytes. **Histopathology**: Necrosis of catheterised blood vessels; Lymphoid cell depletion in spleen.<br>**1.67: Histopathology**: Thymic oedema. | | | |
| GLR030561 / GLP | SD Rat / 11-12 / sex / group | 0.1, 0.3, 1.0 / IV 4 hour infusion / Q7Dx3<br><br>Recovery: 2 weeks (all groups) | ND |
| **≥ 0.1: Histopathology**: Necrosis of lymphocytes in thymus.<br>**0.1 and 0.3: Histopathology**: ↑Tingible body macrophages in bone marrow in femur and sternum.<br>**≥ 0.3: Haematology**: ↓ WBC; Occurrence of fibroblasts; ↑PT; ↑APTT. **Clinical chemistry**: ↑ AST; ↑ LDH; Ossification of femoral bone marrow. **Histopathology**: Extramedullary haematopoiesis in liver; ↓ Ovarian follicles; ↓ Corpora lutea size.<br>**1.0: Mortality** 4♂ & 5♀. **Clinical signs**: ↓ Spontaneous movement; Tremor and clonic/tonic convulsions. **Haematology**: ↓ Haematocrit; ↓ RBC; ↓ MCV; ↓ HGB; ↓ Reticulocytes; Occurrence of erythroblasts); ↑ Fibrinogen. **Clinical chemistry**: ↑ ALT; ↑ ALP; ↑ CPK; ↑ T.CHOL; ↑ TG; ↑ PL; ↑ Amyl; ↓ K; ↓ Ca. **Histopathology**: Necrosis of lymphatic tissues; Degeneration / necrosis of submandibular gland, adrenal, mammary gland, colon and stomach; ↓ Lymphocytes in spleen; ↓ Haematopoietic cells in bone marrow of femur and sternum; Degeneration, necrosis, atrophy and mineralisation in organs/tissues in digestive system; Congestion in heart.<br>**After recovery**<br>**≥ 0.3: Histopathology**: ↓Ovarian follicles; ↓Corpora lutea size. | | | |
| 501650 / GLP | SD Rat / 20 / sex / group | 0.10, 0.33, 0.67/1.0 [a] / IV bolus / 3 times per month for 26 weeks<br><br>No recovery period | ND |
| **≥ 0.10: Haematology**: ↓ WBC; ↓ Neutrophiles; ↓ Lymphocytes; ↓ Monocytes; ↓ Eosinophiles; ↓ Basophiles; ↓ PLT; ↑ MPV; ↑ Reticulocytes; ↑ haematopoietic hypocellularity. **Organ weight**: ↓ Rel. & abs. ovary; ↓ Rel. & abs. thymus. **Gross pathology**: Small thymus; Small ovaries; Minimal to severe atrophy in ovaries and uterus; Pituitary enlargement. **Histopathology**: Lymphoid atrophy/necrosis (thymus); ↑ Pigment deposits in spleen; ↓ Prominence and/or activity of glandular elements (atrophy) of mammary gland.<br>**0.10 and 0.33: Gross pathology**: ↑ Cysts (ovary).<br>**≥ 0.33:** Body weight ↓. **Organ weight**: ↑ Rel. & abs. pituitary. **Haematology**: ↑ Reticulocytes; ↑ Haematopoietic hypocellularity. **Histopathology**: ↑ Pigment deposits in bone marrow and hepatocytes and/or Kupffer cells; Lymphoid atrophy/necrosis (spleen); Minimal to slight hyperplasia in pituitary; ↑ Extramedullary haematopoiesis in spleen.<br>**0.67/1.0: Clinical signs**: ↑ Dry, red and/or scabbed skin at injection site. **Organ weight**: ↓ Rel. & abs. uterus. **Histopathology**: ↑ Megakaryocytes in bone marrow; ↑ Extramedullary haematopoiesis in spleen; Focal and/or multifocal degeneration/atrophy of testicular seminiferous epithelium. | | | |
| GLR910296 / GLP | Beagle dog / 3 / sex / group | 0.0032, 0.01, 0.032 / IV bolus / Q1Dx28<br><br>Recovery: 4 weeks (all groups) | ND |
| **≥ 0.0032: Histopathology**: Tingible body macrophages in thymus and spleen; Degeneration & necrosis of lymphocytes in lymph nodes.<br>0.01: Food consumption ↓. **Organ weight**: ↓Thymus. **Histopathology**: Slight atrophy of thymus.<br>**0.032:** Body weight ↓; Food consumption↓. **Haematology**: ↓WBC; ↓Erythroblasts; ↑M/E. **Clinical chemistry**: ↑APTT; ↓AST; ↓ALT; ↓K; ↑Glucose; ↓AP. **Histopathology**: Slight haemosiderin deposition in red pulp of spleen in recovery animals; Slight atrophy of thymus. | | | |

| Study ID / GLP | Species / Number / Sex / Group | Dose / Route / Regimen | NOAEL (mg/kg/day) |
|---|---|---|---|
| GLR910295 / Non-GLP | Beagle dog / 2♂ + 1♀ / group | 0.1, 0.32, 1.0 / IV bolus / Q1Dx14<br><br>No recovery period | ND |
| **0.1: Histopathology**: Necrosis in kidney.<br>**≥ 0.1: Haematology**: ↑Neutrophiles; ↓Lymphocytes; ↓Eosinophiles; ↓Monocytes; ↑ Fibrinogen. **Clinical chemistry**: ↑Total protein; ↑Total cholesterol; ↑GOT; ↑AST; ↑ALT; ↑LDH; ↓Inorganic phosphate; ↓Ca; ↓Na; ↓K; ↓Cl; ↓Fe. **Clinical signs**: Vomiting; abnormal faeces; Swelling at injection site. **Bone marrow**: ↓Erythroblastic cells; ↑M/E. **Gross pathology:** Softening and small size of thymic, spleen and mandibular lymph nodes; Dark-red foci in heart, kidney and digestive tract. **Histopathology**: ↑Granulocytic cells in bone marrow; Haemorrhage on corticomedullary border in kidney; Perivascular haemorrhage & inflammatory cellular infiltration at injection site; Atrophy of lymph follicle & neutrophilic infiltration in thymus, spleen & mandibular lymph nodes.<br>**≥ 0.32:** Body weight ↓; Food consumption↓. **Clinical signs**:↓Spontaneous motility**. Haematology**: ↓Reticulocytes; ↑APTT; ↑GPT. **Histopathology**: Heart haemorrhage; Myocardial atrophy or necrosis; Dilation of renal tubule.<br>**1.0: Mortality** 3/3. **Haematology**: ↑LDH. **Histopathology**: Dark red foci or depressed mucosa of lung, gallbladder & GI tissues; Congestion & haemosiderosis in spleen, liver, or kidney. | | | |
| GLR910297 / non-GLP | Beagle dog / 1♂ / group | 0.1, 0.5, 1.0 / IV slow bolus / Q7Dx4 or 2Wx4<br><br>No recovery period | ND |
| **≥ 0.1:** Food consumption↓. **Haematology**: ↑Neutrophiles; ↓Lymphocytes; ↓Monocytes; ↑Fibrinogen. **Clinical chemistry**: ↓BUN; ↓ Inorganic phosphate; ↓Ca; ↓Fe. **0.1 (2W): Clinical signs**: ↑ Body temp. **Gross pathology**: Diffuse red spots in thymus.<br>**≥ 0.5: Organ weight**: ↓Abs. and rel. thymus. **Gross pathology**: Small thymus.<br>**0.5 (Q7D): Clinical signs**: ↑ Body temp.<br>**1.0 (Q7D): Clinical signs**: Abnormal faeces. ↑ Body temp. **Clinical chemistry**: ↑GOT. | | | |
| GLR910298 / non-GLP | Beagle dog / 1♂ / group | 1.0, 2.0 / IV slow bolus / 2Wx4<br><br>No recovery period | ND |
| **≥ 1.0:** Body weight ↓; Food consumption↓. **Clinical signs**: Vomiting; abnormal faeces. **Haematology**: ↑Neutrophiles; ↓Lymphocytes; ↓Monocytes; ↑Fibrinogen; ↓Reticulocytes; ↓Haematocrit; ↓Haemoglobin; ↓RBC. **Bone marrow**: ↓Erythroblastic cells. **Clinical chemistry**: ↑GOT; ↓Fe; ↓Inorganic phosphorus; ↓Ca; ↓K. **ECG**: ↑ QT time. **Gross pathology**: Small thymus; Multiple dark red spots in thymus. **Organ weight**: ↓ Thymus.<br>**2.0: Clinical signs**: Swelling of right hind-paw interdigit; Swelling at injection site; Decreased spontaneous motility. **Clinical chemistry**: ↑LDH; ↑ BUN. **Gross pathology**: Dark red spots; White coloration and thickening of heart epicardium and pericardium with retention of dark red fluid in pericardial cavity; Dark red foci on corticomedullary zone of kidney; Node of splenic abscess; Dark red/yellowish white foci in left anterior lobe of lung; Hepatisation in part of lung. **ECG**: ↑ST time. | | | |
| SRI-Chm-93-2-6464-XCV / Non-GLP | Beagle dog / 2 / sex / group | 0.5, 1.0, 2.0 / IV injection (day 1), slow bolus (day 12) or 1 hour infusion (day 19) / QWx3<br><br>No recovery period | ND |
| **≥ 0.5:** Body weight ↓; Food consumption↓. **Clinical signs**: Severity was greatest following IV bolus injections (Pallor and/or emesis; Eye discharge; Hypoactivity; Red gums; Red ears; Red skin; Swollen and/or red injection site; Swollen ventral thorax; Swollen right shoulder; Discharge from a sore at injection site; Decreased ability to use the back legs; Cyanotic tongue; Fast breathing; Laboured breathing; Sunken eyes; and bloody diarrhoea). **Haematology**: Neutrophilic leukocytosis; ↑WBC (greatest after slow bolus or 1-hour infusions); ↓RBC. **Clinical chemistry**: ↑LDH.<br>**≥ 0.5: Clinical signs:** ↑ Body temp. **Water consumption** ↓.<br>**≥ 1.0: Mortality**: 1.0: 2/4; 2.0: 3/4. **Clinical chemistry**: ↑ CK (bolus only); ↑ AST. **Clinical signs**: Localised tissue damage and swelling at injection site. **Gross pathology**: Red areas in mucosa or lymphoid tissue of the intestines, tonsils, or lymph nodes. **Histopathology**: Oedema, congestion, haemorrhage, suppurative inflammation, necrosis, and thrombosis at injection site. | | | |
| SRI-Chm-93-3-6464-XCVI / GLP | Beagle dog / 1 / sex / group | 0.5, 1.0, 2.0 / IV 1-1.5 hour infusion / Q7Dx4 or 2Wx4<br><br>Recovery: 4 weeks (all groups) | ND |
| **≥ 0.5:** Body weight ↓ (2W only). **Clinical signs**: Hypoactivity; Emesis; Pallor; Red ears; Red gums; Injected sclera; Eye discharge; Squinted eyes; Diarrhoea; Abnormal respiration. **Haematology**: Mild anaemia; Leukocytosis; Neutrophilia; Lymphopenia; ↑WBC; ↑Segmented neutrophiles; ↓ Lymphocytes. **Clinical chemistry**: ↓HCT; ↑AST; ↑CK; ↑LDH. **Gross pathology**: Dark foci / mottling of right atrium of the heart; Thin carcass; Red lymphoid tissue of jejunum; Dark or mottled lungs; Red, dark, and/or enlarged lymph nodes; Thick, red, and/or mottled skin, with crust formation, at injection site; Red bilateral tonsils. **Histopathology**: Cellular depletion of bone marrow; Focal necrosis, cellular depletion, haemorrhage, and/or pigment in lymphoid tissues; Focal necrosis of liver; Haemorrhage and/or inflammation in lungs; Inflammation, haemorrhage, necrosis, and/or ulceration of skin or SC tissue at injection site; Subepicardial haemorrhages of right atrium of the heart.<br>**≥ 0.5 (Q7D only): Clinical signs**: ↑ Body temp.<br>**2.0 (2W only): Mortality**: 1/2. **Clinical chemistry**: ↑BUN; ↑Creatinine; ↑ALP. | | | |

| Study ID / GLP | Species / Number / Sex / Group | Dose / Route / Regimen | NOAEL (mg/kg/day) |
|---|---|---|---|
| SRI-CBE-94-318-8000-XXXVIII / GLP | Beagle dog / 2 / sex / group | 1.0 (4 hour infusion), 2.0 (4 or 24 hour infusion) / IV / Q4Dx3<br><br>Recovery: 30 days (all groups) | ND |
| **≥ 1.0 (4h and 24h): Clinical signs**: Swelling associated with indwelling catheter; Emesis and diarrhoea. **Clinical chemistry**: ↑ AST; ↑ CK; ↑ LDH; ↑ ALP. **Gross pathology**: Small thymus; Heart focus; Lung focus; Dark lymph node; Enlarged lymph node; Dark small intestine; Thick/dark skin at injection site; Gelatinous skin and dark urinary bladder; Dark large intestine; Dark lung; Thick/dark mammary gland; Dark mesentery; Haemorrhage in heart, lung, brain, large intestine, urinary bladder, mammary gland, lymph node and mesentery.<br>**1.0 (4 h only):** Body weight ↓; Food and water consumption ↓. **Clinical signs**: Acute or chronic inflammation and/or oedema at injection site. **Haematology**: Neutrophilic leukocytosis; Lymphopenia. **Histopathology**: Hypospermia and seminiferous tubular degeneration; Mild mucosal cystic degeneration in small intestines.<br>**2.0 (4 h only): Mortality:** 1/4. Body weight ↓; Food and water consumption ↓. **Clinical signs**: Acute or chronic inflammation and/or oedema at injection site. **Haematology**: Neutrophilic leukocytosis; Lymphopenia. **Clinical chemistry**: ↑ PT. **Histopathology**: Mild mucosal cystic degeneration in small intestines; Mild to moderate mucosal necrosis in small and/or large intestines; Mild dilatation in mucosal glands of intestine; Hypospermia and seminiferous tubular degeneration.<br>**2.0 (24 h only): Mortality** 4/4. **Clinical signs**: Bloody diarrhoea; Mild oedema at injection site. **Haematology**: Neutrophilic leukocytosis and lymphopenia. **Clinical chemistry**: ↑ PT. **Histopathology**: Mild to moderate cellular depletion and haemorrhage in bone marrow; Mild to moderate mucosal necrosis in small and/or large intestines.<br>**After recovery**<br>**≥ 1.0 (4h only): Clinical signs**: Chronic inflammation at injection site. **Haematology**: Neutrophilic leukocytosis; Lymphopenia.<br>**1.0 and 2.0 (4h only): Histopathology**: Hypospermia | | | |
| SRI-CBE-95-63-8000-LX / GLP | Beagle dog / 1 / sex / group | 0.5, 1.0 / IV 4 hour infusion / Q4Dx3<br><br>Recovery: 21 days (all groups) | ND |
| **≥ 0.5:** Body weight ↓; Food and water consumption ↓. **Clinical signs**: Emesis; Swelling and drainage at catheter entry site; ↑ Body temp. **Clinical chemistry**: ↑ ALP; ↑ ALT; ↑ AST. **Gross pathology**: Lesion at injection site; Small thymus; Lung focus; Lymphoid atrophy; Inflammation of injection site; Haemorrhage and necrosis of lung.<br>**0.5: Haematology**: ↑ WBC.<br>**1.0: Clinical signs**: Diarrhoea. **Haematology**: ↑ WBC. **Histopathology**: Testis degeneration; Epididymal atypical cells. | | | |
| SRI-LIF-95-660-8000-LXXVIII / GLP | Beagle dog / 1 / sex / group | 0.05, 0.1, 0.5, 1.0 / IV 4 hour infusion / Q4Dx3<br><br>Recovery: 21 days (all groups) | ND |
| **≥ 0.05:** Body weight ↓. **Haematology**: ↑Fibrinogen. **Gross pathology**: Thick jugular injection site; Lung focus; Small thymus; Gelatinous mediastinum; Small testis; Small prostate.<br>**≥ 0.1: Clinical signs**: Swelling at catheter entry site. **Haematology**: Neutrophilic leukocytosis; Lymphopenia; ↑ WBC; ↑ Segmented neutrophiles.<br>**≥ 0.5: Clinical signs**: Emesis; Diarrhoea / bloody diarrhoea; Inflammation in catheterised jugular veins/skin. **Clinical chemistry**: ↑ AST. **Histopathology**: Lymphoid depletion of thymus, tonsil, spleen and lymph nodes.<br>**1.0:** Body weight ↓. **Clinical chemistry**: ↑ALP; ↑CK. **Gross pathology**: Necrosis in catheterised jugular veins/skin; Haemorrhage and necrosis in lung; Degeneration in seminiferous tubule. **Histopathology**: Hypospermia in seminiferous tubule; Hypospermia and atypical cells in epididymis; prostate atrophy. | | | |
| GLR030590 / GLP | Beagle dog / 4 / sex / group | 0.3, 1.0 / IV 4 hour infusion / Q7Dx3<br><br>Recovery: 2 weeks (all groups) | ND |
| **≥ 0.3:** Body weight ↓; Food consumption ↓. **Clinical signs**: Vomiting; Abnormal faeces. **Haematology**: ↓Erythrocytic parameters; ↓ WBC; ↓ Lymphocyte ratio; ↑ Neutrophil ratio; ↑ APTT; ↑ Fibrinogen. **Clinical chemistry**: ↓ Albumin; ↓ BUN; ↓ Electrolytes; ↑ Total cholesterol; ↑ GOT; ↑ LDH. **ECG:** ↑QT interval. **Histopathology**: Atrophy or necrosis in bone marrow, lymphoid tissues (lymph node, thymus and spleen) and male reproductive organs; Inflammatory changes in lymphoid tissues and stomach; Haemosiderin deposition and extramedullary haematopoiesis in spleen; Basophilic tubules and interstitial infiltration of mononuclear cell in kidney.<br>**1.0: Clinical signs**: Tachypnoea; ↑ Muscle tone. **Haematology**: ↓ Monocyte ratio. **Clinical chemistry**: ↑ GPT; ↑ CPK. **Histopathology**: Epithelial mucosal degeneration in small and large intestines.<br>**After recovery**<br>**≥ 0.3: Haematology**: ↓Erythrocytic parameters; ↓WBC **Clinical chemistry**: ↓Albumin. **Histopathology**: Haemosiderin deposition and extramedullary haematopoiesis in spleen; Atrophy or necrosis in male reproductive organs. | | | |

## *Genotoxicity*

A standard battery of genotoxicity studies has been performed with romidepsin. The studies are summarised in Table 10 below.

**Table 10: Genotoxicity studies conducted with romidepsin**

| Type of test/study ID/GLP | Test system | Concentrations or doses/ Metabolising system/ Purity | Results |
|---|---|---|---|
| Gene mutations in bacteria/ IRN25107/ GLP | Ames test/ *S. typhimurim:* TA98, TA100, TA 1535, TA 1537, *E. coli :* WP2uvrA | 17-5000µg/plate±S9/ 100% Purity | **Negative**<br>(Cytotoxicity at ≥500 µg/plate)<br>(Precipitation at ≥1667 µg/plate) |
| Gene mutations in mammalian cells/ IRN25590/ GLP | Mouse lymphoma cell mutation assay/L5178 $tk^+tk^-$cell line | 6.25-225 ng/mL-S9 25-350 ng/mL+S9/ 98.6% Purity | **Weakly positive**<br>(Cytotoxicity at ≥1.56 ng/mL-S9, 24 hours)<br>(Cytotoxicity at ≥100 ng/mL±S9, 4 hours)<br>(Precipitation at ≥333.3 ng/mL) |
| Chromosomal aberrations *in vivo*/ IRN25561/ GLP | CD Rats, micronuclei in bone marrow | ♂: 0.25, 0.5, 1 mg/kg IV<br>♀: 0.75, 1.5, 3 mg/kg IV | **Negative**<br>Signs of bone marrow toxicity (i.e., suppressed PCE/NCE ratios) most pronounced in females receiving 3 mg/kg |

PCE/NCE - Polychromatic erythrocyte to normochromatic erythrocyte ratio

## *Carcinogenicity*

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

## *Reproduction Toxicity*

Reproductive and developmental toxicity has been investigated in rats only. The data are summarised in Table 11.

**Table 11: Embryo-foetal developmental toxicity studies**

| Study ID / GLP | Species; Number Female / group | Dose (mg/kg/day) / Route / Dosing period | NOEL (mg/kg/day) |
|---|---|---|---|
| IRN25115 / GLP | SD rats; 6/group | 0.0032, 0.01, 0.032, 0.1 / IV slow bolus / GD 6-16 | F0: 0.01<br>F1: 0.01 |
| **Major findings**<br>**≥ 0.032:** F0: Body weight gain ↓ (76-88% of control gain); Food consumption ↓.<br>**0.1:** F1: Foetal weight ↓. | | | |
| IRN25501 / GLP | SD rats; 19-20/group | 0.006, 0.02, 0.06 / IV slow bolus / GD 6-16 | F0: 0.006<br>F1: 0.02 |
| **Major findings**<br>**≥ 0.02:** F0: Food consumption ↓.<br>**0.06:** F0: Body weight gain ↓ (87% of control gain). F1: ↓Incidence of locally thinned tendinous region of diaphragm with protrusion of median liver lobe. | | | |
| ROMI-TOX-005/ GLP | SD rats; 21-25/group | 0.1, 0.2, 0.5 / IV bolus / GD 6-17 | F0: ND<br>F1: 0.1 |
| **Major findings**<br>**≥ 0.1:** F0: Body weight gain ↓ (89-13% of control gain); Food consumption ↓.<br>**≥ 0.2:** F0: ↓Gravid uterine weight. F1: ↓Foetal body weight; Delayed foetal ossification (caudal vertebrae, metatarsals, and/or hind limb phalanges); ↑ Folded retina (foetal and litter).<br>**0.2:** F1: ↑ Early resorption; ↑ Rotated hind limbs (foetal and litter).<br>**0.5:** F0: Mortality (1/25). White raised areas on one or both kidneys; ↓ Motor activity; Piloerection; Chromodacryorrhea; Ptosis; Ungroomed coat; Urine-stained abdominal fur; Ataxia; Vocalisation; Hyperreactivity / hyperactivity; Hyperpnoea; Abnormal faeces; Perivaginal substance (brown or red); Hunched posture; Dehydration; Swollen tail; Pale ears; Pale extremities. F1: Completely resorbed litters (24/25); ↑ Supernumerary thoracic ribs. | | | |

ND: not determined.

## *Toxicokinetic data*

Toxicokinetic exposure data from the embryo-foetal developmental toxicity testing in rats are shown in the following Table 12.

**Table 12: Toxicokinetic data**

| Study ID / GLP | Species / N / Sex | Dose (mg/kg) | Cmax (ng/ml) | AUC (ng/mL*hr) | Animal: Human $C_{max}$ Multiple [a] | Animal: Human AUC Multiple [a] |
|---|---|---|---|---|---|---|
| ROMI-TOX-005 / GLP | Rat Crl:CD(SD)/ 21-25/group / Female | 0.1 | 6.64 | 2.44 | 0.02 | 0.002 |
| | | 0.2 | 14.5 | 4.99 | 0.04 | 0.003 |
| | | 0.5 | 44.5 | 17.2 | 0.1 | 0.01 |

[a] Based on approximate human maximum steady-state AUC and Cmax values at 14 mg/m2 of 1549 ng.h/ml and 377 ng/mL, respectively.

## *Local Tolerance*

Local tolerance studies have assessed the delayed contact hypersensitivity potential of romidepsin in mice and the acute dermal irritation of the compound in rabbits. The studies are summarised in Table 13 below.

**Table 13: Local tolerance studies conducted with romidepsin**

| Study type/ Study ID/ GLP | Species/ Number/sex/ group | Dose/ route | Major findings | Conclusion |
|---|---|---|---|---|
| Local Lymph Node Assay/ IRN25565/ GLP | Mice (CBA/Ca)/ 5♀/group | 25 µl/ 0.025, 0.05, 0.1, 0.25 (%)/ Topical | Romidepsin was considered a sensitiser in mice although the measured results was just below the criterion for a positive response<br><br>Clinical signs at 0.25% were more severe than at other dose levels. | Sensitiser in mice |
| Acute Dermal Irritation Test/ IRN25564/ GLP | New Zealand White rabbits/ 2♂/group | 0.5 g/ Topical (4 hours) | No erythaema or oedema. | Non-irritating to rabbit |

## *Other toxicity studies*

Romidepsin was tested *in vitro* for myelotoxicity in CFU-GM progenitor cells from mouse, dog and human. Bone marrow cells were exposed continuously to romidepsin (0.001-10 nM); however, the duration of application was not stated in the study report. The IC50 values were 1, 0.35 and 0.03 nM in murine, canine and human bone marrow cells, respectively (data not shown).

Romidepsin was also tested for cytotoxicity towards cardiac myocytes derived from two strains of rat neonates (F344 and Sprague Dawley), canine (beagle pup) and a transformed human foetal cardiac myocyte cell line (W1). The myocytes were prepared in monolayers and exposed to romidepsin (0.1-100 µM), doxorubicin (0.001-10 µM) or minoxidil (0.1-100 µM). The myocytes were analysed for viability by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) dye conversion and by lactate dehydrogenase release (LDH) at 24, 48, and 72 hours after the start of treatment. Romidepsin produced both cytotoxic effects and elevated LDH levels that were greater than doxorubicin (data not shown).

