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EMA/249101/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Aplidin

International non-proprietary name: plitidepsin

Procedure No. EMEA/H/C/004354/0000

Note

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

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Table of contents

List of abbreviations

AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
API	Active pharmaceutical ingredient
ASCT	Autologous stem cell transplant
AST	Aspartate Aminotransferase
ASMF	Active substance master file
AUC	Area Under the Concentration Time Curve
BM	Bone Marrow
BSA	Body Surface Area
BTZ	Bortezomib
^{t}BuOH	tert-butanol
C	Censored
CFU	Colony forming units
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CL	Clearance
CLCR	Clearance of Creatinine
Cmax	Maximum Plasma Concentration
conf	Confirmed
CPK	Creatine Phosphokinase
CPP	Critical process parameter
CQA	Critical quality attribute
CR	Complete Response
D	Day
Diff	Difference between Arms
DLT	Dose-limiting Toxicity
DR	Duration of Response
DSC	Differential scanning calorimetry

DXM	Dexamethasone
EBMT	European Group for Blood and Marrow Transplantation
EC	European Commission
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eEF1A2	Eukaryotic Elongation Factor 1A2
EFS	Event free survival
EMA	European Medicines Agency
ER	Endoplasmic Reticulum
ERK	extracellular signal–regulated kinases
ESMO	European Society of Medical Oncology
EU	European Union
F	Female
FDA	Food and Drug Administration (USA)
GC	Gas chromatography
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GLP	Good Laboratory Practice
HDAC	Histone Deacetylase Inhibitor
HDPE	High density polyethylene
HPLC	High performance liquid chromatography
HR	Hazard Ratio
IA	Investigator's Assessment
IC50	Half Maximal Inhibitory Concentration
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IDMC	Independent Data Monitoring Committee
IMiDS	Immunomodulatory Drugs
IMWG	International Myeloma Working Group
IPC	In-process control
IR	Infrared

IRC	Independent Review Committee
ISS	International Staging System
ITT	Intention-to-treat
i.v.	Intravenous (ly)
JNK	c-Jun N-terminal Kinase
KF	Karl Fischer titration
LC	Liquid chromatography
LC-MS/MS	Liquid Chromatography with Spectrometric Detection
LR	Log-rank test
LVEF	Left Ventricular Ejection Fraction
M	Male
MAPK	Mitogen Activated Protein Kinase
MedDRA	Medical Dictionary for Regulatory Activities
MGUS	Monoclonal Gammopathy of Undetermined Significance
miR-663	Micro RNA-663
MM	Multiple myeloma
MR	Minor Response
MTC	Medullary Thyroid Carcinoma
MTD	Maximum Tolerated Dose
NA	Not available/not applicable
NCA	Non-compartmental Analysis (Pharmacokinetics)
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
nconf	Not Confirmed
ND	Not Done
NE	Not Evaluable
NLT	Not less than
NMT	Not more than
NHL	Non-Hodgkin Lymphoma

NOAEL	No Observed Adverse Effect Level
nr	Not Reached
NSCLC	Non-small Cell Lung Cancer
OOPD	Office of Orphan Products Development
OOS	Out of specification
ORR	Overall Response Rate
OS	Overall Survival
P	Plitidepsin
PD	Disease Progression/Progressive Disease/Pharmacodynamics
PDE	Permitted daily exposure
PE	Polyethylene
PES	Polyethersulfone
PFS	Progression-free Survival
Ph. Eur.	European Pharmacopoeia
PIs	Proteasome Inhibitors
PK	Pharmacokinetic(s)
PK/PD	Pharmacokinetic/Pharmacodynamic
Pop-PK	Population-PK model
PP	Polypropylene
PR	Partial Response
PRAC	Pharmacovigilance Risk Assessment Committee
Prdx-I	Peroxiredoxin I
PS	Performance Status
PT	Preferred Term
PTFE	Polytetrafluoroethylene
q2wk	Every Two Weeks
q3wk	Every Three Weeks
q4wk	Every Four Weeks
qs	*quantum satis*
QTc	QT Corrected (Electrocardiogram)
QTPP	Quality target product profile

RD	Recommended Dose
RH	Relative humidity
RPSFT	Rank Preserving Structural Failure Time
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAWP	Scientific Advice Working Party
s.c.	Subcutaneous (ly)
SCLC	Small Cell Lung Cancer
sCR	Stringent Complete Response
SCT	Stem Cell Transplantation
SD	Stable Disease
SLAMF-7	Signalling Lymphocytic Activation Molecule F7
SMPC	Summary of product characteristics
SOC	System Organ Class
t1/2	Half Life
TAMC	Total aerobic microbial count
th.	Therapy
TTP	Time to Progression
TYMC	Total combined yeasts/moulds count
ULN	Upper Limit of Normal
USA	United States of America
UV	Ultraviolet
VAD	Vincristine, Doxorubicin and Dexamethasone
VGPR	Very Good Partial Response
Vss	Volume of Distribution at Steady State
WFI	Water for injections
XRPD	X-ray powder diffraction
y.o.	Years Old

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Pharma Mar, S.A. submitted on 21 September 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Aplidin, 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 17 December 2015.

Aplidin, was designated as an orphan medicinal product EU/3/04/245 on 16 November 2004 in the following condition: ''Treatment of multiple myeloma''.

The applicant applied for the following indication:

Aplidin is indicated in combination with dexamethasone for the treatment of relapsed/refractory multiple myeloma (MM) in adult patients who have received at least three prior regimens including bortezomib, and either lenalidomide or thalidomide.

The legal basis for this application refers to:

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

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 tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision CW/1/2011 on the granting of a class 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 submit a critical report addressing the possible similarity with authorised orphan medicinal products.

New active Substance status

The applicant requested the active substance plitidepsin contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 23 July 2009. The Protocol Assistance pertained to clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Jorge Camarero Jiménez Co-Rapporteur: Bjorg Bolstad

- The application was received by the EMA on 21 September 2016.
- The procedure started on 27 October 2016.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 23 January 2017. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 12 January 2017. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 26 January 2017
- During the meeting on 23 February 2017, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 10 July 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 25 August 2017.
- During the PRAC meeting on 1 September 2017, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP.
- During the CHMP meeting on 14 September 2017, the CHMP agreed on a list of outstanding issues to be sent to the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 10 October 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Outstanding Issues to all CHMP members on 26 October 2017.
- During the CHMP meeting on 7 November 2017, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- CHMP adopted a second List of Outstanding Issues via written procedure on 16 November 2017 to be sent to the applicant. The CHMP adopted questions to be addressed by the BSWP as part of this step.
- The applicant submitted the responses to the CHMP second List of Outstanding Issues on 21 November 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the second List of Questions to all CHMP members on 1 December 2017.
- BSWP members completed a report addressing the questions raised by the CHMP on 6 December 2017. The CHMP considered the views of the Working Party (as appropriate) as presented in the minutes of this meeting.
- During the meeting on 14 December 2017, 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 Aplidin.

2. Scientific discussion

2.1. Problem statement

Disease or condition

Aplidin (plitidepsin) is proposed for treatment in combination with dexamethasone for relapsed/refractory multiple myeloma (MM) in adult patients who have received at least three prior regimens including bortezomib, and either lenalidomide or thalidomide.

Epidemiology and risk factors

Multiple myeloma (MM) accounts for approximately 1 percent of all cancers and slightly more than 10 percent of all hematologic malignancies. The incidence in Europe is 4.5 -6.0 per 100,000/year. The median age at diagnosis is around 70 years and many patients are older than 75 years. The mortality is 4.1/100,000/year (Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, 2013). At present, more than 80,000 new cases are reported worldwide each year, and the disease prevalence is increasing due to both, increasing diagnosis cases and improvement of the survival of the patients with the new therapeutic approaches (1).

Biological features/Aetiology and pathogenesis

Multiple myeloma is characterized by marrow plasmacytomas (plasma cell tumours) and overproduction of monoclonal immunoglobulins (IgG, IgA, IgD or IgE) or Bence-Jones protein (monoclonal K or h light chains), while the production of normal immunoglobulin is impaired.

The cause of a myeloma cell's failure to differentiate is unknown. However, translocations between chromosome 14q32 and its neighbours (involving the immunoglobulin heavy chain region) and deregulation of the c-myc oncogene appear to play a role in the initial stages of the disease; additionally, mutations in N-ras and K-ras are seen in up to 15% of patients at the time of diagnosis. Conversely, mutations in p53 are rarely seen at diagnosis but instead are noted in extramedullary relapses, along with phenotypic and cytological changes. With the exception of chromosome 13q deletions, which are consistently associated with a poor prognosis, the role of other changes in the pathogenesis and severity of the disease have yet to be defined.

Clinical presentation, diagnosis and stage/prognosis

The clinical features of MM are varied and can arise from the effects of the tumour itself, or the toxicity of the tumour products, or the host's own response.

The most common symptoms include persistent unexplained skeletal pain (especially pain in the back or thorax), recurrent or persistent bacterial infection, anaemia, renal impairment, fractures and vertebral collapse, hypercalcaemia and, in some patients, hyper-viscosity syndromes, neurological disease and clotting abnormalities. Approximately 20% of patients are symptom free and are diagnosed by chance (2).

The most common criteria used in diagnosis of symptomatic MM are the presence of neoplastic plasma cells comprising greater than 10% of BM cells or presence of a plasmacytoma; paraprotein (M protein) in the serum and/or urine; and evidence of related organ or tissue impairment due to plasma cell disorder.

A clinical staging system, developed by Durie and Salmon (3), is useful for predicting survival of multiple myeloma patients and is used for prognosis (4). Combining a number of biological parameters of prognostic importance with serum albumin has led to a new International Staging System (ISS) (5).

The prognosis depends on a variety of factors including age and stage of MM at time of diagnosis. Due to the availability of new agents in recent years including thalidomide, bortezomib and lenalidomide, and autologous stem cell transplant (ASCT), the 5-year survival rate has improved to 40% - 50%.

Despite progress in its current treatment and management, MM remains incurable. Although ASCT has extended survival in newly diagnosed MM, practically all patients eventually relapse (3) (6). In addition, approximately two thirds of newly diagnosed patients aged > 65 years are ineligible for this treatment. The treatment option for the majority of the MM population, i.e., the more fragile and elderly patients, is associated with low response rate and short survival.

In relapsed/refractory MM, despite salvage therapy, median overall survival (OS) remains poor (in the range of 30 months). Although patients with relapsed disease can achieve responses to subsequent anti-myeloma regimens, the duration of response typically decreases with successive relapses until resistant disease develops.

Management

The management of patients with relapsed/refractory disease represents a clinical challenge, as these patients suffer from continuing symptoms, complications of the disease (including renal failure, blood cytopenia or recurrent infections) and decreased quality of life. These patients typically receive salvage therapy until the next relapse or progression of disease or the development of intolerable toxicity and then go onto the next salvage option.

For the treatment of relapsed/refractory MM, conventional-dose chemotherapy and high-dose chemotherapy with stem cell support remain the current standard of care, along with supportive care including bisphosphonates (7).

Treatment options are limited to the following classes of agents (chemotherapy, IMiDs, proteasome inhibitors, monoclonal antibodies, histone deacetylase inhibitors) used in various combinations and schedules. Depth and duration of response are shorter than for newly-diagnosed patients and decrease with each line of therapy as drug resistance develops (NCCN 2014):

- Vincristine, doxorubicin and dexamethasone (VAD) or a related infusional regimen such as vincristine, adriamycin, methotrexate and prednisone (VAMP) or VAMP + cyclophosphamide (C-VAMP) have been most widely used.
- Pegylated doxorubicin (Caelyx) is approved in combination with bortezomib for the treatment of progressive multiple myeloma in patients who have received at least one prior therapy and who have already undergone or are unsuitable for bone marrow transplant.
- Bortezomib (Velcade) is indicated as monotherapy for the treatment of progressive multiple myeloma in patients who have received at least 1 prior therapy and who have already undergone or are unsuitable for bone marrow transplantation. It is used either alone or in combination with dexamethasone or chemotherapy although these combinations are not approved.
- Lenalidomide (Revlimid) in combination with dexamethasone is indicated for the treatment of multiple myeloma patients who have received at least one prior therapy.
- Carfilzomib (Kyprolis) in combination with either lenalidomide and dexamethasone or dexamethasone alone is indicated for the treatment of adult patients with multiple myeloma who have received at least one prior therapy.

- Elotuzumab (Empliciti) is indicated in combination with lenalidomide and dexamethasone for the treatment of multiple myeloma in adult patients who have received at least one prior therapy.
- Daratumumab (Darzalex) as monotherapy is indicated for the treatment of adult patients with relapsed and refractory multiple myeloma, whose prior therapy included a proteasome inhibitor and an immunomodulatory agent and who have demonstrated disease progression on the last therapy.
- Ixazomib (Ninlaro) in combination with lenalidomide and dexamethasone is indicated for the treatment of adult patients with multiple myeloma who have received at least one prior therapy.
- Pomalidome (Imnovid) in combination with dexamethasone is indicated in the treatment of adult patients with relapsed and refractory multiple myeloma who have received at least two prior treatment regimens, including both lenalidomide and bortezomib, and have demonstrated disease progression on the last therapy.
- Panobinostat (Farydak) in combination with bortezomib and dexamethasone, is indicated for the treatment of adult patients with relapsed and/or refractory multiple myeloma who have received at least two prior regimens including bortezomib and an immunomodulatory agent.

About the product

Plitidepsin is a cyclic depsipeptide originally isolated from a Mediterranean marine tunicate, *Aplidium albicans.*

Plitidepsin interacts with eEF1A2, a protein described to have oncogenic properties. The compound triggers the generation of early oxidative stress, which induces the sustained activation of MAPK signalling cascades that finally lead to apoptosis.

The applicant applied for the following indication: Aplidin is indicated in combination with dexamethasone for the treatment of adult patients with relapsed/refractory multiple myeloma (MM) who have received at least three prior regimens including bortezomib, and either lenalidomide or thalidomide.

Type of Application and aspects on development

In July 2009, CHMP provided protocol assistance (EMEA/H/SA/929/2/2009/PA/II) for the development of plitidepsin as a treatment of relapsed and refractory multiple myeloma. This advice focused on the design of a Phase 2b/3 study as follows:

- The applicant sought advice for a planned adaptive designed study. However, during the scientific advice meeting, the applicant declared that they no longer wished to pursue a conditional approval on completion of a Phase 2b of their study.

- The CHMP agreed that dexamethasone as proposed control arm was acceptable. However, CHMP advised the applicant to consider the use of the "investigators preferred choice of treatment option" which may serve to provide an enhanced evidence of efficacy.

- The CHMP endorsed the choice of the applicant's proposal of stratification according to ECOG-PS score and prior treatment with high-dose chemotherapy plus bone marrow transplant. It was advised that data related to relevant prognostic factors (serum ß2-microglobulin, serum albumin and cytogenetic abnormalities) should also be collected.

- Regarding choice of an appropriate primary endpoint of the Phase 3 of the study, the CHMP referred to the Guideline on the Evaluation of Anticancer Medicinal Products in Man (CPMP/EWP/205/95/Rev.3/Corr.2). The Guideline states that "If major differences in toxicity are

expected in favour of the control regimen, OS should normally be selected as the most appropriate primary endpoint. Similarly, if there are no evidence based next line therapies available and if the period of time from disease progression to death is expected to be short, OS is considered to be the most appropriate endpoint; in most cases even if crossover is foreseen according to protocol." According to CHMPs scientific advice, for the currently proposed protocol, these differences in toxicity are expected, as the toxicity of the experimental drug will be added to dexamethasone's toxicity, and there are no evidence-based next line treatments. Therefore, at least a clear positive trend for this key secondary endpoint OS is essential when PFS is accepted as a minimum requirement for the primary endpoint. In order to grant a marketing authorisation, a treatment effect that is both statistically significant and clinically relevant will need to be demonstrated.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as a powder for concentrate for solution for infusion containing 2 mg of plitidepsin as active substance.

The other ingredient is D-mannitol.

The powder is accompanied by a "solvent for plitidepsin" which is used for initial dissolution of plitidepsin to form a concentrate. The solvent consists of macrogolglycerol ricinoleate, anhydrous ethanol and water for injections.

The powder is available in a type I clear glass vial with a grey butyl rubber stopper covered by a flip-off aluminium cap whilst the solvent is packaged in type I glass ampoules, as described in section 6.5 of the SmPC.

2.2.2. Active Substance

General information

The chemical name of plitidepsin is (-)-(3*S*,6*R*,7*S*,10*R*,11*S*,15*S*,17*S*,20*S*,25a*S*)-11-hydroxy-3-(4-methoxybenzyl)-2,6,17-trimethyl-15-(1-methylethyl)-7-[[(2*R*)-4-methyl-2-[methyl[[(2*S*)-1-(2-oxopropanoyl)pyrrolidin-2-yl]carbonyl]amino]pentanoyl]amino]-10-[(1*S*)-1-methylpropyl]-20-(2-methylpropyl)tetradecahydro-15*H*-pyrrolo[2,1-f]-[1,15,4,7,10,20]dioxatetrazacyclotricosine-1,4,8,13,16,18,21(17*H*)-heptone corresponding to the molecular formula $C_{57}H_{87}N_7O_{15}$. It has a relative molecular mass of 1110.34 g/mol and the following structure:

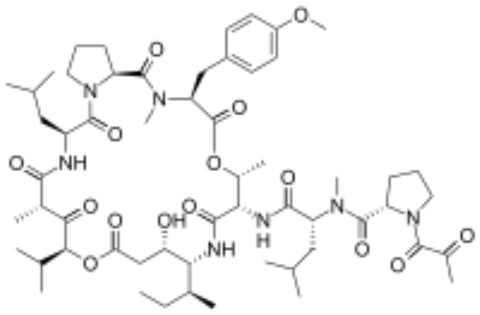


Figure 1: active substance structure

Plitidepsin is a naturally occurring substance, first isolated and characterised in 1989. Subsequently, a synthetic process was developed and published in 1997, the publication containing analytical data which

confirmed the structure and both absolute and relative stereochemistry. The active substance in Aplidin is manufactured via a fully synthetic route. The ASMF holder provided data from elemental analysis, infrared spectroscopy, ^{1}H, ^{13}C and 2D nuclear magnetic resonance spectroscopy, UV spectroscopy, mass spectrometry, X-ray crystallography and optical rotation, confirming the chemical structure in relation to the published data. The solid state properties of the active substance were measured by differential scanning calorimetry (DSC), thermogravimetric analysis and x-ray powder diffraction (XRPD).

The active substance is a partially crystalline, hygroscopic white to pale yellow powder, practically insoluble in aqueous media between pH 1-13 although it is slightly soluble in polar organic solvents mixed with water. Since the active substance is fully dissolved in water/^{t}BuOH in the first step of the finished product manufacturing process, the lack of control of both polymorphic form and particle size is deemed acceptable.

Plitidepsin exhibits stereoisomerism due to the presence of 12 chiral centres. All but one of the stereocentres originate from and are controlled in the starting materials which are mostly derived from chiral pool materials. The specifications of the starting materials contain suitable controls for stereoisomeric impurities. The 12th stereocentre is epimerisable until the macrocycle is formed at which point, a single diastereomer is formed. Another stereocentre has been found to partially epimerise under the processing conditions and a limit has been set for the minor diastereomer in the relevant intermediate specification.

Manufacture, characterisation and process controls

Detailed information on the manufacturing process of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory. A single manufacturer carries out the entire process.

Plitidepsin is synthesized in nine main steps using five well-defined starting materials with acceptable specifications. One of the originally proposed starting materials was re-defined as an intermediate during the procedure in response to a major objection from CHMP, so that enough of the route is included in the process description. The revised route comprises a series of alternating peptide coupling reactions and deprotections.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. Critical steps were identified and process parameters and raw material stoichiometries were defined. The control strategy has been demonstrated to prevent formation of key impurities and ensure the quality of the active substance.

The characterisation of the active substance and its impurities is in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Plitidepsin is a naturally occurring substance and initial studies used material isolated from its natural source. Subsequent studies used semi-synthetic material before development of a fully synthetic route which was used to supply the main clinical studies. Changes in the synthetic route such as purification method were introduced in order to improve manufacturability and active substance quality. Changes introduced have been presented in sufficient detail and have been justified.

The active substance is packaged in type I borosilicate vials sealed with polypropylene screw caps with PTFE-lined silicone septa. The materials comply with the EC directive 2002/72/EC and EU Regulation

10/2011 as amended. The vials are further stored in amber HDPE bottles containing calcium bentonite desiccant.

Specification

The active substance specification includes tests for appearance and colour (visual inspection), identity (IR, HPLC), water content (KF), related substances (HPLC), residual solvents (GC), sulphated ash (Ph. Eur.), assay (LC), bacterial endotoxins (Ph. Eur.) and microbial tests (Ph. Eur.).

Impurities thresholds were set in line with Ph. Eur. 2034 and appropriate specifications have been set, in line with batch analysis data. The palladium content is controlled in upstream intermediates so no further control is considered necessary in the active substance specification. The enantiopurity is controlled in the chiral starting materials and the impurities method is able to detect any diastereomers which form during processing, so no specific optical rotation test is included.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data from 14 batches of the active substance were provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from 3 production scale batches of active substance from the proposed manufacturer stored in a smaller version of the intended commercial package for up to 48 months under long term conditions (5 ± 3 ºC) and for up to 6 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. Samples were tested for appearance, colour, identity, impurities, residual solvents and assay. Other than the impurities and assay HPLC methods, the analytical methods used were the same as for release. The impurities and assay methods were those in use at the time of the studies and had been fully validated. All analytical methods are stability indicating. No significant changes or trends were observed to any of the measured parameters other than 1 out of specification (OOS) result for assay which was too high for 1 batch after 36 months. However, the batch was well within specification after 48 months and the result can be attributed to analytical variability. The lack of degradation under accelerated conditions indicates that short temperature excursions, for example during handling or transport, should not impact the quality of the active substance.

Photostability testing following the ICH guideline Q1B was performed on 1 batch which resulted in significant degradation. Plitidepsin is photosensitive. Samples of plitidepsin were also exposed to stressed conditions (heat, acid, alkali and oxidative conditions). Samples were stable under dry and moist heat conditions, moderately stable under acidic and oxidative conditions, but degraded rapidly under aqueous alkaline conditions.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 48 months at 5 ± 3 ºC in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

Plitidepsin powder for concentrate for solution for infusion is a white to off-white lyophilised powder stored in glass vials. In addition to the powder, a solvent is also provided which is used to dissolve plitidepsin and generate the concentrate.

The aim of development was to obtain a formulation of plitidepsin suitable for intravenous infusion following dilution with a suitable infusion medium. A quality target product profile (QTPP) was defined as a sterile lyophilised powder for concentrate for intravenous infusion with the correct amount of plitidepsin in each vial, before and after recostitution. The product should be sterile, and the infusion solution should have acceptable viscosity and tonicity an be in the required pH range. The storage conditions and shelf-life should be appropriate for clinical use and the product should meet the pharmacopoieal requirements for intravenous adminstration.

Plitidepsin is practically insoluble in water but is more soluble in certain water-miscible solvents. Therefore, a solution for intial dissolution of plitidepsin was developed and is provided as part of the finished product. Preparation of the infusion solution therefore occurs in 2 steps: dissolution of the lyophilisate in the "solution for plitidepsin" followed by dilution with a suitable infusion solution.

In order to improve dissolution characteristics, plitidpesin is lyophilised with mannitol as a bulking agent. A mixture of water and ^{t}BuOH was suitable to dissolve both components and also facilitated the freeze-drying process. Plitidepsin is hygroscopic, photosensitive and degrades in basic aqueous media. It is however, sufficiently stable in acidic aqueous media and so a slightly acidic pH (4-7.5) is stipulated for the infusion solution. The bulk plitidepsin was shown to be both physicochemically and microbiologically stable for up to 24 hours under ambient temperature and lighting conditions without an impact on the finished product quality. Nonetheless, formulation and analysis are carried out protected from exposure to light as a precautionary measure.

Since plitidepsin is intended to be infused and thus needs to be sterile, different sterilisation methods were investigated for the lyophilised powder. Terminal sterilisation by autoclaving was ruled out as the active substance degrades at high temperature. Therefore, the bulk solution, prior to lyophilisation, is sterile filtered followed by aseptic filling and freeze drying.

Critical quality attributes of the finished product were identified based on the QTPP as appearance, identity, colour of reconstituted solution, reconstitution time, subvisible particles, water content, ^{t}BuOH content, assay, uniformity of dosage units, degradation products, endotoxins and sterility. These are reflected in the finished product specification tests.

Raw materials have the potential to impact the endotoxin content and sterility of the finished product. Therefore, the mannitol and active substance are tested for bioburden and endoxtoxins. Vials and stoppers are washed, sterilised and depyrogenised prior to filling according to validated procedures.

The formulation used during clinical studies is the same as that intended for marketing. The different steps of the process were optimized to ensure the quality of the finished product whilst maintaining manufacturability. The order of addition of materials for the compounding step was investigated. Plitidepsin is added last to ensure complete dissolution and minimize processing time. For the lyophilisation step, time, temperature and pressure were investigated and appropriate set-points defined to prevent generation of crystalline material.

The "solvent for plitidepsin" needs to ensure both complete dissolution of the lyophilisate, and prevent precipitation when it is subsequently diluted with an aqueous infusion solution. Solubility studies were undertaken, where it was found that an alcoholic solvent/water mixture combined with a surfactant offers

the required properties. A mixture of water for injections (WFI), ethanol and macrogolglycerol ricinoleate was found to afford suitable dissolution characteristics and the relative amounts of each component were optimised. It was found that this solution can be terminally sterilised although re-homogenisation is required afterwards as it become biphasic at high temperature.

The solution was exposed to stressed conditions including light, heat and oxygen. Some degradation of the surfactant was observed when heated at 40 ^{o}C with an air-filled headspace. This is prevented by using a nitrogen headspace. The solvent mixture is not photosensitive.

Studies were undertaken to assess the stability of the concentrated solution, as well as following dilution in several common infusion solvents. The concentrated solution is stable for up to 72 hours under ambient temperature and light conditions. Following dilution with either 0.9% NaCl solution or 5% glucose solution, solutoins were stable for up to 6 hours under ambient conditions and for 24 hours under refrigerated conditions. Nonetheless, it is recommended to use the reconstituted solutions immediately.

No plitidepsin absorption is observed when the solutions are generated in PE or PP infusion bags. Similarly, the reconstituted solutions were found to be compatible with common infusion lines and catheters with the excpetion of polyurethane infusion lines which absorbed too much plitidepsin.

The applicant originally proposed to include a routine filtration step prior to infusion to remove any subvisible particles. CHMP raised a major objection, which stated that management of uncontrolled formation of subvisible particles by routine filtration was unacceptable. The applicant carried out a series of experiments to investigate the cause and nature of the particles. It was concluded that an increased number of manipulations was responsible for the increase in number of particles. It was also concluded that the majority of subvisibe particles are either air bubbles, stabilised by the surfactant, silicone oil droplets from standard syringes, or fragments of rubber from piercing of septa with a needle. The number and nature of particles is not considered an issue, and filtration is not considered necessary. Nonetheless, the applicant proposed to maintain the routine in-line filtration protocol, in accordance with the reflection paper on "pharmaceutical development of intravenous medicinal products containing active substances solubilized in micellar systems (EMA/CHMP/QWP/799402/2011)" although Aplidin is not a micellar product. The CHMP accepted this approach. Compatibility of the reconstituted diluted infusion solution with both polyethersulfone (PES) and nylon filters was demonstrated.

All excipients in both the lyophilisate and the solution are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. Since the product is sterile, endotoxins and bioburden are controlled in the excipients, including those where the tests are not included in the Ph. Eur. monograph. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

The primary packaging for the powder is a type I clear glass vial with a grey butyl rubber stopper covered by a flip-off aluminium cap which provides sufficient protection from both light and moisture. The solution is packaged in a type I hydrolytic glass ampoule. The materials comply with Ph. Eur. and EC requirements. The choice of the container closure systems has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process for the lyophilisate consists of seven main steps: sterilisation/depyrogenation of primary packaging materials and equipment; compounding; sterile filtration; filling; lyophilisation and stoppering; capping and inspection; bulk packaging. The process is considered to be a non-standard manufacturing process.

Critical process parameters (CPPs) have been identified for several steps. Similarly, in-process controls (IPCs) are in place to check parameters impacting the finished product CQAs including bioburden, filter integrity, assay, appearance and closure integrity.

The process has been fully validated on three consecutive batches at the proposed production scale at both listed manufacturers. It has been demonstrated that the manufacturing process is capable of producing the lyophilisate of intended quality in a reproducible manner. The IPCs are adequate for this type of manufacturing process and pharmaceutical form.