Romidepsin did not elicit an effect on uterine weights (wet and blotted) and no effect on vaginal patency in the uterotrophic assay (data not shown).

Finally, the applicant conducted studies in rats with *tert*-butanol (2-methyl-2-propanol) which is an impurity found in the finished product. An acceptance criterion of ≤1.2 mg/vial for residual *tert*-butyl alcohol is included in the finished product specification. The rat studies were aimed to determine the NOAEL and a safety margin (data not shown, see discussion of non-clinical aspects).

## 2.3.5. Ecotoxicity/environmental risk assessment

The maximum daily dose of romidepsin for the treatment of patients with PTCL is 25 mg/day. Using the Fpen for orphan drugs of 0.0005 (0.05%), the calculated $PEC_{surfacewater}$ for romidepsin was 0.006 µg/L.

**Table 14: Summary of main study results**

| **Substance (INN/Invented Name): Romidepsin** | | | |
|---|---|---|---|
| **CAS-number (if available): 128517-07-7** | | | |
| ***PBT screening*** | | Result | **Conclusion** |
| *Bioaccumulation potential*- log $K_{ow}$ | $K_{ow}$win module in EpiSuite software | -0.40 | Potential PBT (N) |
| ***Phase I*** | | | |
| ***Calculation*** | **Value** | **Unit** | **Conclusion** |
| $PEC_{surfacewater}$ , default or refined (e.g. prevalence, literature) | 0.006 | µg/L | < 0.01 threshold (N) |
| Other concerns (e.g. chemical class) | | | (N) |

## 2.3.6. Discussion on non-clinical aspects

Romidepsin showed at least a 10-fold higher inhibitory activity in vitro towards HDAC class I enzymes as compared to class IIa/b. The activity for the HDAC class I enzymes was highly variable depending on the enzyme assay used (ranging from picomolar to nanomolar). The effect of romidepsin on HDAC class III and IV enzymes have not been investigated as the enzymatic activity of these enzymes could not be determined.

To understand more precisely the mechanism underlying the anti-tumour activity of romidepsin, both in vitro and in vivo studies dealing with its impact on cell cycle and gene expression were conducted. The main results were that romidepsin induced cell cycle arrest at either G1, G2/M or G0/G1 phase, increased acetylation of histones associated with some regions of p21WAF1/Cip1 and VEGF promoters, induced of cell apoptosis or differentiation, and modulated the expression of some genes (notably - p21WAF1/Cip1 , VEGF, bFGF). Based on its ability to inhibit HDACs, it is not unexpected that romidepsin modulates gene expression through increased acetylation of histones, and impacts on the biological properties of some proteins known to be substrates for HDAC (e.g. transcription factors). However, the exact mechanism of action of romidepsin in relation to its anti-tumour activity is not fully characterised at the gene expression level. Overall, both in vitro and in vivo studies support the anti-tumour activity of romidepsin, and thus its use in the proposed therapeutic indication.

*In vitro*, romidepsin was shown to inhibit the proliferation of numerous human lymphoma cell lines at concentrations lying in the nanomolar range. Inhibition of the proliferation of various human solid tumour cell lines (e.g., renal, prostate, colon, lung and stomach cancers) was also observed at similar concentrations. The IC50 values determined *in vitro* are well below the mean Cmax of 377 ng/mL measured in patients treated at the recommended dose of 14 mg/m2 on days 1, 8, and 15 of a 28-day cycle. Of note, romidepsin was shown to inhibit the proliferation of renal tumours ACHN and A-498 *in*

*vitro* with IC50 up to 5.98 ng/mL, but these cells not considered to be sensitive to romidepsin *in vivo* in mice treated at up to 3.2 mg/kg Q4D x 3 (one dose every 4 days for a total of 3 doses).

Romidepsin had significant effects in mice inoculated with leukemia (both non-resistant and several drug-resistant tumours), lymphoma, melanoma, sarcoma, carcinomas (lung, stomach, colon and mammary) and some renal and prostate tumours. The antitumour effect of romidepsin was investigated using different treatment schedules and routes of administration.

SCID mice were used to evaluate the anti-tumour activity of romidepsin against human lymphoma U-937 cells inoculated (both U-937 cells inoculation and treatment were via IP route). Romidepsin at doses ranging from 0.1 to 1 mg/kg given once or twice weekly significantly prolonged the median survival time of mice vs. controls. Maximal effect was observed at 0.56 mg/kg (1.68 mg/m2) once weekly and 0.32 mg/kg (0.96 mg/m2) twice weekly. The 1 mg/kg (3 mg/m2) dose level caused some toxicity since mice of this group started to die earlier than controls.

Romidepsin caused CNS-related effects in rats and dogs, e.g., change in activity, and decline in the limb tone and grip strength. The clinical relevance of these effects has not been discussed by the applicant. Nevertheless, a high incidence of asthenic conditions (fatigue, lethargy, malaise) has been reported for romidepsin in the clinical setting.

Regarding the cardiovascular system, in vitro studies showed a weak inhibitory potential for hERG channel current at concentrations above the clinical Cmax (-18% at 1 µg/mL, clinical Cmax = 377 ng/mL). In addition, romidepsin shortened the action potential duration on guinea pig papillary muscle at 10 µg/mL (300-fold higher than Cmax). *In vivo* in dogs, increased heart rate was observed at 0.3 mg/kg (6 mg/m2, +52% vs. baseline) and at 1 mg/kg (20 mg/m2,+34% vs. baseline). Baseline values were higher in dogs dosed at 1 mg/kg, which may explain that the increase was less important than that reported at 0.3 mg/kg. Some changes in blood pressure were observed but were weak, and thus of poor biological significance. Regarding ECG parameters, there was no change in QTc interval at the low dose (0.1 mg/kg), but QTc (Bazett) interval was prolonged at 0.3 mg/kg: +20 ms and + 27 ms at 8 and 10 hours following initiation of the 4-hour infusion. At this dose level, the lack of statistical significance mentioned by the applicant was probably due to the low number of animals (n=4). At 1 mg/kg, QTc interval was significantly prolonged (+8% QTc Bazett, +5% QTc Fridericia), sporadically according to the applicant. This was observed at 5, 6, 8, 10 and 12 hours following initiation of the 4-hour infusion, and corresponded to increases of 8, 21, 20, 23 and 18 ms, respectively. The QTc prolongations observed at 0.3 mg/kg and 1 mg/kg are of similar intensity. Overall, some inconsistencies are noted between the weak inhibition of hERG current, the shortening of APD on guinea pig papillary muscle, and the prolongation of QTc interval in dogs. It should also be mentioned that the guinea pig papillary muscle model is not considered sufficiently predictive of QT interval prolongation in humans. The Purkinje fiber model is much more predictive. In addition, inhibition of hERG currents is not the only mechanism which causes QT prolongation.

In vivo, romidepsin caused an increase in the body temperature of dogs treated at 0.1 mg/kg and above.

*In vitro* pharmacodynamic drug-drug interaction studies indicated that romidepsin may have synergistic antitumour effects when combined with other anticancer agents. However, these data were only partially confirmed in vivo, which suggests that caution should be taken when extrapolating to the clinical setting.

The analytical methods used for determination of romidepsin exposure in plasma of the species (rat and dog) used in the pivotal toxicity studies were appropriately validated in compliance with GLP (data not shown).

The pharmacokinetics has been studied in SD rats and in beagle dogs following IV administration. The pharmacokinetic of romidepsin seemed to decline in a multiexponential manner with rapid initial distribution phase. The terminal half-life was 2.92 hours in human patients. The elimination phase was rapid in rats (t1/2 < 1 hour) and slower in dogs (estimated t1/2 > 4 hours). This discrepancy may be related to the bolus vs. 4-hour infusion administration regimen. Also, dogs were much more exposed than rats at similar dose levels expressed in mg/kg. Clearance and volume of distribution were not determined in animal species but were estimated to be 10.96 L/hr and 5.37 L, respectively, in humans.

Dose-proportionality was observed in both animal species. In repeated-dose toxicokinetic studies, no accumulations were found except for one rat study (5016500), where the exposure of romidepsin appeared to be increased in all groups on Day 176 when compared to Day 1. However, the applicant states that it is not clear if this observation is meaningful and that the observation of accumulation in a single repeat-dose toxicity study may be related to a longer sampling period at Day 176 (up to 6 hours) as compared to Day 1 (up to 4 hours). Nevertheless, accumulation has not been observed in the clinical studies following repeated dosing.

Overall, the pharmacokinetic data obtained in animals are considered weak. In accordance with the current ICH S9 guideline on anticancer pharmaceuticals, information on absorption of the pharmaceutical in animals should normally be generated in parallel with clinical development. Further elaboration is not considered necessary as it will not affect the conclusions of the toxicity studies as 1) there are no indications of huge differences in the pharmacokinetics between the animal toxicity species and humans, and 2) additional toxicity testing with higher exposures are neither feasible nor ethically justifiable.

The distribution study performed in rats showed that exposure of most tissues to drug-derived radioactivity was in general higher than that of plasma at up to 24 hours (plasma radioactivity could not be detected thereafter). At 5 minutes post-dose, the concentration of radioactivity was highest in organs of excretion (i.e. kidney and urinary bladder, jejunum, and liver) while the concentration of radioactivity in the brain, testis, white fat, eyeball, and thigh bone was all very low (tissue/plasma ratio range 0.02-0.21). At 168 hours post dose, romidepsin-related radioactivity was still detected in most tissues (except for the brain, white fat, and prostate) with the highest levels detected in the pituitary gland, adrenal gland, spleen and mesentery lymph node. As indicated by the applicant, radioactivity concentrations were lower at 168 hours than at 5 minutes postdose. However, some tissues were characterised by high tissue: plasma concentrations at 24 hours post-dose, and slow elimination of drug-derived radioactivity from these tissues (79% to 92% concentration decline at 168 hours post-dose vs. peak concentration at 5 minutes post-dose), suggesting accumulation of drug-related material in these tissues. This may be sought in some lymphoid tissues in relation to the pharmacological activity of romidepsin (spleen, lymph nodes, thymus); however, both adrenal gland and pituitary were also concerned. Pituitary was shown to be a target organ in the 26-week rat toxicity study. Another target organ identified in rats was the ovary, and distribution of drug-related material in this tissue could not be investigated in this study since only males were included.

In vitro, the blood/plasma ratios were comparable between species (0.58-0.75) with little concentration-dependency. Hence, plasma is considered an appropriate matrix for the bioanalytical methods. The contrary was observed in vivo in rats administered a single 0.3 mg/kg intravenous dose of [14C]-romidepsin. This suggests that distribution of radioactivity in blood cells in vivo may involve romidepsin metabolites rather than romidepsin itself.

The metabolism of romidepsin was studied *in vivo* in rats only and *in vitro* in microsomes from rats, dogs and human. Hence, the conclusions rest heavily on the *in vitro* data. The results suggest that the metabolic pathways of romidepsin involve at least a rapid non-enzymatic GSH-mediated reduction of disulfide bonds to thiols and/or phase I reaction mediated by CYP3A4 to produce mono- or di-oxidised

metabolites and mono- or di-oxidised and reduced metabolites. In studies conducted on liver microsomes, romidepsin disappeared more rapidly in humans, followed by rats, and then by dogs. In rat and dog liver microsomes, the disappearance rates of romidepsin were 1.6- and 3.9-fold lower than in human liver microsomes.

No significant qualitative and quantitative differences in romidepsin metabolism in liver microsomes were found between male rats, male dogs, and humans (see Clinical Pharmacology section for human liver microsome studies). Hence, the applicant concludes based on these data that humans are expected to handle romidepsin in a manner similar to rats and male dogs. However, the available data are presently insufficient for the exclusion of the existence of unique human metabolites due to lack of clinical data. It is acknowledged that a qualification of metabolites is generally not warranted for the proposed patient population (advanced cancer) in accordance with the current ICH S9 guideline. Nevertheless, an assessment of the *in vivo* metabolic profile in the pivotal toxicity species and humans is essential for understanding the limitation of the non-clinical package for both the proposed and future indications. In principle, an evaluation of the human metabolic profile should be based on the circulating metabolites (plasma metabolic profile). Urine and faecal metabolic profiles might be of limited value, since the urine/faecal metabolites might be formed in the excretion organs yielding little systemic exposure. The Applicant will qualitatively assess the profile of circulating metabolites in plasma samples from the ongoing rifampicin drug-drug interaction study.

In rats, the primary route of elimination of romidepsin and its metabolites is in the bile with subsequent excretion in faeces. In rats, more than 90% of administered romidepsin was excreted into faeces 48 hours after administration. In bile duct cannulated rats, 66% of the dose was recovered from the bile and an additional 5% was recovered from faeces 48 hours post-dose. Approximately 20% of the total radioactivity was recovered from urine, suggesting that the hepatic clearance of romidepsin and its associated metabolites is the most important route of elimination. Data on the excretion of romidepsin in human patients and dogs have not been submitted. In accordance with the current ICH S9 guideline on anticancer pharmaceuticals, information on excretion of the pharmaceutical in animals should normally be generated in parallel with clinical development. However, further elaboration is not deemed necessary considering that the liver and the kidney have been identified as potential target organs of toxicity in the repeat-dose toxicity studies in rats and dogs. Hence, no potential targets of toxicity are disregarded on the basis of the absence of excretion data obtained in dogs.

*In vitro*, romidepsin was shown to be a substrate for CYP3A4 and to a much lesser extent for CYP3A5, 1A1, 2B6, and 2C19 (< 16% of CYP3A4). At therapeutically relevant concentrations, romidepsin did not cause significant inhibition or induction of CYP P450 enzymes in human liver microsomes. No inhibitory potential for CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 was found at clinically relevant concentrations of romidepsin. However, the inhibitory effect of romidepsin on CYP2B6 should be assessed, in line with the EMA draft guidance on interaction studies and the Applicant has committed to conduct further in vitro studies assessing the inhibitory effect of romidepsin on CYP2B6 and CYP2C8 by 1Q2013.

Romidepsin is a substrate of CYP3A4, therefore drug-drug interactions with co-administered CYP3A4 inducers or inhibitors cannot be excluded. As regards P-gp and MRP-1, literature data show that romidepsin is a substrate of P-glycoprotein. However, further in vitro studies to investigate the inhibitory potential of romidepsin towards P-gp are needed. As regards other transporters, since romidepsin is mainly excreted by bile, further in vitro studies assessing the potential of romidepsin to be a substrate and/or inhibitor of MRP2, OATPs (organic anion transporting polypeptide), and BSEP (Bile salt export pump) are needed.

The applicant has only investigated the ability of romidepsin to induce the CYP enzymes CYP1A2, CYP2B6 and CYP3A4/5. However, it has been shown that CYP2C8, CYP2C9, CYP2C19 and P-

glycoprotein are co-inducible with CYP3A enzymes (via PXR) and that CYP3A is a sensitive marker for all inducers (Pelkonen *et al*, 2008; Marchetti, 2007). Since romidepsin had no notable inductive effects on CYP1A2, CYP2B6 and CYP3A enzymes, it can be concluded that romidepsin does not induce CYP2C family of enzymes and P-glycoprotein. CYP2D6 is not an inducible enzyme. The reported induction of CYP2E1 occurs through protein stabilisation via inhibition of proteosomal degradation pathway and mainly by substrates of CYP2E1 (Pelkonen *et al*, 2008). Therefore, the induction of CYP2D6 and CYP2E1 were not examined.

Single-dose toxicity was investigated in rats and dogs. In rats, lethality was observed at 3.6 mg/kg following IV administration and at 55 mg/kg following PO administration. 3.6 mg/kg is approximately 1.5-fold higher than intended clinical dosing based on allometric scaling using the recommended human dose of 14 mg/m2 and a 60-kg person with a body surface area of 1.62 $m^2$.

The repeat dose toxicity studies were conducted using IP bolus in mice, IV bolus in mice, rats and dogs and IV infusion in rats and dogs. The infusion time was approximately 1-24 hours in dogs and 4 hours in rats. IV infusion is the clinical route of administration. The planned infusion time in man is 4 hours.

In the pivotal repeat-dose toxicity studies, rats (501650 & GLR030561) were dosed 0.1-1.0 mg/kg and dogs (GLR030590) were dosed 0.3-1.0 mg/kg. The treatment regimens in the studies corresponded approximately to the proposed clinical treatment regimen. The durations of the pivotal studies are in accordance with ICH S9.

In rats included in the 3-week study, mortality was observed in 9/23 rats one or two days following the administration of the first 1 mg/kg dose. Clinical signs reported in these animals were indicative of CNS toxicity (tremors, decreased spontaneous movements, tonic or clonic convulsions) and were similar to those seen in animals that survived this first dose. In the latter animals, these signs were of lower incidence and severity after the second dose, and not reported after the last dose indicating that some tolerance develops. This is also supported by the results of the 26-week rat study, in which no mortality was seen in animals receiving 2 weekly doses of 0.67 mg/kg and switched to 1 mg/kg/dose for the remaining of the study (18 doses). In the 3-week study, ophthalmic examinations in animals dosed at 0.3 and 1 mg/kg revealed treatment-related irreversible focal opacity of the lens. However, this finding seems to be study-specific since it was reported neither in the 26-week rat study, nor in the 3-week dog study. Haematological changes in both rat studies were generally consistent with decreased WBC and lymphocyte counts, increased relative neutrophils, and decreased erythrocytic parameters. Data obtained in the 26-week study showed however that these changes were of decreasing intensity over time. Serum chemistry changes were noted in the 3-week rat study only at 0.3 mg/kg and above. They consisted notably in increased liver transaminase levels without histopathological correlate, and increased CK and LDH levels. Examination of isoenzymes fractions suggest that CK increases originate from skeletal muscle (no increase in CK-MB), while all LDH fractions were increased. In the 3-week study, the main target organs identified at histopathological examination were the lymphoid tissue (thymus, spleen, lymph nodes, intestine), the bone marrow (haematopoietic hypocellularity), the stomach, the mammary gland of males (atrophy of acinar elements), and the ovaries. These changes were reversible or showed trend for reversibility, except for the ovaries due to the nature of the lesion (decreased number of follicles). Most occurred at 0.3 mg/kg and above; the only effect noted in rats treated at 0.1 mg/kg was lymphatic necrosis in the thymus of males. Target organs identified in the 26-week study were the same but changes occurred more consistently from the low dose level of 0.1 mg/kg. Additional target organs were also identified after 26-week exposure. In fact, romidepsin-related effects in the pituitary of females at 0.33 mg/kg and above were reported, and consisted in hyperplasia and increased cytoplasmic vacuolation of the pars distalis portion. Golden brown pigment deposition, of unknown nature, was noted in several tissues including the liver. Finally, degeneration or atrophy of testicular seminiferous epithelium was observed

in 0/20 low-dosed, 1/20 mid-dosed rat, and 10/20 high dosed rats. Such effects on the testes were not reported in previous rat toxicity studies. Romidepsin-related toxicity on gonads thus appears much lower in male than in female rats. Indeed, ovarian atrophy was seen in a reproducible manner during the non-clinical development of romidepsin, and occurred at a greater incidence (in the 26-week study: 15/20, 20/20 and 20/20 in low, mid and high dosed rats). In addition, the effect male testis was seen with significant incidence in animals dosed at 1 mg/kg, and this dose level is well-above the MTD of 0.3 mg/kg determined from results of the 3-week study. No NOAEL could be determined in any rat study.

The main findings reported in the 3-week dog study consisted in clinical signs of emesis and diarrhoea, reversible haematological changes at ³ 0.3 mg/kg in line with these described in rats, and ECG findings at ³ 0.3 mg/kg in line with the safety pharmacology data (- heart rate and QTc). At histopathological examination, treatment related effects were seen at ³ 0.3 mg/kg in the lymphoid tissue (thymus, lymph nodes, spleen), the bone marrow (single cell necrosis and hypocellularity), the intestine (degeneration of mucosal epithelium), the kidney (basophilic tubules and inflammation). In addition, minimal changes were reported in the male reproductive organs at ³ 0.3 mg/kg. Most changes were reversible or showed trend to recovery, except for splenic haemosiderin deposition that did not affect the organ function, and changes in male reproductive organs considered of minimal severity by the applicant.

Toxicokinetic assessment was performed in the 26-week rat study only. No safety margin could be determined due to a lack of NOAEL, and the data show that rats were much less exposed than patients at all dose levels. At the MTD (0.3 mg/kg or 1.8 mg/m2), the animal-to-human exposure ratios based on Cmax and AUC levels reached 0.2-0.3 and 0.01-0.02, respectively. In dogs, such data were not generated in the pivotal study. Supportive data show that higher exposure levels were reached in this species, although they remained below those reached in patients.

Although the relevance of the cardiotoxic findings in mice and rats may be questioned, similar events were observed in all three animal species. The observations included congestion and haemorrhage in the heart, myocardial atrophy or necrosis, and prolonged QT interval (in dogs only). Furthermore, an in vitro assay of cytotoxicity using rat, dog and human cardiac myocytes indicated that cardiotoxicity could be considered a relevant finding in these species. Nevertheless, in patients it has been shown that romidepsin does not affect the T-wave, ST segment and QT interval, and does not cause myocardial damage as measured by serum cardiac troponin I levels and left ventricular ejection fraction (LVEF). Hence, further elaboration is not needed. Nevertheless, the cardiotoxicity observed in animals was included in section 5.3 of the proposed SmPC.

The non-clinical toxicology data give sufficient reassurance that toxicity to be expected from romidepsin treatment is mainly pharmacologically mediated and is well-known for anti-cancer drugs and can be accordingly monitored clinically.

Romidepsin was tested in the standard test battery for genotoxicity. Romidepsin was negative in the Ames' test. Romidepsin was weakly positive in the in vitro mouse lymphoma cell mutation assay following 4 hours incubation. The applicant did not perform the experiments with continuous treatment (i.e., 24 hours without metabolic activation) due to severe cytotoxicity of romidepsin under these conditions. This is considered acceptable. The biological significance of this positive finding in the in vitro mouse lymphoma cell mutation assay is questionable as the results were either equivocal or the increases in mutant fraction were of very low magnitude and usually within the historical control values. Romidepsin was considered non-genotoxic in the in vivo micronucleus study for which significant exposure to the bone marrow was demonstrated. Furthermore, the concentration in bone marrow was above the plasma concentration at all time points in the rat distribution study. However, the exposure margins in the in vivo micronucleus study only comparable to the exposure observed in humans (based on allometric scaling using the recommended human dose of 14 mg/$m^2$ and a 60-kg

person with a body surface area of 1.62 m2). Furthermore, another HDAC inhibitor has been reported as weakly genotoxic both in vitro and in vivo (EMEA/CHMP/559066/2008). The lack of carcinogenicity testing is acceptable considering the proposed patient population in accordance with the ICH S9 guideline.

Dedicated fertility, and prenatal- and postnatal developmental toxicity studies have not been performed, which is acceptable in accordance with the ICH S9 guideline. Testicular toxicity was observed in mice, rats and dogs in the repeat-dose toxicity studies. Male mice exhibited testicular atrophy that persisted for at least 2 weeks after the last dose of romidepsin. In rats, romidepsin caused testicular atrophy or degeneration following daily administration for 26 weeks; as mentioned above, the effect was significant at the high dose level, which exceeds the MTD. In dogs, romidepsin administration (≥ 1.0 mg/kg) was associated with hypospermia in the testes and epididymides, and seminiferous tubule degeneration; recovery was not demonstrated. In female rats, microscopic findings in the reproductive organs included minimal-to-severe atrophy in the ovary and a decrease in ovarian follicular activity with decreases in corpora lutea to complete inactivity of the ovary; recovery was not demonstrated. Atrophy was observed in the rat uterus. Hence, the data from the repeat-dose toxicity studies indicated that romidepsin has a potential for causing irreversible infertility in humans.

The studies on embryo-foetal toxicity were performed at doses comparable to (but slightly lower than) those used in the repeat-dose toxicity studies (the highest dose in the rat embryo-foetal toxicity study was 0.5 mg/kg; the lowest dose in the dog and rat pivotal repeat-dose toxicity studies was 0.1 mg/kg). This is acceptable since dosing in the embryo-foetal toxicity was daily, while the pivotal repeat-dose toxicity study was performed with dosing every 4 or 7 days.

Toxicokinetics was only performed in the pivotal rat embryo-foetal toxicity study. Exposures at the high dose 0.5 mg/kg was Cmax= 44.5 ng/ml and AUC= 17.2 ng•h/ml. These values are 10 and 100 times lower than clinical exposure, respectively.

In the pivotal rat embryo-foetal toxicity study (ROMI-TOX-005), teratogenic effects (i.e. rotated hind limbs, folded retina and supernumerary thoracic ribs) and embryo-foetal effects (early resorptions, post-implantion losses, delayed foetal ossification of caudal vertebrae, metatarsals and/or hind limb phalanges) were seen from 0.2 mg/kg, and maternal toxicity was seen from 0.1 mg/kg.

The calculated $PEC_{surfacewater}$ for romidepsin was 0.006 µg/L. It should be noted that the calculated $PEC_{surfacewater}$ was likely to be a worst-case overestimation of environmental exposure, as romidepsin is metabolised extensively. The calculated $PEC_{surfacewater}$ was below the action limit of 0.01 $\mu$g/L. Hence, a Phase II environmental fate and effects analysis was not triggered. Romidepsin is not considered a risk for the environment.

## 2.3.7. Conclusion on the non-clinical aspects

The nonclinical package submitted by the applicant to support the marketing authorisation application of Istodax for the second line treatment of adult patients with peripheral T-cell lymphoma is generally in line with the current regulatory requirements for such products. Outstanding minor issues include

1. the qualitative assessment of the profile of circulating metabolites, which the Applicant proposed to address using plasma samples from the ongoing rifampicin drug-drug interaction study;
2. an investigation of the effect of romidepsin on CYP2B6 and CYP2C8; and
3. an investigation of the potential for romidepsin to be a substrate and/or an inhibitor of MRP2, OATPs and BSEP.

These would become recommendations for future development.

## *2.4. Clinical aspects*

### 2.4.1. Introduction

The clinical data supporting this application were derived primarily from the pivotal study GPI-06-0002 and the supporting study NCI-1312-PTCL.

The Applicant received Scientific Advice from the CHMP. The clinical advice related to cardiac safety and adequacy of the safety database as well as other issues outside the claimed indication.

### *GCP*

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.

A routine GCP inspection was conducted for study GPI-06-0002 on three study sites. Overall, no critical findings but 14 major findings were detected. The inspection team concluded for two of the three sites that, based on the samples reviewed, the trial was conducted in compliance with the provisions laid down in the GCP ICH guideline and data generated are robust and reliable. For the third site, the inspection team concluded that, based on the deviations observed during the site inspection, the trial was not conducted fully in compliance with ICH GCP and this might have an impact on the investigator's reported response and duration of response.

The overall recommendation of the inspection team was to accept the submitted data from this trial, provided that the CHMP does not consider the major issues identified to cause invalidation of the trial result.

An overview of the main studies is provided below

- Tabular overview of clinical studies

**Table 15: Main clinical studies with romidepsin**

| Study ID | Design | Study Posology | Study Objective | Subjs by arm entered / compl. | Duration | Gender M/F Median Age | Diagnosis Incl. criteria | Primary Endpoint |
|---|---|---|---|---|---|---|---|---|
| GPI-06-0002<br><br>Efficacy & Safety | Phase 2, Open-label, Single-arm | 14 mg/m$^2$ (4-hr infusions on Days 1, 8, and 15 of 28-day cycles) | Evaluate the activity of romidepsin in patients with progressive or relapsed peripheral T cell lymphoma (PTCL) following prior systemic therapy | 131/130 | 6 cycles or until disease progression or other withdrawal criteria were met | 88/42<br><br>61 | Progressive PTCL following prior systemic therapy. | Complete response (CR+CRu) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| NCI-1312-PTCL<br><br>Efficacy & Safety | Phase II Multicentre Open label | The initial dose of 18mg/m$^2$ i.v. on Days 1 and 5 of a 21 Day cycle was reduced to 14mg/m$^2$ i.v. on Days 1, 8 and 15 of a 28 Day cycle | Determine the response rate and toxicity profile of romidepsin in T-Cell Lymphoma | 47 | A median of three cycles | | Relapsed/refractory patients with T-Cell lymphomas | Response rate |

Moreover, the applicant has submitted the following early phase clinical studies

**Table 16: Early phase clinical studies with romidepsin**

| Study Number | Title |
|---|---|
| **Clinical Studies and Analyses** | |
| T-95-0077 | Pharmacokinetic report: Phase 1 trial of a 4-hour infusion of depsipeptide (NSC630176) given on days 1 and 5 of a 21-day cycle in patients with refractory neoplasms |
| T-95-0022 | Pharmacokinetic report: Phase 1 trial of a 4-hour infusion of depsipeptide given on days 1, 8, and 15 of a 28-day cycle in patients with advanced cancers, solid tumors |
| AN10018a | Development of a non-compartmental model pharmacokinetics of romidepsin and assessment of dose proportionality |
| NCI 1312 (Protocol 01-C-0049) | Non-compartmental pharmacokinetics of romidepsin (Phase II trial of depsipeptide (NSC 630176) in patients with cutaneous T-cell lymphoma and relapsed peripheral T-cell lymphoma) |
| AN10022 | Development and validation of an integrated population pharmacokinetics model for romidepsin |
| GPI-06-0005-QT | Romidepsin exposure-QTc response analysis, study GPI-06-0005 |
| AN10019 | Integrated romidepsin exposure-QTc response population analysis |

## 2.4.2. Pharmacokinetics

PK samples were obtained from Phase 1 dose-ranging studies and Phase 2 studies (see Table 16 above) that evaluated a 4-hour romidepsin infusion of 14 mg/m2 administered on Days 1, 8, and 15 every 28 days.

**T-95-0077: Phase 1 trial of a 4-hour infusion of romidepsin given on Days 1 and 5 of a 21-Day cycle in patients with refractory neoplasms**

Romidepsin was intravenously administered over a 4-hour period. The dosages were 1.0, 1.7, 2.5, 3.5, 6.5, 9.1, 12.7, 17.8, and 24.9 mg/m$^2$. Dosing was performed on Days 1 and 5 of a repeating 21-day cycle. Blood samples for plasma romidepsin concentration analysis were collected at 0 (predose), 1, 2, 3, and 4 hours (end of infusion), and at 4.25, 4.5, 5, 7, 10, 16, 24 and 48 hours post infusion.

Additional blood samples for plasma romidepsin analysis collected on Day 5 were 96 hours (trough), 100 hours (end of infusion, Day 5), and 103 hours (3 hours post infusion, Day 5).

A total of 38 patients were enrolled in the study and each received at least one dose of study drug. Thirty-five of these patients had evaluable PK data. In many cases, more than one PK profile was obtained from a patient at the same dose level. In addition, there were six patients that had PK profiles collected at two distinct dose levels.

**T-95-0022: Phase 1 trial of a 4-hour infusion of romidepsin given on Days 1, 8, and 15 of a 28-Day cycle in patients with advanced cancers, solid tumours**

Romidepsin was intravenously administered over a 4-hour period. The dosages were 1.0, 2.0, 3.25, 5.0, 7.5, 13.3, 17.7, and 23.5 mg/$m^2$. Dosing was performed on Days 1, 8, and 15 of a repeating 28-day cycle. Blood samples for plasma romidepsin concentration analysis were scheduled to be collected at 0 (predose), 3.75 and 4.0 hours (end of infusion); and at 4.08, 4.17, 4.25, 4.5, 4.75, 5.0, 6, 8, 10, 12, and 28 hours post infusion.

**AN10022: Development and Validation of an Integrated Population Pharmacokinetics Model for Romidepsin**

An integrated analysis of PK data, termed a PPK study, was undertaken to investigate the PK of romidepsin in patients with advanced cancer, including patients with CTCL. The secondary objectives were to determine the effect of renal function and hepatic function on romidepsin PK, and to develop separate predictive PPK models for PK data from a subset of studies to support exposure-QTc analysis.

Data for PPK analysis were available from six studies: NCI 1312, FJ-228-0001, FJ-228-0002, GPI-04-0001, GPI-06-0005, and T-95-0077.

PPK model development was performed through a combination of exploratory data analysis and nonlinear mixed effects modeling. Pairs plots (scatterplot matrices), with and without superimposed smoothing lines, were used to examine relationships between parameters and continuous covariates and categorical/binary covariates. Boxplots of PK parameters, sorted by categorical/binary covariates, were also used to examine potential relationships between parameters and such covariates.

The results of the PPK study are not shown but the main findings are discussed below (see discussion on clinical pharmacology).

## *Absorption*

Romidepsin is administered intravenously; hence this section is not applicable.

## *Distribution*

From non-compartmental analysis, the mean $V_{ss}$ values ranged from 7.1 to 76.0 L/$m^2$ over the dose range investigated. In compartmental analysis, romidepsin PK was characterised with a 3 compartment linear PK model. The volume of central compartment (V1), was estimated to 5.37 l. The volumes of the first peripheral compartment (V2) and of the second peripheral compartment (V3) were estimated to be 12.87 l and 2.14 l, respectively.

Romidepsin was highly protein bound in both human serum (94 to 95%) and in human plasma (92 to 94%) over the concentration range of 50 to 1000 ng/mL (concentrations in the range that is observed clinically). Radiolabeled romidepsin was 19.9% bound to human albumin and 93.5% bound to human α1-acid glycoprotein.

### *Elimination*

The mean terminal t1/2 value across all dose groups was 8.1 ± 5.1 hours with a range of 1.2 to 23.7 hours. The mean plasma $Cl_{tot}$ values for romidepsin ranged from 159 to 604 mL/min/$m^2$ across the 1.0 to 24.9 mg/m2 dose range investigated.

No mass balance studies have been performed in humans. Liver microsomal studies demonstrated no significant qualitative and quantitative differences in romidepsin metabolism in liver microsomes between male rats, male dogs, and humans. Human excretion is likely to be similar. Rats excrete about 79.4%, 16.5%, and 0.1% of radioactivity in faeces, urine, and expired air, respectively.

### *Dose proportionality and time dependencies*

In study T-95-0077 a number of patients received multiple intravenous doses of romidepsin at various doses. Data from this study suggest dose-proportionality with respect to AUC and Cmax within a dosing range of 2-25 mg/square meters (data not shown).

In patients with more than one evaluable PK profile, the pharmacokinetics of romidepsin did not change appreciably with repeated administration (data not shown).

### *Special populations*

Based on an integrated population PK (PPK) model, age, race, gender, mild to severe renal impairment and mild to moderate hepatic impairment had no significant effect on romidepsin PK. Study effect and weight were the 2 most significant predictors of romidepsin CL in the integrated PPK model. Weight accounted for approximately 2% of the variability in romidepsin CL and study effect explained 4% of the variability. Dosing of romidepsin was based on BSA, as is common in oncology patients.

### *Pharmacokinetic interaction studies*

No clinical drug-drug interaction studies have been conducted with romidepsin, but two are planned, one with ketoconazole and one with rifampin:

ROMI-ADVM-001 and ROMI-ADVM-002 studies are designed to evaluate the effect of ketoconazole, a known inhibitor of CYP3A4, or rifampin, a known inducer of CYP3A4, on the pharmacokinetic of romidepsin following a single IV 4-hour infusion in patients with advanced malignancies.

When romidepsin was incubated aerobically with human liver microsomes in the presence of a nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) -generating system, at least 20 different metabolites were produced. Of 14 recombinant human P450s examined, romidepsin disappearance was greatest in the presence of CYP3A4, followed to a much lesser extent by 3A5, 1A1, 2B6, and 2C19 and the rate of disappearance of romidepsin produced by CYP 3A5, 1A1, 2B6, and 2C19 was 16.8, 5.2, 1.4, and 1.3%, respectively, that of CYP 3A4. Other P450 isoforms tested showed no significant metabolic activity toward romidepsin.

In human hepatocyte cultures, under conditions where positive controls caused anticipated induction, romidepsin treatment (0.5 to 10 µM) for two consecutive days did not induce the catalytic activities or mRNA levels of CYP2B6, or CYP3A4/5. Romidepsin had a weak inductive effect on CYP1A2 (< 10% of positive control), which is not anticipated to be clinically relevant.

In a study using pooled human liver microsomes from 16 individuals, romidepsin did not exhibit any inhibitory effects on CYP1A2, 2C9, 2C19, 2D6, 3A4, 2E1 activities at concentrations of 10 µmol/L or less. At a concentration of 100 µmol/L that far exceeds the expected plasma Cmax concentration of

0.7 µmol/l, romidepsin inhibited the marker activities of CY3A4, CYP2C19, and CYP2D6 to some degree, which is not expected to be clinically relevant.

When 0.1 µmol/L, 1 µmol/L, and 10 µmol/L of ketoconazole (a specific CYP3A4 inhibitor) was added, romidepsin disappearance activity in human liver microsomes was 41.2%, <10%, and <10% of the control value, respectively. Additionally, an anti-CYP3A4 antibody inhibited romidepsin disappearance in human liver microsomes by >90%.

No study addressing the interaction of romidepsin with P-gp was submitted, results from a published study were submitted and discussed. These demonstrate that romidepsin is a substrate for P-gp and MRP1.

### *Pharmacokinetics using human biomaterials*

Please refer to section on Distribution for binding studies to human serum and plasma and to section on Pharamacokinetic interaction studies for description of studies using human liver microsomes.

## 2.4.3. Pharmacodynamics

### *Mechanism of action*

No clinical studies addressing the mechanism of action were submitted.

### *Primary and Secondary pharmacology*

No clinical primary pharmacology studies were submitted. In terms of secondary pharmacology, an integrated romidepsin exposure-QTc population analysis (report AN10019) was submitted. This was a PK-PD analysis with the aim to develop a descriptive nonlinear mixed effects model characterising the relationship between romidepsin concentration and heart rate-corrected QTc interval duration, change from baseline QTc, and HR derived from the RR interval.

Electrocardiogram and PK data were obtained from two oncology studies of romidepsin, Study GPI-06-0005 and Study NCI 1312.

Data from 29 patients who received romidepsin at 14 mg/m$^2$ IV over 4 hours were included in the PK analyses. Pharmacokinetic data from 16 of these patients who also received a lower dose of romidepsin over a 1-hour infusion interval were also analysed.

Centrally-read triplicate ECG data were available for 26 of the 29 patients who received romidepsin at 14 mg/m$^2$ IV over 4 hours; these 26 patients comprise the ECG evaluable population. Fourteen of these patients also received a lower dose of romidepsin over a 1-hour infusion interval. All patients in the ECG evaluable population had at least 1 matched concentration-QTc pair obtained at baseline and 1 matched concentration-QTc pair obtained post dose.

Evaluation of the relationship between romidepsin plasma concentration and QTc used all replicate ECGs, therefore, 1227 concentration-QTc pairs obtained from 26 patients were available for analysis of QTcF (QT interval corrected for HR according to Fridericia's method) and QTcI (QT interval corrected for HR based on data from study GPI-06-0005 only). The dataset included both the pre-antiemetic and post antiemetic pre-romidepsin dose ECGs. For the analysis of ΔQTcF, 1037 concentration-QTc pairs were used.

No concentration-dependent effect of romidepsin on the duration of the QTc interval was identified, including at exposures of up to more than 2.5-fold higher on average than that observed with the

currently approved and clinically used dose regimen of 14 mg/m2 administered as a 4-hour infusion. Central tendency and categorical analyses also showed no effect of dosing with romidepsin on the heart rate-corrected QTc interval (QTcF). Romidepsin was shown to have no PD effect on the change from baseline QTcF interval and the QTcI interval (Study GPI-06-0005 study data only), but it was associated with an indirect concentration dependent increase in heart rate of about 20-25% (data no t shown).

No studies addressing the relationship between plasma concentration and romidepsin effect and no pharmacodynamic interaction studies with other medicinal substances were submitted.

## 2.4.4. Discussion and conclusions on clinical pharmacology

*In vitro* data indicate high plasma and serum protein binding of romidepsin in human (>92%). Clinically relevant displacement interactions are unlikely as romidepsin is a low clearance-drug.

Romidepsin was primarily metabolised by the cytochrome P450 3A4 isozyme in human liver microsomes in vitro. At least 20 different metabolites were produced. A large number of metabolites of romidepsin were found in plasma, urine, faeces, and bile, and no single metabolite predominated. Several of these metabolites found in plasma appear to have been bound to plasma proteins. Human *in vivo* quantitative and qualitative assessment of metabolites remains unsatisfactory.

An integrated population pharmacokinetic (PPK) model (from six studies) was developed to examine potentially important covariates affecting romidepsin metabolism. The effect of demographic factors (age, gender and race), renal and hepatic impairment on the pharmacokinetics of romidepsin was explored. According to the Applicant age, race, gender, mild to severe renal impairment, and mild to moderate hepatic impairment have no meaningful effect on romidepsin PK. An open-label study to assess the PK of single-dose romidepsin in patients with advanced cancer and moderate or severe hepatic impairment is planned and the effect of hepatic impairment will therefore be elucidated further.

The population model developed appears well validated and consistent across studies. No covariates parameters were included in the final model other than weight and study type accounting for 2 and 4% of PK variability, respectively. The low number for body weight is inherent as dosing was related to BSA which in turns correlates well to body weight. Study factor is most likely related to patient factors related to selection.

With respect to P-gp and MRP-1, no formal in vitro studies were submitted by the Applicant. The Applicant provides evidence from other clinical trials with romidepsin and concomitant use of inducers of P-gp and CYP3A4, which, while not designed as a DDI studies, could not demonstrate an effect of these drugs on romidepsin PK. A pharmacogenetic analysis of clinical data did not find any association between ABCD1 genotypes and romidepsin. These human data confirm preclinical studies in knock-out mice, and clinically relevant DDI based on P-gp appears unlikely.

No clinical drug-drug interaction in vivo studies have been conducted with romidepsin, but two are planned, one with ketoconazole and one with rifampin.

The quality of the pharmacodynamic data was globally poor: results derived from the literature were submitted but not summarised or discussed by the Applicant. No discussion on plasma concentration and romidepsin effect and no PD interaction studies with other medicinal substances were submitted. Taken together, the provided pharmacodynamic data do not allow an adequate characterisation of the pharmacodynamic properties of romidepsin.

## *2.5. Clinical efficacy*

In terms of efficacy the application was supported by the pivotal GPI-06-0002 study with supportive efficacy data from the NCI 1312 Study:

| Study ID | Design | Primary Objective | Study population | | | Primary Endpoint |
|---|---|---|---|---|---|---|
| | | | Entered/ treated | Median age | Diagnosis | |
| GPI-06-0002 | Phase II, non-randomised, open-label | Determine efficacy and assess safety in PTCL | 131/131 | 61 yrs (20-83) | Relapsed/ refractory PTCL | CR+CRu rate |
| NCI study 1312 | Phase II, non-randomised, open-label | Evaluate activity and tolerability in PTCL and CTCL | 47/47 | 59 yrs (27-84) | Relapsed/ refractory PTCL (47) and CTCL (84) | Response rate |

### 2.5.1. Dose response study

The early clinical development of romidepsin included 2 Phase I dose-escalation safety and tolerability studies that were completed by the NCI in 2000 (Study T-95-0022 and Study T-95- 0077). In these studies, the MTD of romidepsin was determined to be 17.8 mg/m2 administered on Days 1 and 5 every 21 days (Study T-95-0077) and 13.3 mg/m2 administered on Days 1, 8, and 15 every 28 days (Study T-95-0022) and responses were observed in patients with T-cell lymphoma. Based on tolerability, NCI adopted a dose and regimen of 14 mg/m2 administered on Days 1, 8, and 15 every 28 days for the Phase 2 NCI 1312 study in patients with T-cell lymphoma. Interim data from this study demonstrated a manageable safety profile for romidepsin administered at this dose and schedule and meaningful responses were observed in patients with CTCL and PTCL. Based on these observations, the same dose and schedule were adopted for the pivotal study GPI-06-0002.

### 2.5.2. Main study

**Study GPI 06 0002**

This was a phase II, open-label, single-arm, international study designed to determine the efficacy and to assess the safety of romidepsin in patients with PTCL who had received prior therapy.

#### *Methods*

#### *Study Participants*

Males and females, 18 years of age or older with histopathologically confirmed PTCL who had progressive disease following, or were refractory to, at least one prior therapy, were eligible. Eligible histologies as defined in the WHO classification included PTCL NOS, AITL, extranodal NK/T-cell lymphoma nasal type, EATL, subcutaneous panniculitis-like T-cell lymphoma, cutaneous $\gamma\delta$ T-cell lymphoma (excludes mycosis fungoides or Sézary syndrome), transformed mycosis fungoides, hepatosplenic T-cell lymphoma, and ALK-1 negative ALCL. Patients with ALK-1 positive ALCL must have had relapsed disease after ASCT.

Patients were required to have measurable disease according to the IWC and/or measurable cutaneous disease, ECOG PS of 0, 1 or 2, adequate bone marrow function (haemoglobin ≥9 g/dL, absolute neutrophil count >1×109/l, and platelet count ≥100×109/l) and no known significant cardiac

abnormalities (e.g., congenital long QT syndrome, QTc >480 msec, cardiac arrhythmia requiring anti-arrhythmic medication, myocardial infarction within the previous 6 months).

### *Treatments*

Patients received romidepsin 14 mg/m2 intravenously over four hours on days 1, 8, and 15 of each 28-day cycle. Treatment was planned for six cycles of treatment or until a study discontinuation criterion was met. Responding patients had the option of continuing treatment 6 cycles at the discretion of the patient and the investigator, until a discontinuation criterion was met. Patients receiving 12 cycles or more were permitted to reduce the treatment from 3 doses to 2 doses per cycle.

### *Objectives*

Primary objective: To evaluate the activity of romidepsin in patients with progressive or relapsed PTCL following prior systemic therapy.

Secondary objectives: To estimate the response rates, the duration of response, and the time to objective disease progression; To assess tolerability and safety of romidepsin; To estimate the change in ECOG performance status.

Exploratory objective: To collect and analyse data for an exploratory study using the IWC + PET response criteria for NHL.

### *Outcomes/endpoints*

The primary efficacy parameter was the rate of complete response defined as the proportion of patients with CR and CRu according to the IWC for response assessment for NHL. Disease assessments were conducted at baseline (within 4 weeks prior to study entry), at every other cycle starting with Cycle 2, and at the final visit. Radiological assessments were performed every 8 weeks during the study. Disease assessments were carried out according to the IWC criteria (Cheson *et al*, 1999). Response was assessed independently by an IRC. The IRC assessment was conducted as a 2-stage process, with an initial radiology review (with no access to clinical information) followed by an overall IRC review with access to the clinical patient data.

Secondary endpoints were Time to response, Duration of response, Time to disease progression, Progression-free survival and change in ECOG performance status.

Exploratory endpoints were response rate based on the best response as determined by the radiology IRC review using CT/MRI scans alone and response rate including FDG-PET data, at sites where this technology was available and if the site agreed to participate.

### *Sample size*

The study was planned so that a sufficient number of patients would be screened and enrolled in order to obtain 65 patients who met all major protocol requirements and received at least 2 of 3 planned doses in 2 consecutive cycles of therapy. It was anticipated that in order to meet these sample size criteria, more than 100 patients would be enrolled and treated under this study protocol.

All statistical sample size calculations were based on the primary endpoint of complete response (CR and CRu). The one-stage design sample size of N=65 was calculated based on the need to obtain a robust estimate of the proportion of complete responders including 95% lower confidence limits, for a reasonable range of true response rates. This sample size would yield lower 95% confidence limits that would range from 2.2% to 7.7% if the observed rate of CR + CRu ranged from 8% to 15%.

### *Randomisation*

This was a single-arm study so that randomisation was not applicable.

### *Blinding (masking)*

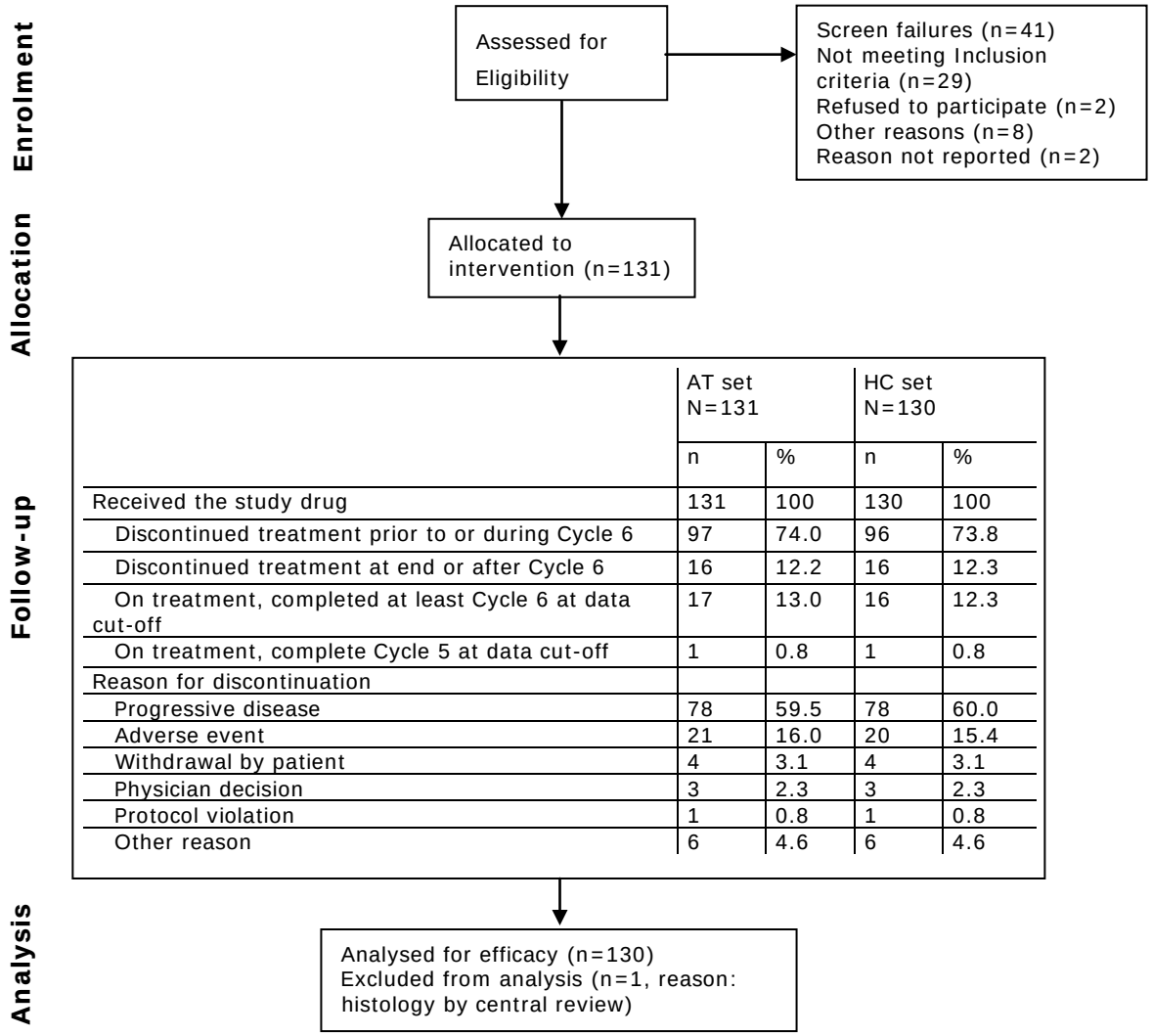
This was an open-label study.