The manufacturing process for "solvent for plitidepsin" consists of eight main steps: sterilisation/depyrogenation of primary packaging materials and equipment; compounding; filtration; filling and sealing; terminal sterilisation; homogenisation; inspection; bulk packaging. The process is considered to be a standard manufacturing process.

CPPs have been identified for several steps. IPCs are in place to check parameters impacting the finished product CQAs including bioburden, filter integrity and appearance.

The process has been fully validated on three consecutive batches at the proposed production scale at the proposed manufacturer. It has been demonstrated that the manufacturing process is capable of producing the solution of intended quality in a reproducible manner. The IPCs are adequate for this type of manufacturing process and pharmaceutical form.

The final step in the process is to co-package 1 vial and 1 solvent ampoule which is carried out at separate manufacturing site.

Product specification

The finished product (plitidepsin lyophilisate) release and shelf-life specifications include appropriate tests for this kind of dosage form including appearance (visual), reconstitution time (visual time), appearance and colour of reconstituted solution (Ph. Eur.), subvisible particles (Ph. Eur.), identity (UV, LC), water content (Ph. Eur.), ^{t}BuOH content (GC), degradation products (LC), assay (LC), uniformity of dosage units (Ph. Eur.), sterility (Ph. Eur.) and endotoxins (Ph. Eur.).

Since the lyophilisate is a solid, the risk of interaction with components of the container closure system is considered low so no test for extractables is included. Since the lyophilisate is dissolved in "solvent for plitidepsin, no control of particle size is needed. A risk assessment was carried out in line with ICH Q3D to determine the likelihood of contamination with elemental impurities. Metals deliberately introduced during manufacture (Pd) or which could be leached from the manufacturing vessels and pipework during compounding were considered. Since all were found well below the permitted daily exposure (PDE) levels, then no control of elemental impurities is considered necessary.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Compendial methods have been used wherever possible. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for 15 production scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product (solvent for plitidepsin) release and shelf-life specifications include appropriate tests for this kind of dosage form including appearance (visual, Ph. Eur.), colour (Ph. Eur.), subvisible particles (Ph. Eur.), identity (IR, GC), ethanol content (GC), macrogolglycerol ricinoleate content (UV titration), extractable volume (Ph. Eur.), acid value (Ph. Eur.), sterility (Ph. Eur.) and endotoxins (Ph. Eur.).

The "acid value" test was shown during forced degradation studies to be indicative of macrogolglycerol ricinoleate degradation. Extractables and leachables studies showed that no relevant impurity is

introduced during manufacture or storage in the type I glass vials. The specifications as listed are considered adequate.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Compendial methods have been used wherever possible. Satisfactory information regarding the reference standards used for identity and solvent content testing has been presented.

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

Stability of the product

Stability data from 4 production scale batches of the lyophilisate, representing both manufacturing sites, stored for up to 60 months under long term conditions (5±3 ºC) and for up to 36 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. The batches of lyophilisate are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance and colour, reconstitution time, appearance and colour of reconstituted solution, particulate matter, identity, water content, assay, degradation products and sterility. The analytical procedures used are stability indicating. No significant trends to any of the measured parameters were observed under long term conditions, other than a small, within specification, increase in total impurities for 2 batches. Under accelerated conditions, degradation products increased over time but with the exception of 1 batch, were still within specification after 12 months.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The sample underwent virtually complete degradation, indicating that plitidepsin lyophilisate is highly photosensitive. Samples were also exposed to other stressed conditions including heat, oxidant, acid and base. Samples degraded to an extent under all conditions, more so under basic conditions.

Based on available stability data, the proposed shelf-life of 48 months stored at 5±3 ºC in the original packaging in order to protect from light as stated in the SmPC (section 6.3) is acceptable.

Stability data from 4 production scale batches of "solvent for plitidepsin" stored for up to 60 months under long term conditions (5±3 ºC) and for up to 30 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. The batches of solvent are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, pH, particulate matter, identity and sterility. The analytical procedures used are stability indicating. No significant trends to any of the measured parameters were observed under long term conditions or accelerated conditions.

Since the solvent is co-packaged with the lyophilisate, then the same storage conditions are mandated. Based on available stability data, the proposed shelf-life of 48 months stored at 5±3 ºC in the original packaging in order to protect from light as stated in the SmPC (section 6.3) is acceptable.

In-use stability was investigated under conditions likely to be encountered in a clinical setting, using three different batches of finished product reconstituted with solvent from three different batches. Solutions were analysed for appearance and colour, subvisible particels, assay and degradation products. Stability of the reconstituted concentrated solution was demonstrated under ambient light at both room temperature and 5±3 ºC for 24 hrs, with no trends in the analysis data.

Studies were undertaken to assess the stability of the concentrated solution, as well as the infusion solution following dilution in several common infusion solvents. The concentrated solution is stable for up to 72 hours under ambient temperature and light conditions. Following dilution with either 0.9% NaCl solution or 5% glucose solution, stability was demonstrated for up to 6 hours under ambient conditions and for 24 hours under refrigerated conditions. Nonetheless, from a microbiological point of view, it is recommended to use the reconstituted solutions immediately since they contain no preservative.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.2.6. Recommendations for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

Safety pharmacology, toxicology and toxicokinetics studies were conducted in accordance with GLP principles.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Mechanism of action

Interaction with eukaryotic elongation factor A2 eEF1A2 (PMAR16-CB003)

The eukaryotic elongation factor eEF1A2 is considered to be the primary target for plitidepsin. The eukarotic elongation factor A2 (eEF1A2) is one of the two isoforms of eEF1A in mammalian cells. Expression of both proteins is mutually exclusive in different mammalian tissues, indicating that they should have distinctive functions: eEF1A1 is present almost ubiquitously and eEF1A2 expression is restricted to brain, muscles, heart, islet cells in the pancreas and endocrine cells in the gut of healthy individuals. In addition, many human tumours and transformed cell lines abnormally express eEF1A2, including multiple myeloma and plasmacytoma cells as well as prostate, pancreas, ovary, breast, lung and liver cancers, and eEF1A2 have pro-oncogenic activities, such as inhibition of apoptosis, alteration of cytoskeleton dynamics and modulation of signalling pathways. It was recently described that

down-regulation of eEF12 with miR-663 in pancreatic cancer cell lines reduced the level of the protein and attenuated their proliferation and invasion both in vitro and *in vivo*.

A comparative analysis was conducted in order to evaluate the differences in protein expression in a plitidepsin-resistant cell line (HeLa-APL-R) and the wild type HeLa cell line. HeLa-APL-R cells are highly resistant to plitidepsin compared to their parental HeLa cells (IC50 values of >100 nM vs.1 nM). eEF1A2 was about 8-fold under-expressed in HeLa-APL-R resistant cells as compared to wild type HeLa cells. This difference might provide an explanation for their different response to plitidepsin treatment. Ectopic expression of eEF1A2 in HeLa-APL-R restored the sensitivity to plitidepsin, as well as some of the signalling events induced by plitidepsin in tumour cells and absent in HeLa-APL-R, such as p38 and ERK phosphorylation.

In addition, it was determined that plitidepsin binds to eEF1A2 with high affinity (K_D = 80 nM) and the resulting complex dissociates with moderately slow off rate, suggesting that the residence time of the molecule in eEF1A2 is in the range of several minutes.

Generation of oxidative stress (8)

Cellular oxidative stress plays a crucial role in regulating the activation of MAPK and the downstream events leading to apoptosis. The proposed mechanism involves an alteration of glutathione (GSH) homeostasis by plitidepsin. GSH is crucial in the control of the cellular redox status. As seen in figure 2, plitidepsin increased the GSSG/GSH (oxidised/reduced glutathione) ratio in MDA-MB-231 cells only 5 minutes after starting the treatment, indicating the induction of an oxidising environment, thus generating oxidative stress. The increase of the GSSG/GSH ratio caused an increase in the levels of reactive oxygen species (ROS) and DNA oxidation. The main ROS source has not been clearly identified but it is likely related to the mitochondria respiratory chain.

Activation of Rac1 and MAPK family: effect on mitochondria and caspases activation (8-10)

In several tumour cell models, plitidepsin induced a specific cellular stress response program involving the rapid and sustained activation of two members of the MAPK family: the kinases JNK and p38 MAPK. This event was related to the induction of apoptosis and was observed in several tumour cell lines such as breast, kidney, and cervix carcinoma, as well as in leukemia and multiple myeloma cells, where a rapid and strong phosphorylation of both kinases was detected after treatment with plitidepsin, without altering their basal protein levels.

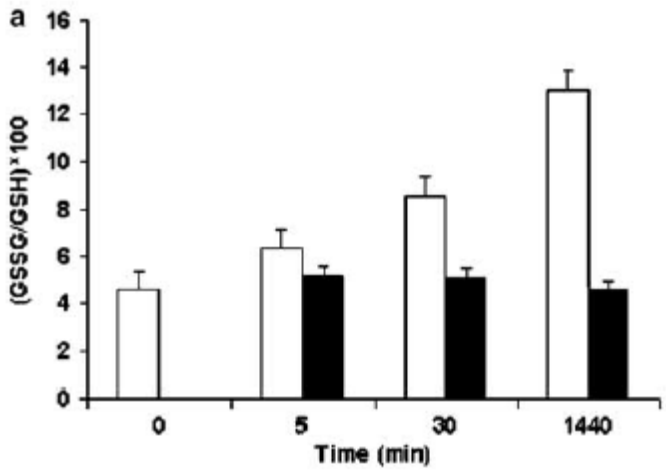


Figure 2 Ratio of the levels of GSSG and GSH in cells treated with 500 nM plitidepsin in the absence (white columns) or presence (black columns) of 40 mM Ebselen for the indicated times

Subsequent intracellular events include the activation of a protein-kinase C (PKC) and mitochondrial dysfunction with cytochrome c release observed in Jurkat, multiple myeloma 5T33MM, HeLa and HL-60 cells treated with plitidepsin. This suggests that plitidepsin may induce apoptosis via JNK-mediated mitochondrial stress and is also consistent with the activation of both the upstream pro-caspase 9 and the downstream effector pro-caspase 3. Comparable evidences were detected in a panel of multiple myeloma cells (figure 3), where active caspase-cleaved fragments were generated after cell exposure to plitidepsin. The drug-induced apoptotic effect is attenuated in the presence of caspase inhibitors.

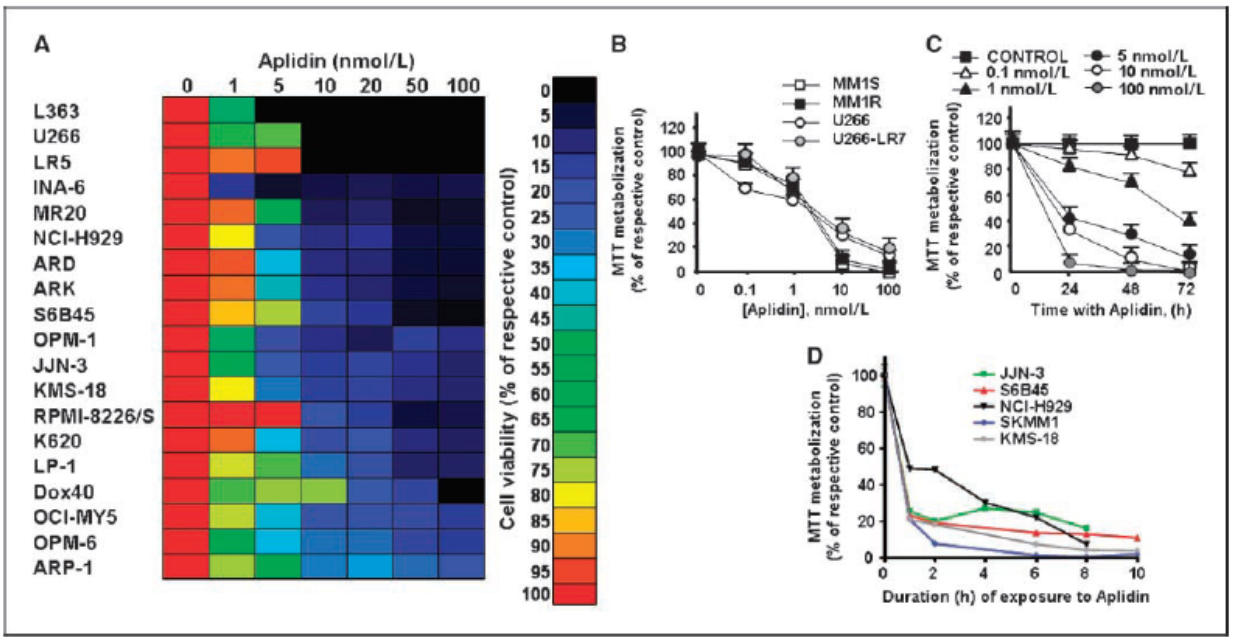


Figure 3 Activity of Aplidin on MM cell lines

A, dose-response matrix of human MM cell lines to Aplidin in vitro (0–100 nmol/L for 72 h). The viability of Aplidin-treated MM cells (expressed for each cell line as the percent of its respective control)is visualized in color format according to their values on a linear scale (0–100%). B, dose effect of Aplidin after 48 h of treatment in MM1S, MM-1R, U266, and U266-LR7 cell lines using the MTT uptake assay. The mean cell viability values are presented for each condition as percent of the respective control of each cell line. A similar pattern of decrease in cell viability in a dose-dependent manner was observed for the four cell lines tested. C, time course analysis of Aplidin-induced death of MM1S cells. D, results of cell death commitment experiments of five MM cell lines. MM cells were cultured in the presence of Aplidin (40 nmol/L)for 0 to 10 h and, after washout of the drug, were cultured for an additional 72 h in the presence of drug-free media to assess the shortest duration of exposure to Aplidin that could commit MM cells to eventual death. A 1-h exposure to Aplidin was sufficient, despite the subsequent absence of drug in the culture medium, to trigger a significant decrease in viability in the five MM cell lines tested.

Induction of endoplasmic reticulum stress (PMAR16-CB004)

As plitidepsin induces a strong oxidative stress, it was studied whether the drug could also induce and endoplasmic reticulum (ER) stress. ER is the site for folding of secreted and transmembrane proteins, and accumulation of unfolded or misfolded proteins in the ER induces stress, triggering the "unfolded protein response" (UPR) through the induction of its targets genes: genes for ER chaperones, ER-associated protein degradation genes, and genes involved in autophagy and apoptosis. Plitidepsin was able to activate several key molecular components of UPR, including the phosphorylation of eIF2α and JNK1, the protelytic processing of ATF6 and the alternative splicing of XBP1 in the cancer cell lines HeLa, MDA-MB-241 and MM1S. However, no induction was reported of the two pro-apoptotic proteins Noxa and CHP and the proteolytic activation of caspase 4.

Other effects at the molecular level

HeLa cells incubated with a fluorescent derivative of plitidepsin showed a drug accumulation at membrane level, corresponding to the formation of a complex plitidepsin-binding site (11). Plitidepsin is probably aggregated at the membrane in a system including stabilised lipid domains (lipid rafts) and a membrane protein. The formation of plitidepsin/eEF1A2 complexes was observed at the cell membrane of HeLa cells.

The action of plitidepsin at the cell membrane level showed evidence of Fas/CD95 involvement in plitidepsin-induced apoptosis. Proliferating lymphocytes, in which the expression of Fas/CD95 is significantly high, show a high degree of susceptibility to plitidepsin treatment and require low drug concentration to undergo apoptosis. In Jurkat cells, the incubation with anti-Fas monoclonal antibody inhibited cell apoptosis (12) .

In addition, the involvement of membrane cholesterol in the apoptotic cascade of events was investigated . In MDA-MB-231 human adenocarcinoma cells, the pre-treatment with a cholesterol depleting drug decreased plitidepsin binding to the cells, an effect reverted by the addition of exogenous cholesterol. Cholesterol depletion was correlated with a lack of activation of Rac1 GTPase, JNK and p38 kinases induced by plitidepsin, that was reversed by the addition of exogenous cholesterol.

Effects at gene level

In primary multiple myeloma tumour cells and in stromal cells, the gene expression profiling identified a number of transcripts that were significantly up- or down-regulated by plitidepsin treatment. These genes were involved in apoptosis/response to stress, control of the cell cycle/proliferation, kinases participating in cell cycle and survival signalling and cell adhesion (9).

Anticancer effects: *In vitro* studies

Non-comparative studies (13) (P62N-2008, P62N-2009, PUSA-SR007)

As shown in Table 1 and Table 2, antitumour activity was observed in several cancer cell lines exposed to plitidepsin on a short and long-term treatment schedule.

Table 1 Antiproliferative effects of plitidepsin after short-term exposure (1 hour)

Tumour	Plitidepsin (µM)			
	0.01	0.05	0.1	1.0
Breast	3/11	9/11	10/11	11/11
Melanoma	1/11	6/11	9/11	11/11
Ovarian	5/11	10/11	11/11	11/11
Stomach	1/3	2/3	3/3	3/3
NSCLC	1/4	4/4	4/4	4/4
NHL	3/3	3/3	3/3	3/3
Colon	2/3	3/3	3/3	3/3
Hodgkin Lymphoma	1/1	1/1	1/1	1/1
Total (%)	17/49 (35%)	40/49 (82%)	46/49 (94%)	49/49 (100%)

Number of inhibited specimens (< 50% survival of tumour colony forming units) over number of evaluable specimens.

Table 2 Antiproliferative effects of plitidepsin after long-term exposure (21-28 days)

Tumour	Plitidepsin (μM)				
	0.0001	0.001	0.01	0.1	1.0
Breast	0/7	3/7	12/13	12/12	12/12
Melanoma	0/10	6/10	11/12	12/12	12/12
Ovarian	0/3	0/3	11/11	11/11	11/11
Stomach	0/1	1/1	3/3	3/3	3/3
NSCLC	1/4	3/4	4/4	3/3	3/3
NHL	1/3	1/3	4/4	3/3	3/3
Colorectal	0/1	0/1	3/3	3/3	3/3
Sarcoma	ND	ND	2/2	2/2	2/2
SCLC	ND	ND	1/1	1/1	1/1
Hodgkin Lymphoma	0/1	1/1	1/1	1/1	1/1
Total (%)	2/30 (7%)	15/30 (50%)	52/54 (94%)	51/51 (100%)	51/51 (100%)

Number of inhibited specimens (< 50% survival of tumour colony forming units) over number of evaluable specimens.

A large study was carried out in 18 different tumour types derived from 115 human xenografts to assess the antiproliferative effect of plitidepsin in a clonogenic assay (Table 3). Tumour specimens were grown as cell suspension and treated for 20 consecutive days. The most plitidepsin-sensitive tumours were multiple myeloma, leukaemia, lymphoma, bladder cancer, lung cancer, breast, melanoma, pleural mesothelioma, renal cancer and sarcoma.

Table 3 Influence of plitidepsin on the viability of different tumour cell lines

Tumour	Antitumour Selectivity	
	More Sensitive[a]	Less Sensitive[b]
Bladder	3/6	2/6
Glioblastoma	1/1	0/1
Colon	1/11	5/11
Stomach	1/4	2/4
Head and neck	0/2	0/2
Leukaemia	2/2	0/2
Liver	1/1	0/1
NSCLC	7/21	4/21
SCLC	3/5	0/5

Tumour	Antitumour Selectivity	
	More Sensitive[a]	Less Sensitive[b]
Lymphoma	2/2	0/2
Breast	3/11	1/11
Melanoma	9/18	1/18
Myeloma	1/1	0/1
Ovary	1/4	2/4
Pancreas	2/9	3/9
Pleural mesothelioma	2/2	0/2
Kidney	4/11	1/11
Sarcoma	2/5	0/5
Total	45/115	21/115

[a]Individual IC_{70} > 1/3-fold mean IC_{70} of all tumours.
[b]Individual IC_{70}> 3-fold mean IC_{70} of all tumours.

Another set of experiments with cells exposed to plitidepsin for 72 h confirmed the activity of plitidepsin against a panel of 18 solid and non-solid human tumour cell lines, with the most sensitive being prostate, thyroid, lung, neuroblastoma, bladder, leukaemia and lymphoma.

Antiproliferative activity of plitidepsin was investigated in a wide panel of haematological tumour models Table 4). In this study, multiple myeloma and NHL were highly sensitive. Additional studies on acute myelogenous leukaemia cells provided confirmation of plitidepsin dose-dependent antiproliferative activity and comparable IC50 values were observed in samples from paediatric leukaemia patients.

Table 4 Plitidepsin antiproliferative effect on the viability of different haematologic cell lines

Tumour Type	Name	IC_{50} (µM)	Geom. Mean IC_{50} (µM)
ALL	CCRFCEM	0.0438	0.028
	CCRFCEM/VCR*	0.1571	
	JURKAT	0.0174	
	MOLT4	0.0052	
AML	HL60	0.0663	0.024
	KG1	0.0268	
	MV411	**0.0033**	
	NOMO1	0.0186	
	OCIAML2	0.0278	
	PL21	0.0652	
CML	EM2	0.1853	0.074
	JURLMK1	0.0299	
	K562	0.0572	
	KCL22	0.0511	
	MEG01	0.1387	
MM	IM9	**0.0029**	0.0077
	L363	**0.0032**	
	LP1	0.0166	
	MM1RL	**0.0026**	
	MM1S	**0.0033**	
	NCIH929	0.0503	
	RPMI8226	0.0245	
NHL	HUT78	**0.0036**	0.0078
	MYLA	**0.0015**	
	RAJI	0.0262	
	U937	0.0262	

Tumour Type	Name	IC_{50} (µM)	Geom. Mean IC_{50} (µM)
All cell lines		0.0190	0.0190

*Vincristine resistant subline of CCRFCEM.
Cells were continuously exposed to plitidepsin for 4 days.
Bold numbers indicate 5- to 13-fold value lower than mean IC_{50}.

Comparative studies (9, 13-15)

The antiproliferative effects of plitidepsin were compared to those of antitumour drugs currently used in chemotherapy in different human haematologic tumour cell lines treated for 24 h. Results are shown in table 5. Plitidepsin was 2 to 100-fold more potent than all other chemotherapeutic agents tested, with IC50 values in the range 1.5-63 nM. Comparative cytotoxicity tests were carried out on mononuclear cells from patients with acute myelogenous leukaemia, in which plitidepsin showed a significantly higher activity than idarubicin.

Table 5. Plitidepsin and other chemotherapeutic agents activity in haematologic tumour cell lines

Cell lines	IC_{50} (nM)				
	CCRF-CEM	SKI-DLCL	K-562	HL-60	HEL
Plitidepsin	1.5	1.5	15	34	85
Methotrexate	10	30	5	--	--
Doxorubicin	4	5	18	--	--
Idarubicin	--	--	75	184	122
Ara-C	10	30	30	--	--
Vinblastin	5	4	10	--	--
Mitoxantrone	30	5	7.5	--	--
Methylprednisolone	80	160	--	--	--
5-Fluorouracil	--	--	--	--	--
Paclitaxel	--	--	--	--	--

The antiproliferative activity of plitidepsin was also compared to that of doxorubicin an bortezomib. In clonogenic haematolopietic stem cells, plitidepsin showed 7-fold lower IC50 than doxorubicin. In multiple myeloma cells, plitidepsin inhibited cell proliferation with IC50 5-fold lower than bortezomib. The antiproliferative effect of plitidepsin did not impact the viability of bone marrow stromal cells.

Activity against resistant cells (9, 16)

The antiproliferative effect of plitidepsin was tested in human tumour cell lines poorly responsive to standard regimens or selected for resistance to other antineoplastic agents. In the MM1R multiple myeloma cell line resistant to dexamethasone, the coadministration of plitidepsin restored the sensitivity to dexamethasone and the activity was comparable to that of the parental, sensitive lines. In addition, plitidepsin -responsive cell lines included cells resistant to conventional anti-MM agents such as melphalan, mitoxantrone, and doxorubicin as well as cells with low sensitivity to immunomodulatory anti-MM agents such as thalidomide derivatives.

Cross-resistance tests in leukemia samples of plitidepsin with 20 cytotoxic drugs commonly used in the treatment of leukaemia only showed cross resistance with gemcitabine.

Plitidepsin-resistant cell lines (PMAR16-CB003, PMAR16-CB004, (17, 18)

Two human cancer cell lines (cervix and ovarian cancer) were treated with sub-lethal drug concentrations of plitidepsin and the corresponding resistant sublines were isolated and stabilised. Comparative analysis of plitidepsin-resistant cervix cancer cells and the sensitive parental HeLa line indicated an association between acquired resistance and absence of perturbations in the cell cycle as well as in signs of induced apoptosis. Acquired resistance to plitidepsin in HeLa cells is also correlated to altered expression of eEF1A2. Low levels of this protein characterize the resistant phenotype and restoration of plitidepsin sensitivity can be achieved in plitidepsin-resistant HeLa cells via transfection with an eEF1A2-containing plasmid.

In addition, marginal and transient activation of JNK and p38 MAPK, but no decrease drug accumulation could be detected in the plitidepsin-resistant cells. Decreased drug accumulation is often associated with resistance of tumour cells to anticancer drugs and frequently involves overexpression of drug efflux proteins, P-glycoprotein being the most common pump related to drug resistance. No involvement of the mechanism typically related to P-gp expression could be determined in plitidepsin-resistant HeLa cells.

On the contrary, in human ovarian IGROV-1 cells, cross-resistance with other drugs substrates of multidrug resistance has been reported. Plitidepsin-resistant IGROV-1 cells showed reduced sensitivity to anticancer agents VP16, doxorubicin, vinblastine, taxol and colchicine. The overexpression of the

multidrug transporter P-gp is proposed as the most relevant alteration responsible for the decreased response to plitidepsin treatment. Sensitivity was restored with P-gp inhibitor cyclosporine A, therefore supporting that resistance in this cell line could be related to a typical multidrug resistance phenotype.

In vivo studies (19, 20)

The antineoplastic activity of plitidepsin alone or in combination with antitumour agents was studied in different in vivo tumour models of human origin (combination studies are commented below). Plitidepsin was administered to a primary myelofibrosis model mice at 0.1 mg/kg/day for 5 consecutive days for 2 or 4 cycles 21 days apart. Treatment was well tolerated with acceptable toxicity (death) and morbidity (body weight reduction) and prevented development of myelofibrosis by partially restoring progenitor cell (megakaryocytes) and microenvironmental functions in the marrow.

Another study assessed the activity of plitidepsin in 44 xenograft models. Aplidin demonstrated limited activity against the in vivo solid tumour panels. While there were clearly treatment effects as demonstrated by significant differences in event free survival (EFS) distribution between treated and control animals, these effects were modest and in no case did the time to event for a treated group exceed that for a control group by a factor of two or greater. Aplidin showed greater activity against the acute lymphoblastic leukemia panel compared to the solid tumor panels, although the level of activity observed for Aplidin was less than that previously noted for standard agents (e.g., vincristine and cyclophosphamide).

Pharmacodynamic drug combinations

In vitro studies (9, 21, 22)

In multiple myeloma established cells and in cells isolated from patients, the interaction between plitidepsin and standard anti-myeloma agents showed synergistic (lenalidomide, thalidomide, bortezomib) or additive (dexamethasone, melphalan) effects. No increased toxicity was observed with concomitant administration of doxorubicin.

The addition of plitidepsin to traditional anticancer agents in myelogenous (HL-60) and lymphoblastic (CEM) leukaemia cell lines resulted in a synergistic combination with most of the tested drugs, particularly with Ara-C, daunorubicin, VP-16, dexamethasone and L-asparaginase.

In diffuse large cell lymphoma SKI-DLCL, the combination of plitidepsin with Ara-C, vinblastine or prednisolone showed a better efficacy than monotherapy. In all cell lines, plitidepsin potentiated the cytotoxicity of Ara-C and lowered its IC50 from 30 nM to 1 nM. An increased plitidepsin uptake promoted by the Ara-C concomitant treatment might be the basis of this synergistic interaction, which was confirmed in CEM and SK1-DLCL cells (Table 6).

Table 6 Effect of combination treatments with plitidepsin and different agents in leukaemic cell lines (CI values)

Drug	CEM	SKI-DLCL
Cytosine arabinoside.	0.49	0.31
Methotrexate	0.95	-
Mitoxantrone	0.91	0.65
Vinblastine	2.05	0.76
Doxorubicin	1.95	0.48
Methylprednisolone	0.35	0.30

CI < 1: synergism; CI = 1: additive effect; CI > 1: antagonism.

An increase in the cytotoxic effect of several antitumour agents such as doxorubicin, methotrexate, daunorubicin or Ara-C in the presence of plitidepsin was also observed in the acute myelogenous leukaemia K562 cells and acute myelogenous leukaemia cells from paediatric patients. The most relevant effect was observed when plitidepsin was added as post-incubation treatment.