### *Statistical methods*

Standard statistical methods were used in the analysis of the data.

### *Results*

## Participant flow




| | AT set N=131 | | HC set N=130 | |
|---|---|---|---|---|
| | n | % | n | % |
| Received the study drug | 131 | 100 | 130 | 100 |
| Discontinued treatment prior to or during Cycle 6 | 97 | 74.0 | 96 | 73.8 |
| Discontinued treatment at end or after Cycle 6 | 16 | 12.2 | 16 | 12.3 |
| On treatment, completed at least Cycle 6 at data cut-off | 17 | 13.0 | 16 | 12.3 |
| On treatment, complete Cycle 5 at data cut-off | 1 | 0.8 | 1 | 0.8 |
| Reason for discontinuation | | | | |
| Progressive disease | 78 | 59.5 | 78 | 60.0 |
| Adverse event | 21 | 16.0 | 20 | 15.4 |
| Withdrawal by patient | 4 | 3.1 | 4 | 3.1 |
| Physician decision | 3 | 2.3 | 3 | 2.3 |
| Protocol violation | 1 | 0.8 | 1 | 0.8 |
| Other reason | 6 | 4.6 | 6 | 4.6 |

## Recruitment

Patients were treated at a total of 48 study centres; in the US (20 study centres), Europe (25 study centres including 3 in the Czech Republic, 7 in France, 4 each in Germany, Poland and Spain, 1 each in Sweden, the United Kingdom and Ukraine) and Australia (3 study centres).

Among the 131 patients treated in this study, 61 (46.6%) were enrolled at study centres in the US, 23 (17.6%) in France, 15 (11.5%) in Australia, 10 (7.6%) in Spain, 9 (6.9%) in Germany, 6 (4.6%) in Poland, 3 (2.3%) in the Czech Republic, 2 (1.5%) in Sweden, and 1 (0.8%) each in the United Kingdom and Ukraine.

The first patient was enrolled in the study on 19 June 2007 and the last patient was enrolled on 5 January 2010. As of the cut-off data for the primary efficacy analysis of 31 March 2010, 18 patients were still on treatment in the study. An efficacy update with a cut-off date of 31 October 2010 was conducted and submitted at the request of the CHMP.

## Conduct of the study

The initial protocol, dated 27 October 2006, was amended once on 1 October 2008. The amendment did not modify study design, endpoints or planned analyses. A number of changes to the planned analyses were made. Most importantly, the protocol stipulated that the 'per protocol' population (termed 'Evaluable for Response' population in the protocol) was to be used for primary statistical inferences. However, in order to provide a more conservative approach, the 'As-treated' population was defined in the statistical analysis plan and was to be used for analyses of efficacy. This population more closely adheres to the principles of intent to treat. Finally, most of the efficacy results presented were based on the 'histolopathologically confirmed' population. This population (N=130) is, however, almost identical to the 'As-treated' population (N=131).

Furthermore, a few protocol addenda were written to meet country-specific requirements. These addenda had no relevance for the analysis of efficacy endpoints.

## Baseline data

Baseline demographic and disease characteristics are presented in the following Table 17.

**Table 17: Baseline demographic and disease characteristics, HC population, GPI-06-0002**

| Characteristic | Total Patients (N=130) |
|---|---|
| Males, (%) | 67.7% |
| Age, Median (Range) (years) | 61.0 (20-83) |
| Age, (%) | |
| < 65 years | 62.3% |
| ≥ 65 years | 37.7% |
| ECOG PS, (%) | |
| 0 | 35.4% |
| 1 | 50.8% |
| 2 | 13.1% |
| Duration of PTCL, Median (Range) (years) | 1.3 (0.24-17.0) |
| Stage at Diagnosis, (%) | |
| I/II | 28.5% |
| III/IV | 70.0% |
| Not reported/Not available | 1.5% |
| Lactate Dehydrogenase Elevated > ULN at Baseline, (%) | 54% |
| International Prognostic Index at Study Baseline, (%) | |
| <2 | 23.8% |
| ≥2 | 76.2% |
| PTCL Subtype Based on Central Diagnosis, (%) | |
| PTCL Unspecified (NOS) | 53.1% |
| Angioimmunoblastic T-cell lymphoma (AITL) | 20.8% |
| ALK-1 negative ALCL | 16.2% |
| Other | 10.0% |

Bone marrow biopsies were conducted at screening in 115 (89%) of the 130 patients; 36 of these patients (31%) had bone marrow involvement with the disease at study entry, a poor prognostic factor. Skin involvement by disease was noted by the investigator in 34 (26%) of the 130 patients.

The median number of prior systemic therapies for PTCL was 2; 37% of the 130 patients had received 3 or more systemic therapies prior to study entry. The most commonly administered prior combination chemotherapy was anthracycline based (e.g., CHOP, either alone or in combination with other chemotherapies or monoclonal antibodies). A total of 16% of patients had received prior ASCT and had relapsed prior to study entry.

Overall, 49 (38%) of the patients were refractory to their last prior therapy (i.e., had a best response of PD reported for this therapy); SD was reported as best response for last prior therapy for 15% of patients, PR for 18%, and CR for 23%. Median time since most recent prior systemic therapy was 2.1 months indicating rapid progression of disease for the majority of patients following their last therapy.

## Numbers analysed

One hundred and thirty-one (N=131) patients were enrolled and received at least one dose of romidepsin (as-treated (AT) analysis population). This population was used for the analyses of safety endpoints. The histopathologically confirmed (HC) Analysis Set (N=130) excluded 1 patient found by the central pathology review to have DLBCL. The HC was used for the analyses of efficacy endpoints.

Finally, the per protocol (PP) analysis set (N=78) comprised patients who received at least 2 cycles of treatment and had histopathologically confirmed disease and no major protocol violations. This was used as supportive for the analysis of efficacy endpoints.

## Outcomes and estimation

### Primary endpoint

Results with regard to the primary endpoint of independently assessed response rate are presented in the following Table 18.

**Table 18: Summary of IRC-assessed best response, HC population, GPI-06-0002**

| Efficacy Endpoint | Original Analysis[1] | Efficacy Update[2] |
|---|---|---|
| **Best Response Category, n (%) [95% CI][3]** | | |
| Objective Disease Response (CR+CRu+PR) | 34 (26.2) [18.8, 34.6][3] | 33 (25.4) [18.2, 33.8][3] |
| Complete Response (CR+CRu) | 17 (13.1) [7.8, 20.1][3] | 19 (14.6) [9.0, 21.9][3] |
| Partial Response (PR) | 17 (13.1) | 14 (10.8) |
| Stable Disease (SD) | 32 (24.6) | 33 (25.4) |
| Progressive Disease (PD) | 35 (26.9) | 35 (26.9) |
| Not Evaluable[4] | 29 (22.3) | 29 (22.3) |

[1] data cut-off: 31 March 2010, [2] data cut-off 31 October 2010, [3] two-sided 95% confidence interval, [4] insufficient efficacy data to determine response due to early termination; included as non-responders in the analysis

A comparison of baseline prognostic factors between patients who achieved a CR or CRu and patients with PR, SD, PD as best response (including non-evaluable patients) did not reveal any striking imbalance between the two groups (data not shown).

### Secondary endpoints

Secondary endpoints were Time to response, Duration of response, Time to disease progression, Progression-free survival and change in ECOG performance status.

Duration of response data are summarised in the following Table 19.

**Table 19: Summary of IRC-assessed duration of response, HC population, GPI-06-0002**

| Efficacy Endpoint | Original Analysis[1] | Efficacy Update[2] |
|---|---|---|
| **Duration of Response (Days)** | | |
| Patients with Objective Disease Response | | |
| N | 34 | 33 |
| Median [95% CI] | 353 [130, NE] | 505 [353, NE] |
| Minimum, Maximum | 1.0+[6], 801+ | 1.0+[6], 1035+ |
| Censored Observations[5], n (%) | 26 (76.5) | 25 (75.8) |
| Patients with Complete Response | | |
| N | 17 | 19 |
| Median [95% CI] | NE [353, NE] | 505 [353, NE] |
| Minimum, Maximum | 1.0+[6], 801+ | 1.0+[6], 1035+ |
| Censored Observations[5], n (%) | 16 (94.1) | 17 (89.5) |

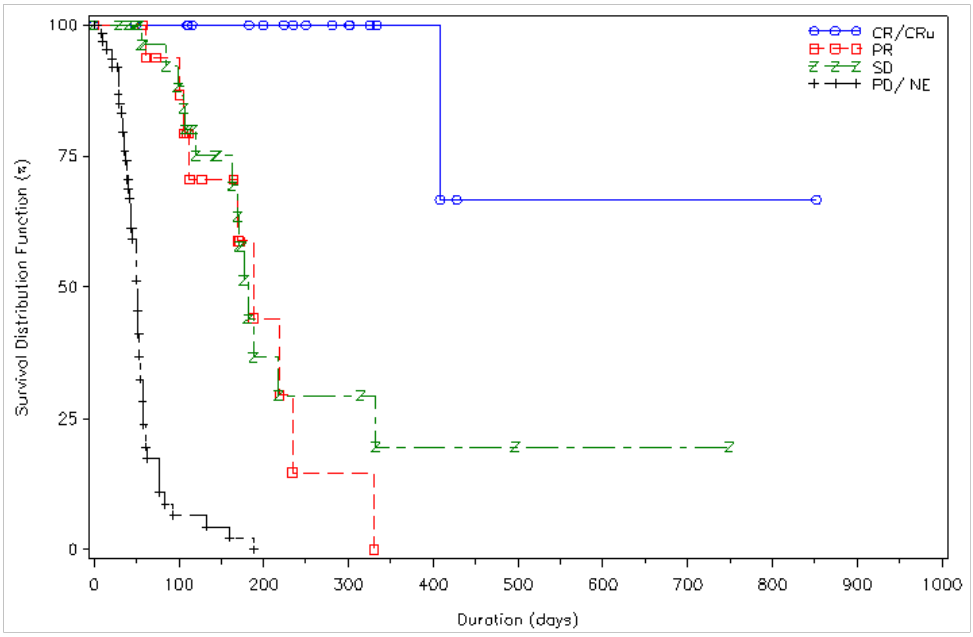
[1] data cut-off: 31 March 2010, [2] data cut-off 31 October 2010, [5] cencoring for the overall IRC analysis was conducted based on the last clinical assessment date, [6] one patient elected to go to transplant following the first response assessment of CR

Time to response: Median time to objective disease response based on Overall IRC review was 1.8 months and ranged from 1.4 to 5.6 months. Median time to complete response was longer at 3.5 months, ranging from 1.6 to 9.2 months, indicating that in some patients, complete response occurred during continued treatment after a period of sustained PR.

Time to disease progression: Median TTP was 177 days (6 months) based on the Overall IRC review and 85 days (3 months) based on the Investigators' assessments. Clinical IRC reviewers were unaware of the reasons why patients went off study. Therefore, a higher number of patients were reported to have a date of progression based on the study termination record in the Investigator-assessed TTP analyses (68%) compared to the IRC-reported TTP (42%), likely leading to the observed difference in TTP.

Progression-free survival: Median PFS based on the Overall IRC review was 107 days (4 months) and 77 days (3 months) based on the Investigators' assessments. PFS data by response category are presented in the following Figure 1.

**Figure 1: Progression-free survival by response category**




Change in ECOG performance status data are presented in the following Table 20.

**Table 20: Shifts from Baseline in ECOG Performance Status by Response to Romidepsin**

| Change in ECOG PS: | CR/CRu n/N (%) | PR n/N (%) | SD n/N (%) | PD/NE n/N (%) |
|---|---|---|---|---|
| Improvement at any time on study | 9/11 (81.8) | 7/12 (58.3) | 11/20 (55.0) | 7/38 (18.4) |
| Worsening at any time on study | 3/17 (17.6) | 8/16 (50.0) | 13/31 (41.9) | 33/61 (54.0) |

## Ancillary analyses

Exploratory endpoints were response rate based on the best response as determined by the radiology IRC review using CT/MRI scans alone and response rate including FDG-PET data, at sites where this technology was available and if the site agreed to participate.

Investigator-assessed response rates and duration of responses are presented in Table 21 below.

**Table 21: Summary of investigator-assessed best response and duration of response, HC population (N=130), GPI-06-0002**

| Efficacy Endpoint | Original Analysis[1] | Efficacy Update[2] |
|---|---|---|
| **Best Response Category, n (%) [95% CI]** | | |
| Objective Disease Response (CR+CRu+PR) | 38 (29.2) [21.6, 37.8][3] | 38 (29.2) [21.6, 37.8][3] |
| Complete Response (CR+CRu) | 21 (16.2) [10.3, 23.6][3] | 21 (16.2) [10.3, 23.6][3] |
| Partial Response (PR) | 17 (13.1) | 17 (13.1) |
| Stable Disease (SD) | 22 (16.9) | 22 (16.9) |
| Progressive Disease (PD) | 59 (45.4) | 59 (45.4) |
| Not Evaluable[4] | 11 (8.5) | 11 (8.5) |
| **Duration of Response (Days)** | | |
| Patients with Objective Disease Response | | |
| N | 38 | 38 |
| Median [95% CI] | 353 [176, NE] | 353 [234, NE] |
| Minimum, Maximum | 16+, 811+ | 16+, 1035+ |
| Censored Observations[5], n (%) | 24 (63.2) | 20 (52.6) |
| Patients with Complete Response | | |
| N | 21 | 21 |
| Median [95% CI] | 429 [353, NE] | NE [353, NE] |
| Minimum, Maximum | 36, 811+ | 36, 1035+ |
| Censored Observations[5], n (%) | 16 (76.2) | 15 (71.4) |

[1] data cut-off: 31 march 2010, [2] data cut-off 31 October 2010, [3] two-sided 95% confidence interval, [4] insufficient efficacy data to determine response due to early termination; included as non-responders in the analysis,
[5] cencoring for the overall IRC analysis was conducted based on the last clinical assessment date

With regard to response assessment including FDG-PET: 110 of the 130 patients in the HC population had FDG-PET at baseline. When assessed according to the revised response criteria including the information from FDG-PET (Cheson *et al*, 2007), the CR+CRu rate was 16.4% and the objective disease response rate (CR, CRu, PR) was 30.0%.

Finally, although Overall Survival was not pre-specified as a secondary or exploratory endpoint, OS data at the time of the updated efficacy analysis (cut-off date 31 October 2010) were as follows: with 76 (58.5%) events, median OS was 345 days (95% CI: 253-671) in the HC population (N=130).

## Summary of main study

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

**Table 22: Summary of Efficacy for trial GP-06-0002**

| | | | |
|---|---|---|---|
| **Title:** GPI-06-0002 | | | |
| Study identifier | not applicable | | |
| Design | Phase II, multicentre, open-label trial evaluating the activity and tolerability of Romidepsin (Depsipeptide, FK228) in progressive or relapsed Peripheral T-Cell Lymphoma following prior systemic therapy | | |
| | Duration of main phase:<br>Duration of Run-in phase:<br>Duration of Extension phase: | | 6 months (6 cycles)<br>not applicable<br>until disease progression or other withdrawal from treatment |
| Hypothesis | Exploratory: evaluation of Romidepsin activity in patients with PTCL | | |
| Treatments groups | Romidepsin group | | 14 mg/m2 of Romidepsin IV on Days 1, 8, and 15 of each 28-day treatment cycle, N=131 |
| Endpoints and definitions | Primary endpoint: | Complete Response Rate (CR+CRu) | Based on overall review of radiological and clinical data by an Independent Review Committee (IRC) |
| | Secondary endpoint | Objective Response Rate (ORR=CR+CRu +PR) | Based on overall review of radiological and clinical data by an Independent Review Committee |
| | Secondary endpoint | Complete Response Rate (CR+CRu) | Based on overall review of radiological and clinical data by the Investigator |
| | Secondary endpoint | Objective Response Rate (ORR=CR+CRu +PR) | Based on overall review of radiological and clinical data by the Investigator |
| | Secondary endpoint | Time to complete response | time from first dose date to the first date of CR or Cru (IRC overall review) |
| | Secondary endpoint | Duration of complete response | time from the date of the first complete response (CR or Cru, IRC overall review) until the date of progression |
| | Secondary endpoint | Time to progression | Time from the date of the first study drug dose to the date of progression |
| Database lock | 31 March 2010 | | |

| **Results and Analysis** | | |
|---|---|---|
| **Analysis description** | **Primary Analysis** | |
| Analysis population | other: histopathologically-confirmed (HC) population, patients that received at least 1 dose of romidepsin and who had histopathologically confirmed PTCL based on central histopathology review | |
| Descriptive statistics and estimate variability | Treatment group | Romidepsin group |
| | Number of patients | 130 |
| | Complete response rate (CR +CRu) | 13.1% (17) = 7.7% (10) + 5.4% (7) |
| | One-sided 95% lower bound of CI | 8.5% |
| | Objective Response Rate (ORR=CR+CRu+PR) | 26.2% (34) = 7.7% (10) + 5.4% (7) + 13.1% (17) |
| | One-sided 95% lower bound of CI | 19.9% |

| | | |
|---|---|---|
| | Complete response rate (CR +CRu) | 16.2% (21) = 13.8% (18) + 2.3% (3) |
| | One-sided 95% lower bound of CI | 11.1% |
| | Objective Response Rate (ORR=CR+CRu+PR) | 29.2% (38) 13.8% (18) + 2.3% (3) + 13.1% (17) |
| | One-sided 95% lower bound of CI | 22.7% |
| | Time to Complete Response (median, in days) | 105 |
| | range | 49, 281 |
| | Duration of Complete Response (median, in days) | NE |
| | 95% CI | 353, NE |
| | Time to progression (median, in days) | 177 |
| | 95% CI | 106, 219 |
| Notes | This was an uncontrolled trial | |

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

No analysis of efficacy results across trials was submitted.

## Clinical studies in special populations

No clinical study in special populations was submitted.

## Supportive study

**NCI Study 1312** was a phase 2, open-label, multicentre, international study designed to evaluate the activity and tolerability of romidepsin in separate cohorts of patients with CTCL and PTCL who had received prior systemic therapy. The study was initiated in 2000, 6 years prior to the start of the applicant-sponsored study GPI- 06-0002. Initially, the PTCL cohort in NCI Study 1312 was restricted to patients with relapsed or refractory PTCL NOS or primary cutaneous ALCL who had not received more than 2 systemic cytotoxic chemotherapy regimens. Observed activity in the early phase of the trial led to a protocol amendment allowing inclusion of patients with other mature T-cell lymphoma subtypes and patients who had previously received more than 2 cytotoxic therapies.

Male and female patients 18 years of age or older with measurable disease, ECOG PS or 0, 1 or 2, and life expectancy of >12 weeks were eligible for enrolment. Central histopathologic confirmation of PTCL was required. Exclusion criteria included CNS involvement, QTc >480 msec, and prior therapy with an HDAC inhibitor.

Romidepsin was administered as a 4-hour infusion on Days 1, 8 and 15 of a 28-day cycle with a starting dose of 14 mg/m$^2$. Treatment of the first 2 patients enrolled with PTCL was initiated at 18 mg/m$^2$ on Days 1 and 5 of a 21-day cycle, the schedule originally studied at the NCI. The schedule was changed to the 14 mg/m$^2$ regimen to improve tolerability. Dose reductions were permitted during the study for toxicity; furthermore, the study drug dose could be increased by 25% to 17.5 mg/m$^2$ in patients benefitting from treatment. Patients with a measurable response continued to be treated with

romidepsin, with increasing intervals allowed between cycles after completion of 6 cycles, if needed, to improve patient tolerance and compliance. Study patients previously treated with romidepsin who experienced clinical benefit as evidenced by either complete or partial response and who discontinued therapy for reasons other than disease progression or dose-limiting toxicity could be retreated with romidepsin.

The primary efficacy endpoint in this study was the objective disease response rate as determined by the site Investigators. Responses in patients with nodal disease were assessed using the IWC (Cheson *et al*, 1999). Responses in patients with skin or visceral lesions were assessed using Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000). Response assessments were performed every 2 cycles; for patients who achieved CR, assessments were then conducted every 3 cycles. Complete response required clearing of known sites of disease. Radiotherapy of non-responding lesions was allowed for patients with evidence of overall response. Irradiated lesions were not included in response assessment following radiation.

Forty-seven patients with PTCL were enrolled and treated across multiple study centres in the US and Australia. Of the 47 patients, 28 patients had PTCL NOS, seven patients had AITL, and five patients had ALK-negative ALCL. Other PTCL subtypes occurred in only one or two patients. Central histopathology review confirmed the diagnosis in 46 patients; one PTCL NOS patient developed new nodules after Cycle 5, and a biopsy led to a revision of the diagnosis to DLBCL. The median age of the 47 patients was 59 years and the majority of patients were male (53%). At study entry, 72% of patients had Stage IV disease, including bone marrow involvement in 30%. The median number of prior regimens for treatment of PTCL was 3 and ranged from 1 to 11. All 47 patients had received at least one cytotoxic chemotherapy, with 17 patients (36%) having undergone HD+ASCT.

Forty-five patients were available for response assessment (the patient with DLBCL was excluded from the analysis, along with a patient who received a single dose before exclusion due to prolonged QTc, which is a violation of the entry criteria).

A summary of responses in NCI Study 1312 is given in Table 23. Objective disease responses based on site investigators' assessments were achieved in 17 (37.8%) of the 45 patients, with complete responses noted in 8 patients (17.8%). Five (11.1%) of the 45 patients had SD as a best response on treatment.

**Table 23: Summary of best response, Evaluable population (N=45), study NCI 1312**

| Best Response Category | n (%) |
|---|---|
| Objective Disease Response (CR+PR) | 17 (37.8) |
| Complete Response (CR) | 8 (17.8) |
| Partial Response (PR) | 9 (20.0) |
| Stable Disease (SD) | 5 (11.1) |
| Progressive Disease (PD) | 18 (40.0) |
| Not Evaluable (NE) | 5 (11.1) |

Median time to response was 1.8 months, with most patients achieving a response within 2 months; responses were noted at 4, 8, and 11 months for 3 of the responding patients. The overall median duration of objective disease response was 9 months and ranged from 2 to 74 months. Six (75.0%) of the 8 patients with CR had durations of response of 6 months or longer, including 5 patients with

durations of 12 months or longer (12, 23+, 17, 49+, 74 months). Five of the 9 patients who achieved a PR had response durations of 6 months or longer, 2 of them had responses longer than 12 months.

Median duration of CR calculated by NCI was 29.7 months. Based on the Applicants review of the data included in the efficacy narratives, median duration of response for patients with CR was 17 months.

Objective disease response was observed in patients with PTCL NOS (12 of 28, 42.9%), AITL (1 of 6, 16.7%), ALK-1 negative ALCL (3 of 3, 100%), and enteropathy associated T-cell lymphoma (1 of 1, 100%). Of the 17 patients who had undergone prior transplant, 6 (35.3%) experienced an objective response to therapy, including 3 patients with CR (17.6%).

One patient who achieved a complete response with romidepsin was successfully retreated twice with romidepsin after relapse.

## 2.5.3. Discussion on clinical efficacy

### Design and conduct of clinical studies

The main efficacy results are derived from the pivotal Study GPI-06-0002 (n=130 evaluable patients) and the supportive NCI Study 1312 (n=45 evaluable patients with PTCL). The pivotal study seems well conducted, with no problematic protocol amendments and relatively few protocol violations.

Both trials are single-arm Phase II trials with response rate being the primary endpoint. This endpoint was considered appropriate by the applicant as complete response is a direct measure of activity in a single-arm study and induction of complete response is considered the primary aim in the treatment of T-cell lymphomas (Weidmann *et al*, 2004) and is a likely predictor of clinical benefit, including resolution of symptoms and delaying the requirement for additional therapy. Furthermore, the survival of patients attaining complete response has been shown to be longer than the survival of those attaining less than complete response (Gallamini *et at*, 2004; Raina *et al*, 2010; Yang *et al*, 2010). Finally, the single-arm design was also considered by the applicant appropriate due to the rarity of the disease, the pattern of continuous relapse and the lack of treatment consensus in relapsed/refractory PTCL; at the time the study was initiated, there were no agents approved for second-line in patients with PTCL.

While these arguments are fully acknowledged, the value of objective responses per se is not determined in this clinical setting and a single-arm design does not allow estimation of its translation into clinical benefit in terms of PFS and OS. On the issue of the single arm design, concomitant use of any other anti-cancer therapy was prohibited in the pivotal trial because it was considered as potentially effective for PTCL. Therefore, 'physician's choice' or a list of limited choice would have been a conceivable option for a comparative arm in a randomised phase III study (or at least a comparative phase II study).

### Efficacy data and additional analyses

The complete response rate (CR+Cru) in Study GPI-06-0002 (IRC assessed) was 13.1%, and the ORR was 26.2%. Updated efficacy data that included 7 additional months of follow-up showed complete response and overall response rates of 15% and 25%, respectively. The CR rate in NCI Study 1312 (investigator assessed) was 17.8% and the ORR 37.8%. In the pivotal study, the median duration of response was not reached for the 17 patients who achieved a CR. The median time to response was 56 days and the median time to CR was 150 days. Median duration of response for all patients with an objective response (n=34) was 12 months in the primary analysis and 17 months in the updated analysis. Both CR and ORR rate were slightly higher when assessed by the investigators (16.2%,

29.2%) and the duration of response was also assessed as slightly longer by the investigators (14 months). Median TTP was 177 days, median PFS was 107 days (in both primary and updated analysis) and median OS was 11.3 months. The activity of romidepsin was apparently not dependent on histological subtype, risk factors, the number of previous therapies, or the response to previous therapies.

The Applicant acknowledged the challenges in assessing the benefit/risk profile of a compound using only non-comparative data. To that end, the Applicant submitted a comparison with external control data with currently used therapies in patients with relapsed or refractory PTCL. As noted in the 'Guideline for clinical trials in small populations' (CHMP/EWP/83561/2005), the use of external control data as a comparator may be acceptable under exceptional circumstances. Based on this comparison, the applicant concluded that treatment with romidepsin led to higher complete and overall response rates and a similar duration of response compared to other monotherapies administered in the External Control population. Moreover, despite the fact that 66% of patients in the External Control dataset received combination chemotherapy or high-dose therapy with bone marrow transplant in 3rd line, for patients receiving 3rd line or higher therapy, romidepsin appeared similar or better than other therapies in terms of complete and overall response rates and duration of response. Furthermore, overall survival from the time of front-line therapy for patients treated with romidepsin in Study GPI-06-0002 appeared favourable with a median OS of 34.9 months; median OS in the External Control population from time of frontline therapy was 24.1 months. Finally, review of published data showed that the safety profile of romidepsin compares favourably with the safety of currently administered combination regimens used in the relapsed refractory setting (data not shown).

The CHMP considered that differences in the types and intensity of treatments administered (combination therapy vs. monotherapy) created challenges in interpretation of the data across the datasets (romidepsin vs external control). Even if interpretation of external control data should be taken with caution, it can be outlined that romidepsin is as effective as external controls, at least for 3$^{rd}$ line treatment: ORR = 29.2 % versus 27.1 %, respectively. This similarity is far from demonstrating outstanding efficacy and does not exclude inferiority of romidepsin, given the high level of uncertainty associated with such analyses.

## Additional expert consultation

The CHMP convened a Scientific Advisory Group-Oncology (SAG-O) meeting to consult clinical experts on the Marketing Authorisation Application for Istodax. A number of questions on the efficacy of romidepsin based on the data submitted were posed to the experts. The questions and the SAG-O responses were as follows:

1. The SAG is asked to describe how impressive they view the efficacy data for romidepsin (to be based on response rate, duration of response, PFS, OS) in light of the methodological limitations of the study, in particular the absence of a randomised control group.

Romidepsin has shown antitumour activity in the study population with PTCL. The observed results can be considered hypothesis-generating. The activity in terms of response rate appears to be in the same range of other single-agent or combination regimens which are currently used in this setting, although the efficacy of such treatment options cannot be considered established according to conventional scientific or regulatory standards. Concerning response duration, there are concerns about the high rate of early censoring and the potential for informative censoring.

The antitumour activity observed for romidepsin cannot be considered impressive or otherwise outstanding, and based on this it is not possible to establish the clinical efficacy of this agent in the proposed indication.

There are serious concerns from the point of view of external validity in view of the design of the pivotal study, especially the non-randomised nature of the study. Such design does not allow establishing the efficacy of romidepsin in terms of relevant clinical benefit endpoints such as PFS or OS in this population. Overall, the clinical benefit cannot be considered established and therefore the benefit cannot be considered to outweigh the risks.

2. Does the SAG consider the results of the planned study of Romidepsin in combination with CHOP (Ro-CHOP) versus CHOP alone in patients with previously untreated PTCL of value and supportive/confirmatory in terms of efficacy for the current indication applied for?

The results of the planned study may be of limited value for the current application, due to important differences in terms of line of therapy, patient selection, and treatment schedule. This study is not expected to be of sufficient support or to confirm the efficacy in the target indication.

3. Is there another patient group which is considered relevant for the indication applied for, where a controlled study would be considered feasible to conduct?

A controlled study in the target indication is considered feasible and the best way to establish clinical efficacy, due to heterogeneity of the patient populations in different stages of the disease. Considering the design and size of the study, different possible approaches are considered feasible (e.g., randomised Phase 2 trial, underpowered Phase 3 study). Scientific Advice is recommended.

### 2.5.4. Conclusions on the clinical efficacy

The results are clearly and lucidly presented. The activity of romidepsin in the rather heavily pre-treated and highly treatment-refractory groups of patients is high, and the responses seem more durable than could be expected in this setting. This shows that romidepsin has activity in a clinical situation where other available therapeutic options fail to induce lasting responses. However, without an active comparator arm, it is difficult to make a fully meaningful assessment of the clinical efficacy. The design of the pivotal study precludes further interpretation of magnitude of response and its possible translation into clinical benefit in terms of PFS and OS. The absence of control arm makes it especially difficult to evidence a true clinical benefit beyond the observed response rates.

## *2.6. Clinical safety*

The primary analysis of safety for the proposed PTCL indication was based on data from the pivotal Study GPI-06-0002, in which a total of 131 patients with PTCL were enrolled. The study is a Phase 2, open-label, single-arm, international study designed to determine the efficacy and to assess the safety of romidepsin in the treatment of patients with PTCL who had received prior therapy.

Supporting data were provided from NCI Study 1312, in which a total of 47 patients with PTCL were enrolled. NCI Study 1312 is a Phase 2, multicenter, open-label study designed to evaluate the activity and tolerability of romidepsin in separate cohorts of patients with CTCL and PTCL who had received prior systemic therapy.

Furthermore, Romidepsin was approved in the US by FDA on 05 November 2009 for treatment of patients with cutaneous T-cell lymphoma (CTCL) who have received at least one prior systemic therapy. Post-marketing data collected via spontaneous reports between March 2010 and 04 August 2010 were included.

## Patient exposure

Overall, a total of 891 patients had received at least 1 dose of romidepsin as monotherapy through 30 September 2010 in clinical studies sponsored by the Applicant or the US NCI. Of the 447 patients with haematologic malignancies, 178 were patients with PTCL or other T-cell lymphomas treated in studies GPI-06-0002 (N=131) and NCI Study 1312 (N=47) and 186 were patients with CTCL.

**Table 24: Patients Treated with Romidepsin as Monotherapy**

| | Applicant Studies | NCI Studies | Total |
|---|---|---|---|
| **Indication** | **(N=327) n (%)** | **(N=564) n (%)** | **(N=891) n (%)** |
| Haematologic Malignancies (including T-cell Lymphomas) | 233 (71.3) | 214 (37.9) | 447 (50.2) |
| T-cell Lymphomas | | | |
| CTCL | 102 (31.2) | 84 (14.9) | 186 (20.9) |
| PTCL and other T-cell Lymphomas | 131 (40.1) | 48[1] (8.5) | 179[1] (20.1) |
| Solid Tumours | 94 (28.7) | 350 (62.1) | 444 (49.8) |
| Patients Aged > 22 years | 325 (99.4) | 536 (95.0) | 861 (96.6) |

[1] One additional paediatric patient with PTCL each was enrolled in NCI 1312 and NCI ADVL0212; data from the NCI ADVL0212 patient are not presented in the pool of 178 adults with PTCL in GPI-06-0002 and NCI 1312

In both the pivotal GPI-06-0002 study and in the NCI 1312 study, all patients were to receive romidepsin 14 mg/m2 on Days 1, 8, and 15 every 28 days, with the exception of the 2 initial patients with PTCL in NCI 1312 who received romidepsin at a starting dose of 18 mg/m2 on Days 1 and 5 every 21 days. In NCI 1312, it was permitted by protocol to increase the study drug dose by 25% to 17.5 mg/m2 for patients who were experiencing treatment benefit with minimal toxicity. Five patients received romidepsin at this higher dose level of 17.5 mg/m2. In GPI-06-0002, 1 patient received 2 doses of romidepsin 20 mg/m2 in error. Thus, a total of 8 patients received romidepsin at doses >14 mg/m2.

**Table 25: Exposure to Romidepsin, by Study: Patients with PTCL (N=178)**

| | GPI-06-0002 (N=131) | NCI 1312 (N=47) | Overall (N=178) |
|---|---|---|---|
| Number of Cycles Treated Overall | | | |
| Mean (±SD) | 4.3 (4.72) | 7.9 (10.67) | 5.2 (6.97) |
| Median | 2.0 | 3.0 | 2.0 |
| Minimum, maximum | 1, 31 | 1, 57 | 1, 57 |
| Duration of Treatment (days) | | | |
| Mean (±SD) | 105.3 (136.37) | 245.8 (398.83) | 142.4 (242.60) |
| Median | 44.0 | 85.0 | 46.0 |
| Minimum, maximum | 1, 862 | 1, 1883 | 1, 1883 |
| Total Dose of Romidepsin (mg) | | | |
| Mean (±SD) | 276.3 (310.40) | 491.4 (609.83) | 333.1 (420.07) |
| Median | 144.4 | 196.4 | 150.9 |
| Minimum, maximum | 26, 1616 | 25, 2391 | 25, 2391 |

| | GPI-06-0002 (N=131) | NCI 1312 (N=47) | Overall (N=178) |
|---|---|---|---|
| Patients who received, n (%): | | | |
| No Dose Reduction | 83 (63.4) | 18 (38.3) | 101 (56.7) |
| < 100% of expected dose[1] | 41 (31.3) | 21 (44.7) | 62 (34.8) |
| < 80% of expected dose[1] | 7 (5.3) | 8 (17.0) | 15 (8.4) |
| < 50% of expected dose[1] | 0 | 0 | 0 |
| Completed the study through Cycle 6 | | | |
| Yes | 33[2] (25.2) | 15 (31.9) | 48 (27.0) |
| No | 97 (74.0) | 32 (68.1) | 129 (72.5) |
| Treatment ongoing at cut-off date | 18 (13.7) | 5 (10.6) | 23 (12.9) |
| If No, Reason for Discontinuation | | | |
| Progressive Disease | 71 (54.2) | 24 (51.1) | 95 (53.4) |
| Adverse Event | 19 (14.5) | 2 (4.3) | 21 (11.8) |
| Protocol Violation | 1 (0.8) | 0 | 1 (0.6) |
| Physician Decision | 1 (0.8) | 0 | 1 (0.6) |
| Withdrawal by Patient | 3 (2.3) | 0 | 3 (1.7) |
| Complicating Disease/Illness | 0 | 2 (4.3) | 2 (1.1) |
| Switched to Alternative Treatment | 0 | 0 | 0 |
| Death | 0 | 2 (4.3) | 2 (1.1) |
| Lost to Follow-Up | 0 | 0 | 0 |
| Other | 2 (1.5) | 2 (4.3) | 4 (2.2) |

[1] The expected dose over the course of the study was defined as the original dose multiplied by the number of doses given. To obtain the % expected dose received, the sum of the actual dose was calculated, then divided by the expected dose (and multiplied by 100). [2] One additional patient remained on treatment, but had only completed Cycle 5 as of data cut-off.

## Adverse events

The summary of adverse events in patients with PTCL is found in the following Table 26.

**Table 26: Summary of treatment-emergent Adverse Events**

| | GPI-06-0002 (N=131) n (%) | NCI 1312 (N=47) n (%) | |
|---|---|---|---|
| Treatment-emergent AE (TEAE) | 126 (96.2) | 47 (100.0) | 173 (97.2) |
| Treatment-related TEAE | 120 (91.6) | 47 (100.0) | 167 (93.8) |
| ≥Grade 3 TEAE | 86 (65.6) | 40 (85.1) | 126 (70.8) |
| ≥Grade 4 TEAE[2] | 26 (19.8) | 22 (46.8) | 48 (27.0) |
| Serious TEAE | 60 (45.8) | 30 (63.8) | 90 (50.6) |
| TEAE leading to study drug discontinuation | 22 (16.8) | 13 (27.7) | 35 (19.7) |
| TEAE resulting in death | 7 (5.3) | 8 (17.0) | 15 (8.4) |

The most common adverse events (i.e., incidence ≥20% in either Study GPI-06-0002 or NCI Study 1312) and the Grade 3 and Grade 4 incidences of these common adverse events among patients with PTCL are summarised in the following Table 27. Adverse Drug Reactions are summarised in Table 28.

**Table 27: Treatment-emergent Adverse Events in at least 20% of patients in either study**

| MedDRA Preferred Term[1] | GPI-06-0002 (N=131) n (%) | | | NCI 1312 (N=47) n (%) | | | Overall (N=178) n (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Any Grade | Grade 3 | Grade 4 | Any Grade | Grade 3 | Grade 4 | Any Grade | Grade 3 | Grade 4 |
| At Least One TEAE | 126 (96.2) | 60 (45.8) | 23 (17.6) | 47 (100.0) | 18 (38.3) | 14 (29.8) | 173 (97.2) | 78 (43.8) | 37 (20.8) |
| Nausea | 77 (58.8) | 3 (2.3) | 0 | 35 (74.5) | 3 (6.4) | 0 | 112 (62.9) | 6 (3.4) | 0 |
| Infections[2] | 71 (54.2) | 19 (14.5) | 3 (2.3) | 24 (51.1) | 10 (21.3) | 3 (6.4) | 95 (53.4) | 29 (16.3) | 6 (3.4) |
| Asthenic conditions[3] | 71 (54.2) | 11 (8.4) | 0 | 36 (76.6) | 9 (19.1) | 0 | 107 (60.1) | 20 (11.2) | 0 |
| Vomiting | 51 (38.9) | 5 (3.8) | 1 (0.8) | 19 (40.4) | 4 (8.5) | 0 | 70 (39.3) | 9 (5.1) | 1 (0.6) |
| Thrombocytopenia | 50 (38.2) | 23 (17.6) | 9 (6.9) | 34 (72.3) | 14 (29.8) | 3 (6.4) | 84 (47.2) | 37 (20.8) | 12 (6.7) |
| Diarrhoea | 46 (35.1) | 3 (2.3) | 0 | 17 (36.2) | 1 (2.1) | 0 | 63 (35.4) | 4 (2.2) | 0 |
| Pyrexia | 45 (34.4) | 6 (4.6) | 0 | 22 (46.8) | 8 (17.0) | 0 | 67 (37.6) | 14 (7.9) | 0 |
| Neutropenia | 39 (29.8) | 18 (13.7) | 8 (6.1) | 31 (66.0) | 12 (25.5) | 10 (21.3) | 70 (39.3) | 30 (16.9) | 18 (10.1) |
| Constipation | 37 (28.2) | 0 | 0 | 19 (40.4) | 1 (2.1) | 0 | 56 (31.5) | 1 (0.6) | 0 |
| Anorexia | 37 (28.2) | 2 (1.5) | 0 | 21 (44.7) | 0 | 1 (2.1) | 58 (32.6) | 2 (1.1) | 1 (0.6) |
| Anaemia | 31 (23.7) | 11 (8.4) | 2 (1.5) | 29 (61.7) | 13 (27.7) | 0 | 60 (33.7) | 24 (13.5) | 2 (1.1) |
| Dysgeusia | 27 (20.6) | 0 | 0 | 13 (27.7) | 0 | 0 | 40 (22.5) | 0 | 0 |
| *Cough* | *23 (17.6)* | *0* | *0* | *10 (21.3)* | *0* | *0* | *33 (18.5)* | *0* | *0* |
| *Headache* | *19 (14.5)* | *0* | *0* | *16 (34.0)* | *1 (2.1)* | *0* | *35 (19.7)* | *1 (0.6)* | *0* |
| *Dyspnoea* | *17 (13.0)* | *3 (2.3)* | *0* | *10 (21.3)* | *1 (2.1)* | *1 (2.1)* | *27 (15.2)* | *4 (2.2)* | *1 (0.6)* |
| *Leukopenia* | *15 (11.5)* | *5 (3.8)* | *3 (2.3)* | *26 (55.3)* | *15 (31.9)* | *6 (12.8)* | *41 (23.0)* | *20 (11.2)* | *9 (5.1)* |
| *Hypotension* | *11 (8.4)* | *2 (1.5)* | *0* | *13 (27.7)* | *5 (10.6)* | *0* | *24 (13.5)* | *7 (3.9)* | *0* |
| *Hypomagnesaemia* | *8 (6.1)* | *0* | *0* | *15 (31.9)* | *0* | *0* | *23 (12.9)* | *0* | *0* |
| *Alanine aminotrans-ferase increased* | *5 (3.8)* | *0* | *0* | *17 (36.2)* | *6 (12.8)* | *1 (2.1)* | *22 (12.4)* | *6 (3.4)* | *1 (0.6)* |
| *Aspartate aminotrans-ferase increased* | *5 (3.8)* | *1 (0.8)* | *0* | *18 (38.3)* | *4 (8.5)* | *1 (2.1)* | *23 (12.9)* | *5 (2.8)* | *1 (0.6)* |
| *Lymphopenia* | *5 (3.8)* | *3 (2.3)* | *1 (0.8)* | *19 (40.4)* | *18 (38.3)* | *0* | *24 (13.5)* | *21 (11.8)* | *1 (0.6)* |
| *Hypocalcaemia* | *5 (3.8)* | *0* | *0* | *28 (59.6)* | *7 (14.9)* | *0* | *33 (18.5)* | *7 (3.9)* | *0* |
| *Hyponatraemia* | *4 (3.1)* | *4 (3.1)* | *0* | *10 (21.3)* | *3 (6.4)* | *0* | *14 (7.9)* | *7 (3.9)* | *0* |
| *Hyperglycaemia* | *4 (3.1)* | *1 (0.8)* | *0* | *18 (38.3)* | *4 (8.5)* | *0* | *22 (12.4)* | *5 (2.8)* | *0* |
| *Hypophosphataemia* | *3 (2.3)* | *1 (0.8)* | *0* | *10 (21.3)* | *5 (10.6)* | *1 (2.1)* | *13 (7.3)* | *6 (3.4)* | *1 (0.6)* |
| *Hypoalbuminaemia* | *2 (1.5)* | *0* | *0* | *24 (51.1)* | *5 (10.6)* | *0* | *26 (14.6)* | *5 (2.8)* | *0* |
| *Electrocardiogram T wave amplitude decreased* | *1 (0.8)* | *0* | *0* | *32 (68.1)* | *0* | *0* | *33 (18.5)* | *0* | *0* |
| *Hyperbilirubinaemia* | *1 (0.8)* | *0* | *0* | *14 (29.8)* | *3 (6.4)* | *1 (2.1)* | *15 (8.4)* | *3 (1.7)* | *1 (0.6)* |
| *Hyperuricaemia* | *0* | *0* | *0* | *14 (29.8)* | *0* | *3 (6.4)* | *14 (7.9)* | *0* | *3 (1.7)* |

[1] Terms occurring at an incidence ≥20% in NCI Study 1312 and <20% in Study GPI-06-0002 are presented in italicised font, [2] MedDRA SOC [3] MedDRA High-level term (HLT)

**Table 28: ADRs reported in clinical studies in patients with PTCL treated with romidepsin**

| System Organ Class | CTCAE v3 all Grade ADRs | | CTCAE v3 grade 3-4 ADRs | |
|---|---|---|---|---|
| | Very common | Common | Very common | Common |
| **Infections and Infestations** | Infections 55% | | | Sepsis 5%, Pneumonia 5%, Cellulitis 4%, Staphylococcal Infection 2%, Upper respiratory tract infection 2% |
| **Blood and Lymphatic System Disorders** | Thrombocyto-penia 41%, Neutropenia 30%, Anaemia 24%, Leukopenia 12% | | Thrombocyto-penia 24%, Neutropenia 20%, Anaemia 11% | Febrile neutropenia 3%, Leukopenia 6%, Lymphopenia 3% |
| **Metabolism and Nutrition Disorders** | Anorexia 28%, Hypokalaemia 11% | | | Tumour lysis syndrome 2%, Anorexia 2%, Dehydration 3%, Hypokalaemia 2%, Hyponatraemia 3% |
| **Nervous System Disorders** | Dysgeusia 21%, Headache 15% | | | |
| **Cardiac disorders** | Tachycardia 10% | Electrocardiogram QT prolonged 3%, Electrocardiogram T wave amplitude decreased 1%, Electrocardiogram T wave inversion 1% | | |
| **Vascular disorders** | | | | Deep vein thrombosis 3%, Hypotension 2% |
| **Respiratory, thoracic and mediastinal disorders** | Cough 18%, Dyspnoea 13% | | | Pulmonary embolism 2%, Dyspnoea 2%, Hypoxia 2%, Pleural effusion 2% |
| **Gastrointestinal Disorders** | Vomiting 39%, Nausea 59%, Diarrhoea 36%, Constipation 30%, Abdominal pain 14%, Stomatitis 10% | | | Vomiting 5%, Nausea 2%, 0Diarrhoea 2%, Constipation 1%, Abdominal pain 2%, Colitis 2% |
| **Musculoskeletal and connective tissue disorders** | | | | Arthralgia 2% |
| **Renal and urinary disorders** | | | | Renal failure 2% |
| **General Disorders and Administration Site Conditions** | Asthenia 16%, Fatigue 41% Pyrexia 35%, Chills 11%, Oedema peripheral 10% | | | Chest pain 3%, Asthenia 3%, Fatigue 6%, Pyrexia 5%, Chills 1%, Oedema peripheral 1% |
| **Investigations** | Weight decreased 10% | | | Blood lactate dehydrogenase increased 2% |

**Adverse Events of special interest**

The principal adverse events observed with romidepsin in patients with PTCL were: GI disturbances (83%), haematologic toxicities (66%), asthenic conditions (60%), infections (53%) and clinical chemistry abnormalities (43%).

***Gastrointestinal disturbances***

Romidepsin was frequently associated with GI events (83% in the pooled PTCL data), primarily nausea (63%), vomiting (39%), diarrhoea (35%), and constipation (32%). GI events reported with romidepsin were generally mild to moderate in intensity and non-serious, and most patients continued romidepsin despite the occurrence of GI events. However, severe (Grade 3 or 4) nausea and vomiting may occur (3% and 6%, respectively) in patients receiving nausea and vomiting prophylaxis at the time of onset. In accordance with the study protocol, anti-emetic support was very common in the GPI-06-002 study, but less common in the NCI-1312 study. Dehydration concurrent with vomiting and/or diarrhoea was uncommon.

***Asthenic conditions***

Treatment of PTCL with romidepsin was associated with asthenic conditions (60% in the pooled PTCL data) such as fatigue (50%), asthenia (12%), and malaise (<1%). Most asthenic conditions were Grade 1 or 2 in intensity and non-serious. However, Grade 3 asthenic conditions were reported for 11% of patients; no Grade 4 cases were noted. Asthenic conditions were not associated with anaemia for most patients who experienced these events. Most patients continued romidepsin despite the persistence of asthenic conditions: <1% of patients with PTCL discontinued study drug because of asthenic conditions.

***Tumour Lysis Syndrome***

In Study GPI-06-0002, tumour lysis syndrome was reported for 2 patients (2%). Disease stage at diagnosis was III for 1 patient and IV for the other. For both patients, the event was assessed as Grade 3 in intensity and study drug-related and was reported as serious; however, both cases resolved within 24 hours after appropriate treatment and neither led to study drug discontinuation.

***Pulmonary embolism and deep vein thrombosis***

Venous thromboembolism events (VTE) have been reported in patients with NHL, occurring at incidences from ~7% up to 20%. A total of 8 (4%) of 178 patients with PTCL receiving romidepsin experienced a venous thromboembolism event (VTE), including deep venous thrombosis (4 patients; 2%), pulmonary embolism (3 patients; 2%), and embolism venous (2 patients; 1%). For 1 patient, the event (deep venous thrombosis [DVT]) was assessed as Grade 2 in intensity; for the remaining 7 patients, the event was assessed as Grade 3 or 4 in intensity. The VTE was considered serious for all but one patient. The VTE was considered to be study drug-related for 4 of these 8 patients. One patient required a dose interruption and 2 patients required study drug to be discontinued.

***Electrocardiographic abnormalities***

Electrocardiographic changes have been reported in clinical trials with romidepsin and a number of the other HDAC inhibitors and they may be considered as a class effect.

An independent cardiology review of 4910 ECGs in 135 patients treated with romidepsin in Phase II studies determined a 5.0 ± 13.9 ms QT prolongation (including the effect of concomitant antiemetics accounting for approximately 50% of the observed prolongation) following infusion with a return to baseline within 48 hours. Furthermore, review of the data suggests no QTc interval above 480 msec and no increase in QTcF greater than 60 msec.

A subsequent analysis of the exposure-response of QTc in a phase I study revealed no concentration-dependent effect of romidepsin on the duration of the heart rate corrected QTc interval at exposures up to 2.5 × Cmax of therapeutic doses (Report GPI-06-0005-QT). The analyses of QTcF, the individually-corrected QTc, and the change from baseline QTcF each demonstrated the lack of a direct pharmacodynamic effect of romidepsin.