The effects of the combination of plitidepsin and rituximab were assessed in different B-cell lymphoma cell lines, including Diffuse Large Cell Lymphoma (DLCL) lines and Burkitt cell lines. An enhanced antitumor activity compared with either agent alone was observed. Low levels of plitidepsin and rituximab when used alone induced apoptosis in Ramos (Burkiitt's lymphoma cell line) and the individual doses that produced partial apoptosis produce almost complete apoptosis in combination.

In vivo studies (FSR-3-9-09, SRI-11800-01, PUSA00634, (21, 23)

The combination of plitidepsin with dexamethasone was evaluated in three murine MM models (5 doses/week for 3 weeks). The activity of the combination was greater than that of each single drug in monotherapy.

In addition, more than an additive effect was described in RPMI-8226 MM xenografts treated with plitidepsin and bortezomib. However, the combination of plitidepsin with melphalan resulted in higher toxicity. In the acute leukaemia CCRF-CEM model, plitidepsin increased the antitumoural effect of Ara-C.

When evaluated in Ramos (Burkitt lymphoma) xenograft bearing mice, rituximab and plitidepsin alone were able to inhibit tumor growth and the combination produced additive effects, using a low minimally effective dose of rituximab (200 µg/kg). Of interest, the low dose of plitidepsin (200 µg/kg) with rituximab was more effective than the combination with the higher plitidepsin dose (400 µg/kg). With such low doses (200 µg/kg rituximab, 200 µg/kg, significant clinical toxicity would be unlikely from this combination.

Similar additive effects have been described with the combination of plitidepsin and sorafenib in three human renal xenografts, including A498 renal cancer refractory to plitidepsin treatment. The combination produced higher antitumour activity than monotherapy particularly when using the highest sorafenib dose.

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies have been conducted (see discussion on non-clinical aspects).

Safety pharmacology programme

Cardiovascular system

Treatment with plitidepsin at a target concentration of 1 µM for 15 min produced no inhibition of HERG tail current in HEK293 cells stably transfected with HERG cDNA (SPH03-032, GLP).

In dog isolated cardiac Purkinje fibers, exposure to 10, 100, and 1000 nM plitidepsin had no effect on action potential morphology. Plitidepsin is not expected to cause QT prolongation in this concentration range (SPP03-014, GLP).

In spontaneously beating guinea pig hearts (Langendorff preparation), infused with increasing concentrations (0.01 µM-10 µM) of plitidepsin, lengthening of PQ, QT and QTc, decreases of contractility and lusitropy, as well as an increase in coronary resistance were seen (SPLG06-003, GLP). The changes were, in general, rather modest, beginning at 1 µM and increasing through the maximal concentration 10 µM. NOAEL = 0.1 µM, although the changes at 1 µM were small.

The effect of plitidepsin on cardiovascular parameters was evaluated in conscious, telemetered dogs (4 male) (SPT03-020, GLP). Animals received plitidepsin (0.01, 0.03 mg/kg) according to the following schedule:

Table 7 Description of study SPT03-020

Dose Day	Number of animals	Route of administration	Dose-level, mg/kg (mg/m^2)
1	1	iv bolus [a]	0.0
2	1	iv, 3h infusion[b]	0.0
3	3	iv, 3h infusion[b]	0.0
4	4	iv, 3h infusion[b]	0.01 (0.2)
5	4	iv, 3h infusion[b]	0.03 (0.6)
6	4	iv, 3h infusion[b]	0.1 (2)[c]

[a]Vehicle, polyoxyl 35 castor oil/ethanol/water (15/15/70): saline (10:90; v:v).
[b]Vehicle, polyoxyl 35 castor oil/ethanol/water (15/15/70): saline (1:99; v:v).
[c]Dose level originally scheduled but not administered.

On each dose day, collection of hemodynamic and ECG data commenced 30 min before dosing and was continuously recorded for the first 8 h after dosing. Data acquisition was then restarted to capture the 24 and 48 h post-dose time points. The endpoints were systolic blood pressure, diastolic blood pressure, heart rate, ECG variables (QRS duration, RR, QT, PR intervals).

Plitidepsin at 0.01 and 0.03 mg/kg did not affect blood pressure. Tachycardia was observed form 2 to 48 h after administration of the 0.03 mg/kg dose and consequently, decreases in the PR interval, QRS duration, RR interval and uncorrected QT interval were also observed. There were no significant effects on QTcF at any of the doses, only small decreases in QTcQ were detected in the high dose group at 2 and 6 h post-dose. ECG did not show abnormalities consistent with CV toxicity during plitidepsin infusion or up to 8 h after drug administration.

The effects on blood pressure, heart rate, electrocardiogram and body temperature after single intravenous administration to conscious dogs was evaluated in study 20140077PCCP (GLP). Male and female beagle dogs implanted with telemetry transducers were treated with plitidepsin at MTD (0.13 mg/kg) according to the following design:

Table 8 Description of study 20140077PCCP

Dose Day	Number of animals	Route of administration	Dose-level, mg/kg (mg/m^2)
1	3M+3F	iv, bolus [a]	0.0 [b]
3	3M+3F	iv, bolus [a]	0.0 [c]
8	3M+3F	iv, bolus [a]	0.13 (2.6)

M, male; F, female.
[a] Dosing time, 3 min.
[b] Saline.
[c] Vehicle, polyoxyl 35 castor oil/ethanol/water (15/15/70): saline (10:90; v:v).

Animals were monitored for blood pressure, heart rate, ECG, conduction times (PR, PQ, QT intervals and QRS complex) and ST-segment, as well as for clinical signs, including body weight and temperature.

During the first 15-30 min after dosing, plitidepsin produced transient, marked hypotension, bradycardia and ST-segment depression. These findings could be caused by initial marked hypotension with tachycardia that lead to subendocardial ischaema due to hypoperfusion during the hypotension period, and ST-segment depression is a sign of ischaemia. During the 30 min to 4 h post-dose interval, blood pressure returned to baseline values; however tachycardia and ST-segment depression were still recorded up to 4 h post-dosing. During the 4-8 h post-dose period, findings were a moderate tachycardia and short term QT interval variability, likely caused to the former ischemic event, as well as QT prolongation with no signs of arrhythmia, slight amplitude of QT prolongation and PR and PQ lengthening. An increased HF/LF rhythm ratio was also recorded, suggesting an increase in vagal tone and/or reduction in sympathetic activity. During the 8-24 h post-dose interval, plitidepsin-treated dogs experienced moderate hyperthermia, tachycardia, QT, PR and PQ shortening and reduction in parasympathetic HF rhythms; all these changes are consistent with a suppression of normal variations on these parameters.

Respiratory system

Sprague Dawley rats (8 male/group) were administered plitidepsin as a single IV bolus injection at 0.15, 0.50, 1.50 mg/kg (SPR04-001, GLP). The effects of plitidepsin on respiratory function were assessed by recording changes in respiratory rate and/or tidal volume at 15, 60 min and 24 h post-administration. No effects were observed at the lower doses, but significant decreases in tidal volume and respiratory rate were detected at 24 hours post-dosing in rats treated with 1.50 mg/kg of plitidepsin.

Neurotoxicity and CNS

The rat clonal pheochromocytoma cell line PC12 can be induced to display neurite outgrowth by nerve growth factor and it is typically inhibited by neurotoxic agents. Plitidepsin was incubated with PC12 cells at doses of 4-1000 ng/mL(Geldof 1999, non GLP). A concentration-dependent neurotoxic effect was observed, although it was considered not significant at drug concentrations of plitidepsin showing antitumour activity in vitro.

Sprague Dawley rats (6 male/group) were treated with a single IV dose of plitidepsin at 0.15, 0.50 and 1.50 mg/kg and their gross behavioural and physiological state was assessed by recording changes based on the Irwin test different times after dosing (SPI04-001, GLP). No effects were observed at 0.15 and 0.5 mg/kg. In rats treated at the highest dose of 1.5 mg/kg, occasional cage dispersion, increased cutaneous blood flow, diarrhoea and vocalization were observed up to 120 min post-dose. At 24 h post-dose, decreased grooming, piloerection, abnormal gait, decreased touch response, passivity, hypothermia and decreased locomotor activity were observed. In general, the clinical signs were slight and appeared to be treatment-related.

Haematoxicity

The potential haematoxicity of plitidepsin was evaluated *in vitro* in samples from human bone marrow and cord blood, which were exposed to increasing concentrations of plitidepsin Albella 2002, non GLP). The clonogenic ability of myeloid (CFU-GM), erythroid (BFU-E), megakaryocytic (CFU-Meg) and pluripotent (CFU-Mix) haematopoietic progenitors was then assayed. Results suggested that the CFU-GM and BFU-E progenitors were the least sensitive to plitidepsin. Regardless of the origin of the haematopoietic progenitors, the toxicity of plitidepsin in human haematopoietic progenitors (IC50 = 150-350 nM) was higher than that observed with tumoural cell lines.

Hepatotoxicity

To explore the potential *in vitro* hepatotoxicity of plitidepsin, normal human hepatocytes obtained from liver biopsies were exposed to 10 or 100 nM of plitidepsin for 24 h (Gajate 2003, non GLP). At drug concentrations toxic for cancer cells, the hepatocyte viability remained at 100 and 99%, respectively, suggesting that plitidepsin would not likely be toxic for human hepatocytes.

Pharmacodynamic drug interactions

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

2.3.3. Pharmacokinetics

All drug metabolism and pharmacokinetics (DMPK) studies were conducted in accordance with the applicable Standard Operating Procedures of the respective test facilities. Pivotal toxicokinetic studies were conducted in accordance with GLP.

Blood and plasma levels of plitidepsin have been evaluated in mouse (PMAR13-NC008), rat (PMAR13-NC007, PMAR15-NC008) and dog (PMAR13-NC0110) following i.v. bolus dosing. Also, relevant data were obtained from companion TK analyses conducted in support of single (dogs, 8293-093) or repeated dose toxicology studies in rats and dogs (RTC70760, 8293-094, 8293-095).

See Table 9 for overview of representative blood and plasma single dose PK parameters in animals, and Table 10 and Table 11 for PK parameters following repeated i.v. dosing in rat and dog, respectively.

Table 9 Overview of plitidepsin pharmacokinetic parameters determined in blood and plasma from mouse, rat and dog following single i.v. dose administration

Study ID	Species	N M/F (no per timepoint)	Dose (mg/kg)	Anal.	Cmax (ng/ml)	AUC_{0-48h} (ng.h/ml)	Cl (l/h.kg)	t1/2 (h)	Vdss (l/kg)
PMAR13-NC008	Mouse/ CD-1	50 F (5)	0.5	Plasma	24.9	148.5	0.8	135.5	149.7
				Blood	519.8	3829.1	0.1	16.3	3.0
PMAR13-NC007	Rat/SD	48 F (5)	0.2	Plasma	6.5	38.4	2.1	58.7	183.3
				Blood	341	2194.5	0.0.8	19.3	1.9
PMAR15-NC008	Rat/SD	48 M (5)	0.2	Plasma	4.3	13.4	9.5	31.6	423.3
				Blood	1047.2	7801.9	0.02	15.8	0.5
PMAR13-NC011	Dog/ Beagle	2 M, 3 F	0.01	Plasma	0.7/0.8	1.1/1.1[a]	7.1/6.6	3.9/4.6	36.1/39.6
				Blood	7.2/6.5	28.9/31.0[a]	0.2/0.3	38.8/33.3	12.8/9.9

[a]: AUC_{0-t}

Table 10 Mean plasma plitidepsin pharmacokinetic parameters in SD rat (n=9/group/gender) after 12 repeated cycles (one dose every 2 weeks) of plitidepsin i.v. bolus administration (RTC70760)

Gender	Dose Group	Dose (mg/kg/mg/m^2)	Dose Day[a]	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)
Male	Low Dose	0.1/0.6	Day 1	0.8	3.5
			Day 71	0.9	4.5
			Day 155	1.1	5.2
	Mid Dose	0.2/1.2	Day 1	1.5	7.1
			Day 71	1.8	9.2
			Day 155	1.6	10.4
	High Dose	0.4/2.4	Day 1	2.7	14.0
			Day 71	4.3	23.0
			Day 155	3.1	21.4
Female	Low Dose	0.1/0.6	Day 1	0.7	3.6
			Day 71	1.0	3.9
			Day 155	0.9	3.8
	Mid Dose	0.2/1.2	Day 1	1.3	6.0
			Day 71	1.8	8.2
			Day 155	1.4	9.0
	High Dose	0.4/2.4	Day 1	2.4	13.3
			Day 71	3.8	22.3
			Day 155	3.3	23.7
[a]Dose given on days 1, 15, 29, 43, 57, 71, 85, 99, 113, 127, 141 and 155. Cmax at 0.5 h post-dose.					

Table 11: Mean (±SD) pharmacokinetic parameters in Beagle dogs (n=6/group/gender) after 6 repeated cycles (one dose every 2 weeks) of plitidepsin i.v. bolus administration (8293-094)

Sample	Gender	Dose Group	Dose (mg/kg)	Dose Day[a]	Cmax (ng/mL)	AUC0-t (ng·h/mL)	AUC0-∞ (ng·h/mL)
Plasma	Male	Low dose	0.1	Day 1	11.8±1.1	8.3±2.8	11.0±3.5
				Day 71	$_5$8.8±1.6	$_5$13.2±2.2	$_5$31.0±15.4
		Mid dose	0.13	Day 1	11.5±1.9	15.4±6.0	26.7±15.7
				Day 71	$_4$10.7±3.3	$_4$18.7±5.5	$_4$53.2±34.9
		High dose	0.16	Day 1	21.1±8.9	26.3±1.3	47.2±13.1
				Day 71	$_1$14.7	$_1$29.4	$_1$46.0
	Female	Low dose	0.1	Day 1	9.2±1.2	10.8±5.3	21.5±18.2
				Day 71	12.3±2.6	20.3±9.0	36.9±20.8
		Mid dose	0.13	Day 1	11.7±2.9	16.4±4.3	27.7±9.4
				Day 71	$_5$8.6±1.7	$_5$12.4±6.6	$_5$20.8±11.8
		High dose	0.16	Day 1	16.2±7.4	25.7±6.6	48.1±15.2
				Day 71	10.6±2.8	17.5±2.8	28.0±9.0
Blood	Male	Low dose	0.1	Day 1	26.8±8.3	93.8±20.9	215.3±64.7
				Day 71	$_5$25.9±14.3	$_5$113.7±21.6	$_5$192.2±47.8
		Mid dose	0.13	Day 1	29.0±10.0	133.7±28.1	189.9±54.8
				Day 71	$_4$53.0±27.1	$_4$207.2±76.3	$_4$354.1±222.7
		High dose	0.16	Day 1	27.2±22.9	114.7±69.6	190.4±76.8
				Day 71	$_1$11.3	$_1$148.6	NC
	Female	Low dose	0.1	Day 1	29.0±11.5	136.7±47.8	235.2±104.3
				Day 71	25.4±7.6	133.4±22.0	254.2±49.4
		Mid dose	0.13	Day 1	24.7±8.4	103.9±31.6	202.3±128.3
				Day 71	$_5$36.4±22.8	$_5$190.2±67.0	$_5$282.7±124.6
		High dose	0.16	Day 1	38.1±12.5	209.1±58.1	326.9±90.9
				Day 71	39.1±15.4	300.5±60.1	472.5±115.8[b]

[a]Dose given on days 1, 15, 29, 43, 57 and 71.
[b]One animal was excluded from group mean calculations.
Subscripts indicate the sample size (n) when different from that given.
NC, Not Calculated

A study was performed by using $^{14}C_2$-plitidepsin and liver microsomal fractions from male mouse (CD-1), rat (SD), dog (Beagle), non-human primate (NHP; Cynomolgus), mini-pig (Göttingen), rabbit (New

Zealand) and human (mixed pool from both genders). Microsomes were incubated with $^{14}C_2$-plitidepsin (at 5 µM, 5500 ng/mL); human microsomes were also incubated with 0.5 and 1 µM (550 and 1,000 ng/mL, respectively).

Three metabolites of $^{14}C_2$-plitidepsin were identified in humans and NHP although other not relevant minor peaks were identified (Table 12). In addition, the presence of other very minor peaks was confirmed (but not quantified) in all animal species. This data confirmed that the product undergoes microsomal metabolization.

Table 12 Summary of the remaining quantity (as percentages) of ^{14}C2-plitidepsin and its metabolites found in several animal species

	Human	NHP	Mini-pig	Dog	Rabbit	Rat	Mouse
^{14}C-plitidepsin	69.9	83.8	76.6	91.1	79.9	97.4	95.2
Metabolite 1	11.3	8.3	13.3	6.1	10.7	2.6	4.8
Metabolite 2	12.9	2.0	-	-	-	-	-
Metabolite 3	6.0	5.9	10.1	2.8	9.4	-	-

In the rat, the excretion of 14C-plitidepsin-related radioactivity occurred principally via the faeces (ca. 61 % of total radioactivity in both genders) with only a minor fraction recovered from the urine (circa 5 %). Biliary excretion of 14C-plitidepsin-related radioactivity was confirmed in bile duct cannulated Sprague-Dawley rats.

In patients, the radioactivity related to 14C-plitidepsin administration was mainly found in faeces (circa 70 %) and, to a lesser extent, in urine (circa 6%).

No studies have been conducted to assess the excretion of plitidepsin into milk.

2.3.4. Toxicology

Single dose toxicity

An overview of single dose toxicity studies conducted with plitidepsin is displayed in Table 13

Table 13 Overview of single dose toxicity studies performed with plitidepsin

Study ID: 170/1/2/97 **GLP: Yes**	**Species: MF1 mouse** **Sex & number: 4M/group**	**Dose: 4.8-5.4-6.0-6.6-7.2 mg/m²** **Route: i.v. bolus**
MTD: 5.4 mg/m² All: piloerection, inactivity, tail lesions		
Study ID: 3170/1/2/97 **GLP: Yes**	**Species: CD1 mouse/** **Sex & number: 20M/group**	**Dose: 0 (saline)–2.7–5.4 mg/m²** **Route: i.v. bolus**
Evaluation day 3 5.4: Inactivity, ↓bw, ↑RBC, ↑Haemoglobin, ↑Haematocrit, ↑WBC, ↑neutrophils, ↑ASAT, ↑ALAT, ↑ASAT, ↑creatinine, ↓kidney wt, ↓testes wt, bone marrow depletion, liver cell necrosis, bone marrow congestion, lymphocyte necrosis in LN and spleen, thymus (lymphocyte necrosis, atrophy cortical) ≥2.7: Unscheduled death, tail lesions, ↓ platelets, ↓lymphocytes, ↓eosinophils, ↓ALKP, ↓spleen wt, ↓thymus wt, stomach (crypt cell necrosis, ulcer glandular mucosa, dilated glands) All: Piloerection		
Evaluation day 14 2.7: ↓RBC, ↓haemoglobin, ↓haematocrit, ↓MCV, ↑MCHC, ↓WBC, ↓Lymphocytes, ↑Eosinophils		
Evaluation day 28 2.7: ↑testes wt		
Study ID: 3170/2/2/98 **GLP: Yes**	**Species: MF1 mouse** **Sex & number: 4M/group**	**Group 1:** **Dose: 1.8-3.0-4.2-5.4-6.6 mg/m²** **Batch A** **Vehicle: PET:saline** **Route: i.v. bolus**

		Group 2: **Dose: 1.8-3.0-4.2-5.4-6.6 mg/m²** **Batch BVehicle: PET:saline** **Route: i.v. bolus**
		Group 3: **Dose: 1.8-3.0-4.2-5.4-6.6 mg/m²** **Batch CVehicle: Cremophor EL:Etanol:WFI** **Route: i.v. bolus**
Group 1: LD50: 5.97, MTD:4.875 Group 2: LD50:4.14, MTD:3.0 Group 3: LD50:4.74, MTD:3.75		
3170/3/2/98 **GLP: Yes**	**Species: MF1 mouse** **Sex & number: 20M/group**	**Dose: 0 (saline)-1.875-3.75 mg/m²** **Route: i.v. bolus**
Evaluation day 3 ≥0.1875: Haematology (↑monocytes), ↓ALKP, ↓ALAT, ↓Urea, ↑liver wt, stomach (crypt cell necrosis, dilated glands) 3.75: Haematology (↑MCV, ↓platelets, ↓lymphocytes), ↓spleen wt, ↓thymus wt, bone marrow depletion, bone marrow congestion, lymphocyte necrosis Peyer's patches, centrilobular hypertrophy in liver, lymphocyte necrosis in LN and thymus, atrophy cortical in thymus		
Evaluation day 14 ≥0.1875: haematology (↑eosinophils) 3.75: haematology (↓RBC, ↓Haemoglobin, ↓haematocrit, ↑neutrophils, ↓lymphocytes)		
Evaluation day 28 ≥0.1875: ↑ASAT 3.75: haematology (↓WBC, ↓lymphocytes)		
Study ID: 3170/4/2/98 **GLP: Yes**	**Species: Wistar Rat** **Sex & number: 4M+4F/group**	**Dose: 1.8-3.0-4.2-5.4-6.6 mg/m²** **Route: i.v. bolus**
≥1.8: Red staining around nose (F), ≥3.0: Piloerection, Inactivity (F), Red staining around nose and on the neck/head ≥4.2: Inactivity		
MTD: 3.42		
Study ID: 3170/4/2/98 **GLP: Yes**	**Species: Wistar Rat** **Sex & number:10M+10F/group**	**Dose: 0-0.342-3.42 mg/m²** **Route: i.v. bolus**
Evaluation day 3 ≥0,342: Clinical chemistry (↓ALKP (M)) 3.42: ↓BW, Haematology (↓WBC, ↓Lymphocytes, ↓Basophils (M)), Clinical chemistry (↓ALKP, ↓ALAT, ↓ASAT, ↑Urea), ↓Spleen wt, ↓Thymus wt, ↓Ovaries (F), Histopathology (Bone marrow congestion, lymphocyte necrosis Peyer's patches, lymphocyte necrosis in mesenteric LN, lymphocyte necrosis white pulp in spleen, crypt cell necrosis in stomach, lymphocyte necrosis in thymus)		
Evaluation day 14 ≥0,342: 3.42: Haematology (↓RBC, ↓haemoglobin, ↓haematocrit, ↑platelets, ↓WBC, ↓lymphocytes, ↑monocytes (F)), Clinical chemistry (↓creatinine (M)), ↓Liver wt (M)		
Evaluation day 28 ≥0,342: Haematology (↓platelets (M)) 3.42: Haematology (↓RBC (M), ↓haemoglobin, ↓haematocrit (M)		

Study ID: 8293-092 **GLP: No**	**Species: Beagle dog** **Sex & number: 1M+1F/group**	**Dose: 0-0.4-0.8-1.6-2.0-2.2-2.4-2.6-3.6 g/m²** **Route: i.v. bolus**
≥0.4: Vomiting (M), red hind leg (M) ≥0.8: Soft/liquid faeces (M), ≥1.6: Red, swollen and indurated hind leg. ↓BW ≥2.0: Swollen hind leg (M), indurated hind leg (M), ↑AST, ↑ALT, ↑Total bilirubin, ↓Cholesterol, ↓Food consumption ≥2.2: Reduced activity (M), ↓platelets, ↓reticulocytes, ↑PT, ↑APTT, ↑Fibrinogen ≥2.4: Vomiting, posterior paresis (M) ≥2.6: Tremors (M) 3.6: Reduced activity, cold to touch, pale ears, gums and eyes, reduced activity, thinness, ↑WBC, ↑neutrophils, ↑monocytes, ↓lymphocytes, ↓eosinophils, ↑Total bilirubin, ↑lung wt		
MTD: 2.6		
Study ID: 8293-093 **GLP: Yes**	**Species: Beagle dog** **Sex & number: 4M+4F/group**	**Dose : 0-2.0-2.6-3.2 mg/m²** **Route : i.v. bolus**
≥0: Red appearance, swollen appearance, head shaking, head wrinkled, depressed ST wave, Inflammatory cell foci (atrial epicardium) ≥2.0: Vomiting, tachycardia (minor), ↑APTT (M), ↑Fibrinogen (M), ↓Cholesterol (M), ↓Triglycerides, ↓Thymus wt, Red injection site (F), Thymus (small), Injection site (chronic inflammation, focal minimal necrosis) (F), Acinar cell degeneration and degranulation in pancreas, Atrophy of thymus (F), Centrilobular clear cell change in liver (M), Atrophy of thymus ≥2.6: ↑APTT, ↓platelets (F), ↓Reticulocytes, ↑Fibrinogen, ↓Calcium (M), ↓ALT (M), Injection site (chronic inflammation, focal minimal necrosis), Centrilobular clear cell change in liver 3.2: Posterior paresis (M), reduced activity, tremors of forelegs (M), ↓BW, ↓Food consumption, ↑HR, ↓platelets, Mucosal haemorrhage (F), Red jejunum (M), Red Thymus (F)		
MTD: 2.6		

Repeat dose toxicity

An overview of repeat dose toxicity studies performed with plitidepsin is displayed in **Table 14**

Table 14 Overview of repeat dose toxicity studies performed with plitidepsin

Study ID	Species	Dose/Route	Duration	MTD (mg/kg)	Major findings
3170/5/5/98 GLP	Mice 20♂/group	IV 0.11, 0.22 mg/kg/day	5 days 28 recovery	0.625 mg/kg/day	Piloerection, tail lesions ↓body weight,. ↓ALP, urea, RBC, MCV, haemoglobin, haematocrit, ↑neutrophil. ↑liver, ↓thymus. Extramedullar haematopoiesis, lymphocyte necrosis in lymph nodes and thymus, lymphocyte depletion, thymus atrophy.
3170/6/2/98 GLP	Rats 10/sex/group	IV 0.014, 0.140 mg/kg/day	5 days 28 recovery	0.14 mg/kg/day	Piloerection, hunched posture, tail lesions, ↓body weight. ↓RBC, WBC, haemoglobin, haematocrit. ↑liver, ↓thymus, spleen, testes/ovary. Bone marrow depletion, lymphocyte necrosis and depletion in ileum, lymph nodes, spleen, thymus.

TRL 421 GLP	Rats 10/sex/group	IV 0.035, 0.070, 0.140 mg/kg/day	3 cycles of 5 days, 2 weeks washout	0.035 mg/kg/day	Mortality at the highest dose. Ataxia, letargy, dehydration, hunched posture, piloerection, tremours, laboured breathing. Lesions in the site of injection. ↓body weight and food consumption. ↓ALP, ALT, cholesterol, triglycerides, creatinine, RBC, reticulocyte, haemoglobin, haematocrit, MCV, WBC. ↓testes/epididymis. Histopathology: findings in pancreas, thymus, spleen, mesenteric lymph nodes, bone marrow, stomach, liver, mammary glands, adrenal glands, parathyroid glands, salivary glands, pituitary glands, testes/epididymis, skeletal muscle, heart, injection site.
RTC 70760 GLP	Rats 15/sex/group	IV 0.1, 0.2, 0.4 mg/kg/dose	1 dose/2 weeks for 24 weeks, 4 week recovery	0.2 mg/kg/dose	Mortality. Ataxia, letargy, dehydration, hunched posture, piloerection, leaning to one side. Lesions in the site of injection. ↓body weight and food consumption. ↑thrombocytes, reticulocytes, eosinophils. ↓PTT. Anemia, leukopenia. ↑Cholesterol, triglycerides, albumin, phosphorus, eleectrolytes, BUN. ↓Globulin, glucose, creatinine. ↓Kidneys, testes, thymus. ↑Adrenals, brain, heart, liver. Histology findings in liver (periportal fibrosis), thymus (lymphoid depletion), spleen (extramedullary haematopoiesis), pancreas (acinar cell degranulation and apoptosis).
TRL 291 GLP	Dogs 2/sex/group	IV 0.008, 0.020, 0.040 mg/kg/day	5 days, 28 recovery	MTD>0.04 mg/kg NOEL= 0.008 mg/kg	GI damages, ↑BUN, ALT, AST. ↓Cholesterol, glucose, total serum protein, albumin. ↑APTT, PT. Bone marrow atrophy, thrombocytopenia, anaemia. Thymus: necrosis, atrophy. Pancreas: necrosis, inflammation. Axonal swelling.
TRL 419 GLP	Dogs 3/sex/group	IV 0.01, 0.02, 0.03 mg/kg	3 cycles of 5 days, 2 weeks washout	MTD=0.02 mg/kg NOEL= 0.010 mg/kg	Hypersensitivity, vomit, diarrhoea, dehydration, letargy. ↓Body weight. ↓triglycerides, cholesterol, creatinine. Pancreas: necrosis, material depletion. Tonsils: necrosis. Liver: periportal vacuolation, cholestasis, apoptotic necrosis. Bone marrow: haematopoietic tissue depletion. Axonal atrophy. Degeneration in testes and sperma in the epididymis.
8293-094 GLP	Dogs 6/sex/group	IV 0.10, 0.13, 0.16 mg/kg	1 dose/2 weeks for 6 weeks, 4 week recovery	MTD < 0.10 mg/kg/2 weeks	Mortality: pancreatic injury, thymus atrophy, myocardial degeneration. Emesis, salivation, inflammatory lesions at the injection site. ↓Body weight, food consumption. Tachycardia, ST depression. ↓RBC, haematocrit, haemoglobin, MCV, ↑ RDW. Thrombocytopenia, leukocytosis. ↑PT, fibrinogen. ↓Cholesterol, triglycerides, urea, creatinine, total protein, albumin. ↓Ca, K, ↑P. ↑AST, ALT, CPK. ↑urinary volume. ↓thymus, pancreas weight. ↑spleen, kidney. Atrophy of acinar cells and islets of Langerhans in pancreas, reduction of acinar cells. Thymic atrophy, bile duct proliferation, hepatocytic vacuolation. Fatty infiltration in skeletal muscle or heart. Vacuolation of retina and distal tubular cells in the kidneys. Chronic inflammatory reaction at injection site.