Of note, patients with significant cardiac history, a baseline QTc interval >450 msec, a history of congenital long QT syndrome, ventricular tachycardia, torsade de points (TdP), ventricular fibrillation, bradycardia <50 bpm, congestive heart failure ≥ Grade 3 NYHA, or myocardial infarction within 6 months before entry have been excluded from clinical studies.

In Study GPI-06-0002, single 12-lead ECGs were to be performed at screening and baseline and at all study drug administration visits. If the QTc was ≥500 msec, ECGs were to be repeated twice for a mean of 3 QTc values; the romidepsin dose was not to be administered if the repeat QTc remained ≥500 msec.

Review of descriptive statistics for QTcF and QTcB on Day 1 of Cycles 1 through 4, as determined by the central ECG laboratory, revealed no clinically meaningful changes in central tendency across treatment cycles for these parameters. Eight patients (6%) had an ECG abnormality reported as a TEAE. The most common abnormality was ECG QT prolonged, reported in 4 (3%) patients. Other ECG abnormalities included T wave changes in 2 patients (inversion and decreased amplitude) and repolarisation abnormality in 1 patient.

A special review of cardiac rhythm disturbances and ECGs in Study GPI-06-0002 showed that 17% of patients had a cardiac rhythm disturbance or ECG abnormality reported as an adverse event, with the most common such events including tachycardia (10%) and ECG QT prolonged (3%). All other cardiac rhythm and ECG abnormalities were reported as adverse events in 1 patient each (<1%). The majority of these events were assessed as unrelated to study drug.

***Infections***

In Study GPI-06-0002, the overall incidence of infections was 54%. As was seen in the population of patients with PTCL as a whole, no particular type occurred in Study GPI-06-0002 at a frequency ≥10%. The most commonly reported types of infections included upper respiratory tract infections (8%), urinary tract infections (7%), and pneumonia (6%). For approximately two-thirds (46 of 71 patients; 65%) of patients who experienced infections, all such events were mild or moderate (Grade 1 or 2) in intensity. Overall, 19 (15%) patients experienced a Grade 3 infection and 3 (2%) patients experienced a Grade 4 infection. Five patients had an infection with a fatal outcome, with the event presenting 11 to 29 days after the last dose of study drug for 4 of these 5 patients and occurring in the setting of known PD for 2 of those 4 patients. Overall, 25 (19%) patients had an infection reported as an SAE. Infections were more common in patients who had received prior monoclonal antibody therapy than in patients who had not (80% versus 50%, respectively) and in patients with previous stem cell transplantation. There was no correlation between leucopenia, neutropenia or their intensity and increased risk of infection. Study drug discontinuation due to an event of infection was infrequent (3%).

The incidence of infections in NCI Study 1312 was similar to that seen in Study GPI-06-0002 (51% versus 54%, respectively). In NCI Study 1312, an infection was Grade 3 and Grade 4 in intensity for 21% and 6% of patients, respectively, and serious for 23% of patients. 15% of patients had a dose held or reduced because of infection, and 2% of patients discontinued study drug because of such events.

## Serious adverse event / deaths / other significant events

### SAEs

An overview of all SAEs occurring in at least 1% of patients in studies GPI-06-0002 and NCI 1312 can be found in the following Table 29.

**Table 29: Serious Adverse Events reported by at least 1% of patients**

| | Overall SAEs | | | Treatment-related SAEs[1] | | |
|---|---|---|---|---|---|---|
| **MedDRA SOC / Preferred Term** | **GPI-06-0002 (N=131) n (%)** | **NCI 1312 (N=47) n (%)** | **Total (N=178) n (%)** | **GPI-06-0002 (N=131) n (%)** | **NCI 1312 (N=47) n (%)** | **Total (N=178) n (%)** |
| At Least One SAE[2] | 60 (45.8) | 30 (63.8) | 90 (50.6) | 33 (25.2) | 26 (55.3) | 59 (33.1) |
| **Blood and lymphatic system disorders** | **15 (11.5)** | **10 (21.3)** | **25 (14.0)** | **9 (6.9)** | **7 (14.9)** | **16 (9.0)** |
| Anaemia | 2 (1.5) | 5 (10.6) | 7 (3.9) | 2 (1.5) | 3 (6.4) | 5 (2.8) |
| Anaemia haemolytic autoimmune | 2 (1.5) | 0 | 2 (1.1) | 0 | 0 | 0 |
| Febrile neutropenia | 4 (3.1) | 2 (4.3) | 6 (3.4) | 3 (2.3) | 2 (4.3) | 5 (2.8) |
| Leukopenia | 2 (1.5) | 2 (4.3) | 4 (2.2) | 2 (1.5) | 2 (4.3) | 4 (2.2) |
| Lymphopenia | 0 | 3 (6.4) | 3 (1.7) | 0 | 3 (6.4) | 3 (1.7) |
| Neutropenia | 3 (2.3) | 3 (6.4) | 6 (3.4) | 2 (1.5) | 2 (4.3) | 4 (2.2) |
| Thrombocytopenia | 2 (1.5) | 5 (10.6) | 7 (3.9) | 1 (0.8) | 4 (8.5) | 5 (2.8) |
| **Cardiac disorders** | **2 (1.5)** | **4 (8.5)** | **6 (3.4)** | **0** | **4 (8.5)** | **4 (2.2)** |
| Ventricular arrhythmia | 0 | 2 (4.3) | 2 (1.1) | 0 | 2 (4.3) | 2 (1.1) |
| **Gastrointestinal disorders** | **15 (11.5)** | **3 (6.4)** | **18 (10.1)** | **6 (4.6)** | **3 (6.4)** | **9 (5.1)** |
| Abdominal pain | 4 (3.1) | 1 (2.1) | 5 (2.8) | 0 | 1 (2.1) | 1 (0.6) |
| Colitis | 2 (1.5) | 0 | 2 (1.1) | 1 (0.8) | 0 | 1 (0.6) |
| Diarrhoea | 1 (0.8) | 1 (2.1) | 2 (1.1) | 0 | 1 (2.1) | 1 (0.6) |
| Vomiting | 6 (4.6) | 2 (4.3) | 8 (4.5) | 4 (3.1) | 2 (4.3) | 6 (3.4) |
| **General disorders and administration site conditions** | **17 (13.0)** | **12 (25.5)** | **29 (16.3)** | **6 (4.6)** | **6 (12.8)** | **12 (6.7)** |
| Chest pain | 3 (2.3) | 0 | 3 (1.7) | 1 (0.8) | 0 | 1 (0.6) |
| Disease progression | 0 | 5 (10.6) | 5 (2.8) | 0 | 0 | 0 |
| Pyrexia | 9 (6.9) | 8 (17.0) | 17 (9.6) | 4 (3.1) | 5 (10.6) | 9 (5.1) |
| **Hepatobiliary disorders** | **0** | **3 (6.4)** | **3 (1.7)** | **0** | **2 (4.3)** | **2 (1.1)** |
| Hyperbilirubinaemia | 0 | 3 (6.4) | 3 (1.7) | 0 | 2 (4.3) | 2 (1.1) |
| **Immune system disorders** | **2 (1.5)** | **2 (4.3)** | **4 (2.2)** | **1 (0.8)** | **2 (4.3)** | **3 (1.7)** |
| Hypersensitivity | 1 (0.8) | 2 (4.3) | 3 (1.7) | 1 (0.8) | 2 (4.3) | 3 (1.7) |
| **Infections and infestations** | **25 (19.1)** | **11 (23.4)** | **36 (20.2)** | **6 (4.6)** | **8 (17.0)** | **14 (7.9)** |
| Catheter related infection | 1 (0.8) | 2 (4.3) | 3 (1.7) | 0 | 0 | 0 |
| Cellulitis | 5 (3.8) | 1 (2.1) | 6 (3.4) | 3 (2.3) | 1 (2.1) | 4 (2.2) |
| Infection | 1 (0.8) | 4 (8.5) | 5 (2.8) | 0 | 3 (6.4) | 3 (1.7) |
| Pneumonia | 7 (5.3) | 0 | 7 (3.9) | 1 (0.8) | 0 | 1 (0.6) |
| Sepsis | 6 (4.6) | 1 (2.1) | 7 (3.9) | 2 (1.5) | 1 (2.1) | 3 (1.7) |
| Staphylococcal infection | 2 (1.5) | 0 | 2 (1.1) | 0 | 0 | 0 |

| MedDRA SOC/Preferred Term | Overall SAEs | | | Treatment-related SAEs[1] | | |
|---|---|---|---|---|---|---|
| | GPI-06-0002 (N=131) n (%) | NCI 1312 (N=47) n (%) | Total (N=178) n (%) | GPI-06-0002 (N=131) n (%) | NCI 1312 (N=47) n (%) | Total (N=178) n (%) |
| Urinary tract infection | 2 (1.5) | 1 (2.1) | 3 (1.7) | 1 (0.8) | 1 (2.1) | 2 (1.1) |
| **Investigations** | **7 (5.3)** | **6 (12.8)** | **13 (7.3)** | **5 (3.8)** | **5 (10.6)** | **10 (5.6)** |
| Alanine aminotransferase increased | 0 | 5 (10.6) | 5 (2.8) | 0 | 4 (8.5) | 4 (2.2) |
| Aspartate aminotransferase increased | 1 (0.8) | 6 (12.8) | 7 (3.9) | 1 (0.8) | 5 (10.6) | 6 (3.4) |
| **Metabolism and nutrition disorders** | **8 (6.1)** | **9 (19.1)** | **17 (9.6)** | **4 (3.1)** | **9 (19.1)** | **13 (7.3)** |
| Dehydration | 3 (2.3) | 4 (8.5) | 7 (3.9) | 0 | 4 (8.5) | 4 (2.2) |
| Hyperuricaemia | 0 | 2 (4.3) | 2 (1.1) | 0 | 2 (4.3) | 2 (1.1) |
| Hypoalbuminaemia | 0 | 2 (4.3) | 2 (1.1) | 0 | 2 (4.3) | 2 (1.1) |
| Hypocalcaemia | 0 | 3 (6.4) | 3 (1.7) | 0 | 2 (4.3) | 2 (1.1) |
| Hyponatraemia | 2 (1.5) | 0 | 2 (1.1) | 2 (1.5) | 0 | 2 (1.1) |
| Tumour lysis syndrome | 2 (1.5) | 0 | 2 (1.1) | 2 (1.5) | 0 | 2 (1.1) |
| **Nervous system disorders** | **5 (3.8)** | **4 (8.5)** | **9 (5.1)** | **1 (0.8)** | **1 (2.1)** | **2 (1.1)** |
| Dizziness | 1 (0.8) | 1 (2.1) | 2 (1.1) | 0 | 0 | 0 |
| Syncope | 1 (0.8) | 2 (4.3) | 3 (1.7) | 0 | 1 (2.1) | 1 (0.6) |
| **Psychiatric disorders** | **2 (1.5)** | **0** | **2 (1.1)** | **1 (0.8)** | **0** | **1 (0.6)** |
| Mental status changes | 2 (1.5) | 0 | 2 (1.1) | 1 (0.8) | 0 | 1 (0.6) |
| **Renal and urinary disorders** | **7 (5.3)** | **0** | **7 (3.9)** | **1 (0.8)** | **0** | **1 (0.6)** |
| Renal failure | 2 (1.5) | 0 | 2 (1.1) | 1 (0.8) | 0 | 1 (0.6) |
| Renal failure acute | 2 (1.5) | 0 | 2 (1.1) | 0 | 0 | 0 |
| **Respiratory, thoracic and mediastinal disorders** | **7 (5.3)** | **7 (14.9)** | **14 (7.9)** | **1 (0.8)** | **5 (10.6)** | **6 (3.4)** |
| Dyspnoea | 3 (2.3) | 4 (8.5) | 7 (3.9) | 0 | 2 (4.3) | 2 (1.1) |
| Hypoxia | 2 (1.5) | 3 (6.4) | 5 (2.8) | 0 | 2 (4.3) | 2 (1.1) |
| Pneumonitis | 0 | 2 (4.3) | 2 (1.1) | 0 | 2 (4.3) | 2 (1.1) |
| Pulmonary embolism | 3 (2.3) | 0 | 3 (1.7) | 1 (0.8) | 0 | 1 (0.6) |
| **Surgical and medical procedures** | **0** | **2 (4.3)** | **2 (1.1)** | **0** | **1 (2.1)** | **1 (0.6)** |
| Packed red blood cell transfusion | 0 | 2 (4.3) | 2 (1.1) | 0 | 1 (2.1) | 1 (0.6) |
| Platelet transfusion | 0 | 2 (4.3) | 2 (1.1) | 0 | 1 (2.1) | 1 (0.6) |
| **Vascular disorders** | **6 (4.6)** | **8 (17.0)** | **14 (7.9)** | **5 (3.8)** | **6 (12.8)** | **11 (6.2)** |
| Deep vein thrombosis | 4 (3.1) | 0 | 4 (2.2) | 3 (2.3) | 0 | 3 (1.7) |
| Hypotension | 1 (0.8) | 6 (12.8) | 7 (3.9) | 1 (0.8) | 5 (10.6) | 6 (3.4) |

[1] Treatment-related adverse events are those indicated by the Investigator as having a possible, probable, or definite/very certain/likely relationship to study drug. Events missing a relationship assessment are also included.
[2] Events with a missing assessment for seriousness are included.

### Deaths

Overall, treatment-emergent deaths (i.e., patients for whom the cause of death was considered study drug-related or who died within 30 days after the last study drug dose and/or with treatment-emergent adverse events resulting in death [i.e., Grade 5 events or events with an outcome of death]) were reported for 10 (8%) patients in Study GPI-06-0002.

**Table 30: Summary of deaths**

| Age at time of Death (yrs) | Sex | AE Leading to Death (MedDRA Preferred Term) | Day of Death | | Total No. Study Drug Doses |
|---|---|---|---|---|---|
| | | | Relative to First Study Drug Dose | Relative to Last Study Drug Dose | |
| **Study GPI-06-0002** | | | | | |
| 77 | Male | Multi-organ failure[1] | 29 | +22 | 2 |
| | | Muscular weakness | | | 2 |
| | | Sepsis | | | 2 |
| 50 | Male | Pneumonia | 38 | +23 | 3 |
| | | Renal failure acute | | | 3 |
| 65 | Male | Septic shock | 62 | +26 | 4 |
| 59 | Male | Candida sepsis | 37 | +30 | 2 |
| | | Pneumonia | | | 2 |
| 75 | Male | Cardiogenic shock | 9 | +2 | 2 |
| | | Gastrointestinal haemorrhage | | | 2 |
| | | Sepsis | | | 2 |
| | | Subendocardial ischaemia | | | 2 |
| 66 | Female | Death due to progression of cancer (not reported as adverse event) | 75 | +25 | 6 |
| 64 | Male | Death due to progression of cancer (not reported as adverse event) | 43 | +25 | 3 |
| 57 | Male | Neoplasm malignant | 57 | +50 | 2 |
| 58 | Female | Death due to progression of cancer /T-cell lymphoma (not reported as adverse event) | 51 | +22 | 3 |
| 48 | Female | Hydronephrosis[2] | 55 | +45 | 2 |
| **Study NCI 1312** | | | | | |
| 67 | Male | Infection[3] | 38 | +31 | 2 |
| 71 | Male | Death[4] | 124 | +3 | 14 |
| 78 | Male | Disease progression | 62 | +20 | 6 |
| 66 | Male | Disease progression | 11 | +11 | 1 |
| | | Pericardial effusion | | | 1 |
| 57 | Male | Disease progression | 37 | +15 | 2 |
| | | Oedema | | | 2 |
| 61 | Female | Disease progression | 27 | +27 | 1 |
| 84 | Male | Disease progression | 27 | +17 | 2 |
| 42 | Male | Aspartate aminotransferase increased | 68 | +13 | 41 |

[1] This was the only death considered possibly related to romidepsin. Relationship to study drug was determined by the Investigator

[2] Per the SAE report, hydronephrosis occurring in the setting of progressive disease, with progressive disease identified as the cause of death.

[3] Per the SAE report, the infection was considered by the Investigator to be a recurrence of Pneumocystic carinii pneumonia.

[4] A specific cause of death was not reported. Per the SAE report, the Investigator considered the patient's death to be probably related to vascular disease but possibly related to study drug, underlying disease, and immobilisation. No autopsy was performed.

## Laboratory findings

A summary of the proportion of patients who entered the study with normal or Grade 1 or 2 haematologic abnormalities shifting on study to Grade 3 or 4 abnormalities is provided in Table 31.

**Table 31: Proportion of patients with normal or Grade 1 or 2 haematology values at baseline who shifted to Grade 3 or 4 abnormalities as worst value on study**

| | GPI-06-0002 Shift to: | | NCI 1312 Shift to: | |
|---|---|---|---|---|
| **Haematology Parameter:** | **Grade 3 n/N (%)** | **Grade 4 n/N (%)** | **Grade 3 n/N (%)** | **Grade 4 n/N (%)** |
| ALC | 30/ 95 (31.6) | 8/ 95 (8.4) | 16/ 36 (44.4) | 11/ 36 (30.6) |
| ANC | 20/128 (15.6) | 6/128 (4.7) | 12/ 45 (26.7) | 11/ 45 (24.4) |
| WBC | 13/126 (10.3) | 2/126 (1.6) | 12/ 46 (26.1) | 12/ 46 (26.1) |
| Haemoglobin | 12/129 (9.3) | 1/129 (0.8) | 8/ 46 (17.4) | 2/ 46 (4.3) |
| Platelet count | 27/129 (20.9) | 10/129 (7.8) | 10/ 45 (22.2) | 11/ 45 (24.4) |

ALC: absolute leucocyte count, ANC: absolute neutrophil count, WBC: white blood cells

A summary of the proportion of patients who entered the study with normal clinical chemistry parameters or Grade 1 or 2 abnormalities and who shifted on study to Grade 3 or 4 abnormalities is provided in Table 32.

**Table 32: Proportion of patients with normal (Grade 0) or Grade 1 or 2 clinical chemistry values at baseline who shifted to Grade 3 or 4 abnormalities as worst value on study**

| | GPI-06-0002 Shift to: | | NCI 1312 Shift to: | |
|---|---|---|---|---|
| **Clinical Chemistry Parameter:** | **Grade 3 n/N(%)** | **Grade 4 n/N (%)** | **Grade 3 n/N (%)** | **Grade 4 n/N (%)** |
| Magnesium | | | | |
| Shift to high | 27/120 (22.5) | 0/120 (0.0) | 15/ 45 (33.3) | 1/ 45 (2.2) |
| Shift to low | 0/129 (0.0) | 0/129 (0.0) | 0/ 45 (0.0) | 0/ 45 (0.0) |
| Calcium | | | | |
| Shift to high | 0/129 (0.0) | 1/129 (0.8) | 0/ 44 (0.0) | 0/ 44 (0.0) |
| Shift to low | 2/129 (1.6) | 1/129 (0.8) | 11/ 44 (25.0) | 3/ 44 (6.8) |
| Albumin | | | | |
| Shift to low | 3/123 (2.4) | 0/123 (0.0) | 7/ 45 (15.6) | 0/ 45 (0.0) |
| Potassium | | | | |
| Shift to high | 3/129 (2.3) | 0/129 (0.0) | 0/ 0 | 0/ 0 |
| Shift to low | 3/129 (2.3) | 1/129 (0.8) | 0/ 0 | 0/ 0 |
| Sodium | | | | |
| Shift to high | 2/129 (1.6) | 0/129 (0.0) | 1/ 45 (2.2) | 0/ 45 (0.0) |
| Shift to low | 7/123 (5.7) | 1/123 (0.8) | 9/ 45 (20.0) | 0/ 45 (0.0) |
| ALK Shift to high | 0/128 (0.0) | 0/128 (0.0) | 2/ 44 (4.5) | 0/ 44 (0.0) |
| ALT Shift to high | 3/124 (2.4) | 0/124 (0.0) | 6/ 45 (13.3) | 0/ 45 (0.0) |
| AST Shift to high | 3/127 (2.4) | 0/127 (0.0) | 4/ 45 (8.9) | 2/ 45 (4.4) |
| Total Bilirubin Shift to high | 0/128 (0.0) | 0/128 (0.0) | 6/ 43 (14.0) | 2/ 43 (4.7) |

## Safety in special populations

No studies in special populations were submitted.

## Safety related to drug-drug interactions and other interactions

No drug-drug interaction studies were submitted. In *in vitro* studies, romidepsin was shown to be a substrate for metabolism by the cytochrome P450 (CYP) isoform 3A4. Romidepsin does not have any known CYP3A4 inhibitory or CYP3A4 induction activity and is therefore not expected to affect the plasma concentrations of other drugs metabolised by CYP3A4. The subgroup analysis comparing adverse event rates in patients with PTCL in Study GPI-06-0002 who did and did not receive concomitant treatment with CYP3A4 inhibitors suggested (although not conclusively) that certain of the more common adverse events are more frequently reported by recipients of concomitant moderate to strong CYP3A4 inhibitors.

Prolongation of prothrombin time (PT) and International Normalized Ratio (INR) was observed in a patient receiving romidepsin concomitantly with coumadin-derivative anticoagulants. Romidepsin and coumadin or coumadin derivatives are highly protein-bound and may alter the pharmacokinetics (PK) or bioavailability of each other if administered in combination.

A 5 msec increase (90% CI upper bound 7.7 msec) in QTcF after romidepsin infusion has been previously reported in patients with advanced cancer and interactions could be expected in patients taking anti-arrhythmic medicines or medicinal products that lead to significant QT prolongation.

## Discontinuation due to adverse events

**Table 33: Adverse events leading to study drug discontinuation in at least 1% of patients**

| | Overall AEs | | | Treatment-related AEs | | |
|---|---|---|---|---|---|---|
| **MedDRA SOC/Preferred Term** | **GPI-06-0002 (N=131) n (%)** | **NCI 1312 (N=47) n (%)** | **Total (N=178) n (%)** | **GPI-06-0002 (N=131) n (%)** | **NCI 1312 (N=47) n (%)** | **Total (N=178) n (%)** |
| At Least One TEAE Leading to Discontinuation | 22 (16.8) | 13 (27.7) | 35 (19.7) | 11 (8.4) | 6 (12.8) | 17 (9.6) |
| **Blood and lymphatic system disorders** | **5 (3.8)** | **3 (6.4)** | **8 (4.5)** | **5 (3.8)** | **3 (6.4)** | **8 (4.5)** |
| Thrombocytopenia | 3 (2.3) | 3 (6.4) | 6 (3.4) | 3 (2.3) | 3 (6.4) | 6 (3.4) |
| **General disorders and administration site conditions** | **2 (1.5)** | **5 (10.6)** | **7 (3.9)** | **0** | **1 (2.1)** | **1 (0.6)** |
| Disease progression | 0 | 4 (8.5) | 4 (2.2) | 0 | 0 | 0 |
| **Infections and infestations** | **4 (3.1)** | **1 (2.1)** | **5 (2.8)** | **1 (0.8)** | **1 (2.1)** | **2 (1.1)** |
| Pneumonia | 2 (1.5) | 0 | 2 (1.1) | 0 | 0 | 0 |
| Sepsis | 2 (1.5) | 0 | 2 (1.1) | 1 (0.8) | 0 | 1 (0.6) |
| **Investigations** | **4 (3.1)** | **3 (6.4)** | **7 (3.9)** | **2 (1.5)** | **1 (2.1)** | **3 (1.7)** |
| Alanine aminotransferase increased | 0 | 2 (4.3) | 2 (1.1) | 0 | 0 | 0 |
| Electrocardiogram QT prolonged | 1 (0.8) | 1 (2.1) | 2 (1.1) | 1 (0.8) | 1 (2.1) | 2 (1.1) |
| **Respiratory, thoracic and mediastinal disorders** | **3 (2.3)** | **1 (2.1)** | **4 (2.2)** | **0** | **0** | **0** |
| Dyspnoea | 2 (1.5) | 1 (2.1) | 3 (1.7) | 0 | 0 | 0 |

## Post marketing experience

Romidepsin was approved in the US on 05 November 2009 by the FDA for the treatment of patients with CTCL who have received at least one prior systemic therapy. Romidepsin is not currently marketed outside the US. Post-marketing data for romidepsin are based on 4 Periodic Adverse Experience Reports submitted to FDA through 30 October 2010; through this date, 5867 vials of romidepsin have been sold, corresponding to 652-978 treatment cycles at 6-8 vials per cycle. No new unexpected safety signals have been identified in the post-marketing setting.

## 2.6.1. Discussion on clinical safety

This safety assessment is based on the safety results from the pivotal study, GPI-06-0002 (n=131) with supporting data provided from the NCI study 1312 (n=47). In both studies the patients enrolled were diagnosed with PTCL and had received at least one prior treatment. The dose schedule was identical in the two studies. However, the two studies differed with regard to AE collection and reporting. In the NCI study 1312 routine safety evaluations were performed more frequently (particularly cardiovascular safety evaluation). Furthermore, clinical test abnormalities in the NCI 1312 were routinely reported as AEs with corresponding CTC grade without consideration of clinical significance. This contributed to a distortion of the frequency of some reported AEs in the two studies.

The size of the safety database is considered sufficient taking into account that PTCL is a rare disease.