8239-095 GLP	Dogs 6/sex/group	IV 0.050, 0.075, 0.100 mg/kg	1 dose/2 weeks for 12 weeks, 4 week recovery	MTD= 0.075 mg/kg/2 weeks	Emesis, lesions at the injection site. ↓Body weight and food consumption. Tachycardia, bradychardia, ST depression. Anaemia, ↓haemoglobin, haematocrit, MCV, ↑RDW. Thrombocytopenia, leucocytosis, fluctuations in reticulocytes. ↑PT, fibrinogen. . ↓Cholesterol, triglycerides, creatinine, total protein, albumin. ↓Ca, ↑ K. ↑AST, ALT, CPK, ↓alkaline phosphatase, trypsinogen. ↑urinary volume. ↓thymus, pancreas weight. Exocrine and endocrine atrophy and chronic inflammatory reaction in pancreas. Thymic atrophy and lymphoid depletion of spleen. Liver: centrilobular hepatocytic clear cell changes and bile duct proliferation. Kidneys: vacuolation of tubular cells and tubular cell degeneration.

Genotoxicity

An overview of genotoxicity studies conducted with plitidepsin is displayed in Table 15.

Table 15 Genotoxicity studies conducted with plitidepsin

Test system	**Endpoint**	**Dose/Concentration range**	**Result**	**Study number** **GLP status**
In vitro				
Salmonella typhimurium TA98, TA100, TA1535 and TA1537 *E.coli* WP2 uvrA	Gene mutation	10 to 5000 µg/plate (minus and plus S9) Presipitation at 5000 µg. 5000 µg/plate: only *S.typhimurium*	No mutagenicity	GA98AK67.502 GLP
Salmonella typhimurium TA98, TA100, TA1535 and TA1537 *E.coli* WP2 uvrA	Gene mutation	10 to 5000 µg/plate (minus and plus S9) Presipitation at 5000 µg.	No mutagenicity	RTC 73010 GLP
TK +/- Mouse lymphoma L5178Y cells	Gene mutation	3h incubation: 21.9 to 5600 ng/ml (minus and plus S9) 24h incubation: 0.00153 to 25.0 ng/ml (minus S9)	Increased mutagenicity frequency with and without S9 metabolic activation. Cytotoxicity: Severe from 1400 and 350 ng/ml for S9+ and S9-, resepctively	RTC 73020 GLP

Carcinogenicity

No carcinogenicity studies have been conducted with plitidepsin (see discussion on non-clinical aspects).

Reproduction Toxicity

Table 16 Reproductive and developmental toxicity studies conducted with plitidepsin

Study type/ Study ID / GLP	Species; Number Female/ group	Route & dose	Dosing period	Major findings	NOEL (mg/kg)
Male fertility 2716-001 GLP	Rats, 25/sex/group	IV 0.005, 0.015, 0.03 mg/kg/day	28 days before cohabitation until sacrifice	↓weight of testes, epididymis, prostate and seminal vesicle.	0.005 mg/kg for general toxicity 0.015 mg/kg for male reprotox
Female fertility 2716-001 GLP	Rats 25/sex/group	IV 0.005, 0.015, 0.03 mg/kg/day	15 days before cohabitation – GD7	↓ litter averages for corpora lutea, implantations and viable embryos.	0.015 mg/kg
Embryo-fœtal development RTC X0090 GLP	Rats 6 ♀/group	0.6 mg/kg	Single dose on Day 10 post coitum	Totally resorted foetuses. Maternal toxicity.	F0: MTD < 0.6 mg/kg F1: NA

Toxicokinetic data

The exposures expressed as the relative ratio of Cmax and AUC in rat and dog in plasma and blood at their respective MTD, compared to the exposure values in patients following a 5 mg/m^2 dose of plitidepsin are shown in Table 17. In both species the exposure in plasma and blood was much lower than in patients.

Table 17 Plitidepsin exposure in animals relative to humans (animals/patients)

Species	Study	No. of Cyclesa	MTD (mg/m2)	Cmax Ratiob		AUC Ratioc	
				Plasma	Blood	Plasma	Blood
Rat d	RTC70760	12	1.2	0.06	NA	0.03	NA
Dog d	8293-093	1	2.6	0.41	0.50	0.07	0.30
	8293-094	6	2.0	0.40	0.48	0.05	0.14
	8293-095	12	1.5	0.24	0.61	0.04	0.16

a A cycle was defined as a bolus i.v. dose of plitidepsin administered once every 2 weeks.
b Maximum concentration Cmax calculated based on human values (ng/mL) of 29.1 (plasma) and 56.2 (blood) at a dose of plitidepsin of5 mg/m2 (3-hour infusion) (APL-B-014-03).
c Exposures calculated based on total drug AUC values and a human AUC (ng·h/mL) of 256 (plasma) and 875 (blood) at a dose ofplitidepsin of 5 mg/m2 (3-hour infusion) (APL-B-014-03).
d Mean data (combined from both genders and cycles) are presented.

NA = not available.
Note: to obtain dose in mg/kg, divide by 6 and 20 in rat and dog, respectively.

Local Tolerance

The irritation potential of plitidepsin was studied in male New Zealand white rabbits after single i.v. and paravenous administration at 0.07 mg/mL in the posterior ear veins and adjacent tissues (Study ML-PMI-3N77-98-136, GLP). Tissues were examined for up to 24 h post-dose. No clinical signs of systemic toxicity were observed. Barely perceptible erythema and oedema were seen 24 h following injection. Histologically, these changes were due to mild thrombophlebitis, subcutaneous oedema and/or perivascular/subcutaneous haemorrhage. Perivascular injection of plitidepsin resulted in subcutaneous acute inflammation and oedema, and subcutaneous haemorrhage.

Other toxicity studies

The potential phototoxicity of plitidepsin was assessed in an *in vitro* cytotoxicity assay with BALC/c 3T3 fibroblast cells (Study RTC97060, GLP). Cell cultures, exposed to a range of plitidepsin concentrations (8 dilutions, 2.73-350 ng/mL), were incubated for 50 minutes either in the dark or irradiated (1.7 mW/cm^2), then washed and incubated overnight in fresh medium. Cell viability was subsequently assayed by neutral red (NR) uptake. No precipitation of the test item was noted at any concentration tested.

Results showed that the IC50 values of plitidepsin in the absence and presence of UV irradiation were 15.4 and 16.5 ng/mL, respectively, with a Photo Irritation Factor (PIF) calculated as 0.952 and a Mean Photo Effect value (MPE) calculated as -0.010. Therefore, on the basis of the results obtained with plitidepsin (PIF < 2 and MPE < 0.1) the score values are predictive of no phototoxicity.

2.3.5. Ecotoxicity/environmental risk assessment

Plitidepsin PECsurfacewater value is below the action limit of 0.01 µg/L and log Kow does not exceed 4.5. Consequently, neither PBT, nor phase II assessment is required.

Table 18 Summary of main study results

Substance (INN/Invented Name): plitidepsin					
CAS-number (if available):					
PBT screening		Result			**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	3.5			Potential PBT (N)
Sediment dwelling organism		NOEC		mg/kg	species

2.3.6. Discussion on non-clinical aspects

The primary pharmacology studies showed that the eukaryotic Elongation Factor 1A2 (eEF1A2), an important component of the translation machinery, is the primary target of plitidepsin. *In vitro*, plitidepsin inhibited tumour cell growth in most cell lines, with higher efficacy against haematological malignancies. Antitumour activity of plitidepsin in haematological malignancies was also observed *in vivo*. When administered in combination with dexamethasone, bortezomib or rituximab, plitidepsin produced higher antitumour activity than monotherapy in multiple myeloma or lymphoma models.

An *in vitro* receptor binding screening in order to evaluate the selectivity of plitidepsin was conducted. Although the mechanism of the antagonist effect of plitidepsin over acetylcholine on the muscarinic receptor M3 cannot be elucidated with these inconsistent data, the possible relationship between the cardiovascular toxicity of plitidepsin, as it set out below and its effect on M3 cannot be ruled out.

The pharmacokinetics of the product was investigated in mice, rats and dogs. In all species blood and plasma concentration-time profiles indicated multi-compartmental kinetics following IV administration with no apparent gender differences and exposure is proportional to dose for both males and females. The C_{max} was reached very quickly after IV administration and the concentration declined slowly afterwards with an initial marked decline. It was also observed that concentration in blood was higher than in plasma. In plasma, a low clearance was observed as well as a very large volume of distribution suggesting a modest systemic clearance and long terminal half-life were reported. In general non clinical data obtained in animal models is fairly comparable to the plasma kinetics reported in humans.

Plitidepsin showed inhibitory effects on CYP3A4 and it is likely a substrate for transporter MDR1. In addition there were data indicative of the product effect on efflux inhibition (MDR1, MRP2 and BSEP) and uptake (OATP1B1, OATP1B3) of human transporters. However, bearing in mind the low human plasma concentration of plitidepsin, the effects in patients are expected to be very limited.

General toxicity studies were conducted in mice, rats and dogs. Mortality was observed in rats (related to lymphoid depletion and atrophy in the lymphoid and hematopoietic system) and dogs (exocrine pancreas insufficiency). The primary target for toxicity identified in preclinical species (mainly in dogs) was characterised by atrophy of the exocrine pancreas. Atrophy of the exocrine pancreas was also observed in rats. Plitidepsin primary target eEF1A2 is expressed in pancreatic acinar cells and islets of Langerhans in humans. Currently the toxic mode of action and relevance for humans, as well as distribution of plitidepsin to pancreas are unclear. At this time, further non-clinical studies to elucidate the pancreas toxicity, are not considered to be of value.

Other toxicities observed included tachycardia, ST depression, thymic atrophy, liver abnormalities (such as increased liver function markers, bile duct proliferation, vacuolation of the biliary epithelial cells, instances of yellow/brown pigmented hepatocytes and Kupffer cells, clear cell changes or hepatocytic vacuolation), moderate/marked fatty infiltration in skeletal muscle or in the heart, and vacuolation of distal tubular cells in the kidney.

Cardiovascular toxicity was observed in dogs in general toxicity and safety pharmacology studies, even at low single doses, and included tachycardia and occasional bradycardia, as well as QT prolongation and ST-segment depression. According to the applicant, these findings, as well as skeletal muscle toxicity, may be explained by an initial peripheral vasodilation that gives rise to hypotension, leading to tachycardia, as well as heart hypoperfusion, subendocardial ischemia and thus, ST-segment depression. QT prolongation was also observed in patients. However, there is not enough experimental data to propose a mechanism for the cardiovascular toxicity of plitidepsin. It is considered plausible that even though aplidin was partially responsible of the CVS effects seen in animals, those are not totally extrapolable to the clinical scenario since the duration of infusion is different and the appearance non clinical and clinical findings do not correlate in time of appearance.

eEF1A2 is known to be expressed in neurons, and toxicity studies show that plitidepsin has an effect on nerves in the periphery and CNS of rats and dogs, which may be related to the proposed mechanism of action. No relevant neurological events related to the treatment in patients, except for headache, have found in patients. Therefore, taking into account the lack of relevant findings in the clinical studies and the relatively weak signs of peripheral nerve and CNS effects in the animal studies, neurotoxicity should not be considered as a clinical safety concern in patients treated with the combination of plitidepsin and dexamethasone.

Plitidepsin is genotoxic as it produces an oxidative-mediated DNA damage. Long term carcinogenicity studies have not been performed due to the cytotoxic nature of the compound.

Fertility studies with plitidepsin performed in rats, as well as limited histopathological changes observed in the gonads in the repeat dose toxicity studies, suggest that plitidepsin could affect reproductive capacity. This is likely, considering the cytotoxic and mutagenic nature of the compound.

Developmental toxicity studies in rats were conducted, in which the administration of plitidepsin resulted in signs of embryo foetal toxicity at the MTD level.

The active substance is a peptide substance, the use of which will not alter the concentration or distribution of the substance in the environment. Plitidepsin PEC surfacewater value is below the action limit of 0.01 µg/L and is not a PBT substance as log Kow does not exceed 4.5.

2.3.7. Conclusion on the non-clinical aspects

Overall, the non-clinical documentation submitted was considered adequate.

2.4. Clinical aspects

2.4.1. Introduction

Plitidepsin is a marine depsipeptide originally isolated from the Mediterranean tunicate Aplidium albicans with a chemical structure composed of a macrocyclic core and a side chain, typical of the Didemnidae family.

Plitidepsin affects different biochemical pathways, resulting in the induction of apoptosis of tumour cells. Interaction with the eukaryotic elongation factor eEF1A2 is considered the primary target for plitidepsin. Other biochemical events induced by plitidepsin such as early oxidative stress, Rac1 activation and initiation of MAPK cascade and cyclins down-regulation, together with endoplasmic reticulum (ER) stress

induction and activation/deactivation of several cytoplasmic factors contribute to the antiproliferative effects. Plitidepsin`s effects on tumour microenvironment and angiogenesis have also been observed.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Tabular overview of clinical studies

Table 19 Clinical studies evaluating plitidepsin alone or in combination in patients with relapsed/refractory multiple myeloma

Study	Design and study population	Study treatment, dose and regimen	Treated patients
Phase III			
APL-C-001-09 (ADMYRE) (Mod.5.3.5.1.1)	Multicentre, open-label, two-arm, 2:1 randomised, phase III pivotal clinical study in patients with relapsed/refractory MM previously treated with at least three but no more than six therapeutic regimens.	**Arm A:** plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion on Day 1 and 15 q4wk plus DXM 40 mg orally on Day 1, 8, 15 and 22, q4wk. **Arm B:** DXM alone at the same dose and schedule than in Arm A (40 mg orally on Day 1, 8, 15 and 22 q4wk).	**Arm A:** n=167 patients. **Arm B:** n=83 patients.
Phase II			
APL-B-014-03 (Mod.5.3.5.2.2)	Multicentre, open-label, exploratory, phase IIa clinical study in pre-treated patients with relapsed/refractory MM.	Plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion q2wk [a]. Patients with suboptimal response (PD after three plitidepsin cycles or SD after four plitidepsin cycles) in the first stage of the study received in a second stage 20 mg of oral DXM every day on Day 1 to 4 of each q2wk cycle, starting at the same time than plitidepsin infusion.	n=51 patients treated with plitidepsin. Nineteen of these 51 patients were treated with plitidepsin plus DXM in the second study stage.
Phase I			
APL-A-009-08 (Mod.5.3.5.2.1)	Dose-finding, phase I clinical study in patients with relapsed and/or refractory MM.	Plitidepsin 1-hour i.v. infusion on D1 and 8, q3wk, plus 3-5 sec i.v. bolus injection of bortezomib on Day 1, 4, 8 and 11, q3wk, plus DXM orally on Day 1 and 2, 4 and 5, 8 and 9, 11 and 12, q3wk.	n=3 patients [b]
APL-A-012-13 [c] (Mod.5.3.5.2.3)	Dose-finding, phase I clinical study in patients with relapsed and/or refractory MM.	Plitidepsin 3-hour i.v. infusion on Day 1 and 15, q4wk, plus 3-5 sec bolus s.c. injection of bortezomib on Day 1, 4, 8 and 11, q4wk, plus DXM orally on Day 1, 8, 15 and 22, q4wk.	n=22 patients

[a] Two infusions over a 4-week period were defined as two cycles of treatment.
[b] This study was closed due to a Sponsor's decision, with a total of three included and treated patients. All three patients were treated at the first planned dose level: plitidepsin 2.0 mg/m^2+ bortezomib1.0 mg/m^2, and DXM 20 mg.
[c] RD for further phase II studies was plitidepsin 5.0 mg/m^2, bortezomib 1.3 mg/m^2 and DXM 40 mg.
DXM, dexamethasone; i.v., intravenous; MM, multiple myeloma; PD, progressive disease; q2wk, every two weeks; q3wk, every three weeks; q4wk, every four weeks; RD, recommended dose; s.c., subcutaneous; SD, stable disease.

2.4.2. Pharmacokinetics

The clinical pharmacology program for plitidepsin includes the evaluation of pharmacokinetic (PK) and pharmacokinetic-pharmacodynamic (PK/PD) relationships. This has been investigated across five phase I studies and nine phase II studies (age range 19-86 years) during which plitidepsin was given as a single agent, as well as four phase I studies and one phase II study that evaluated plitidepsin in combination

with other oncological agents. The data from these studies were used to conduct a population PK (Pop-PK) analysis of plitidepsin as well as exposure-response analyses of selected safety and efficacy endpoints. Furthermore, the in vivo metabolism and excretion of plitidepsin was evaluated in a human mass balance study after single-dose administration of radiolabelled plitidepsin to patients with solid tumours.

Population PK Modelling: An open, 3-compartment disposition model with linear elimination and linear distribution from the central compartment to two peripheral compartments was used to describe the PK of plitidepsin in plasma. The model was parameterised in terms of systemic clearance (CL), central (V1) and peripheral volumes of distribution for the shallow (V2) and the deep (V3) compartments, and intercompartmental exchange flows for shallow (Q2) and the deep (Q3) compartments. The concentration of plitidepsin bound to the red blood cells was modelled as nonlinear function, and the plitidepsin blood concentration was modelled according to the Equation 1:

$$C_{blood} = C_{plasma} \cdot (1 - HCT) + \frac{B_{\max} \cdot C_{plasma}}{k_d + C_{plasma}} \cdot HCT \qquad \text{Equation 1}$$

where Bmax corresponds to the maximal plitidepsin concentration bound to blood cells, kd is the plitidepsin plasma concentration at which the plitidepsin bound to red blood cells is half-maximal and HCT is the baseline haematocrit of each patient. Between and within subject variabilities, as well as residual variabilities, were assumed to be log-normally distributed. Both model predictions and data observed were log-transformed. The estimates of fixed and random effects were obtained with the FOCE algorithm as implemented in NONMEM software.

The following subject related covariates were evaluated as potential factors that may contribute to the between subject variability of plitidepsin pharmacokinetics in plasma and blood: age, sex, body weight, body surface area, liver function test (AST, ALT, ALP and total bilirubin) and renal function (CLCR), serum concentration of albumin and total plasma proteins, leukocytes counts, performance status, presence of liver metastases and concomitant administration of CYP inducers and inhibitors. The covariate analysis was conducted using the forward inclusion and backward elimination process, after the graphical and statistical exploratory analyses based on Empirical Bayesian Estimates (EBE) of model parameters. The results of the pop-PK analysis are presented in Table 20 below.

Absorption

Since plitidepsin is administered as an i.v. infusion, the assessment of biopharmaceutical profile (e.g., bioavailability, food effect, etc.) is not relevant.

Distribution

Plitidepsin binds extensively to plasma proteins; the mean free (unbound) fraction was approximately 2%, independently of the drug concentration. At physiological concentrations of HSA (50 and 20 mg/mL) and human AAG (1.5 and 0.5 mg/mL), plitidepsin (at 5, 10, 25, 50 and 100 ng/mL) was more extensively bound to HSA (87 to 96 %) than to human AAG (72 to 81 %).

In vitro non-clinical studies show that plitidepsin is likely a substrate of MDR1 human efflux transporter, while likely not a substrate of BCRP and MRP2 human efflux transporters and OATP1B1, OATP1B3, OCT1 and MATE1 human uptake transporters.

For the purpose of the Pop-PK model the distribution of plitidepsin could be described by a 3 compartment model, with a central, a blood cell and an unspecific peripheral compartment. Apparent volumes of distribution, estimated by the Pop-PK model, were 492 and 2053 L in whole blood and plasma, respectively. Blood cells are an important distribution compartment of plitidepsin. The volume of the

peripheral compartment (513 L) exceeded the total body water, reflecting the distribution of plitidepsin to peripheral tissues, which is consistent with its lipophilicity and indicating an extensive tissue binding. No association between body weight and V1 was identified in the population PK analyses.

Table 20 Plitidepsin population pharmacokinetic parameters in patients with relapsed/refractory MM

Pharmacokinetic Parameter	Typical value * (RSE, %)	Variability	
		Between subjects, % (RSE, %)	Within subjects, % (RSE, %)
V_1 (L)	25.0 (10.7)	61.1 (26.5)	--
CL (L/h)	4.41 (8.82)	45.7 (27.1)	53.4 (15.1)
V_2 (L)	79.3 (4.38)	39.5 (20.3)	--
Q_2 (L/h)	29.8 (5.64)	54.[a]	--
V_3 (L)	513 (10.6)	47.5 (33.0)	--
Q_3 (L/h)	4.98 (10.3)	68.9 [b]	80.4 (15.3)
Bmax (µg/L)	487 (28.8)	54.0 (23.2)	30.7 (41.4)
k_d (µg /L)	54.7 (30.7)	--	--

* Results expressed as parameter (RSE: relative standard error of parameter estimate, %).
[a] Correlation between IIV of CL and Q_2 set to 1. Expansion factor of Q_2 is 1.40 (RSE=16.5%).
[b] Correlation between IIV of CL and Q_3 set to 1. Expansion factor of Q_3 is 2.27 (RSE=12.3%).
Residual variability (%): Plasma: 45.4% (RSE =6.01 %); Blood: 25.7% (RSE = 6.15 %).

Legend: systemic clearance (CL), central (V1) and peripheral volumes of distribution for the shallow (V2) and the deep (V3) compartments, and intercompartmental exchange flows for shallow (Q2) and the deep (Q3) compartments. Bmax corresponds to the maximal plitidepsin concentration bound to blood cells, kd is the plitidepsin plasma concentration at which the plitidepsin bound to red blood cells is half-maximal and HCT is the baseline haematocrit of each patient.

Depending on the haematocrit value (30 to 45%), the blood to plasma ratio ranged from 3.37 to 4.55. The plitidepsin distribution to blood cells can be considered linear up to doses of 5 mg/m2 administered as 3-hour infusion, given that 90% of the plitidepsin plasma concentration remained below the kd (half maximal binding to blood cells).

Metabolism and Elimination

Owing to its distribution in blood cells, most of the circulating plitidepsin is unchanged compound, as reflected at the human mass balance study (APL-A-013-13), where the mean AUC ratio in whole blood between total radioactivity and unchanged plitidepsin was 0.95. In plasma, the radioactivity measured is less explained by the parent compound as time after dosing increases, with a resulting ratio of 0.29, thus suggesting a gradual formation of metabolites. The results of the APL-A-013-13 show that after administration of a single dose of 14Cplitidepsin the mean radioactive recovery was 6.09±1.51% in urine and 71.26±2.64% in feces up to 480 h after the start of the infusion. The mean total recovery was 77.35%.

The initial disposition of plitidepsin is characterized by an initial fast distribution half-life, estimated to be 14.5 minutes, followed by the beta elimination half-life of about 7.6 hours. Plitidepsin has a low plasma clearance of 5.4 l/h (coefficient of variation [CV] 46%). The terminal half-life, about 6 days, constituted the majority of the overall area under the plasma concentration vs time curve (54%). The apparent CL were 13 and 52 L/h and terminal t1/2 were 44 and 50 h in whole blood and plasma, respectively.

The excretion of plitidepsin was investigated in the mass-balance study APL-A-013-13 using urine and faeces collected from patients with cancer administered a single-dose of 14C-labelled plitidepsin as a 3-hour infusion. A majority of the radiolabelled material excreted was recovered in the faeces (approximately 70% of the dose) and smaller amounts recovered in urine (approximately 6% of the dose) over a collection interval of up to 20 days and 10 days respectively. In urine, roughly 75% is excreted as the parent compound. This means that about 1.5% of the total administered dose is excreted as metabolites in the urine.

According to identified radioactivity, in urine, unchanged plitidepsin and metabolites accounted for 2.8% and 0.9% (A1: 0.8 % and A2/B10: < 0.1%) of the total administered dose, respectively. In feces, unchanged plitidepsin and metabolites accounted for 2.3% and 48.3% (B1: 11.3%, B8: 8,8%, B4: 5.2%, B7: 4.5%, B9: 4.2%, B5: 4%, B6: 3.1%, B2: 3%, B3 2.7%, A2/B10: 1.5%) of the total administered dose, respectively.

For all above mentioned it seems that plitidepsin is mainly eliminated via hepatic clearance, with renal clearance playing a lesser role. Based on in vitro data, plitidepsin seems to be primarily meabolized by CYP3A4/5. Long t1/2 is apparently consistent with the low hepatic extraction ratio of plitidepsin, high blood to plasma ratio (ranged from 3.37 to 4.55) and low free (unbound) fraction (2%).

A schematic drawing of the human *in vivo* mass balance has been submitted, see Figure 4.

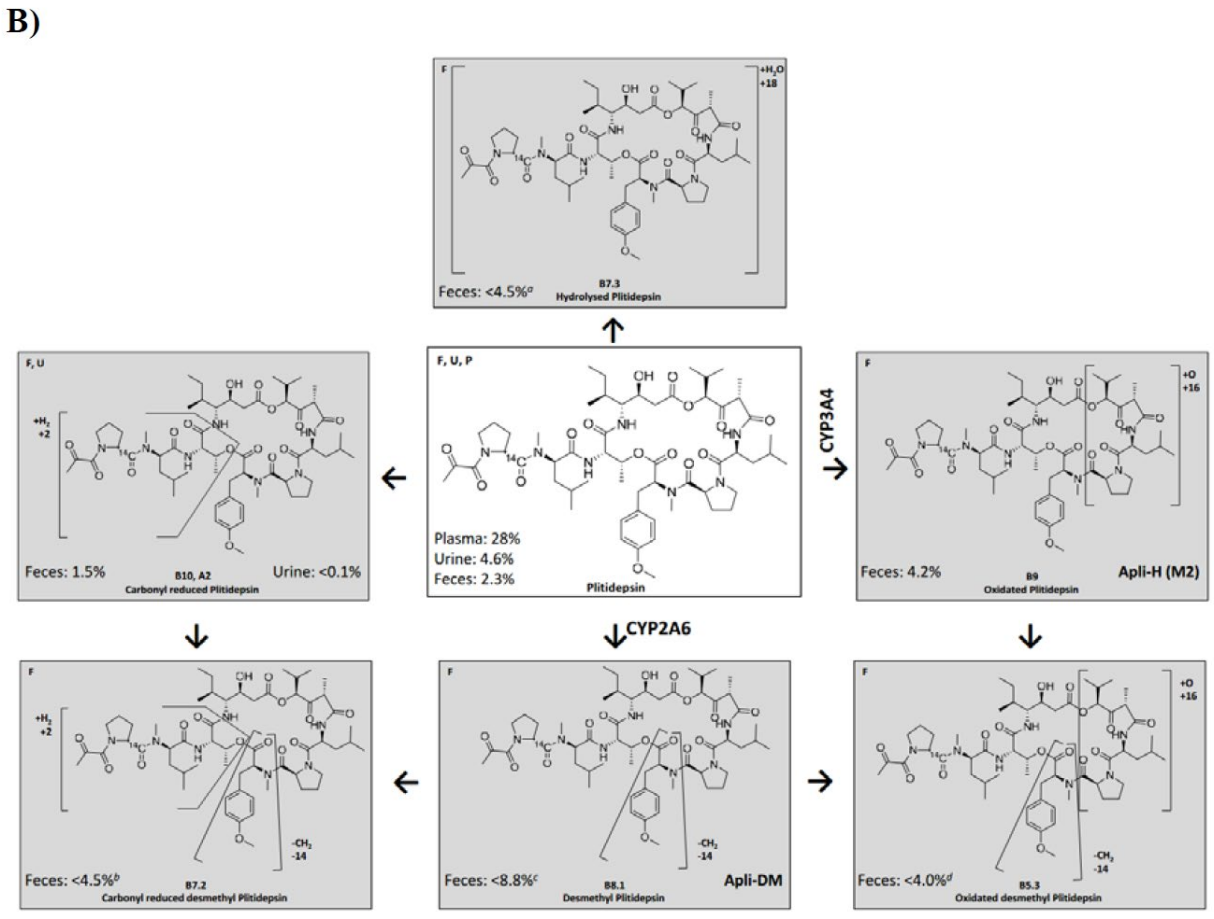


Figure 4. Schematic drawing of the human *in vivo* mass balance.