The pivotal study is a phase II, single-arm, open-label, multicentre trial and the lack of an active control arm is a concern, since it hampers a meaningful interpretation of the safety data as compared to other treatment options for PTCL. Although PTCL is a heterogeneous group of patients for whom no specific treatment is approved in the EU and no single therapy stands out as the treatment of choice a comparison against 'physician's choice' could have been an option.

All together in the two studies 48 patients (27%) completed treatment through cycle 6. The main reason for discontinuation was progressive disease (>70% in both studies). The overall exposure to romidepsin is considered sufficient taking into account the disease course of PTCL.

The principal adverse events observed with romidepsin in patients with PTCL were GI disturbances (83%), haematologic toxicities (66%), asthenic conditions (60%), infections (53%) and clinical chemistry abnormalities (43%). The most common TEAEs >/= grade 3 in the pivotal study were thrombocytopenia, neutropenia and infections. The most common TEAEs >/= grade 3 in the NCI 1312 study were neutropenia, leukopenia, lymphopenia, thrombocytopenia, infections and anaemia.

Overall, a cumulative toxicity throughout the treatment cycles appears not to be evident; however, exceptions to this finding include increased AST and ALT as well as lymphopenia. These findings, however, must be interpreted with caution, as the proportion of the patients decreased by 72% from cycle 1 to cycle 6.

Furthermore, the nature of TEAEs is in accordance with the known class effect and is what could be expected in this heavily pre-treated group of PTCL patients.

Treatment-emergent death was reported for 10 patients (8%) in the pivotal study and for 8 patients (17%). None of these deaths were of concern. The reason for one death, however, was unclear.

In the pivotal study 33 patients (25.2%) experienced at least one treatment related SAE. The most commonly reported treatment related SAEs included vomiting, pyrexia, febrile neutropenia, deep vein thrombosis, and cellulitis. Individual SAEs occurred at a relatively low incidence. In NCI Study 1312, the incidence of treatment related SAEs was 55%. The most commonly reported treatment related

SAEs were pyrexia, hypotension, and AST increased, thrombocytopenia, ALT increased, dehydration, anaemia, lymphopenia and infection.

In the pivotal study, 8 patients experienced a venous thromboembolism event (VTE). No cases of VTE were seen in the NCI 1312 study. It is recognised that the pathogenesis of VTE in cancer is complex. It is furthermore recognised that VTE has been reported in patients with NHL with reported incidences up to 20%. However, the VTE incidence in the PTCL population specifically has not been described. Other HDAC inhibitors have been associated with VTE.

There have been reports of cardiovascular effects with HDAC inhibitors, including, in particular, an effect on QTc prolongation. In study GPI-06-005-QT, a post marketing study, no concentration-dependent effect of romidepsin on the duration of the QTc interval was identified.

In the pivotal study at least one ECG event was seen in 8 patients (6.1%) (4.6% treatment related) with 2.3% serious cases. Three cases (2.3%) led to discontinuation (one case with QT prolongation, one with decreased T-wave amplitude and one with borderline QTc and T-wave abnormality). Four cases (3.1%) of QT prolongation were experienced in the pivotal trial including one case not reported by the investigator. Furthermore, one patient had an 'abnormal ECG' with T-wave abnormality and borderline QTc prolongation.

The incidence of at least one ECG event was much higher in the NCI 1312 study as it was experienced in 33 patients (70.2%). This mainly concerned decreased T-wave amplitude but also prolonged QT and non-specific ST segment changes. All events were considered treatment related, but with no serious cases and only one case led to discontinuation (a case of QT prolongation).

No Torsade de pointes, ventricular fibrillation and flutter or seizures were observed in the pivotal or NCI 1312 study. No sudden deaths were observed. However, one patient had no specific death reported. Two cases of VT and 3 SAEs of syncope (from the narratives causes for syncope other than QTc prolongation can be justified) were observed all together in the two studies. In addition, one patient with chest pain had non-specific repolarisation disturbances.

The pivotal trial did not raise a safety signal with regard to new or unexpected cardiac events. Overall, based on the assessment of the cardiovascular safety data collected, it has been concluded that romidepsin has an average effect on QTc of around 5 msec, with a 95% upper bound CI below 10 msec, which is below the threshold of regulatory concern outlined in the ICH E14 Guideline. However, there are still some uncertainties regarding QT prolongation.

The rate of infections was high. 54 % and 51% experienced at least one infection in the pivotal study and NCI 1312 study, respectively. No particular infection occurred at an incidence of > 10%. All together 20 % were reported as SEAs. The high rate of infections and the types of infections are not unexpected in this heavily pretreated population of patients with PTCL. The nature of the disease, prior therapy and in some cases prior bone marrow transplantation can cause the patient to be immune compromised. A case each of EBV and hepatitis B was observed. With the data presented it is concurred the cases unlikely represented virus reactivation. However, the safety population in the proposed indication is relatively small, which means that uncommon cases of DNA virus reactivation (especially hepatitis B) would not necessarily emerge. Because of the uncertainty on this subject, DNA virus reactivation would be a part of the RMP.

Haematologic abnormalities were common in both studies, in the pivotal study with an overall incidence of 57% and more frequently reported in the NCI 1312 study, with an overall incidence of 94%. Thrombocytopenia was the most common haematologic disorder reported and was mainly considered treatment related. Despite the high number of patients experiencing thrombocytopenia, only 9 patients in the pivotal study were identified with bleeding events and 3 of these had platelet

counts above the lower limit of normal. In the NCI 131 study, 7 patients were identified with bleeding events but only in one patient this was considered drug related. Haematologic abnormalities are not unexpected in this heavily pre–treated population of patients with PTCL. In addition, haematologic abnormalities were common at baseline in both studies. The higher incidence in the NCI 1312 study is probably due to more safety evaluations performed and due to more advanced disease stages compared to the pivotal study. Finally, study drug discontinuations due to haematological abnormalities were infrequent and no obvious signs of cumulative haematologic toxicity appeared. The haematological abnormalities were overall manageable considering this population of PTCL.

The differences in clinical chemistry abnormalities reported between the pivotal study and NCI 1312 study are striking. Most were grade 1 or 2 in intensity, but some grade 3 and less frequently grade 4 was reported.

'ALT increased' and 'AST increased' were each reported in 4% of patients in the pivotal study, and in 36% and 38% of patients, respectively, in the NCI 1312 study. However, there was one SAE of increased AST in the pivotal study and 5 SAEs of increased ALT and 6 of increased AST in the NCI 1312 study. In five patients there were ALT and AST values > 3 x the upper limit of normal range concurrent with bilirubin values >2 x upper limit of normal range. All patients had alternative explanations of their liver function tests abnormality and according to Hy's law there was no evidence of drug induced liver injury.

AEs leading to study drug discontinuation were experienced by 17% of the patients in the pivotal study and by 28% in NCI study 1312. The most common events leading to study drug discontinuation for > 1 patient in the pivotal study were thrombocytopenia, dyspnoea, pneumonia and sepsis. In the NCI 1312 study the most common AEs leading to discontinuation in > 1 patient other than disease progression were thrombocytopenia and increased ALT.

A study in patients with hepatic impairment is ongoing. Patients with renal impairment were routinely excluded from the clinical studies; however, the Applicant has assessed the pharmacokinetics of romidepsin in patients with mild, moderate and severe renal impairment using a population pharmacokinetic model (Report AN10022). Results showed that the clearance of romidespin was not affected by mild, moderate, or severe renal impairment. Moreover, the population PK analysis showed that age had no effect on romidepsin PK. The effect of end stage renal disease on the PK of romidepsin has not been studied.

## Additional expert consultations

As noted previously, the CHMP convened a Scientific Advisory Group-Oncology (SAG-O) meeting to consult clinical experts on the Marketing Authorisation Application for Istodax. One question on the safety of romidepsin based on the data submitted was posed to the experts. The question and the SAG-O response was as follows:

4. The SAG is asked to discuss the severity of the side effects, in particular regarding gastrointestinal disturbances and myelotoxicity and whether these can be managed in clinical practice.

The severity of observed side effects did not raise major concerns in terms of gastrointestinal disturbances and myelotoxicity in this heavily pre-treated population. Although the benefits are currently not established, additional safety data would be useful for a proper assessment of the safety profile when assessing benefit-risk balance, in particular to further clarify causality for treatment-associated deaths, management of cardiotoxicity (particularly in combination) and optimal use of antiemetics.

### 2.6.2. Conclusions on the clinical safety

Romidepsin displays a safety profile that is considered as globally manageable and expected for this heavily pre-treated population of PTCL patients. In addition the safety data are consistent with the known adverse event profile of romidepsin; however, other indications already accepted outside Europe might not be applicable to the claimed indication. Overall, the lack of an active control arm is a major concern since it seriously hampers a meaningful interpretation of efficacy and safety data.

## *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.

### Risk Management Plan

The applicant submitted a risk management plan.

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to consider risk minimisation activities at this time.

## *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.* However, the Applicant should use a landscape layout in line with Europeans readability recommendations.

# 3. Benefit-Risk Balance

## *Benefits*

### Beneficial effects

The present submission is mainly based on a phase II, multicentre, open-Label study designed to assess the activity and tolerability of romidepsin in progressive or relapsed peripheral T-cell lymphoma following prior systemic therapy. Patients were to receive 14 mg/m2 of romidepsin for injection IV over 4 hours on Days 1, 8, and 15 of each 28-day treatment cycle. The treatment was initially planned for 6 months (6 cycles). The primary endpoint was the rate of complete response. Secondary endpoints notably included objective disease response rate, duration of response, time to disease progression, tolerability and, finally, change in ECOG performance status. A total of 131 patients received at least one dose of romidepsin.

Romidepsin shows antitumour activity in terms of complete response in relapsed/refractory PTCL. The complete response rate (CR+Cru) in Study GPI-06-0002 (IRC assessed) was 13.1% with lower limit of the 95% unilateral confidence interval of 8.5%, and the ORR was 26.2%. The CR rate in NCI Study 1312 (investigator assessed) was 17.8% and the ORR 37.8%.

In the pivotal study, the median duration of response was not reached for the 17 patients who achieved a CR. The median time to response was 56 days and the median time to CR was 150 days. Median duration of response for all patients with an objective response (n=34) was 12 months. Both

CR and ORR rate were slightly higher when assessed by the investigators (16.2%, 29.2%) and the duration of response was also assessed as slightly longer by the investigators (14 months). Median TTP was 177 days and the median PFS was 107 days.

The activity of romidepsin was apparently not dependent on histological subtype, risk factors, the number of previous therapies or the response to previous therapies. The activity may be seen as promising since many patients without response to the last previous line of therapy had similar response to romidepsin as patients relapsing from a response to previous therapy.

The applicant presented updated efficacy data from the pivotal Phase 2 Study GPI 06-0002 that include 7 additional months of follow-up allows for more mature data to assess all secondary endpoints, including duration of response and progression-free survival (PFS). These data showed that the efficacy conclusions remained consistent with the original data in the primary analysis population based on Independent Review Committee (IRC). The median duration of response based on overall IRC assessment improved from 12 months to 17 months. Median duration of response was also 17 months for patients with CR. Survival data have now been retrospectively collected and analysed using Kaplan-Meier methodology showing a median overall survival (OS) from the time of enrolment of 11.3 months.

## Uncertainty in the knowledge about the beneficial effects

An antitumour activity in terms of complete response in relapsed/refractory PTCL has been shown; however, the design of the pivotal study precludes further interpretation of magnitude of response and its possible translation into clinical benefit in terms of PFS and OS. Especially the absence of control arm makes it very difficult to substantiate a true clinical benefit beyond the observed response rates. The applicant's effort to provide external (historical) control data specifically for this Marketing Authorisation Application (MAA) and the comprehensive literature review with the aim of characterising the outcomes achieved with currently used or investigational therapies in patients with relapsed or refractory PTCL is appreciated. However, the comparison of activity against external control data gives a very uncertain estimate of relative activity and the CHMP is still of the opinion that a confirmatory comparative study is feasible and necessary for assessment of the beneficial effects of romidepsin. In their response, the applicant suggested to compare gemcitabine + romidepsin with gemcitabine alone and to provide data from such a study as part of a conditional marketing authorisation.

### *Risks*

## Unfavourable effects

The primary analysis of safety for the proposed PTCL indication was based on data from study GPI-06-0002, in which a total of 131 patients with PTCL were enrolled. Supporting data were provided from NCI Study 1312, in which a total of 47 patients with PTCL were enrolled. Safety data from post-marketing experience in the US where romidepsin was granted an approval for treatment of patients with CTCL were also submitted. Overall, the safety data are taken from a total of 891 patients who received at least 1 dose of romidepsin as monotherapy through 30 September 2010 in clinical studies sponsored by the Applicant or the US NCI, including 447 patients with haematological malignancies (including lymphomas) and 444 patients with solid tumours.

Almost all patients (97.2%) with PTCL experienced at least one treatment-emergent adverse event (TEAE). It was reported as 100% in the NCI study. TEAE leading to study drug discontinuation was reported in 20% of patients and TEAE resulting in death was reported in 7 patients (5.3%) in the pivotal study and in 8 patients (17.0%) in the NCI supportive study. The major reasons for

discontinuations roughly correspond to the most common severe toxicities seen in the safety population

In Study GPI-06-0002, the most common adverse events reported by ≥20% of patients included gastrointestinal disturbances (82%), including nausea and vomiting (64%), diarrhoea (35%), and constipation (28%); haematological abnormalities (57%), including thrombocytopenia (38%), neutropenia (30%), and anaemia (24%); and asthenic conditions (54%), generally Grade 1 or 2 in intensity and non-serious. Nearly all of these events were assessed as study drug-related by the investigators.

QT prolongation was reported in 7 patients in the NCI 1312 study. One led to discontinuation. In the pivotal study, 4 cases of QT prolongation were reported. Furthermore, one of the four cases was not reported by the investigator and finally, a case of 'abnormal ECG' included borderline QT prolongation and T-wave abnormality.

Infections are usually commonly reported in patients with advanced PTCL, because the disease and prior therapy can cause the patient to be immune-compromised, with poor T-cell mediated immunity and neutropenia: Overall incidence of infections was 54%. The most commonly reported types of infections in Study GPI-06-0002 were upper respiratory tract infection (8%), urinary tract infection (7%), and pneumonia (6%). For most patients who experienced infections, the event was mild or moderate (Grade 1 or 2) in intensity and non-serious. Other types of adverse events commonly reported in Study GPI-06-0002 included pyrexia, anorexia and taste disturbances.

Serious adverse events: The maximum adverse event severity was Grade 3 for 46% (60 patients) of patients, Grade 4 for 18% (23 patients), and Grade 5 for 2% (3 patients) of patients. The most common treatment-related serious adverse events reported in PTCL patients were: Blood and lymphatic system disorders (9.0%), infections and infestations (7.9%), metabolism and nutrition disorders (7.3%), general disorders and administration site conditions (6.7%), vascular disorders (6.2%), gastrointestinal disorders (5.1%), respiratory, thoracic and mediastinal disorders (3.4%) and cardiac disorders (2.2%).

Other adverse events of interest (pivotal study) included cardiac disorders (15%), hypotension (8%), hypersensitivity (2%), tumour lysis syndrome (2%) and venous thromboembolic event (6%).

Reactivation of DNA viruses in relation to romidepsin treatment has previously been reported. Beside cases of HSV and HZV representing potential virus activation, a case each of EBV and hepatitis B was observed. However, with reference to the narratives, it is recognised that it appears unlikely that the cases of EBV and hepatitis B represent reactivation.

Deaths: Treatment-emergent deaths were reported for 10 (8%) patients in Study GPI-06-0002. The median time to death after starting study drug was 47 days. The primary cause of death was reported as disease progression for 4 patients. For the remaining 6 patients, an infection or an event that occurred in the setting of infection was reported as a cause of death. In NCI Study 1312, treatment-emergent deaths were reported for 8 (17%) of 47 patients, with the death occurring within 30 days after the first study drug dose in 3 of these 8 patients. The median time to death after the first study drug dose among all 8 patients was 38 days.

The safety findings in PTCL patients are generally consistent with those from CTCL patients. Overall, the safety profile of romidepsin among patients with haematological malignancies and with solid tumours was similar to that seen specifically in patients with T-cell lymphomas.

## Uncertainty in the knowledge about the unfavourable effects

In the main phase II study, 15% of patients had at least 1 cardiac disorder reported as an adverse event. A special review of cardiac rhythm disturbances and ECGs showed that 17% of patients had a cardiac rhythm disturbance or ECG abnormality reported as an adverse event, with the most common such events including tachycardia (10%) and ECG QT prolonged (3%). All other cardiac rhythm and ECG abnormalities were reported as adverse events in 1 patient each (<1%). Only two of these events were ≥Grade 3 and the majority were assessed as unrelated to study drug. Even though no clinical relevance has been demonstrated, clinical correlates of these ECG changes would have to be monitored by the Applicant under the Risk Management Plan.

In clinical trials, a prolongation of QTc > 500 msec during therapy has been the threshold of particularly concern. While lower limits increase the false positive rate, higher limit fail to detect a signal of concern. Thus, using different limits are a reasonable approach to this uncertainty. This includes QTc interval >450 msec and QTc interval > 480 msec. In addition, changes from baseline in QTc interval are valuable when assessing QT prolongation. From the presented data on QT-prolongation the CHMP finds the following is unclear: Categorisation of all QTc >450 msec, > 480 msec and > 500 msec; delta QTc concerning all QTc prolongations > 450 msec; missing central reviews of ECG. Additional information on delta QTc on ECGs from C1, where both pre-dose and post-dose ECGs were performed would be valuable.

In addition, safety data for patients with significant cardiac history in the proposed indication are not available since patients with known cardiac abnormalities were excluded from the study.

A higher rate of SAEs in NCI Study 1312 (55% compared to 25% in the pivotal study, treatment related) was reported. More patients in the NCI 1312 study had a disease stage =/>III, had received a higher number of prior systemic therapies, had received prior autologous bone marrow transplantation and had received prior radiation therapy.

The frequency of adverse events according to sex, race, age, baseline liver function, and baseline calculated creatinine clearance was also analysed by the Applicant. Data did not reveal any clear findings that suggested any change in therapeutic approach on the basis of these demographic and baseline factors. Patients with renal impairment were routinely excluded from the clinical studies.

Since the drug is intended for use in elderly patients with generally impaired renal function, a study in this population should be performed. Moreover, a study in patients with impaired hepatic function is ongoing and it could satisfy concerns about missing information in this patient group.

With the data presented, reactivation of DNA viruses did not appear to be a major concern in the pivotal study and study NCI 1312. However, the safety population in the proposed indication is relatively small, which means that uncommon cases of DNA virus reactivation (especially hepatitis B) would not necessarily emerge. Because of the uncertainty on this subject, DNA virus reactivation would be a part of the RMP.

No formal drug to drug interaction study has been performed. A potential drug-drug interaction between CYP3A4 inhibitors and romidepsin has been suggested. MAA has committed to conduct interaction studies with ketoconazole and rifampicin.

Finally, immunological events were not discussed by the Applicant.

## *Benefit-risk balance*

### Importance of favourable and unfavourable effects

There is a clear medical need for new active agents for the treatment of PTCL both in first-line and later lines regimens. In that context the activity of romidepsin with an ORR > 25% and a high rate of CR/CRu is considered potentially clinically meaningful and promising. The duration of CR is in some patients rather long. Moreover, the activity of romidepsin was apparently not dependent on histological subtype, risk factors, the number of previous therapies or the response to previous therapies. The safety profile of romidepsin does not cause particular concern and is considered acceptable and expected for a heavily pre-treated population of PTCL patients. The most common toxicity consists of myelosuppression with neutropenia, anaemia, and thrombocytopenia. As a consequence, there is a risk of infections probably not very different from the risk seen with other salvage regimens. Asthenia and gastrointestinal disturbances with nausea and vomiting are other common side effects. There are some uncertainties as to whether the risks of VTE and QT-prolongation are increased by romidepsin.Even if there is no consensus on standard therapy for PTCL, the choice of an open-label study with no comparator arm is highly questionable. Unfortunately, such data do not allow drawing any conclusion on the efficacy of romidepsin. Physician's choice or a list of limited choice would have been the expected option for a comparative arm in a randomised phase III study or at least a comparative phase II study. The pivotal study GPI-06-0002 and the supportive NCI 1312 study should only be considered as exploratory studies. Confirmatory data are needed to be convincing and to allow a positive recommendation on the granting of marketing authorisation. The evidence provided by these data is below the minimum requirement which is generally one controlled study with statistically compelling and clinically relevant results (Points to consider on application with 1. meta-analyses; 2. one pivotal study [CPMP/EWP/2330/99]).

The inconvenience related to romidepsin administration, due to gastrointestinal disturbance and the expected haematologic toxicities and infections, is also of concern, even if these effects are predictable and consistent with those observed in preclinical studies and clinical studies in other indications. However, taking into account this class of product and most of all this rare form of NHL with many subtypes that share an aggressive clinical behaviour, the safety profile of romidepsin might be considered as globally manageable, even if the known safety profile of romidepsin in other indications already accepted outside Europe might not be applicable to the currently claimed indication.

### Benefit-risk balance

Due to the lack of comparative data the benefit-risk balance is negative.

## *Discussion on the benefit-risk balance*

CHOP is commonly used in first-line treatment for PTCL based on extrapolation from the treatment of aggressive B-cell lymphomas; however, results are not satisfactory with relapse rates between 40 to 70% and short durations of response reflecting refractory disease. There are no widely accepted and effective therapies for patients with relapse or refractory disease.

Antitumour activity of romidepsin has been clearly demonstrated. The main problem with provided data on CR and ORR rates is that the single-arm design does not allow estimation of clinical benefit in terms of PFS and OS as compared to standard of care for patients with relapsed/refractory PTCL.

The Applicant sought an approval based on the provided phase II trials and proposed a comparative phase III study to compare the efficacy of romidepsin administered in combination with CHOP (Ro-

CHOP) versus CHOP alone in patients with previously untreated PTCL. The primary efficacy parameter is PFS. However, it should be noted that the proposed confirmatory study is a study of the effect of romidepsin in a completely different setting. Although the Phase III study will add to the evidence for the drug's anti-tumour activity in PTCL, it will not directly support its use within the proposed indication. In order to address this concern the applicant later proposed a study in relapsed/refractory PTCL patients comparing gemcitabine + romidepsin with gemcitabine alone as part of a proposal for conditional marketing authorisation. However, due to the negative benefit-risk, the CHMP cannot recommend a conditional marketing authorisation in order to consider such a study for the purposes of provision of comprehensive data on the benefit-risk.

Romidepsin displays a safety profile that is considered as globally manageable and expected for this heavily pre-treated population of PTCL patients. In addition the safety data are consistent with the known adverse event profile of romidepsin; however, other indications already accepted outside Europe might not be applicable to the currently claimed indication.

# 4. Recommendations

### *Outcome*

Based on the CHMP review of data on quality, safety and efficacy for Istodax in the treatment of adult patients with peripheral T-cell lymphoma (PTCL) that has relapsed after or become refractory to at least two prior therapies, the CHMP considers by majority decision that the efficacy of the above mentioned medicinal product is not sufficiently demonstrated and the particulars or documents provided in accordance with Article 6 of Regulation (EC) No 726/2004 are incorrect

and, therefore recommends the refusal of the granting of the Marketing Authorisation for the above mentioned medicinal product. The CHMP considers that:

- in the absence of an adequate GMP certificate from the finished product manufacturer, particulars or documents provided in accordance with Article 6 of Regulation (EC) No 726/2004 cannot be considered correct
- in the absence of established benefits, a positive benefit-risk balance cannot be considered established

Furthermore, the CHMP, in light of the negative recommendation, is of the opinion that it is not appropriate to conclude on the new active substance status at this time.

# Re-examination of the CHMP opinion of 19 July 2012

Following the CHMP conclusion that Istodax was not approvable due to the lack of an established benefit as a result of the non-comparative nature of the pivotal efficacy data submitted and due to the lack of an acceptable GMP certificate from the finished product manufacturer, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

# Detailed grounds for re-examination submitted by the applicant

The applicant presented in writing responses to the CHMP grounds for refusal of the marketing authorisation.

**Ground #1** (absence of GMP certificate from the finished product manufacturer): The applicant provided an updated GMP certificate from the proposed finished product manufacturer.

**Ground #2** (absence of established benefit): The applicant considered that a positive benefit to risk ratio for romidepsin can be established for the following reasons:

- Romidepsin has demonstrated efficacy in relapsed refractory PTCL, a highly-aggressive and difficult to treat malignancy. Specifically, romidepsin shows unprecedented high rates of durable complete responses in a group of patients that require salvage therapy and have no satisfactory treatment options available, none of which are approved for all PTCL sub-types. Furthermore, patients who achieve clinical response derive benefit from the response in the form of prolonged progression-free survival (PFS) and OS compared with patients who do not achieve this level of response.
- Romidepsin has a well-defined and manageable safety profile in relapsed refractory PTCL. The safety profile is supported by the largest existing safety database in relapsed refractory PTCL consisting of 178 patients, with additional supportive safety data in more than 800 patients.

More specifically, the applicant presented Overall Survival data from a cohort of 204 PTCL patients following first relapse of their disease from an additional academic centre. These data were submitted in support of the poor prognosis of relapsed-refractory PTCL patients (data not shown).