Only two of four metabolites observed in *in vitro* studies were identified also *in vivo* (*i.e.* Apli-H/M2 and B8.1/APLI-DM corresponding to 4.2% and 8.8% of the administered dose, respectively), potentially due to low *in vivo* plitidepsin and metabolite concentration and hepatic elimination pathways involving both plasma- and cytochrome-mediated metabolism.

Only one metabolite, the *in vitro* identified Apli-DA, has been investigated for antitumor activity – a 10-28% of the corresponding plitidepsin activity was observed.

Dose proportionality and time dependencies

Dose proportionality

In general, an increase in the concentrations of plitidepsin in whole blood and plasma was observed with an increase in the dose administered.

Dose proportionality was observed between 2.0- 5.0 mg/m2. The PK of plitidepsin is characterised by a rapid decline phase observed at the end of the infusion followed by slower exponential phases, with the terminal t1/2 of approximately 6 days. Based on this terminal t ½ accumulation appears limited.

Dose-proportionality in overall plitidepsin exposure (AUC0-inf) was formally assessed in whole blood and plasma after pooling NCA results from the phase I program of single agent plitidepsin dose-escalation studies, because they comprise the largest range of plitidepsin dose levels. This analysis could not be applied to the assessment of maximum concentration because infusion duration of plitidepsin was different among pooled studies (i.e., 1-hour, 3-hour and 24-hour). In whole blood, dose-proportionality could be assessed at a range of doses from 0.5 to 8.0 mg/m² (n=72), while in plasma the available dose levels ranged from 0.08 to 5.0 mg/m² (n=92). The slope of the regression lines and respective 95% confidence intervals are provided in Figure 5.

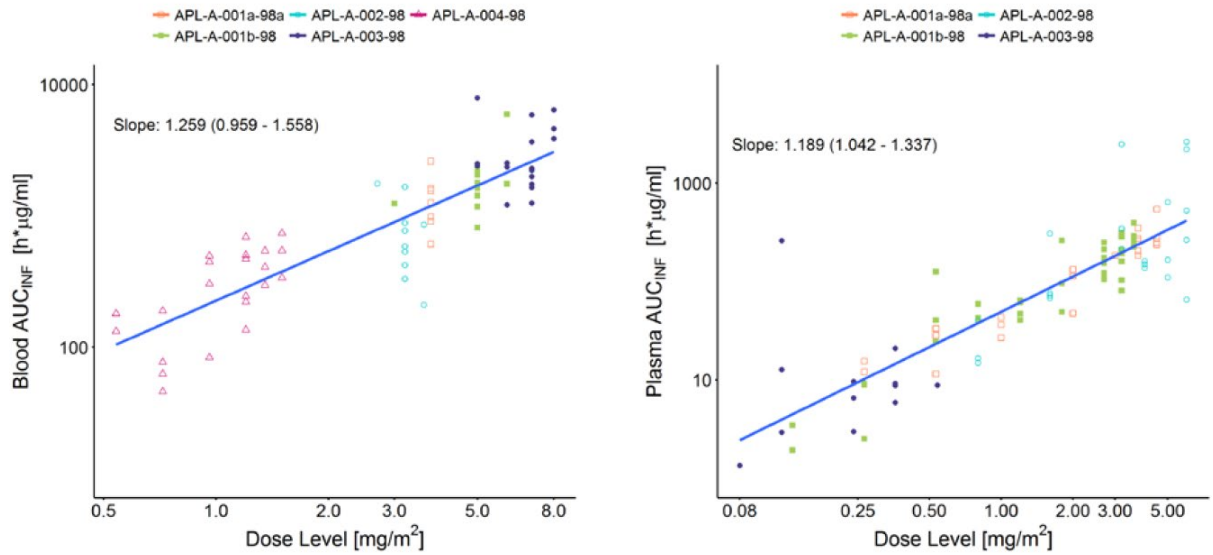


Figure 5. Correlation between whole blood and plasma AUC0-inf values and dose level in patients with plitidepsin administered in the phase I single agent dose-escalation studies.

Time dependency

Studies with PK sampling were phase II studies APL-B-001-01, APL-B-002-02, APL-B-005-02 and APL-B-007-02 and phase III study APL-C-001-09 in patients with relapsed/refractory MM. Phase I study APL-A-004-98 also involved PK sampling in more than one occasion, although its D1-5 regimen is not comparable to the weekly or biweekly regimens. In all these studies, sparse PK sampling was performed, so time dependencies in the PK of plitidepsin were assessed by means of population methodology. The Pop-PK model was characterised by a constant CL, implying time independency. Simulated

concentration-time profiles predicted minimal accumulation (24%) of plitidepsin concentrations when 5.0 mg/m2 was given as a 3 hour infusion every two weeks to patients with relapsed/refractory MM.

The accumulation factor for plitidepsin plasma concentrations after biweekly dosing is expected to be limited (24%), since the biweekly dosing interval is, on average, at least twice as long as the terminal half-life.

Intra- and inter-individual variability

The variability in the PK of plitidepsin was moderate to high. The estimated between-subject variability for plasma CL and V1 were 45.7% and 61.1%, respectively. The within-subject variability for CL was estimated to be lower than the corresponding value of between-subject variability but still high (53.4%).

Pharmacokinetics in target population

Plitidepsin has not been evaluated in healthy volunteer subjects because of its mutagenicity, hence, no PK data are available in healthy subjects.

Plitidepsin has been administered in patients with advanced solid and haematological malignancies, including relapsed/refractory MM, for whom an extensive PK assessment has been performed. Extensive PK sampling in whole blood was performed in several phase II studies with plitidepsin as single agent in specific cancer types; namely, study APL-B-011-02 (prostate cancer), APL-B-013-02 (NHL), APL-B-014-03 (MM), APL-B-015-04 (ALL) and APL-B-016-05 (melanoma).

Special populations

No dedicated pharmacokinetic studies in either renal or hepatic impairment have been conducted. The population PK model developed mainly supports the influence of intrinsic and extrinsic factors on the PK of plitidepsin.

Impaired renal function

A small fraction of plitidepsin dose (6% of the dose) is excreted in the urine, mostly (75%) as unchanged drug, thus indicating minimal effect of renal impairment on the excretion of plitidepsin.

The Pop-PK results show that PK parameters of plitidepsin are not dependent on the creatinine clearance of patients with mild (CLCR of 50-80 mL/min) or moderate (CLCR of 30-50 mL/min) renal impairment, in line with the small contribution of renal excretion to the overall elimination.

In phase I studies APL-A-001a-98, 001b-98 and -002-98 the amount of plitidepsin excreted in urine was consistently low (<4.3%), which was comparable to the results obtained in the mass balance study (6%). However, the urine data from these studies are not considered reliable, and the recovery in the mass balance study was relatively low (77%).

Clearance has been presented for patients from Dataset B (popPK model) according to renal function (estimated GFR). The data include five patients with severely impaired renal function. Glomerular filtration rate has been estimated based on Levey et al. 2009 (CKD-EPI).

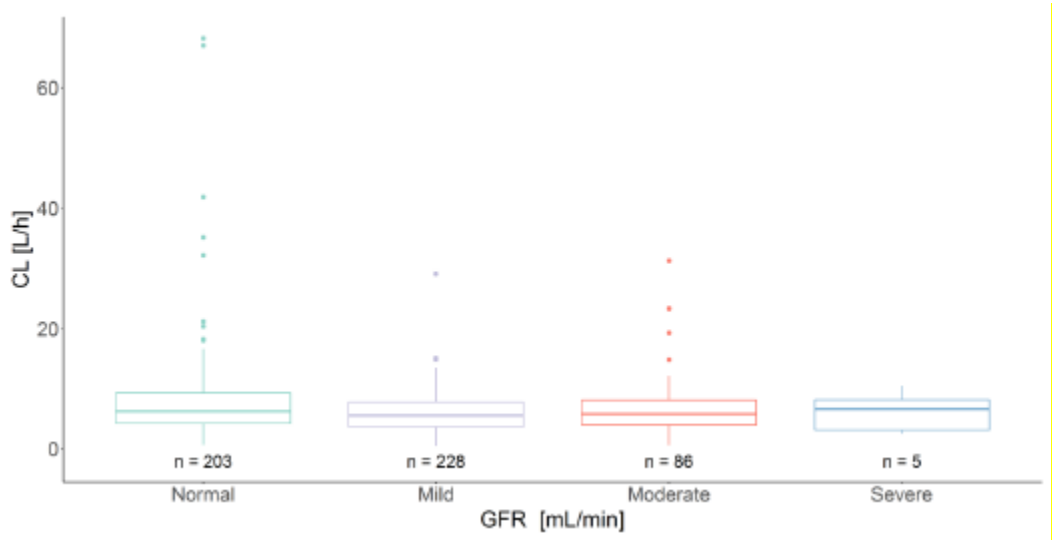


Figure 6. Estimated plitidepsin plasma clearance and GFR group

Table 21. Actual clearance values according to glomerular filtration rate group

	GFR (mL/min)			
	>90	60-<90	30-<60	<30
CL (L/h) [a]	Normal renal function (n=203)	Mild impaired renal function (n=228)	Moderate impaired renal function (n=86)	Severe impaired renal function (n=5)
Median	6.19	5.54	5.76	6.58
Interquartile range (Q1–Q3)	4.21 – 9.29	3.63 – 7.80	3.94 – 8.07	3.10 – 8.14

[a] Data from patients with pharmacokinetic data who received plitidepsin as single agent or in combination with dexamethasone (extended dataset at the pooled Pop-PK database comprising 522 patients).
CL, clearance; GFR, glomerular filtration rate; Pop-PK, population pharmacokinetic analysis.

Impaired hepatic function

A formal clinical study to evaluate the impact of hepatic impairment on the PK of plitidepsin was not performed, although biliary/hepatic excretion constitutes the major pathway of plitidepsin elimination.

Based on the population PK analysis, patients with alterations at baseline in markers of hepatic function (ALT, AST, bilirubin and serum albumin) treated with the compound did not present differences in plasma CL or volume of distribution. In addition, the CL of plitidepsin is not different in patients with and without liver metastasis.

- **Gender**

Covariate analysis based on population PK indicated that gender is not an influential factor on plitidepsin plasma CL.

- **Race**

Patients included in the pooled PK database for whom race was reported were mostly Caucasian (n=200), except for 17 Asians, two Blacks, and five reported as Other. No statistically significant differences in CL were found among these race groups.

- **Weight**

No significant correlation between body size and the PK parameters of plitidepsin were reported in the phase I dose-escalation studies. Furthermore, a lack of clinically meaningful correlation between body weight (range, 44-137 kg), or BSA (range, 1.2-2.5 m²) and the plasma CL of plitidepsin was observed during the Pop-PK analysis, although all plitidepsin dose regimens are based on BSA.

- **Elderly**

Results from the population PK analysis indicated that the CL and distribution volume of plitidepsin are not influenced by patient age (range, 19 to 86 years old), Table 22.

Table 22. Age distribution of patients with PK assessment by study.

PK study	Population	Pop-PK dataset A and/or B	Age<65 years n (%)	Age 65-74 years n (%)	Age 75-84 years n (%)	Age≥85 years n (%)
Phase I Studies In Patients With Solid and Haematological Tumours						
APL-A-001a-98	Solid tumours, NHL	B	32 (82.1)	7 (17.9)	-	-
APL-A-001b-98	Solid tumours, NHL	B	17 (81.0)	4 (19.0)	-	-
APL-A-002-98	Solid tumours, NHL	B	38 (82.6)	7 (15.2)	1 (2.2)	-
APL-A-003-98	Solid tumours, NHL	B	39 (88.6)	5 (11.4)	-	-
APL-A-004-98	Solid tumours, NHL	B	26 (70.3)	10 (27.0)	1 (2.7)	-
Phase II Studies in Patients with Solid Tumours						
APL-B-001-01	Renal cancer, CRC	B	33 (76.7)	10 (23.3)	-	-
APL-B-002-02	Medullary thyroid cancer	B	15 (93.8)	1 (6.2)	-	-
APL-B-005-02	Cancer of the urothelium	A and B	20 (100)	-	-	-
APL-B-006-02	SCLC	A and B	9 (60.0)	5 (33.3)	1 (15)	-
APL-B-007-02	Melanoma	A and B	27 (73.0)	8 (21.6)	2 (5.4)	-
APL-B-011-02	Prostate cancer	A and B	6 (75.0)	2 (25.0)	-	-
Phase II Studies in Patients with Haematological Tumours and Multiple Myeloma						
APL-B-013-02	NHL	A and B	16 (66.7)	4 (16.7)	4 (16.7)	-
APL-B-014-03	MM	A and B	24 (53.3)	11 (24.4)	9 (20.0)	1 (2.2)
APL-B-015-04	ALL	A and B	12 (70.6)	5 (29.4)	-	-
Phase III Study in Patients with Multiple Myeloma						
APL-C-001-09	MM, with DXM	A and B	79 (56.8)	46 (33.1)	14 (10.1)	-

ALL, acute lymphoblastic leukaemia; CRC, colorectal; DXM, dexamethasone; MM, multiple myeloma; NHL, Non-Hodgkin's

- **Children (APL-A-005-02)**

The PK of plitidepsin has been investigated in children (age range: 2 to 17 years) with refractory solid tumours. Based on NCA results, no significant difference in CL was observed in children as compared with adolescents, and Vss and t1/2 were comparable as well.

The plasma PK of plitidepsin was characterised in 20 children and 14 adolescents (APL-A-005-02) with malignant solid tumours or acute leukaemia (**Figure 7**). The starting dose level (4.0 mg/m2) was equivalent to 80% of the RD for this schedule in adults.

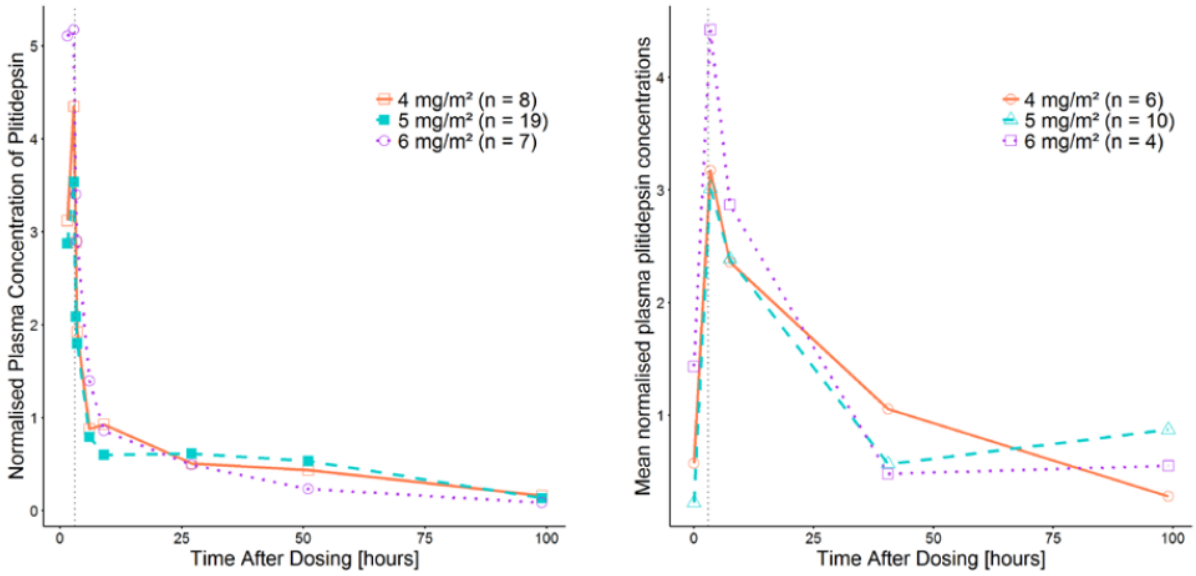


Figure 7. Mean concentration of plitidepsin in plasma following administration as 3-hour intravenous infusion on Day 1 of Cycle 1 (left) and Cycle 3 (right) (APL-A-005-02).

Blood samples (3 ml) for the analysis of plitidepsin in plasma were collected prior to, during and after the first and third cycles. However, NCA evaluation could only be performed on Cycle 1.

There was no significant difference in plitidepsin CL in children (≤12 years of age when entering study) with respect to adolescents (>12 but less than 18 years) as described in **Error! Reference source not found.**. The elimination of the parent compound, as reflected by the t1/2 and Vss, appeared comparable in children and adolescents. These results have also been reported in the literature.

Table 23.Non-compartmental plasma PK parameters of plitidepsin following administration as a 3-hour infusion in children and adolescents (APL-A-005-02).

Dose level (mg/m²)	Age group	n	Cmax (ng/ml)	AUC (hr*ng/ml)	Cl (l/h)	$t_{½}$ (hr)	Vss (l)
4	Children	3	19.2 ± 7.70	267 ± 259	27.3 ± 25.8	35.8 ± 37.4	463 ± 316
	Adolescents	5	23.9 ± 7.03	179 ± 69.3	36.5 ± 17.1	21.8 ± 10.5	722 ± 281
5	Children	12	22.6 ± 10.5	641 ± 851	16.7 ± 13.2	36.3 ± 28.9	517 ± 311
	Adolescents	7	21.6 ± 5.98	432 ± 487	35.5 ± 21.2	44.3 ± 44.1	1122 ± 348
6	Children	5	25.5 ± 16.3	236 ± 128	24.3 ± 12.4	38.5 ± 18.7	762 ± 500
	Adolescents	2	33.5 ± 15.8	362 ± 86.8	22.5 ± 7.75	19.4 ± 10.8	473 ± 275

Note: Results presented as mean ± standard deviation.
AUC, area under the plasma concentration-time curve; CL, clearance; C_{max}, maximum concentration in plasma; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady state.

Plitidepsin is covered by the class waiver condition "treatment of multiple myeloma" for the applied indications.

Pharmacokinetic interaction studies

No formal in vivo DDI studies were conducted.

The clinical program of plitidepsin included 4 phase I trials in which plitidepsin was co-administered with different drugs: APL-A-006-05 (plitidepsin co-administered with carboplatin), APL-A-010-08 (plitidepsin co-administered with sorafenib or

ered with bortezomib) and a Phase II clinical trial APL-B-016-05 (plitidepsin co-administered with dacarbazine). No evidence of drug-drug interaction was found between plitidepsin and any of the administered drugs but for study APL-A-006-05 where an effect of plitidepsin on the exposition to carboplatin could not be ruled out.

Effect of Co-administered Drugs on the Pharmacokinetics of Plitidepsin

The effect of concomitant administration of CYP inhibitors and inducers on the PK of plitidepsin on a pooled database containing 303 patients was evaluated by means of population methodology. No effect of concomitant co-medication on plitidepsin PK was observed.

Effect of Plitidepsin on Other Co-administered Drugs

In vitro studies showed no potential of plitidepsin to inhibit or induce pharmacokinetic drug-drug interactions on co-administered substances. A potential effect of plitidepsin on CYP2B6 has not been studied in an in vivo study (see Discussion).

2.4.3. Pharmacodynamics

No clinical PD studies have been performed. No receptor screening studies (non-clinical secondary pharmacology) were performed that might have elucidated more target candidates regarding the in vivo functioning of plitidepsin.

Efficacy

The area under the plitidepsin plasma concentration vs. time (AUC) after the first dose (5 mg/m2) from phase III study APL-C-001-09 was selected as the exposure metric to conduct the E/R analyses. Plitidepsin plasma AUC was inferred from blood concentrations. A PK/PD model was developed to investigate the relationship between AUC after the first dose of treatment and two efficacy endpoints PFS and ORR. Results did not show a strong relationship between AUC after first dose and PFS (Figure 8) or ORR.

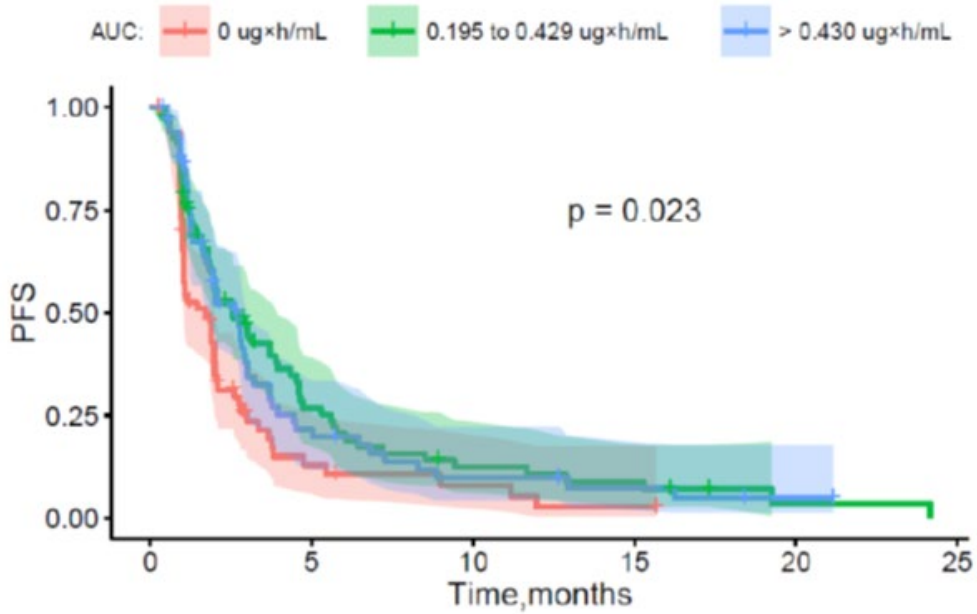


Figure 8. Kaplan Meier plot for progression free survival as a function of plitidepsin plasma AUC.

Table 24 Results of the multivariate logistic regression analysis for overall response rate for the base, extended and alternative model.

Base Model (-2LL: 177.38)	**ORR (95% CI)**	**p-value**
Treatment (D+P vs D)	8.667 (2.955 - 37.06)	0.0005
Extended Model (-2LL: 177.21)	**ORR (95% CI)**	**p-value**
Treatment (D+P vs D)	7.266 (1.812 - 37.90)	0.0087
Plasma AUC (µg.h/mL)	1.462 (0.217 - 8.819)	0.6794
Alternative Model (-2LL: 185.34)	**ORR (95% CI)**	**p-value**
Plasma AUC (µ.h/mL)	8.581 (2.409 - 34.91)	0.0014

AUC, area under the plasma concentration-time curve; CI, confidence interval; ORR, overall response rate.

A multivariate Cox-regression model, including treatment, age, haemoglobin and PCELL as covariates, was considered the base model, the addition of the plitidepsin AUC in plasma into the base model was considered the extended model, and the replacement of treatment effect on PFS by the plitidepsin AUC in plasma was considered the alternative model. The proportion of treatment effect that could be explained by plitidepsin exposure was calculated using Li's method.

Safety

Data from four clinical phase I studies (APL-A-001-98, APL-A-002-98, APL-A-003-98 and APL-A-004-98), nine phase II studies (APL-B-001-01, APL-B-002-02, APL-B-005-02, APL-B-006-02, APL-B-007-02, APL-B-011-02, APL-B-013-02, APL-B-014-03, APL-B-015-04) and one phase III study (APL-C-001-09) were available for the PK/PD analysis of ALT elevation following plitidepsin administration. Plitidepsin population PD (ALT) parameters are shown on Table 25.

Table 25 Plitidepsin population PD (ALT) parameters.

PD Parameter	Typical value* (RSE, %)	Between subjects variability, % (RSE, %)
$ALT.H_0$ (IU/L)	4500 (10.7)	
$ALT.P_0$ (IU/L) in MM	23.4 (4.92)	53.4 (4.95)
k_{out} (d^{-1})	0.0775 (8.15)	99.3 (9.28)
α		64.6 (12.5)
LS (mL/ng)	2.40 (6.08)	
HS (mL/ng)	13.1 (9.6)	
Proportion HS (%)	14.2 (18.7)	

LS: low sensitivity; HS: high sensitivity; Proportion HS: Percentage of population with high sensitivity
The residual variability was estimated to be 41.6 % (RSE: 2.37 %).
* Results, expressed as parameter (RSE: relative standard error of parameter estimate, %), were originally calculated from normalised ALT and converted in IU/L by using ULN = 50 IU.

To explore the impact of plitidepsin in the CPK elevation, a total of 656 patients were pooled from three phase I studies (APL-A-001-98, APL-A-002-98 and APL-A-003-98) including 140 patients (21.3%), nine phase II studies (APL-B-001-01, APL-B-002-02, APL-B-005-02, APL-B-006-02, APL-B-007-02, APL-B-011-02, APL-B-013-02, APL-B-014-03, APL-B-015-04) including 288 patients (43.9%), and one phase III study (APL-C- 001-09) including 228 patients (34.8%). In the target population, at the dose of 5 mg/m2 administered as 3h i.v. infusion bi-weekly, the incidence of grade≥3 CPK elevations was not found to be dependent on plitidepsin exposure (p=0.2614), Table 26.

Table 26. Results of the multivariate logistic regression analysis for CPK toxicity in patients with r/r MM.

Subject Characteristics	OR (95% CI)	p-value
Gender, female	0.502 (0.189 - 1.288)	0.1570
BSA, m^2	0.158 (0.015 - 1.415)	0.1092
CPK baseline, × ULN	3.589 (1.454 - 9.382)	0.0058
Plasma AUC, µg×h/mL	2.224 (0.429 - 10.52)	0.3220

When the multivariate analysis is conducted in the subset of relapsed/refractory MM patients receiving plitidepsin, the effects of gender and BSA were no longer statistically significant, while the effect of baseline CPK remained statistically significant. However, seemingly, analyses showed that the risk of grade ≥3 CPK elevation increase as the baseline CPK increase, regardless of the cancer type.

Primary and secondary pharmacology

The Applicant has submitted a number of primary pharmacology studies regarding plitidepsin primary target eEF1A2 (see Non-clinical part).

A QT substudy within the main clinical trial (ADMYRE) was carried out to assess the potential effects of plitidepsin administered at a therapeutic dose on the duration of the QTc interval and other ECG parameters in patients with relapsed/refractory MM, and to seek for any relationship between whole blood plitidepsin concentration and QTc interval duration.

Seven patients participated; six of them were evaluable for Day 1 measurements and four for Day 15 measurements. A substantial effect of plitidepsin on QTcF interval was not observed at any of the time points with sufficient ECG data. Specifically, the maximum mean increase in QTc from baseline of day 1 occurred 0.5 h after EOI (QTcF = 6.8 ms [LS mean 95% CI –0.2, 13.7]; QTcB = 13.6 ms [LS mean 95% CI 7.2, 20.1]). For the rest of time points of day 1, the mean increase from baseline was ≤5 ms for QTcF. Furthermore, there was only one minor QT increase from baseline (30-60 ms), and the mean PR, QRS, and heart rate remained stable during plitidepsin treatment.

Results showed that no patients had a QTc exceeding 500 ms or an increase from baseline in QTc exceeding 60 ms at any point and there were no meaningful changes in mean heart rate at any time point tested during day 1 and day 15. No patient had a lower than normal post-baseline heart rate (<50 beats/min) at pre-scheduled time points during the study. No clinically significant ECG abnormalities related to plitidepsin treatment were observed.

2.4.4. Discussion on clinical pharmacology

The in vivo mechanism of action of plitidepsin has not been fully elucidated, but recently the elongation factor 1A2 (eEF1A2) was indicated as a target. No receptor screening studies (non-clinical secondary pharmacology) were performed that might have elucidated more target candidates regarding the in vivo functioning of plitidepsin.

The product formulation is a powder for concentrate for solution for infusion. An aqueous based solvent containing polyoxyl castor oil and ethanol is provided to solubilize the lipophylized powder. The same composition of the drug product has been used throughout the development and no absorption, bioequivalence or food effect studies are required.

The PK of plitidepsin is characterized by

- a high level of plasma protein and blood cell binding
- passive distribution over cell membranes (extensively distributed)
- mainly CYP3A4 and plasma esterases involvement in the metabolism
- some contribution of active disposition as plitidepsin is a substrate of P-pg

No relationship between primary endpoint PFS or secondary endpoint objective response rate (ORR) and plitidepsin exposure in terms of high or low Day1 AUC plasma was identified. This was an exploratory ER analysis that included only one dose level (5 mg/m2). Plitidepsin plasma AUC was inferred from blood concentrations. The choice of AUC after first dose appears to be an acceptable exposure metric. However, dose interruptions and reductions were frequent which could have affected the ER analysis. Furthermore, the estimation of AUC is imprecise.

Replacing treatment effect by plasma AUC in the multivariate regression analysis was not associated with better fit, although a significant statistical association between plasma AUC and ORR was found.

Plitidepsin experiences a moderate clearance in plasma from human, suggesting the involvement of plasma esterases as an additional route of its metabolism. Owing to its distribution in blood cells, unchanged plitidepsin was the major circulating component (95% of radioactivity in the systemic

circulation) *in vivo* in patients with cancer who received a single dose of [^{14}C]-plitidepsin (2.2 mg). In plasma, the radioactivity measured was less explained by the parent compound as time after dosing increases, resulting in a 30% of the administered dose, thus suggesting a gradual formation of metabolites. None of them was present at a relevant concentration relative to unchanged plitidepsin or total radioactivity in plasma.

Plitidepsin is mainly hepatically cleared with minimal renal contribution. There is a disease impact on PK, however the mechanism has not been described. Patients with cancer who received a single dose of [14C]-plitidepsin (2.2 mg) showed that 70% of total radioactivity was recuperated in faeces. Population pharmacokinetic analyses included patients with mild hepatic impairment (n=92, total bilirubin > 1 to ≥ 1.5 x ULN or AST > ULN). Based on the population PK analysis, patients with alterations at baseline in markers of hepatic function (ALT, AST, bilirubin and serum albumin) treated with the compound did not present differences in plasma CL or volume of distribution. In addition, the CL of plitidepsin is not different in patients with and without liver metastasis. However, the lack of impact of hepatic impairment of PK of plitidepsin cannot be interpreted as demonstrated due to the limited stages of hepatic impairment included in the pop PK and the sparse sampling. Considering that biliary/hepatic excretion constitutes the major pathway of plitidepsin elimination and the above mentioned limitations on the population PK analysis, plitidepsin should not be used in patients with abnormal bilirubin.

Plitidepsin is an inhibitor of MDR1, BSEP, MRP2, BCRP, OATP1B1 and OAT1B3 at higher concentrations. Some uncertainty remains for plitidepsin as inducer of CYP2B6, although it appears that plitidepsin has little induction potential for the investigated CYP enzymes (CYP1A2, CYP3A4 and CYP2B6).

Alternative hepatic impairment study designs (since study in healthy subjects with hepatic impairment should not be ethical due to mutagenicity of plitidepsin) or follow up measures to obtain data conclude on the impact of all stages of hepatic impairment are needed.

A small fraction of plitidepsin dose (6% of the dose) is excreted in the urine, mostly (75%) as unchanged drug, thus indicating minimal effect of renal impairment on the excretion of plitidepsin. In addition, the plasma exposure of plitidepsin is not dependent on the GFR of patients with mild (GFR of 60-89 ml/min, n=228) or moderate (GFR of 30-59 ml/min, n=86) or severe (15-29 ml/min, n=5) renal impairment. The Pop-PK results show that PK parameters of plitidepsin are not dependent on the creatinine clearance of patients with mild (CLCR of 50-80 mL/min) or moderate (CLCR of 30-50 mL/min) renal impairment. According to Pop-PK analysis, adjustments to the starting dose in these patients are not required. However, since only patients with mild and moderate impairment were included in the data set (n=5), the Pop-PK analysis is not sufficient to conclude that there is no impact of more severe/end stage renal impairment (CLCR <30 mL/min). The pharmacokinetics of plitidepsin in patients with end stage renal disease (GFR of < 15 ml/min) has not been studied, so there are no data on the safety and efficacy of plitidepsin in this subgroup of patients. Based on the presented data it is not considered very likely that severe renal impairment has a relevant impact on plitidepsin PK, although no firm conclusion can be drawn.

No PK data from patients with ESRD are available.

Although the main elimination pathways of plitidepsin have not been fully characterized, in vitro studies suggest that cytochrome CYP3A enzymes are involved in the main elimination pathways of plitidepsin. Therefore, an in vivo study should be conducted to assess the impact of a strong inhibitor (e.g. itraconazole) and, of note, in presence of dexamethasone, on the pharmacokinetics of plitidepsin. Based on in vitro data co-administration with strong CYP3A4 inhibitors and inducers should be avoided and moderate CYP3A4 inhibitors and inducers should be co-administered with caution.

The AUC after the first dose of plitidepsin treatment was selected as the metric to conduct the exposure CPK toxicity analyses, a choice that can be discussed as the most appropriate one, also referring to the

same selected exposure parameter in the exposure-responses analyses, as well as in the analyses on ALT elevation and plitidepsin exposure.

A temporal exposure-dependency of transaminase (ALT)-elevations was shown, and the incidence of severe ALT elevation is seemingly linearly related with the cumulative dose. The incidence of grade ≥3 creatine phosphokinase (CPK) elevations was not found to be dependent on plitidepsin exposure.

ALT peaks following weekly dosing are expected to be higher than those achieved in dosing every second week. According to the applicant, there is an observed tolerance to the elevation in ALT concentrations following subsequent cycles of plitidepsin administration that was investigated.

The applicant suggests a dose reduction in patients developing severe transaminase elevation, however, a high rate (degree) of dose reductions might reflect a starting dose that is too high. A lower dose may have been more appropriate regarding the observed serious treatment-related safety issues like cardiac disorders, myopathies, and liver affection by plitidepsin. Nevertheless, influence on efficacy of lower doses of plitidepsin in MM patients has not been investigated by the applicant.

Based on in vitro results no induction has been observed and it seems that the potential of plitidepsin to in vivo inhibit the enzymatic activities of the main CYP isoforms isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) or transporter function is considered to be remote because of the lowest estimated in vitro IC50, which was far greater than the Cmax values achieved in patients with relapsed/refractory MM.

No induction potential for plitidepsin at 10 ng/mL (for CYP3A4, 1A2 or 2B6) or at 100 ng/mL (for CYP 3A4 or 1A2), although a modest induction (ca. 2.3-fold) was reported for CYP2B6. Consequently, the induction potential of plitidepsin on CYP2B6 cannot be ruled out. However, it is considered unlikely that induction of plitidepsin on CYP2B6 will be of clinical relevance.

Bearing in mind the age of the target population, the use of plitidepsin in fertile women is less likely. Potential effect of plitidepsin on contraceptives has not been studied in an in vivo study, and effective measures to avoid pregnancy must be taken.

Results on the effect of plitidepsin on the QTcF are scarce as the QT substudy was only performed on data from seven patients. The limited sample size therefore precludes ruling out an effect of plitidepsin on the QT interval. Taking into account both preclinical and clinical findings regarding cardiac safety, the lack of clinically significant ECG abnormalities related to plitidepsin treatment can be reasonably attributed to the limited data available from the QT substudy rather than to the lack of effect itself. A safety study with QT evaluation as one of the primary endpoints (included in the RMP) is needed.

The PK analysis indicated no clinically significant influence of gender, race, weight, or patient age hence no dose adjustment is recommended based on those parameters. The PK is somewhat different in the younger patients.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology of plitidepsin is considered reasonably well investigated.

2.5. Clinical efficacy

2.5.1. Dose response study(ies)

The clinical development programme supporting the efficacy of plitidepsin plus DXM combination in the treatment of relapsed/refractory MM includes one primary phase III study (APL-C-001-09, ADMYRE) and one supportive phase II study (APL-B-014-03). Furthermore, supportive information on two phase I studies (APL-A-009-08 and APL-A-012-13) evaluating plitidepsin in the same disease setting (relapsed/refractory MM) but combined with bortezomib and DXM is given.

Table 27 Clinical studies included for the evaluation of efficacy of plitidepsin plus DXM in relapsed/refractory multiple myeloma

Study	Design and study population	Study treatment, dose and regimen	Treated patients
Phase III – Primary study			
APL-C-001-09 (ADMYRE) (Mod.5.3.5.1.1)	Multicentre, open-label, two-arm, 2:1 randomised, phase III pivotal clinical study in patients with relapsed/refractory MM previously treated with at least three but no more than six therapeutic regimens.	**Arm A:** plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion on D1 and 15 q4wk plus DXM 40 mg orally on D1, 8, 15 and 22, q4wk. **Arm B:** DXM alone at the same dose and schedule than in Arm A (40 mg orally on D1, 8, 15 and 22 q4wk).	**Arm A:** n=167 patients. **Arm B:** n=83 patients.
Phase II – Supportive study			
APL-B-014-03 (Mod.5.3.5.2.2)	Multicentre, open-label, exploratory, phase IIa clinical study in pre-treated patients with relapsed/refractory MM.	Plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion q2wk [a]. Patients with suboptimal response (PD after three plitidepsin cycles or SD after four plitidepsin cycles) in the first stage of the study received in a second stage 20 mg of oral DXM every day on D1 to 4 of each q2wk cycle, starting at the same time than plitidepsin infusion.	n=51 patients treated with plitidepsin. Nineteen of these 51 patients were treated with plitidepsin plus DXM in the second study stage.

2.5.2. Dose finding studies

For the treatment of MM, the recommended starting dose of plitidepsin is 5 mg/m², administered as an i.v. infusion on Day 1 and 15 q4wk, combined with 40 mg DXM orally administered on Day 1, 8, 15 and 22, q4wk.

An extensive phase I clinical development programme to define the dose and regimen of single-agent plitidepsin explored five administration regimens, including a total of 215 adult patients with various tumour types, both solid and haematological.

The RD found in the phase I programme for further development of plitidepsin as single agent consistently delivered similar dose intensity (around 2.5 mg/m²/week) and had a similar pattern of DLTs and antitumour activity, without unexpected toxicities, regardless of the schedule. Higher dose intensity could be obtained with the 24-hour biweekly schedule due to an improvement in plitidepsin-induced muscular toxicity when L-carnitine was supplemented. However, such protective effect of L-carnitine was not confirmed when tested in the first phase II study. Hence, this schedule was not further developed. In addition, shorter infusion times are more convenient for the patient and significantly reduce cost and treatment complexity. Since no significant differences were found in the dose intensity and the safety

profile of the long infusion regimens, the clinical development of 24-hour continuous infusion schedules was discontinued in favour of the 1-hour and 3-hour infusion regimens.

Therefore, two dose regimens remained available to be evaluated in the phase II clinical studies, according to specific features of the tested patient population:

1-hour weekly infusion (D1, 8 and 15, q4wk), at a starting dose of 3.2 mg/m²,

3-hour biweekly infusion (D1 and 15, q4wk), at a starting dose of 5.0 mg/m².

Patients with relapsed or refractory MM are heavily pre-treated, and they mostly are elderly patients, with significant tumour burden and associated comorbidities. Under these conditions, even grade 2 toxicities may have a significant impact on quality of life. Therefore, any therapy for this disease should ideally have an acceptable tolerability profile, ensuring similar dose intensity, as well as a simple and convenient administration. In this sense, spacing apart the infusion involves fewer visits, and reduces the risk of infusion site reactions and infections. Consequently, the 3-hour biweekly infusion regimen (D1 and 15, q4wk) was selected for the phase II study APL-B-014-03 in patients with MM.

The 5 mg/m² dose level for the biweekly 3-hour infusion schedule was set in phase I study APL-A-001b-98. After treating a total of 16 patients with solid and haematological tumours at this dose, levels of toxicity were within an acceptable range.

2.5.3. Main study

Study APL-C-001-09 (ADMYRE)

This was a randomized, multicentre, open-label, phase III study of plitidepsin in combination with dexamethasone vs. dexamethasone alone in patients with relapsed/refractory multiple myeloma.

Methods

Study Participants

Main inclusion criteria

ECOG performance status (PS) ≤ 2.

Life expectancy ≥ 3 months.

Patients previously diagnosed with MM based on IMWG diagnostic criteria.

Patients must have relapsed or relapsed and refractory multiple myeloma (MM) after at least three, but not more than six, prior therapeutic regimens for MM, including induction therapy and stem cell transplantation in candidate patients, which were considered as only one regimen.

Patients must have received previous bortezomib-containing and lenalidomide containing regimens (or thalidomide where lenalidomide was not available), unless unable to tolerate either of them.

Patients must have measurable disease defined as:

a) For secretory MM: any quantifiable serum monoclonal protein value and, where applicable, urine light-chain excretion ≥ 200 mg/24 hours.

b) For oligo or non-secretory MM: presence of soft tissue (not bone) plasmacytomas, as determined by clinical examination or applicable radiographs [i.e., magnetic resonance imaging (MRI), computed tomography (CT)-scan], and/or by the presence of abnormal serum free light chains (sFLC): involved free light chain (FLC) level ≥ 10 mg/dL provided the serum FLC ratio was abnormal.

At least two-week washout period since the end of last therapy (six weeks if previous nitrosoureas-containing regimen), given recovery to grade ≤ 1 from any non-hematological related adverse event (AE) derived from previous treatment (excluding alopecia).

Main exclusion criteria

Concomitant diseases/conditions:

a) History or presence of angina, myocardial infarction, clinically relevant valvular heart disease, cardiac amyloidosis or congestive heart failure within the last 12 months.

b) Symptomatic arrhythmia (excluding anemia-related sinusal tachycardia grade ≤ 2) or any arrhythmia requiring ongoing treatment, and/or prolonged QT-QTc grade ≥ 2; or presence of unstable atrial fibrillation. Patients with stable atrial fibrillation on treatment were allowed provided they did not meet any other cardiac or prohibited drug exclusion criterion.

c) Active uncontrolled infection.

d) Morphological or cytological features of myelodysplasia and/or postchemotherapy aplasia on BM assessment.

e) Myopathy > grade 2 or any clinical situation that causes significant and persistent elevation of CPK (>2.5 x ULN in two different determinations performed one week apart).

f) Known human immunodeficiency virus (HIV) infection (HIV testing was not required unless infection was clinically suspected).

g) Known active hepatitis B or C virus (HBV or HCV) infection.

h) Limitation of the patient's ability to comply with the treatment or followup requirements.

i) Any other major illness that, in the Investigator's judgment, substantially increased the risk associated with the patient's participation in this study.

j) Peripheral neuropathy > grade 2.

Concomitant medications that included corticosteroids, chemotherapy, or other therapy that is or may be active against MM, within two weeks prior to Cycle 1 Day 1. Concurrent corticosteroids were allowed, provided they were administered at an equivalent prednisone dose of ≤ 10 mg daily, as premedication for blood products only.

Treatments

The administration of each study medication was as follows:

• Arm A:

DXM: 40 mg orally on Day 1, 8, 15 and 22 q4wk at least one hour before plitidepsin infusion.

Plitidepsin: 5 mg/m^2 i.v. diluted to a total volume of 250 mL in 0.9% saline (or 5% glucose) via a central venous catheter (suggested) or diluted to a total volume of 500 mL in 0.9% saline (or 5% glucose) via a peripheral line. Infusion was performed through a pump device over three hours (fixed rate) on Day 1 and 15 q4wk.

• Arm B:

DXM: 40 mg orally on Day 1, 8, 15 and 22 q4wk. A cycle was defined as a four-week period.

All patients in arm A had to receive the following prophylactic medication 20-30 min before infusion of plitidepsin: Ondansetron 8 mg i.v. or equivalent (granisetron 3 mg i.v. preferred when available); Diphenhydramine hydrochloride 25 mg i.v. or equivalent, and Ranitidine 50 mg i.v. or equivalent.

Oral metoclopramide and/or extended oral ondansetron (or their equivalents) could be used as per Investigator's criteria/institutional guidelines. No prophylactic medication was specified in the arm B.

Patients in the control arm (DXM alone, Arm B) who had documented disease progression according to the Investigator's criteria after a minimum of eight weeks from randomization should be offered crossover to combination arm (plitidepsin plus DXM, Arm A) upon Sponsor's agreement. Patients who were withdrawn from Arm A for any reasons or from Arm B for any reason other than progression must not be re-treated in the context of this study at any time.

The design of the study is displayed in **Figure 9**.

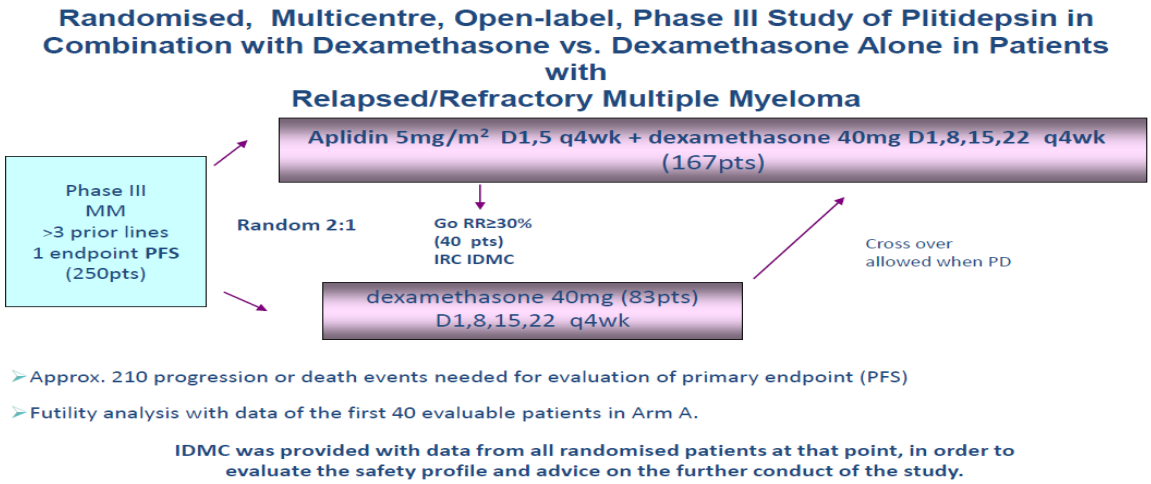


Figure 9. Study APL-C-001-09 design .

Objectives

The primary objective of the study was to compare the efficacy of plitidepsin with DXM vs. DXM alone, as measured by progression-free survival (PFS), in patients with relapsed/refractory MM.

Secondary objectives include the following:

To evaluate tumour response according to the International Myeloma Working Group (IMWG) criteria.

To assess duration of response (DR) and overall survival (OS).

To assess efficacy in patients who undergo crossover from DXM alone to plitidepsin and DXM combination.

To characterize and compare the safety profile on both arms in this population.

To characterize the pharmacokinetics (PK) and pharmacokinetic/ pharmacodynamic (PK/PD) relationship.

Outcomes/endpoints

The primary endpoint was PFS defined as the time from the date of randomization to the date of documented progressive disease (PD) by IMWG criteria or death (regardless of the cause of death).

Progressive disease was defined as:

Increase of 25% from lowest response value in any one or more of the following:

- Serum M-component (absolute increase must be ≥ 0.5 g/100 ml) and/or

- Urine M-component (absolute increase must be ≥ 200mg per 24 h) and/or

- Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels (absolute increase must be > 100 mg/l)

- Bone marrow plasma cell percentage (absolute % must be ≥ 10%)

- Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas

- Development of hypercalcemia (corrected serum calcium > 11.5 mg/100 ml) that can be attributed solely to the plasma cell proliferative disorder

Secondary efficacy endpoints included the following:

Objective response defined as having minor response (MR) or better as best overall response based on the IMWG criteria.

Duration of response will be calculated from the date of first documentation of response (not the confirmation) to the date of disease progression or death with the same censoring rules as PFS. The duration of objective response will be analysed in all patients for whom at least a MR has been observed.

Overall survival defined as the time from the date of randomization to the date of death or last contact.

Intrapatient response and PFS comparison of patients who crossed over from DXM alone (Arm B) to plitidepsin plus DXM combination (Arm A).

Sample size

Approximately 210 progression or death events were needed in this trial to reject the equality of hazard rates between both treatment arms, assuming a HR of 0.625 in favour of the combination arm with 90% power and 1-sided 2.5% significance level. It was estimated that up to 250 randomized patients were needed to achieve the 210 events.

Randomisation

Eligible patients were stratified according to their ECOG PS score (0 and 1 vs. 2) and Durie-Salmon stage (I/II vs. III), and then randomized using a 2:1 randomization procedure to Arm A (plitidepsin plus DXM) or Arm B (DXM alone).

Blinding (masking)

This was an open-label study.

Statistical methods

Patient Populations

All randomized patients analysis dataset was defined as all patients who were randomized to either treatment arm, independent of whether they received the study drug.

All treated patients analysis dataset was defined as all randomized patients who received at least part of one dose or infusion of the study drugs.

All evaluable patients analysis dataset was defined as all randomized patients who completed at least one full cycle of treatment or received two incomplete cycles followed by at least one response assessment not less than eight weeks (± one week) after treatment onset. Patients withdrawn from the study due to early PD or treatment-related toxicity were considered as "early progression" or "treatment failure", respectively, even though they have not received a full cycle. Patients withdrawn due to significant clinical deterioration of unknown reason, hypersensitivity reactions, or refusal to continue on study for any reason or unrelated AEs without any disease assessments after the start of study treatment or those patients with a protocol deviation resulting in an impossibility of drawing conclusions about the efficacy of the study therapy were considered not evaluable for efficacy and their response was categorized as "non-evaluable".

All responder patients analysis dataset was defined as all evaluable patients who had MR or better as overall best response.

All crossover patients analysis dataset was defined as all patients randomized to Arm B who had documented PD after eighth week from randomization and crossed over to Arm A.

PFS Analysis

The final PFS analysis was to be performed when at least 210 progression or death events were observed. The unstratified log rank test was used to compare the PFS of Arm A and Arm B. Both the IRC and Investigator's assessments (IA) were used. Cox regression was used to calculate the risk reduction in PFS.

The stratified log-rank test for PFS was also performed as supportive analysis. Both the IRC and IA were used.

Sensitivity analyses to test the consistency of PFS were done; e.g., PFS in the all evaluable patients dataset according to both IRC and IA, or PFS calculated using different imputation algorithms.

The impact of potential asymmetry of assessments or missing tumour evaluations on PFS analysis was also assessed.

For patients within the all crossover patients dataset, PFS was defined as the time from the day of the last disease assessment, before the first administration of the combination, to the date of PD by IMWG criteria or death (regardless of the cause of death). The same censoring rules described for PFS calculation were considered.

OS analysis

The effect of crossover was assessed by different sensitivity analyses, excluding the patients who crossed over, censoring survival at the time of crossover, by means of rank preserving structural failure time (RPSFT) models, and by means of the two-stage method.

Early Futility Analysis

An early futility analysis was performed when information from 40 patients in Arm A were evaluable for response. A response rate (IMWG criteria) of at least 30% (12 or more responses by IRC review) was taken as threshold for continuation of the study. A minimum response rate of 30% was considered as clinically significant in this setting.

This result ensured that the lower limit of the exact binomial 95% CI for RR would be greater than 15% (95% CI in case of 12 responses would be 16.6%-46.5%).

However, the information from all randomized patients in both arms at that time was used by the IDMC to evaluate the safety profile and to provide the Sponsor with a recommendation for the further study conduct. No claim for superiority in efficacy was to be formulated in this interim analysis and no alpha-spending for the analysis of PFS was foreseen. Accrual was to be on-hold while data for the futility analysis was assessed.

For futility analysis based on objective RR, the all evaluable patients analysis dataset was used. On 9 December 2012, the evaluation by the IDMC of efficacy and safety data from the first 60 evaluable patients included in this study resulted in the recommendation to continue the trial unmodified, as the study met the established efficacy threshold of 30% response rate according to IMWG criteria pre-specified in the protocol (RR in Arm A, plitidepsin plus DXM, was 37.8%).

Results

Participant flow

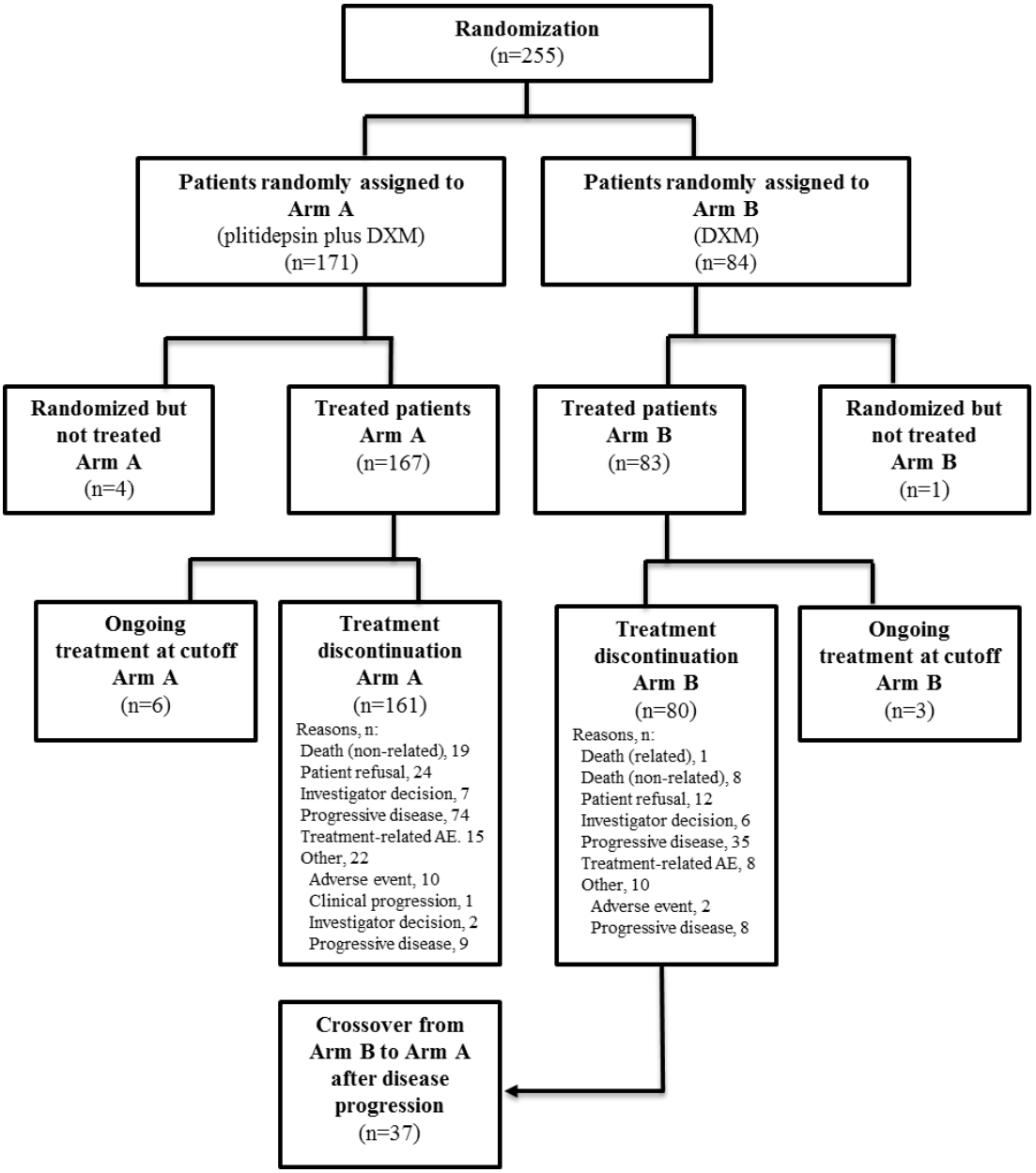


Figure 10 Disposition of patients (APL-C-001-09)

Recruitment

The first randomization took place on 29 June 2010 and the first study treatment administration was on 30 June 2010.

Conduct of the study

The first protocol version (v.1.0) was issued on 8 February 2010. Two substantial amendments to the protocol were implemented. As this was a multinational study, different versions at each step were created.

Key changes under the Amendment No. 1 (28 December 2010)

The exact binomial 95% CI for RR was provided to clarify futility analysis rules, as requested by the IDMC.

Patient eligibility criteria were modified to allow inclusion of patients with stable atrial fibrillation, or patients with controlled infection on antibiotics.

Key changes under the Amendment No. 2 (12 April 2013)

To allow skeletal evaluation at baseline and end of treatment or whenever required, by X-ray or CT-scan, as long as the same procedure is used throughout the study.

The conduct of the QTc substudy to assess the potential effects of plitidepsin on the heart activity of patients enrolled in clinical trial APL-C-001-09. In particular the QTc interval, based on electrocardiogram (ECG) evaluation, was to be recorded and studied.