Moreover, the applicant returned to the comparison between results observed in the pivotal GPI-06-0002 and supportive NCI 1312 study compared to an external control patient data set. These data were submitted in response to the CHMP List of Questions and List of Outstanding Issues and they had shown comparable efficacy with 29.2% (investigator-assessed) and 27.1% Overall Response Rates reported for romidepsin and in the external control dataset, respectively. The applicant focused on the analyses in patients receiving 3$^{rd}$ line of treatment or higher, submitted in response to the List of Outstanding Issues, in line with the latest proposed indication in patients who have failed at least two prior therapies. The external control data set included 205 patients overall and 96 patients receiving 3$^{rd}$ line of treatment or higher. However, data were not provided after 5$^{th}$ line as the data were not considered reliable; consequently, data were analysed for patients in 3$^{rd}$, 4$^{th}$ or 5$^{th}$ line (86 patients). In the pivotal study GPI-06-0002, 90 patients received romidepsin in 3$^{rd}$ line or higher and 79 of them in 3$^{rd}$-5$^{th}$ line.

Results regarding response in the external control patient dataset and in the romidepsin pivotal and supportive trials are presented in the following Tables 34-36.

**Table 34: Response rate and duration of response for patients receiving 3$^{rd}$ line or higher treatment for PTCL (external control dataset, N=86)**

| | External Control | |
|---|---|---|
| **Efficacy Endpoint** | **All Therapy (N=86)** | **Monotherapy (N=44)** |
| **Best Response Category, n (%) [95% CI][d]** | | |
| Objective Disease Response (CR+CRu+PR) | 14 (16.3) [9.2, 25.8] | 4 (9.1) [2.5, 21.7] |
| Complete Response (CR+CRu) | 5 (5.8) [1.9, 13.0] | 1 (2.3) [0.1, 12.0] |
| **Duration of Response (Months)** | | |
| Patients with Objective Disease Response | | |
| N | 14 | 4 |
| Median [95% CI] | N.A. | 3.9 [0.2, 4.7] |

**Table 35: Response rate and duration of response based on IRC assessment for patients receiving romidepsin as 3rd line or higher treatment for PTCL in GPI-06-0002 (N=90)**

| Efficacy Endpoint | Patients who Received ≥2 Prior Therapies (N=90) |
|---|---|
| **Best Response Category, n (%) [95% CI][a]** | |
| Objective Disease Response (CR+CRu+PR) | 23 (25.6) [16.9, 35.8] |
| Complete Response (CR+CRu) | 13 (14.4) [7.9, 23.4] |
| Partial Response (PR) | 10 (11.1) |
| Stable Disease (SD) | 20 (22.2) |
| Progressive Disease (PD)/Not Evaluable | 47 (52.2) |
| **Duration of Response (Days)** | |
| Patients with Objective Disease Response | |
| N | 23 |
| 25th Percentile [95% CI] | 130 [52, 847] |
| Median [95% CI] | 847 [94, NE] |
| 75th Percentile [95% CI] | NE [847, NE] |
| Minimum, Maximum | 1+, 1465+ |
| Censored Observations | 16 (69.6) |
| Events | 7 (30.4) |
| Patients with Complete Response | |
| N | 13 |
| 25th Percentile [95% CI] | 847 [500, NE] |
| Median [95% CI] | NE [500, NE] |
| 75th Percentile [95% CI] | NE [847, NE] |
| Minimum, Maximum | 57+, 1465+ |
| Censored Observations | 11 (84.6) |
| Events | 2 (15.4) |

**Table 36: Response rate and duration of response for patients receiving romidepsin as 3rd line or higher treatment for PTCL in NCI 1312 (N=31)**

| Efficacy Endpoint | Patients who Received ≥2 Prior Therapies (N=31) |
|---|---|
| **Best Response Category, n (%)** | |
| Objective Disease Response (CR+CRu+PR) | 10 (32.2) |
| Complete Response (CR+CRu) | 5 (16.2) |
| **Duration of Response (Days)** | |
| Patients with Objective Disease Response | |
| N | 10 |
| 25th Percentile [95% CI] | 168 [90, 274] |
| Median [95% CI] | 256 [90, 2255] |
| 75th Percentile [95% CI] | 1387 [238, 2255] |
| Minimum, Maximum | 90, 2255 |
| Censored Observations | 2 (20.0) |
| Events | 8 (80.0) |
| Patients with Complete Response | |
| N | 5 |
| 25th Percentile [95% CI] | 519 [182, 2255] |
| Median [95% CI] | 2255 [182, 2255] |
| 75th Percentile [95% CI] | 2255 [182, 2255] |
| Minimum, Maximum | 182, 2255 |
| Censored Observations | 2 (40.0) |
| Events | 3 (60.0) |

The applicant also reminded of previously submitted Kaplan-Meier curves comparing duration of response, progression free survival and overall survival in patients achieving Overall Response with that in patients achieving Complete Response in the pivotal GPI-06-0002 study (and duration of response K-M curves from the supportive study NCI 1312) which showed that duration of response was, as expected, higher in the latter patient subset (data not shown).

Moreover, the applicant focused on an also previously submitted analysis from the pivotal study focusing on patients refractory to previous therapies. Data are presented in the following Table 37.

**Table 37: Response rates based on IRC assessment for patients refractory to prior therapy and receiving romidepsin as 3rd-line or higher treatment for PTCL in GPI-06-0002**

| Patient Subgroup | Overall Response Rate n (%) | Complete Response Rate n (%) |
|---|---|---|
| Refractory (PD) to Last Therapy (N=37) | 11 (29.7) | 7 (18.9) |
| Received ≥2 Prior Combination Therapies and Refractory (PD) to at Least One (N=38) | 10 (26.3) | 7 (18.4) |
| Refractory (PD) to ≥2 Therapies (N=25) | 6 (24.0) | 4 (16.0) |

In support of the benefit-risk assessment, the applicant further submitted safety results from the subset of 90 patients in the pivotal study who received romidepsin as 3$^{rd}$ line therapy or higher. As was observed with the overall population, the most commonly reported types of events (i.e. events reported in 10% or more patients) were GI disturbances, asthenic conditions and haematological toxicities (Table 38). Although individual types of infections were each reported in <10% of patients, the overall incidence of infections in this patient population was 57%, with 21% of patients experiencing ≥Grade 3 infections.

Although these types of events were commonly reported, they were not common causes of treatment discontinuation. Thrombocytopenia led to treatment discontinuation in 3 patients (3%), febrile neutropenia in 2 patients (2%), pneumonia in 2 patients (2%), and dyspnoea in 2 patients (2%); all other events leading to treatment discontinuation were reported in only 1 patient. Of note, none of the 90 patients discontinued treatment due to nausea, vomiting, or diarrhoea.

Grade 3 or 4 events were reported in 63 (70%) of the 90 patients, with the most common Grade 3 or 4 events being haematological toxicities, including thrombocytopenia (31%), neutropenia (20%), anaemia (11%), and infections (21%) (Table 38).

**Table 38: Adverse events reported in 10% or more of patients who had received 2 or more prior systemic therapies, overall and ⩾Grade 3 events (safety population, N=90)**

| MedDRA Preferred Term | All Events n (%) | ≥Grade 3 Events n (%) |
|---|---|---|
| **At Least One TEAE** | **89 ( 98.9)** | **63 ( 70.0)** |
| Nausea | 56 ( 62.2) | 2 ( 2.2) |
| Thrombocytopenia | 43 ( 47.8) | 28 ( 31.1) |
| Fatigue | 38 ( 42.2) | 6 ( 6.7) |
| Vomiting | 36 ( 40.0) | 4 ( 4.4) |
| Pyrexia | 33 ( 36.7) | 4 ( 4.4) |
| Diarrhoea | 32 ( 35.6) | 2 ( 2.2) |
| Neutropenia | 28 ( 31.1) | 18 ( 20.0) |
| Constipation | 25 ( 27.8) | 1 ( 1.1) |
| Anorexia | 24 ( 26.7) | 1 ( 1.1) |
| Anaemia | 23 ( 25.6) | 10 ( 11.1) |
| Cough | 20 ( 22.2) | 0 |
| Dysgeusia | 17 ( 18.9) | 0 |
| Asthenia | 16 ( 17.8) | 3 ( 3.3) |
| Headache | 15 ( 16.7) | 0 |
| Dyspnoea | 14 ( 15.6) | 3 ( 3.3) |
| Abdominal pain | 12 ( 13.3) | 3 ( 3.3) |
| Chills | 11 ( 12.2) | 1 ( 1.1) |
| Tachycardia | 11 ( 12.2) | 0 |
| Muscle spasms | 10 ( 11.1) | 0 |
| Skin lesion | 10 ( 11.1) | 0 |
| Abdominal pain upper | 9 ( 10.0) | 1 ( 1.1) |
| Hypokalaemia | 9 ( 10.0) | 2 ( 2.2) |
| Hypotension | 9 ( 10.0) | 1 ( 1.1) |
| Leukopenia | 9 ( 10.0) | 4 ( 4.4) |
| Pain | 9 ( 10.0) | 0 |
| Pruritus | 9 ( 10.0) | 0 |
| Weight decreased | 9 ( 10.0) | 0 |

A total of 7 (8%) of the 90 patients who received romidepsin as 3rd-line therapy or higher died within 30 days of the last dose of study treatment; 3 of the deaths were due to disease progression. In the other 4 patients (4 of 90, 4%), the deaths occurred in the setting of disease progression with concurrent infection. In 3 of these 4 patients, other concurrent SAEs were reported at the time of death, including renal failure, and GI haemorrhage with cardiogenic shock. One case without concurrent SAEs occurred in a patient who discontinued romidepsin due to progression of disease and then received alemtuzumab-CHOP with subsequent development of Grade 4 leukopenia and death from septic shock. None of the other patients had neutropenia >Grade 2 at the time of the event.

In conclusion, the applicant considered that a positive benefit-risk balance exists for romidepsin in the proposed 3rd line or higher (patients having failed at least two prior therapies) indication in PTCL patients. The applicant also argued that the criteria for a conditional marketing authorisation are fulfilled, as

- the benefit-risk balance is positive,

- an unmet medical need exists in the target population,

- comprehensive data confirming the benefit-risk balance in the form of comparative clinical trial data can be provided within a reasonable timeframe. Towards this end, the applicant proposed the following measures to be considered as Specific Obligations of a conditional Marketing Authorisation:

1. a confirmatory, randomised controlled study in the relapsed/refractory PTCL setting in order to provide comprehensive clinical data
2. a post-approval registry study to include patients receiving romidepsin and those prescribed other therapies within the proposed indication for romidepsin in order to provide additional data from a clinical setting during the period between approval and availability of confirmatory data. The Applicant proposed to seek advice on the study design and provide a proposal to CHMP and PRAC for evaluation as required
3. to restrict the promotion of romidepsin in the authorised indication, in agreement with competent authorities, until confirmatory data are available,

- the benefit of immediate availability in patients requiring 3rd-line or higher therapy outweighs the risks inherent in the fact that additional data are required'

## Overall conclusion on grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant.

The updated GMP certificate from the finished product manufacturer was considered acceptable and it was considered to adequately address the CHMP first ground for refusal of the marketing authorisation application.

On the target condition/population and external control data, the CHMP considered that the historical/external control data further highlight the unmet medical need and the poor outcomes in patients following several lines of therapy. The data provided, specifically deal with the issue in patients receiving 3$^{rd}$ line or higher therapy, with overall response rates of 16.3% with all therapies, and 9.1% with monotherapy; and complete response rates of 5.8% with all therapies, and 2.3% with monotherapy.

Considering the pivotal study results with the cut-off date of 31 December 2011, PTCL patients treated with romidepsin in the 3rd line or higher therapy setting in the pivotal study had an overall response rate of 25.5% (23 of 90 patients) with complete responses seen in 13 patients (14.4%). The duration of response also appeared to be long in the patients with complete response, not having been reached by the cut-off date, compared to a median duration of response in all responding patients of 28 months (847 days). In the supportive study, ORR and CR rates were 32.3 % (n=10) and 16% (n=5), respectively, and the median duration of response was 8.4 months and 6.1 years, respectively.

For the patients with a CR in the pivotal study, the duration of response was associated with a median PFS of ±912 days (i.e. 2.5 years) with the median OS not yet reached. The 1 and 2 year OS was approximately 90% and 80%, respectively (data not shown). To what extent the observed results in overall survival are attributable to romidepsin is unclear as other factors could have played a role, in particular 1) whether patients underwent consolidation therapy upon reaching a CR, 2) the type of consolidation therapy, such as autologous or allogeneic SCT. In addition, due to the non-randomised design of the study, it is not possible to establish to what extent the observed results in terms of progression-free and overall survival are due to romidepsin or to the disease characteristics of the

patients selected. The historical/external control data provided do not allow resolution of this issue because an important selection bias cannot be ruled out in the absence of a dramatic effect.

A further issue regarding the duration of the response is the early censoring in the population used to determine the duration of response. According to the Applicant, eight patients, including 1 patient with CR and 7 with PR, were conservatively censored at the first response assessment (after Cycle 2, i.e. after ~ 2 months). A patient with CR went off study to receive autologous stem cell transplantation; among the 7 patients with PR who were censored early in the Overall IRC analysis, 2 were ongoing on treatment at the time of the data cut-off (i.e. 31 March 2010) and 5 went off study due to disease progression based on Investigator assessment (3 patients), adverse event (1 patient), or withdrawal of consent (1 patient). It has not been possible to rule out informative censoring and to what extent the true duration of response may have been over-estimated.

In summary, the magnitude of response cannot be critically assessed and important selection bias cannot be ruled out without a randomised controlled study. Furthermore, it is not known whether or to what extent a response translates into clinical benefit, and due to the non-randomised design of the study, it is not possible to establish to what extent the observed results in terms of progression-free and overall survival are due to romidepsin, follow-up treatment or the disease characteristics of the patients selected.

With regard to safety and as was previously discussed, the safety profile of romidepsin is considered globally manageable. However, the original CHMP concern that the lack of an active control arm hampers meaningful interpretation of safety data remains unaddressed.

In conclusion and following assessment of the analyses provided in the grounds for re-examination, it is considered that efficacy has not been established. A positive benefit-risk balance of romidepsin has not been established, hence the requirements for a conditional Marketing Authorisation are not met.

# Recommendations following re-examination

Based on the arguments of the applicant and all the supporting data on quality, safety and efficacy, the CHMP re-examined its initial opinion and in its final opinion concluded by majority decision that the efficacy of the above mentioned medicinal product is not sufficiently demonstrated

and, therefore recommends the refusal of the granting of the Marketing Authorisation for the above mentioned medicinal product. The CHMP considers that:

- in the absence of established benefits, a positive benefit-risk balance cannot be considered established

Furthermore, the CHMP, in light of the final negative recommendation, is of the opinion that it is not appropriate to conclude on the new active substance status at this time.

Divergent positions to the majority recommendation are appended to this report.

## DIVERGENT POSITION EXPRESSED BY CHMP MEMBERS

In general, the factual presentation of the efficacy and safety data for Istodax (romidepsin) for treatment of adult patients with peripheral T-cell lymphoma (PTCL) that have relapsed after or become refractory to at least one prior therapy as reflected in the CHMP AR is agreed with. However, another and more positive conclusion on the benefit-risk balance is reached when looking at the possibility of a conditional approval.

**Unmet medical need and PTCL is a life-threatening disease**

It has long been recognised that the majority of PTCLs have an inferior prognosis compared with their B-cell counterparts. The standard therapy for PTCLs is CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or a comparable CHOP-like regimen that incorporates anthracyclines. With the exception of anaplastic lymphoma kinase–positive anaplastic large cell lymphoma (ALK+ ALCL), the cure rate for PTCLs with CHOP is low with a long-term survival of only 10% to 30%. Clinical evidence also demonstrates that the progression free survival after second-line salvage therapy is short (in the range 4-5 months) and median survival after first progression is 7-8 months (Mak V et al. ASH 2011, abstract 96). Thus the need for new active substances and in particular with new mechanisms of action that could be incorporated into combination chemotherapy is highly needed. As stated in the ASH (American Society of Hematology) Education Book p.514-25, December 2011, several experts question the adequacy of regimens building on a CHOP backbone in the first-line setting since neither the shortening of the treatment interval from CHOP21 to CHOP14 or the addition of etoposide to CHOP have improved OS. The addition of alemtuzumab to CHOP has increased toxicity without improving the prognosis. The inclusion of HD-Chemotherapy + HSCT as consolidation has resulted in conflicting results in terms of cure rate.

First-line combinations that bypass the Pgp efflux pump are now being investigated. Istodax may have an interesting mechanism of action in that respect.

In conclusion first-line therapy for PTCL appears to be inadequate in terms of long-term disease-free survival.

If the first-line therapy is inadequate and exhausts most of the available classical cytostatics in that setting, the treatment results of second-line or later line therapy are so dismal that the US National Comprehensive Cancer Network (NCCN) Guideline for relapsed/refractory PTCL (2009) states that clinical trials are the preferred option in both first and second relapse.

Thus, the high medical need for new medicines for the treatment of patients with PTCL cannot be questioned.

**Positive Risk/Benefit Balance**

In contrast to the SAG-O and the CHMP majority, it is the opinion of the divergent CHMP members that antitumour activity of romidepsin has been clearly demonstrated with a complete response rate (CR+CRu) in Study GPI-06-0002 (IRC assessed) of 13.1% with lower limit of the of the 95% unilateral confidence interval of 8.5%, and an ORR of 26.2%. The CR rate in NCI Study 1312 (investigator assessed) was 17.8% and the ORR 37.8%. The ORR for single agent romidepsin in patients with PTCL is comparable to the activity of other classical approved cytostatics when used as single agents, and the relatively high CR+CRu rate is highly indicative of clinical benefit because these responses seem durable and may offer an opportunity for curative high-dose chemotherapy with haematopoietic stem cell support, where other available therapeutic options fail to induce lasting responses. Moreover, romidepsin has a new mechanism of action that most probably makes it suitable for use in combination with other anticancer agents. It is not to be expected that any new single agent therapy will

dramatically change the prognosis in advanced aggressive non-Hodgkin's lymphoma where 4-5 drug combinations have been standard of care for more than 30 years.

A median overall survival of 11.3 months for all patients included in the pivotal phase II trial is a strong indicator of clinical benefit even if it is fully acknowledged that additional [comparative] data are still required. In that context it should be noted that the median for both PFS and OS has not been reached for patients with CR+CRu at the time of submission.

The safety profile of romidepsin, although not trivial, is sufficiently well described from the submitted clinical trials as well as the post-marketing experience and the known adverse event profile of romidepsin from the use outside the EU to allow a benefit/risk judgement, and it is considered as globally manageable and expected for this heavily pre-treated population of PTCL patients.

Therefore, in the opinion of the divergent CHMP members the benefits to public health of making Istodax immediately available on the market outweigh the risk inherent in the fact that additional data are still required.

**Conditional Marketing authorisation**

In accordance with Regulation (EC) No 726/2004, conditional marketing authorisations will be valid for one year on a renewable basis. In the case of the conditional marketing authorisation, authorisation is granted before all data are available. The Applicant now considers a comparison of gemcitabine + romidepsin vs. gemcitabine alone a feasible clinical program, as part of a conditional approval. The divergent CHMP members are of the opinion that this trial together with other trials in the ongoing clinical development programme for romidepsin will provide the required additional data within a reasonable period of time taking into consideration the orphan indication.

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Jens Heisterberg

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Aikaterini Moraiti

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Jean-Louis Robert

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Agnes Gyurasics

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Miloslav Salavec

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Alar Irs

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Jacqueline Genoux-Hames

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Pieter Neels

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Mila Vlaskovska

## References


Anderson JR, Armitage JO, Weisenburger DD. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol. 1998; 9(7):717-20

Armitage JO, Weisenburger DD. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol. 1998; 9(7):717-20

Armitage JO. Peripheral T-cell lymphomas. In: Canellos GP, Lister TA, Young BD (Eds) The lymphomas, Chapter 26, Elsievier Inc; 2006: 437-450

Ascani S, Zinzani PL, Gherlinzoni F, et al. Peripheral T-cell lymphomas: clinico-pathologic study of168 cases diagnosed according to the R.E.A.L. classification. Ann Oncol. 1997; 8:583-92

Bradner J, West N, Grachan M, et al. Chemical phylgenetics of histone deacetylases. Nat Chem Biol. 2010; 6:238-243

Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, Lister TA, Vose J, Grillo-López A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris NL, Armitage JO, Carter W, Hoppe R, Canellos GP. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol. 1999;17(4):1244. Erratum in: J Clin Oncol 2000;18(11):2351

Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, Coiffier B, Fisher RI, Hagenbeek A, Zucca E, Rosen ST, Stroobants S, Lister TA, Hoppe RT, Dreyling M, Tobinai K, Vose JM, Connors JM, Federico M, Diehl V; International Harmonization Project on Lymphoma. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007; 25(5):579-86

Feyler S, Prince HM, Pearce R, Towlson K, Nivison-Smith I, Schey S, et al. The role of highdose therapy and stem cell rescue in the management of T-cell malignant lymphomas: a BSBMT and ABMTRR study. Bone Marrow Transplant 2007; 40(5):443-450

Furumai R, Matsuyama A, Kobashi N, et al. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. Cancer Res. 2002; 62:4916-4921

Gallamini A, Stelitano C, Calvi R, Bellei M, Mattei D, Vitolo U, Morabito F, Martelli M, Brusamolino E, Iannitto E, Zaja F, Cortelazzo S, Rigacci L, Devizzi L, Todeschini G, Santini G, Brugiatelli M, Federico M; Intergruppo Italiano Linfomi. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. Blood. 2004; 103(7):2474-9

Groves FD, Linet MS, Travis LB, Devesa SS. Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995. J Natl Cancer Inst. 2000; 92(15):1240-51.

Horwitz S, Moskowitz C, Kewalramani T, Hamlin P, Straus D, O'Connor C, et al. Second-line therapy with ICE followed by high dose therapy and autologous stem cell transplantation for relapsed/refractory peripheral T-cell lymphomas: minimal benefit when analyzed by intent to treat. Blood (ASH Annual Meeting Abstracts), 2005; 106: 2679

Horwitz S. Management of peripheral T-cell non-Hodgkin's lymphoma. Curr Opin Oncol. 2007; 19(5):438-443

Jaffe ES, Harris NL, Stein H, Vardiman JW. Tumours of haematopoietic and lymphoid tissue. In: Pathology & Genetics. Lyon, France: IARC Press; 2001:189-235

Jemal A, Siegel R, Ward E, Murray T, Xu J and Thun MJ. Cancer statistics, 2007 CA Cancer J Clin. 2007; 57:43-66

Marchetti S, Mazzanti R, Beijnen JH, Schellens JH. Concise review: Clinical relevance of drug drug and herb drug interactions mediated by the ABC transporter ABCB1 (MDR1, P-glycoprotein). Oncologist. 2007; 12(8):927-41

Miller TA, Witter DJ, Belvedere S. Histone deacetylase inhibitors. 2003; 46(24):5097-5116

Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, Raunio H. Inhibition and induction of human cytochrome P450 enzymes: current status. Arch Toxicol. 2008; 82(10):667-715

Raina V, Singhal MK, Sharma A, Kumar L, Kumar R, Duttagupta S, Kumar B, Das P. Clinical characteristics, prognostic factors, and treatment outcomes of 139 patients of peripheral T-cell lymphomas from AIIMS, New Delhi, India. J Clin Oncol. 2010 ASCO Annual Meeting Proceedings (Post-Meeting Edition). 2010; 28(15) suppl (May 20 Supplement): e18549

Rodriguez J, Munsell M, Yazji S, Hagemeister FB, Younes A, Andersson B, Giralt S, Gajewski J, de Lima M, Couriel D, Romaguera J, Cabanillas FF, Champlin RE, Khouri IF. Impact of high-dose chemotherapy on peripheral T-cell lymphomas. J Clin Oncol. 2001; 19(17):3766-70

Rodríguez J, Conde E, Gutiérrez A, Arranz R, León A, Marín J, Bendandi M, Albo C, Caballero MD. The results of consolidation with autologous stem-cell transplantation in patients with peripheral T-cell lymphoma (PTCL) in first complete remission: the Spanish Lymphoma and Autologous Transplantation Group experience. Ann Oncol. 2007; 18(4):652-7

Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000; 92(3):205-16

Vose JM, Armitage J, Weisenburger D, International T-Cell Lymphoma Project. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol. 2008; 26(25):4124-30

Weidmann E, Gramatzki M, Wilhelm M, Mitrou PS. Diagnosis and actual therapy strategies in peripheral T-cell lymphomas: summary of an international meeting. Ann Oncol. 2004; 15(3):369-74

Xiao JJ, Foraker AB, Swaan PW, et al. Efflux of depsipeptide FK228 (FR901228, NSC-630176) is mediated by P-glycoprotein and multidrug resistance-associated protein 1. J Pharmacol Exp Ther. 2005; 313:268-276

Yang DH, Kim WS, Kim SJ, Bae SH, Kim SH, Kim IH, Yoon SS, Mun YC, Shin HJ, Chae YS, Kwak JY, Kim H, Kim MK, Kim JS, Won JH, Lee JJ, Suh CW. Prognostic factors and clinical outcomes of high-dose chemotherapy followed by autologous stem cell transplantation in patients with peripheral T cell lymphoma, unspecified: complete remission at transplantation and the prognostic index of peripheral T cell lymphoma are the major factors predictive of outcome. Biol Blood Marrow Transplant. 2009; 15(1):118-25