In APL-C-001-09 (ADMYRE), the IMWG uniform criteria for MM were initially used to document disease progression or response to treatment. In the Durie et al. version, confirmation of PD in two different assessments is required. An updated version of IMWG criteria (Anderson et al. Leukemia, 2008) includes restoration of the MR category, and confirmation of PD in two different disease assessments is not mentioned. As the protocol was designed in the transition period between both versions, PD was confirmed with two separate assessments during the first stage of the APL-C-001-09 trial. In the second study stage, this protocol amendment implemented the use of the updated IMWG criteria only, and to remove the Durie et al. IMWG version. Therefore, the decision taken at that time was to not confirm PD in two assessments. Due to this reason, the analysis of the primary endpoint (PFS) was to identify the first PD reported, with no further confirmation.

Protocols deviations

None of Major deviations corresponding to the "ineligible patients as per protocol" category resulted in patient withdrawal from the study. Four of these protocol deviations consisting in having received less than three prior therapeutic regimens for MM or not having previously received bortezomib resulted in the exclusion for efficacy analysis in the All Evaluable Patients dataset of four patients from Arm A (plitidepsin plus DXM). Additionally, another patient from Arm A did not previously receive bortezomib and was not evaluable for efficacy, and a protocol deviation form was not signed yet at the cut-off date.

Baseline data

The patient and the disease characteristics at baseline are displayed Table 28 and Table 29.

Table 28 Patient characteristics at baseline (APL-C-001-09)

		Arm A (P+DXM) (n=171)		Arm B (DXM) (n=84)		Total (n=255)	
		n	**%**	**n**	**%**	**n**	**%**
Gender	**Female**	74	43.3	49	58.3	123	48.2
	Male	97	56.7	35	41.7	132	51.8
Age (years)	**Median**	64		65		65	
	Range	36-85		42-85		36-85	
	18-64	88	51.5	36	42.9	124	48.6
	65-74	57	33.3	35	41.7	92	36.1
	75-84	25	14.6	12	14.3	37	14.5
	≥85	1	0.6	1	1.2	2	0.8
ECOG PS	**0**	68	39.8	31	36.9	99	38.8
	1	74	43.3	42	50.0	116	45.5
	2	28	16.4	11	13.1	39	15.3
	3	1	0.6	.	.	1	0.4
Hb (g/dL)	**Median**	10.4		10.1		10.3	
	Range	7.0-14.6		7.4-14.6		7.0-14.6	
Platelets (10^3/L)	**Median**	140		154.0		144.0	
	Range	11.0-517.0		24.0-452.0		11.0-517.0	
CrCL (mL/min)	**Median**	72.9		69.4		71.9	
	Range	21.9-252.2		23.0-137.0		21.9-252.2	
Time from first diagnosis to randomization (months)	**Median**	71.8		70.0		71.3	
	Range	0.1-277.2		19.5-178.9		0.1-277.2	
Time from last PD/relapse to first study dose (weeks)	**Median**	6.1		6.4		6.2	
	Range	-0.4-83.1		1.0-101.7		-0.4-101.7	

Table 29. Disease characteristics at baseline (APL-C-001-09)

		Arm A (P+DXM) (n=171)		Arm B (DXM) (n=84)		Total (n=255)	
		n	%	n	%	n	%
MM type at baseline [a]	Non-secretory	17	10.1	2	2.4	19	7.6
	Secretory	151	89.9	80	97.6	231	92.4
MM proteins (serum) [b]	M-spike (g/dL)	n=168		n=80		n=248	
	Median	2.5		2.6		2.5	
	Range	0.00-7.8		0.00-7.6		0.01-7.8	
	sFLC (ratio)	n=53		n=23		n=76	
	Median	16.8		0.01		13.6	
	Range	0.0-10900		0.0-1955		0.0-10900	
	Total IgA (mg/dL)	n=106		n=56		n=162	
	Median	71.5		88.5		73.0	
	Range	25-7060		25-9560		25-9560	
	Total Ig G(mg/dL)	n=165		n=79		n=244	
	Median	2060		1900		1960	
	Range	103-10800		140-9420		103-10800	
	Total IgM (mg/dL)	n=62		n=27		n=89	
	Median	36.0		33.0		36.0	
	Range	20.0-7510		20.0-73.0		20.0-7510	
	Kappa (mg/dL)	n=144		n=67		n=211	
	Median	73.4		22.6		51.7	
	Range	0.5-10900		1.1-16700		0.5-16700	
	Lambda (mg/dL)	n=150		n=72		n=222	
	Median	9.3		13.3		10.0	
	Range	0.4-7300		0.3-5420		0.3-7300	
MM proteins (urine) [b]	M-spike (Bence Jones) (mg/24h)	n=156		n=76		n=232	
	Median	158.2		114.5		127.2	
	Range	0.0-33122.7		0.0-6047.2		0.0-33122.7	
	Kappa (mg/dL)	n=154		n=75		n=229	
	Median	74.9		33.7		54.6	
	Range	0.3-76100		1.6-133000		0.3-133000	
	Lambda (mg/dL)	n=145		n=70		n=215	
	Median	5.2		6.9		5.8	
	Range	0.1-20900		0.3-19800		0.1-20900	
	Protein 24 hour (mg/24h)	n=155		n=75		n=230	
	Median	375.0		260.0		360	
	Range	23.0-37050.0		18.0-9540		18.0-37050	
Urine immunofixation [b,c]	n	n=152		n=77		n=229	
	Negative	25	16.4	9	11.7	34	14.8
	Positive	127	83.6	68	88.3	195	85.2
Percentage of BM plasma cells [d]	Biopsy	n=86		n=35		n=121	
	Median	50.0		39.9		45.0	
	Range	0.0-100.0		0.0-90.0		0.0-100.0	
	Smears	n=76		n=43		n=119	
	Median	24.5		32.0		30.0	
	Range	0.0-97.2		0.1-100.0		0.0-100.0	
	Total	n=162		n=78		n=240	
	Median	40.0		33.0		40.0	
	Range	0.0-100.0		0.0-100.0		0.0-100.0	
Cytogenetic risk group at baseline	Standard risk	47	27.5	22	26.2	69	27.1
	High risk	45	26.3	20	23.8	65	25.5
	NA/ND/UK [e]	79	46.2	42	50.0	121	47.5
Skeletal sites involved at baseline [f]	No	53	31.0	31	36.9	84	32.9
	Yes	118 [g]	69.0	53	63.1	171	67.1
	Bone (lytic lesion)	114	96.6	51	96.2	165	96.5
	Plasmacytoma	12	10.2	10	18.9	22	12.9
	Plasmacytoma dimension (mm^2) [h]	n=6		n=8		n=14	
	Median	2328		2315		2315	
	Range	504-6156		99-64400		99-64400	

The disease characteristics at diagnosis are displayed in **Table 30**.

Table 30 Disease characteristics at diagnosis (APL-C-001-09)

		Arm A (P+DXM) (n=171)		Arm B (DXM) (n=84)		Total (n=255)	
		n	%	n	%	n	%
MM type at diagnosis	Non-secretory	6	3.5	1	1.2	7	2.7
	Secretory	165	96.5	83	98.8	248	97.3
	IgA	35	20.5	21	25.0	56	22.0
	IgD	1	0.6	1	1.2	2	0.8
	IgG	101	59.1	51	60.7	152	59.6
	IgM	1	0.6	.	.	1	0.4
	Light chain disease	27	15.8	10	11.9	37	14.5
Durie-Salmon stage at diagnosis [a]	A	.	.	1	1.2	1	0.4
	IA	21	12.4	9	10.7	30	11.8
	IB	.	.	2	2.4	2	0.8
	II	3	1.8	1	1.2	4	1.6
	IIA	44	25.9	20	23.8	64	25.2
	IIB	1	0.6	.	.	1	0.4
	III	2	1.2	1	1.2	3	1.2
	IIIA	85	50.0	43	51.2	128	50.4
	IIIB	14	8.2	7	8.3	21	8.3
ISS stage at diagnosis [b]	I	72	52.9	33	50.0	105	52.0
	II	41	30.1	18	27.3	59	29.2
	III	23	16.9	15	22.7	38	18.8
Beta-2 microglobulin (mg/L)	Median	4.1		4.2		4.1	
	Range	0.0-27.3		0.2-65.0		0.0-65.0	
Cytogenetic risk group at diagnosis [c]	Standard risk	34	19.9	21	25.0	55	21.6
	High risk	38	22.2	16	19.0	54	21.2
	NA/ND/UK [d]	99	57.9	47	56.0	146	57.3

Data shown are n of randomized patients (%) except for median (range).
a Patient had no Durie-Salmon stage at diagnosis.
b 53 patients had not ISS stage at diagnosis.
c High risk: Patients with translocations such as t(4;14), t(14;16), t(14;20), del 17, del 13 or single alterations such as +1q or +1p.
Standard risk: Patients with translocations such as t(11;14), t(6;14) or other, as well as patients with single alterations of trisomies 3, 5, 6, 9, 11, 15, 19 or 21.
d Details for patients with missing karyotype or FISH data at diagnosis are shown in Section 14.1, Table 14.8.

Details for response according to the type of prior agent (bortezomib, lenalidomide, thalidomide) or the group of prior agents (IMiDs or PIs) is provided in Table 31.

Table 31. Prior anticancer therapy

		Arm A (P+DXM) (n=171)		Arm B (DXM) (n=84)		Total (n=255)	
		n	%	n	%	n	%
Prior radiotherapy		68	40.0	35	42.0	103	40.0
Number of lines of prior systemic therapy	**Median**	4		4		4	
	Range	2-6		3-7		2-7	
Disease status							
Relapsed and refractory to lenalidomide/thalidomide and bortezomib		65	38.0	33	39.3	98	38.4
Relapsed and refractory to lenalidomide/thalidomide but not to bortezomib		39	22.8	24	28.6	63	24.7
Relapsed and refractory to bortezomib but not to lenalidomide/thalidomide		20	11.7	12	14.3	32	12.5
Other than above		47	27.5	15	17.9	62	24.3
Disease status with respect to last prior therapy	**Relapsed**	34	19.9	15	17.9	49	19.2
	Total refractory	126	73.7	62	73.8	188	73.7
	Refractory	71	41.5	39	46.4	110	43.1
	Relapsed and refractory	55	32.2	23	27.4	78	30.6
	Unknown	11	6.4	7	8.3	18	7.1
Disease status with respect to prior bortezomib	**Relapsed**	64	38.3	32	38.1	96	38.2
	Total refractory	92	55.1	47	56.0	139	55.4
	Refractory	53	31.7	22	26.2	75	29.9
	Relapsed and refractory	39	23.4	25	29.8	64	25.5
	Unknown	11	6.6	5	6.0	16	6.4
Disease status with respect to prior lenalidomide	**Relapsed**	42	25.1	15	18.3	57	22.9
	Total refractory	109	65.3	62	75.6	171	68.7
	Refractory	50	29.9	29	35.4	79	31.7
	Relapsed and refractory	59	35.3	33	40.2	92	36.9
	Unknown	16	9.6	5	6.1	21	8.4
Disease status with respect to prior thalidomide	**Relapsed**	36	31.9	21	39.6	57	34.3
	Total refractory	66	58.4	27	50.9	93	56.0
	Refractory	36	31.9	12	22.6	48	28.9
	Relapsed and refractory	30	26.5	15	28.3	45	27.1
	Unknown	11	9.7	5	9.4	16	9.6
Disease status with respect to prior IMiDs	**Relapsed**	42	24.6	17	20.2	59	23.1
	Total refractory	114	66.7	61	72.6	175	68.6
	Refractory	58	33.9	34	40.5	92	36.1
	Relapsed and refractory	56	32.7	27	32.1	83	32.5
	Unknown	15	8.8	6	7.1	21	8.2
Disease status with respect to prior PIs	**Relapsed**	58	34.7	31	36.9	89	35.5
	Total refractory	98	58.7	48	57.1	146	58.2
	Refractory	54	32.3	23	27.4	77	30.7
	Relapsed and refractory	44	26.3	25	29.8	69	27.5
	Unknown	11	6.6	5	6.0	16	6.4
Best response to last prior therapy	**sCR**	2	1.2	.	.	2	0.8
	CR	9	5.3	3	3.6	12	4.7
	VGPR	19	11.1	7	8.3	26	10.2
	PR	44	25.7	26	31.0	70	27.5
	MR	15	8.8	2	2.4	17	6.7
	SD	42	24.6	23	27.4	65	25.5
	PD	29	17.0	16	19.0	45	17.6
	Not evaluable	1	0.6	1	1.2	2	0.8
	Not applicable	3	1.8	.	.	3	1.2
	Unknown	7	4.1	6	7.1	13	5.1
TTP to last anticancer therapy (months)	**n**	n=161		n=82		n=243	
	Median	7.5		7.0		7.5	
	Range	0.0-52.8		0.5-40.1		0.0-52.8	
Prior SCT	**Number of SCT**						
	0	56	32.7	29	34.5	85	33.3
	1	82	48.0	39	46.4	121	47.5
	≥2	33	19.3	16	19.0	49	19.2
	Type						
	Allogenic	6	5.2	5 [m]	9.1	11	6.5
	Autologous	115	100.0	54	98.2	169	99.4

Numbers analysed

A summary of the populations analyzed is displayed in .

Table 32. Number of patients in analysis sets used to evaluate efficacy endpoints (APL-C-001-09)

	Arm A (P+DXM)		Arm B (DXM)		Total	
	n	%	n	%	n	%
All Randomized Patients [a,c]	171	67.1	84	32.9	255	100.0
All Evaluable Patients [b,c]	145	84.8	75	89.3	220	86.3
All Responder Patients [d]						
According to IRC assessment	39	22.8	3	3.6	42	16.5
According to IA assessment	51	29.8	1	1.2	52	20.4
All Crossover Patients [e]	.	.	37	44.0	37	14.5

Data shown are n of patients (%).
[a] The *All Randomized Patients* analysis set was used for the primary endpoint analysis (PFS) and the main efficacy analyses, as well as for all OS analyses.
[b] The *All Evaluable Patients* analysis set was used for futility analysis based on objective RR.
[c] The *All Randomized Patients* and *All Evaluable Patients* analysis sets were used for the final analysis of RR. In evaluable patients, a low number can be found in some analysis as the number shown here corresponds to the Sponsor's assessment of evaluability and in some cases IRC and IA could differ in opinion. IRC and IA numbers were used for preplanned sensitivity analyses.
[d] The *All Responder Patients* dataset was used for DR calculation.
[e] The *All Crossover Patients* (from Arm B to Arm A) dataset was used for the exploratory intrapatient comparison of response and PFS (before and after crossover).
DR, duration of response; DXM, dexamethasone; IA, Investigator's assessment; IRC, Independent Review Committee; OS; overall survival; P, plitidepsin; PFS, progression-free survival; RR, response rate.

Outcomes and estimation

Primary efficacy endpoint: Progression free survival

PFS (IRC-All Randomized Patients) (Primary Analysis)

The results of PFS presented from the blinded IRC as primary analysis (191 events) are displayed in Table 33 and Figure 11.

Table 33. Progression-free survival: IRC-All Randomized Patients (APL-C-001-09)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
n	171	84	.	.
Number of events	130 (76.0%)	61 (72.6%)	.	.
Censored	41 (24.0%)	23 (27.4%)	.	.
Median (95% CI)	2.6 (1.9-3.0)	1.7 (1.1-2.0)	Log-rank: 7.746 HR [a]: 0.650 95%CI (0.477-0.885)	LR:0.0054 HR [a]:0.0062
PFS at 6 months (95% CI)	20.0% (13.1-26.9%)	10.0% (2.0-18.0%)	Diff: 10.0%	0.0618

Data shown are n (%) of randomized patients except for median (months).
[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.
CI, confidence interval; Diff, difference between Arm A and Arm B; DXM, dexamethasone; HR, hazard ratio; LR, unstratified log-rank test; P, plitidepsin; PFS, progression-free survival.

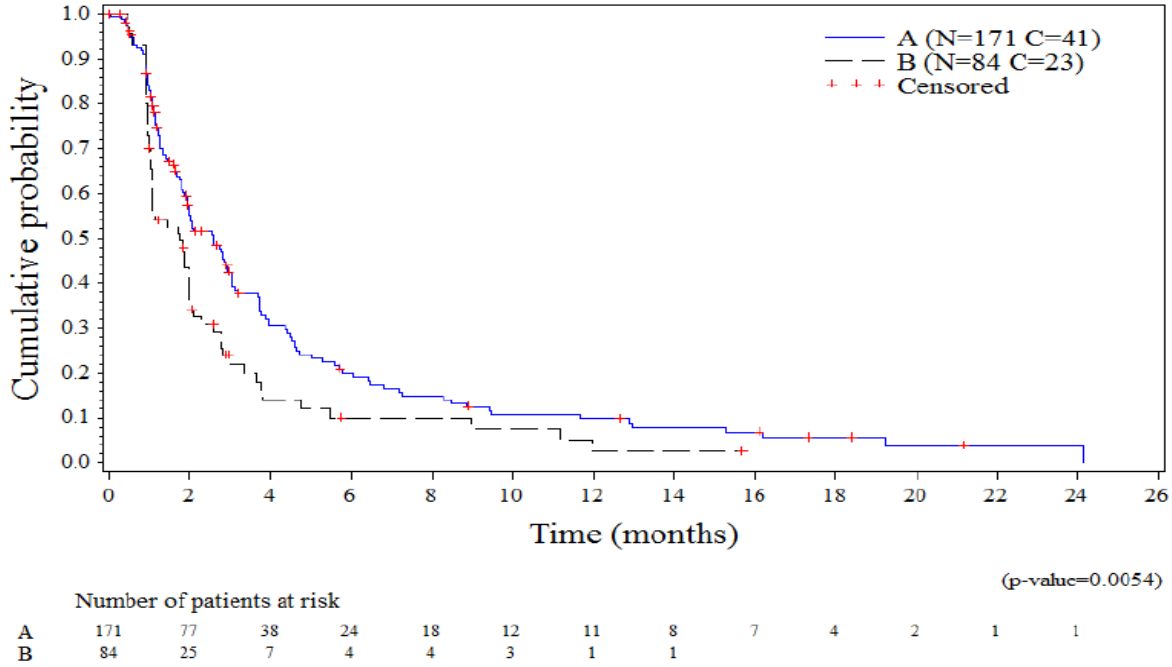


Figure 11 Kaplan-Meier plot of PFS (IRC–All Randomized Patients) (APL-C-001-09)

The results of the stratified supportive analysis of PFS by IRC in all randomized patients dataset is displayed in Table 34.

Table 34 Progression-free survival: IRC-All Randomized Patients (stratified) (APL-C-001-09)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
Stratum 1 - ECOG PS 0-1; Durie-Salmon stage I-II				
n	59	30	.	.
Number of events	42 (71.2%)	19 (63.3%)	.	.
Censored	17 (28.8%)	11 (36.7%)	.	.
Median (95% CI)	2.9 (2.0-3.9)	2.0 (1.4-2.8)	.	.
PFS at 6 months (95% CI)	28.2% (15.0-41.4%)	24.3% (4.9-43.6%)	.	.
Stratum 2 - ECOG PS 2; Durie-Salmon stage I-II				
n	9	4	.	.
Number of events	9 (100.0%)	3 (75.0%)	.	.
Censored	0 (0.0%)	1 (25.0%)	.	.
Median (95% CI)	1.6 (1.1-4.6)	2.2 (0.9-.)	.	.
PFS at 6 months (95% CI)	11.1% (0.0-31.6%)	. (0.0-.%) [a]	.	.
Stratum 3 - ECOG PS 0-1; Durie-Salmon stage III				
n	87	42	.	.
Number of events	66 (75.9%)	34 (81.0%)	.	.
Censored	21 (24.1%)	8 (19.0%)	.	.
Median (95% CI)	2.1 (1.6-2.9)	1.1 (1.0-2.0)	.	.
PFS at 6 months (95% CI)	18.2% (8.7-27.6%)	0.0% (0.0-.%) [a]	.	.
Stratum 4 - ECOG PS 2; Durie-Salmon stage III				
n	16	8	.	.
Number of events	13 (81.3%)	5 (62.5%)	.	.
Censored	3 (18.7%)	3 (37.5%)	.	.
Median (95% CI)	4.3 (0.9-5.3)	1.0 (0.5-3.8)	.	.
PFS at 6 months (95% CI)	7.9% (0.0-22.8%)	0.0% (0.0-.%) [a]	.	.

All	Log-rank:8.977 HR [b]: 0.616 95%CI (0.448-0.848)	LR:0.0027

Data shown are n (%) of randomized patients except for median (months).
Stratum according to Durie-Salmon stage and ECOG PS at time of randomization.
[a] Superior interval could not be calculated as 0 patients had PFS at 6 months.
[b] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.

PFS (IA-All Randomized Patients) (Secondary Analysis)

A total of 203 events of progression or death reported by the Investigator's assessment (IA) form the basis for this analysis. The results are displayed in Table 35 and Figure 12.

Table 35 Progression-free survival: IA-All Randomized Patients (APL-C-001-09)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
n	171	84	.	.
Number of events	131 (76.6%)	72 (85.7%)	.	.
Censored	40 (23.4%)	12 (14.3%)	.	.
Median (95% CI)	2.9 (2.1-3.7)	1.1 (1.0-1.9)	Log-rank:21.362 HR [a]: 0.512 95%CI (0.382-0.686)	LR:<0.0001 HR [a]:<0.0001
PFS at 6 months (95% CI)	26.4% (19.1-33.7%)	8.1% (1.9-14.3%)	Diff: 18.3%	0.0002

Data shown are n (%) of randomized patients except for median (months).
[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.
CI, confidence interval; Diff, difference between Arm A and Arm B; DXM, dexamethasone; HR, hazard ratio; LR, unstratified log-rank test; P, plitidepsin; PFS, progression-free survival.

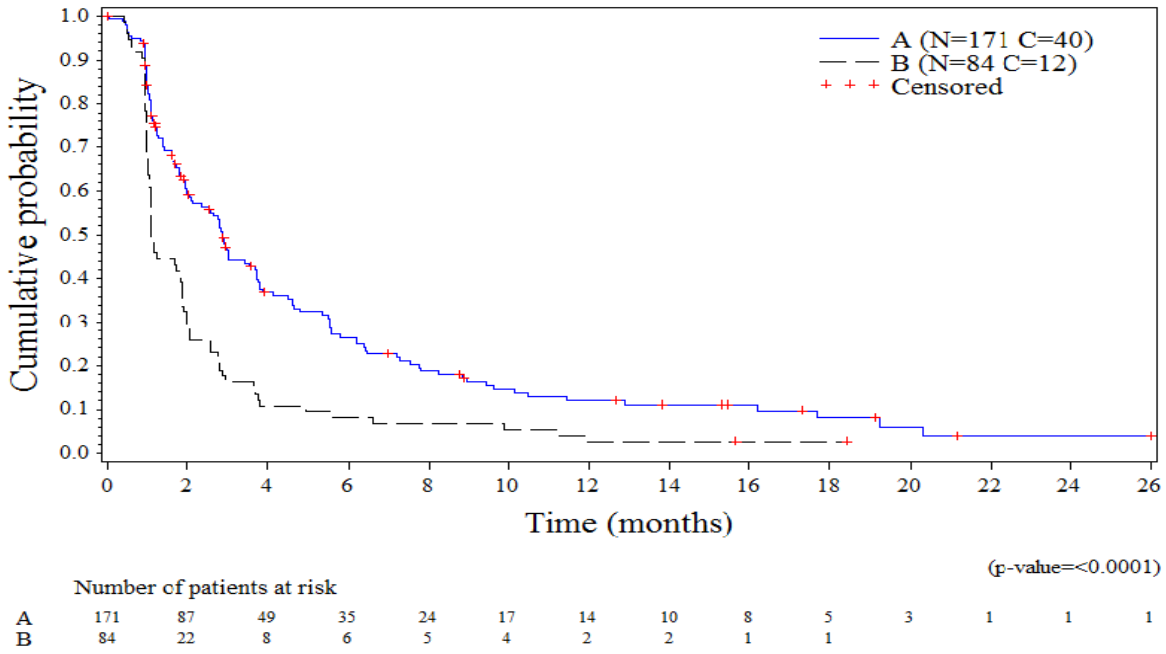


Figure 12 Kaplan-Meier plot of PFS (IA–All Randomized Patients) (APL-C-001-09)

Table 36 Progression-free survival: IA-All Randomized Patients (stratified) (APL-C-001-09)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
Stratum 1 - ECOG PS 0-1; Durie-Salmon stage I-II				
n	59	30	.	.
Number of events	42 (71.2%)	21 (70.0%)	.	.
Censored	17 (28.8%)	9 (30.0%)	.	.
Median (95% CI)	3.5 (2.1-5.4)	1.9 (1.1-2.6)	.	.
PFS at 6 months (95% CI)	29.1% (16.1-42.1%)	21.7% (4.9-38.6%)	.	.
Stratum 2 - ECOG PS 2; Durie-Salmon stage I-II				
n	9	4	.	.
Number of events	8 (88.9%)	4 (100.0%)	.	.
Censored	1 (1.1%)	0 (0.0%)	.	.
Median	1.9	3.0	.	.
(95% CI)	(1.1-7.8)	(0.9-5.5)		
PFS at 6 months (95% CI)	22.2% (0.0-49.4%)	0.0% (0.0-.%) [a]	.	.
Stratum 3 - ECOG PS 0-1; Durie-Salmon stage III				
n	87	42	.	.
Number of events	67 (77.0%)	40 (95.2%)	.	.
Censored	20 (23.0%)	2 (4.8%)	.	.
Median (95% CI)	2.7 (1.8-3.0)	1.1 (1.0-1.8)	.	.
PFS at 6 months (95% CI)	24.8% (14.6-35.0%)	2.5% (0.0-7.3%)	.	.
Stratum 4 - ECOG PS 2; Durie-Salmon stage III				
n	16	8	.	.
Number of events	14 (87.5%)	7 (87.5%)	.	.
Censored	2 (12.5)	1 (12.5%)	.	.
Median (95% CI)	3.3 (1.4-6.2)	1.0 (0.5-1.1)	.	.
PFS at 6 months (95% CI)	28.6% (4.9-52.2%)	0.0% (0.0-.%) [a]	.	.
All			Log-rank:24.421 HR [b]: 0.466 95%CI (0.342-0.635)	LR<0.0001

Data shown are n (%) of randomized patients except for median (months).
Stratum according to Durie-Salmon stage and ECOG PS at time of randomization.
[a] Superior interval could not be calculated as 0 patients had PFS at 6 months.
[b] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.

Concordance between IRC and IA (All Randomized Patients)

Concordance between IRC and IA evaluations in those patients with both assessments is provided in Table 37 and Figure 13.

Table 37 Concordance between IRC and IA (All Randomized Patients) (APL-C-001-09)

		Arm A (P+DXM)		Arm B (DXM)		Total	
		n	%	n	%	n	%
Agreement on status	**Agreement on status**	146	85.4	69	82.1	215	84.3
	Agreement on status and date	84	49.1	55	65.5	139	54.5
Censored by investigator	**Agreement on censoring**	28	70.0	10	83.3	38	73.1
	Different date	9	32.1	.	.	9	23.7
	Same date	19	67.9	10	100.0	29	76.3
Event by investigator	**Agreement on event**	118	69.0	59	70.2	177	69.4
	Earlier date	15	12.7	7	11.9	22	12.4
	Later date	38	32.2	7	11.9	45	25.4
	Same date	65	55.1	45	76.3	110	62.1

Data shown are n (%) of patients evaluable for both IRC and IA.
DXM, dexamethasone; IA, Investigator's assessment; IRC, Independent Review Committee; P, plitidepsin.

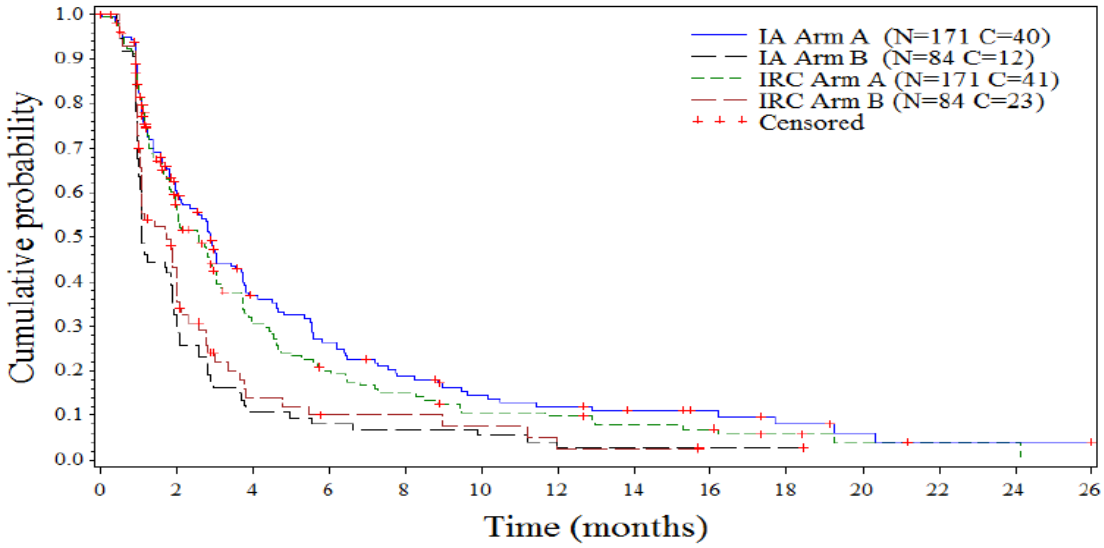


Figure 13 Kaplan-Meier plot of both PFS analyses (IRC and IA– All Randomized Patients) (APL-C-001-09)

- *Sensitivity analyses*

Table 38 Summary of analyses of PFS (APL-C-001-09, ADMYRE)

	Median PFS (95% CI) (months)		Log-rank p-value	HR	p-value
	Arm A (P+DXM)	Arm B (DXM)			
Primary analysis (IRC –All Randomised)	2.6 (1.9-3.0)	1.7 (1.1-2.0)	0.0054	0.650	0.0062
Secondary analysis (IA–All Randomised)	2.9 (2.1-3.7)	1.1 (1.0-1.9)	<0.0001	0.512	<0.0001
Stratified analyses					
IRC –All Randomised	.	.	0.0027	0.616	.
IA –All Randomised	.	.	<0.0001	0.466	.
Multivariate analysis (treatment arm)	.	.	.	0.616	0.0067
Pre-planned sensitivity analyses (All Randomised)					
With confirmation of PD					
IRC –All Randomised	5.0 (3.0-6.4)	2.0 (1.7-3.0)	0.0005	0.517	0.0007
IA –All Randomised	5.6 (3.8-8.9)	2.0 (1.7-3.7)	0.0052	0.581	0.0062
Midpoint imputation of PD dates	2.1 (1.5-2.6)	1.3 (0.5-1.4)	0.0040	0.643	0.0050
Weeks from randomisation imputation of PD dates	2.8 (1.8-2.8)	1.8 (-)	0.0202	0.732	0.0480
Missing assessment imputation of PD dates	2.3 (1.9-2.9)	1.8 (1.1-2.0)	0.0482	0.739	0.0513
Pre-planned sensitivity analyses (All Evaluable)					
IRC –All Evaluable	3.1 (2.8-3.9)	1.9 (1.1-2.6)	0.0024	0.584	0.0030
IA –All Evaluable	3.0 (2.7-3.8)	1.1 (1.0-1.9)	<0.0001	0.484	<0.0001

IA, Investigator's assessment; IRC, Independent Review Committee; PD, progressive disease.

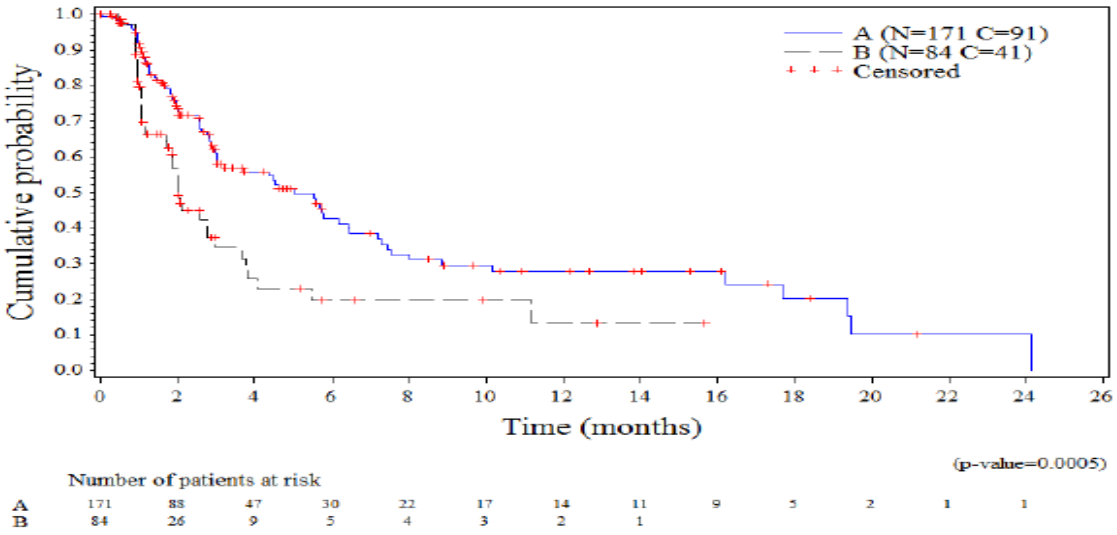


Figure 14 Kaplan-Meier plot of progression-free survival with confirmation of PD: IRC- All Randomized Patients (APL-C-001-09)

Secondary endpoint: Best Overall Response (Including Rate of MR or Better according to IMWG)

Table 39 Response according to IMWG: IRC-All Randomized Patients (APL-C-001-09)

	Arm A (P+DXM) (n=171)		Arm B (DXM) (n=84)	
	n	%	n	%
VGPR	2	1.2	.	.
PR	15	8.8	1	1.2
MR	22	12.9	2	2.4
SD	43	25.1	21	25.0
PD	41	24.0	35	41.7
NE	48	28.1	25	29.8
ORR [a] (95% CI)	22.8% (16.8-29.8%)		3.6% (0.7-10.1%)	
Fisher exact test (p-value)	<0.0001			
ORR (excluding MR) [b] (95% CI)	9.9% (5.9-15.4%)		1.2% (0.03-6.5%)	
Fisher exact test (p-value)	0.0085			

Data shown are n (%) of randomized patients except for ORR.
[a] Includes sCR, CR, VGPR, PR and MR.
[b] Includes sCR, CR, VGPR and PR (excludes MR).
CI, confidence interval; CR; complete response; DXM, dexamethasone; MR; minor response; NE, not evaluable; ORR, overall response rate; P, plitidepsin; PD, progressive disease; PR, partial response; sCR, stringent complete response; SD; stable disease; VGPR, very good partial response.

For all evaluable patients, ORR was 31.7% (95% CI, 23.6-40.7%) in Arm A (plitidepsin plus DXM) and 5.1% (95% CI, 1.1-14.2%) in Arm B (DXM) (p<0.0001). Clinical benefit rate was 66.7% (95% CI, 57.6-74.9%) in Arm A (plitidepsin plus DXM) and 40.7% (95% CI, 28.1- 54.3%) in Arm B (DXM) (p=0.0012).

Table 40 Response according to IMWG: IRC-All Evaluable Patients (APL-C-001-09)

	Arm A (P+DXM) (n=123)		Arm B (DXM) (n=59)	
	n	%	n	%
VGPR	2	1.6	.	.
PR	15	12.2	1	1.7
MR	22	17.9	2	3.4
SD	43	35.0	21	35.6
PD	41	33.3	35	59.3
ORR [a] (95% CI)	31.7% (23.6-40.7%)		5.1% (1.1-14.2%)	
Fisher exact test (p-value)	<0.0001			
ORR (excluding MR) [b] (95% CI)	13.8% (8.3-21.2%)		1.7% (0.04-9.1%)	
Fisher exact test (p-value)	0.0080			
Clinical benefit rate [c] (95% CI)	66.7% (57.6-74.9%)		40.7% (28.1-54.3%)	
Fisher exact test (p-value)	0.0012			

Data shown are n of evaluable patients (%) except for ORR and clinical benefit rate.
[a] Includes sCR, CR, VGPR, PR and MR.
[b] Includes sCR, CR, VGPR, and PR (excludes MR).
[c] Includes sCR, CR, VGPR, PR, MR and SD.
CI, confidence interval; CR, complete response; DXM, dexamethasone; MR, minor response; ORR, overall response rate; P, plitidepsin; PD, progressive disease; PR, partial response; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

Median DR was 3.7 months (95% CI, 2.7-10.5 months) in Arm A (plitidepsin plus DXM) and 1.8 months (9% CI, 1.8-5.5 months) in Arm B (DMX) as determined by the IRC in responder patients. Median DR was 5.1 months in Arm A (plitidepsin plus DXM) and 0.9 months in Arm B (DMX) as determined by the IA. In Arm A (plitidepsin plus DXM), median duration of response by the IRC in patients with response (VGPR, n=2; PR, n=15) was 12.0 months (95% CI, 2.8-23.2 months). In Arm B, only one patient had PR (duration of response was 1.8 months) (p=0.0092).

Median DR was 5.1 months in Arm A (plitidepsin plus DXM) and 0.9 months in Arm B (DMX) as determined by the IA (p=0.0001). Median DR in patients with response, therefore excluding MR (i.e., VGPR, n=4), as determined by the IA, was 7.8 months (95% CI, 4.2 months-not reached) in Arm A (plitidepsin plus DXM). In Arm B, (DXM), one patient had response other than MR with duration of 0.9 months (p<0.0001)

Median duration of response in patients with PD confirmation as determined by the IRC was 18.4 months (95% CI, 5.5-23.2 months) in Arm A (plitidepsin plus DXM; n=16 events) and 5.5 months (95% CI, 1.8-5.5 months) in Arm B (DXM; n=2 events) (p=0.1960)

Secondary endpoint: Overall Survival

At the cut-off date (20 November 2015), 42.1% of patients were censored in Arm A (plitidepsin plus DXM) and 32.1% in Arm B (DXM). Median follow-up was 17.1 months in Arm A and 20.7 months in Arm B.

Table 41 Overall survival: All Randomized Patients (APL-C-001-09)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
n	171	84	.	.
Number of events	99 (57.9%)	57 (67.9%)	.	.
Censored	72 (42.1%)	27 (32.1%)	.	.
Median	11.3	8.1	Log-rank:0.331	LR:0.5649
(95% CI)	(8.7-16.1)	(6.0-18.4)	HR [a]: 0.909	HR [a]:0.5654
			95%CI (0.655-1.260)	
OS at 12 months	47.3%	42.3%	Diff: 5.0%	0.4860
(95% CI)	(39.0-55.6%)	(31.1-53.5%)		
OS at 24 months	28.7%	24.5%	Diff: 4.2%	0.5752
(95% CI)	(19.6-37.8%)	(13.0-36.0%)		

Data shown are n of randomised patients (%) except for median (months) and OS at 12 and 24 months.
Median follow-up for survival was 17.1 months in Arm A and 20.7 months in Arm B.
[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.
CI, confidence interval; Diff, difference between Arm A and Arm B; DXM, dexamethasone; HR, hazard ratio; LR, unstratified log-rank test; OS, overall survival; P, plitidepsin.

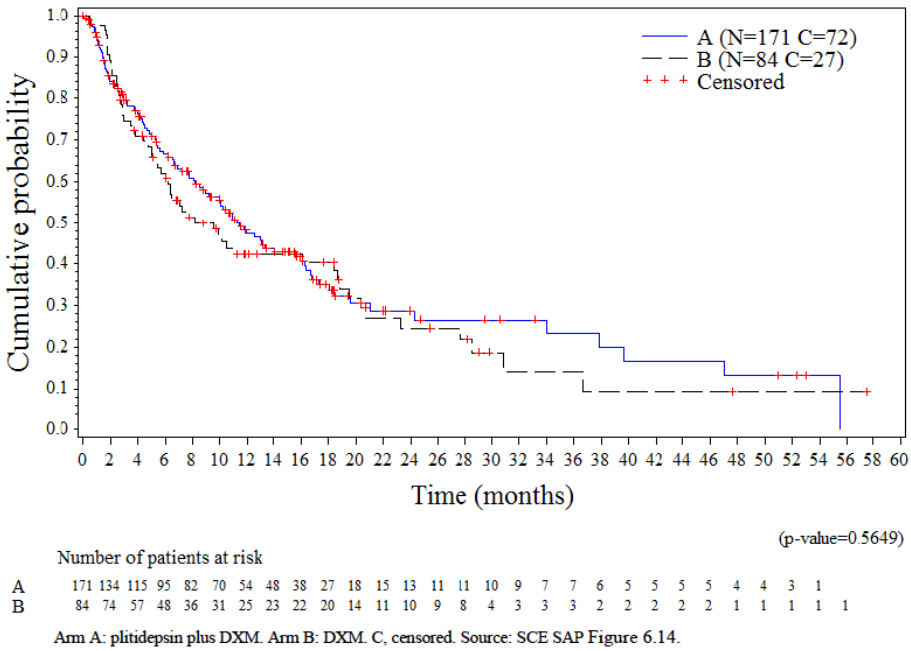


Figure 15 Kaplan-Meier plot of overall survival (All Randomized Patients) (APL-C-001-09)

An updated OS analysis was performed taking into account the planned date per protocol, that is, "when 80% of survival events have been reached or two years after the last patient has been randomised, whichever occurs first". Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on 195 death events.

Table 42. Interim and final unstratified primary analysis of overall survival (all randomised patients) in the APL-C-001-09 study

Overall survival		Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
Interim analysis [b] (cut-off 20 November 2015)	**n**	171	84	-	-
	Events	99 (57.9%)	57 (67.9%)	Log-rank:0.331 HR [a]: 0.909 95%CI (0.655-1.260)	LR:0.5649 HR [a]:0.5654
	Median (95% CI)	11.3 (8.7-16.1)	8.1 (6.0-18.4)		
Final analysis (cut-off 19 May 2017)	**n**	171	84	-	-
	Events	123 (71.9%)	72 (85.7%)	Log-rank:2.339 HR [a]: 0.797 95%CI (0.596-1.067)	LR: 0.1261 HR [a]: 0.1273
	Median (95% CI)	11.6 (9.2-16.1)	8.9 (6.0-15.4)		

Median in months.
[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.
[b] Source: Clinical Study Report (dated 25 July 2016).
CI, confidence interval; DXM, dexamethasone; HR, hazard ratio; LR, unstratified log-rank; P, plitidepsin.

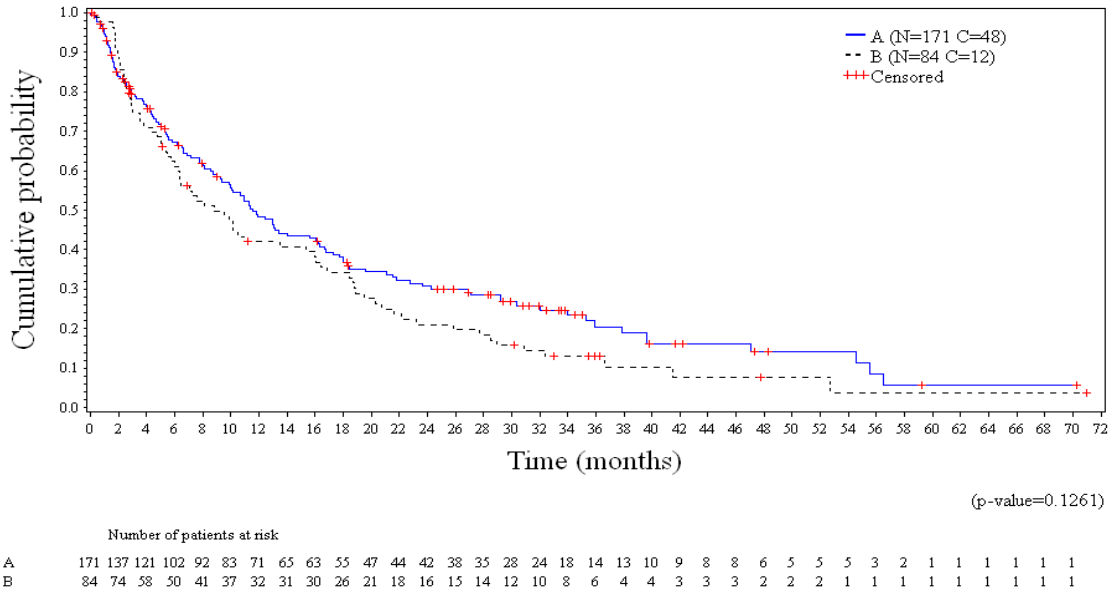


Figure 16: Overall survival (All Randomised Patients) (cut-off date 19 May 2017) (APL-C-001-09 study)

Table 43 Overall survival: All Randomized Patients (excluding patients with crossover) (APL-C-001-09)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
n	171	47	.	.
Number of events	99 (57.9%)	38 (80.9%)	.	.
Censored	72 (42.1%)	9 (19.1%)	.	.
Median (95% CI)	11.3 (8.7-16.1)	5.0 (2.8-6.4)	Log-rank:6.571 HR [a]: 0.614 95%CI (0.421-0.895)	LR:0.0104 HR [a]:0.0112
OS at 12 months (95% CI)	47.3% (39.0-55.6%)	26.0% (12.8-39.1%)	Diff: 21.3%	0.0072
OS at 24 months (95% CI)	28.7% (19.6-37.8%)	14.8% (2.7-27.0%)	Diff: 13.9%	0.0733

Data shown are n (%) of randomized patients except for median (months).Thirty-seven patients who crossed over were excluded in Arm B.

[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.

CI, confidence interval; Diff, difference between Arm A and Arm B; DXM, dexamethasone; HR, hazard ratio; LR, unstratified log-rank test; OS, overall survival; P, plitidepsin.

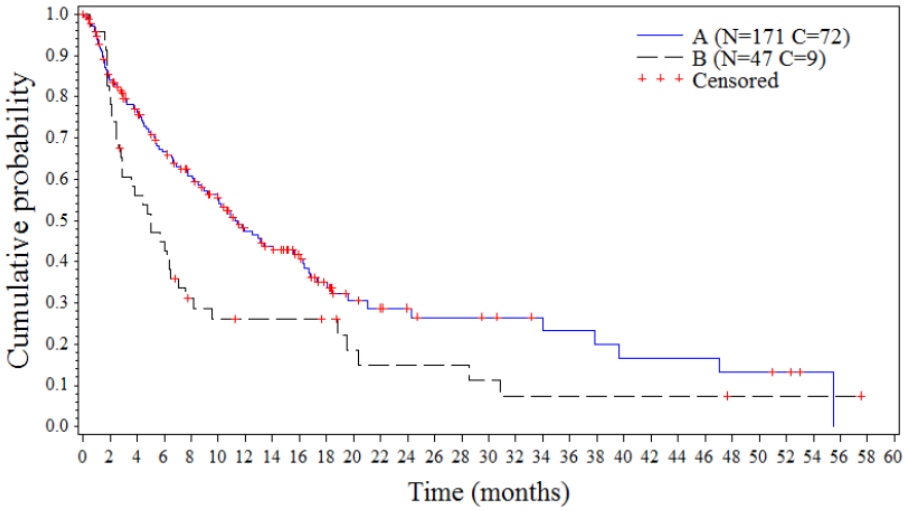


Figure 17 Kaplan-Meier plot of OS in All Randomised Patients (excluding patients with crossover) (APL-C-001-09)

Table 44 Overall survival: All Randomized Patients (censoring patients with crossover at crossover date) (APL-C-001-09)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
n	171	84	.	.
Number of events	99 (57.9%)	38 (45.2%)	.	.
Censored	72 (42.1%)	46 (54.8%)	.	.
Median (95% CI)	11.3 (8.7-16.1)	6.4 (5.0-18.8)	Log-rank:0.940 HR [a]: 0.829 95%CI (0.566-1.213)	LR:0.3324 HR [a]:0.3333
OS at 12 months (95% CI)	47.3% (39.0-55.6%)	35.5% (20.5-50.5%)	Diff: 11.8%	0.1768
OS at 24 months (95% CI)	28.7% (19.6-37.8%)	20.3% (4.7-35.8%)	Diff: 8.4%	0.3613

Data shown are n (%) of randomized patients except for median (months).
[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.
CI, confidence interval; Diff, difference between Arm A and Arm B; DXM, dexamethasone; HR, hazard ratio; LR, unstratified log-rank test; OS, overall survival; P, plitidepsin.

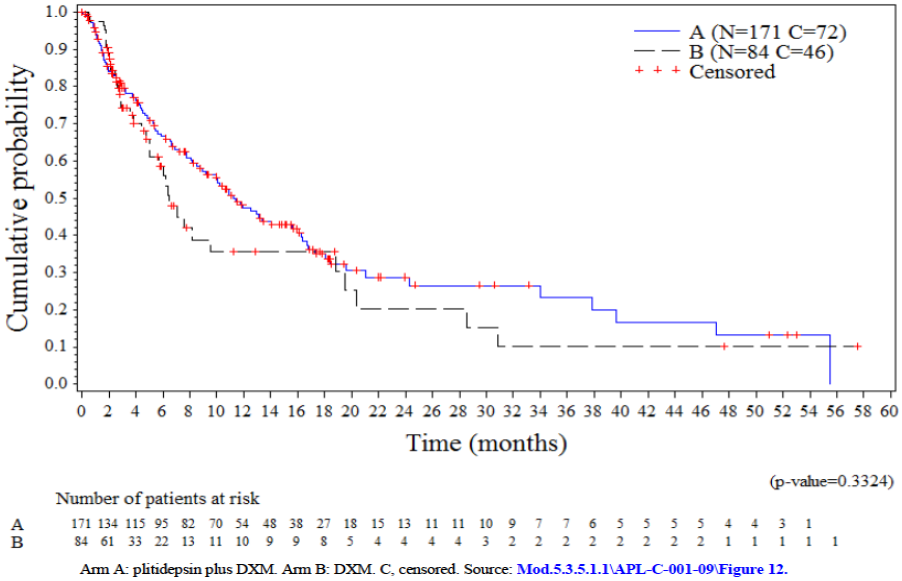


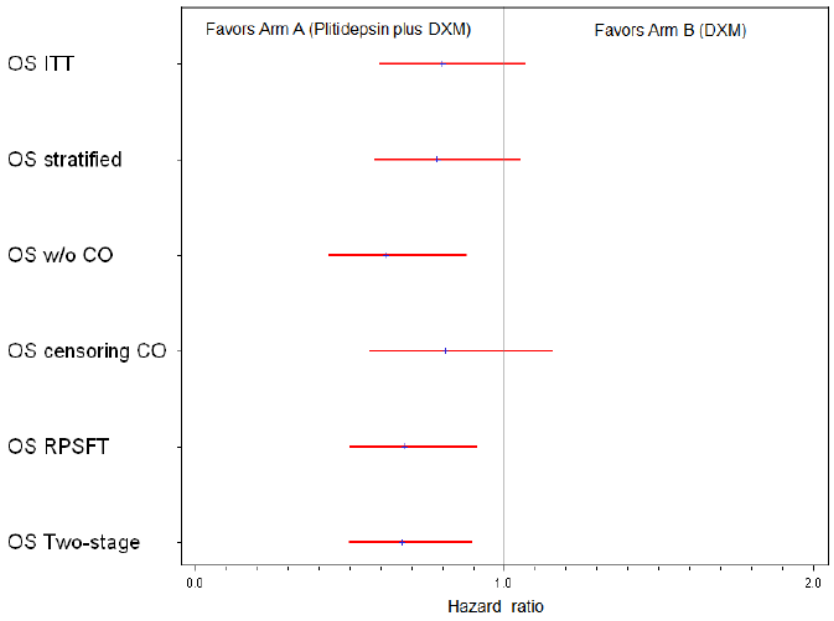
Figure 18 Kaplan-Meier plot of OS in All Randomised Patients (censoring patients with crossover at crossover date) (APL-C-001-09)

The estimated effect of crossover was evaluated in two pre-planned sensitivity analyses excluding the patients who crossed over and censoring survival at the time of crossover. The RPSFT method and the two-stage method proposed by Latimer et al. (the latter was not pre-specified in the protocol) were applied to the group of patients with crossover in the intent-to-treat analysis of OS. Stratified supportive analysis of OS in all randomized patients dataset according to randomization strata and using only treatment arm as covariate are displayed in Table 45:

Table 45. Summary of main results for the final OS analysis (APL-C-001-09 study)

Overall survival (OS) (all randomized patients)	No. of events	HR (95% CI) [a]	HR p-value	LR p-value
Primary Unstratified Analysis	195	0.797 (0.596-1.067)	0.1273	0.1261
Supportive Stratified Analysis	195	0.782 (0.582-1.051)	-	0.1027
Sensitivity Analyses				
Excluding patients with crossover	164	0.616 (0.432-0.879)	**0.0075**	**0.0069**
Censoring patients with crossover at crossover date	164	0.807 (0.563-1.159)	0.2454	0.2443
Rank preserving structural failure time method	192	0.676 (0.500-0.913)	**0.0108**	**0.0103**
Two-stage method	195	0.667 (0.497-0.895)	**0.0069**	**0.0065**

In **bold**, statistically significant results.
[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.
CI, confidence interval; HR, hazard ratio; LR, log-rank.



The central vertical line denotes a HR of 1. Each black mark denotes an individual HR and the horizontal red lines their 95% CI. HR <1 indicates lower risk of death in Arm A (plitidepsin plus DXM) relative to Arm B (DXM). Results reach statistical significance (at a 5% significance level) if the red line does not cross the vertical line.
CO, crossover; DXM, dexamethasone; ITT, intention-to-treat (all randomised patients); OS, overall survival; RPSFT, rank preserving structural failure time method.

Figure 19. Overall survival: hazard ratios in the various analysis subsets (APL-C-001-09 study)

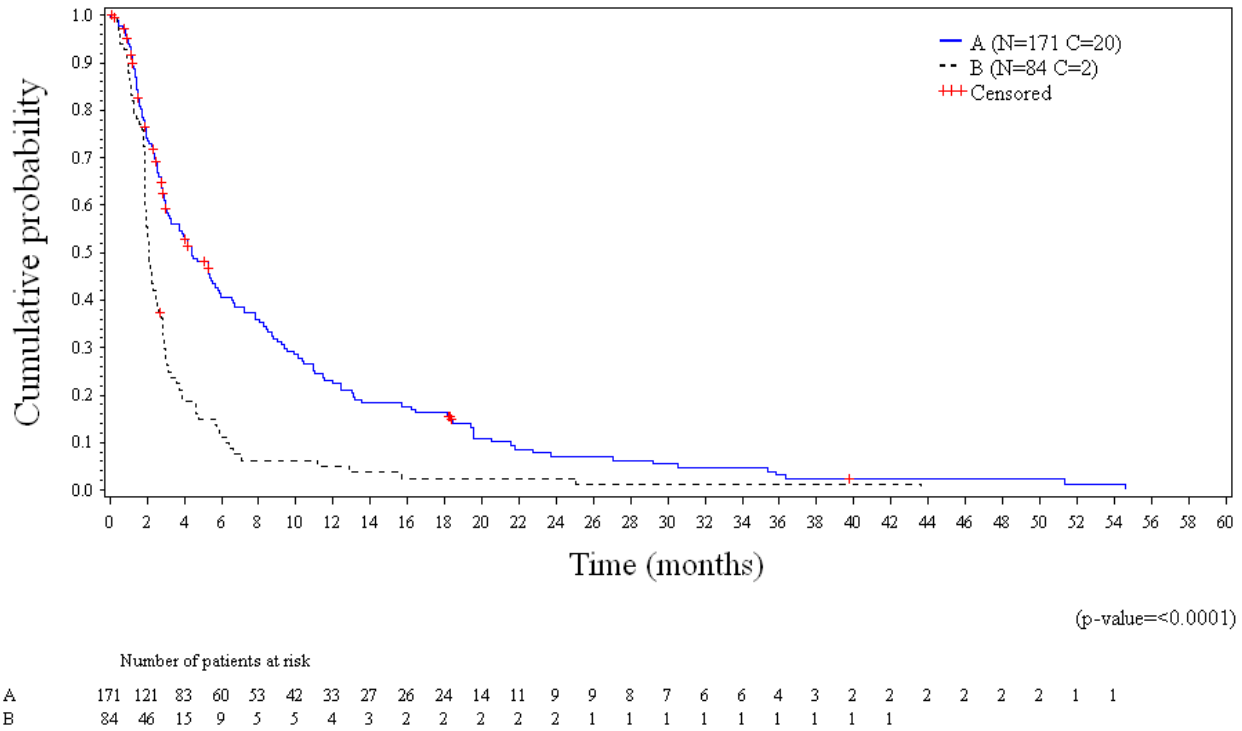
In Arm A (plitidepsin plus DXM), the most common subsequent therapy was antineoplastic agents (39.5% of treated patients), systemic corticosteroids (36.5%) and immunosuppressive agents (13.8%). The most common subsequent agents were DXM (31.7%) and cyclophosphamide (10.8%).

In Arm B (DXM), the most common subsequent therapy was antineoplastic agents (48.2% of treated patients), systemic corticosteroids (45.8%) and immunosuppressive agents (14.5%). The most common subsequent agents were DXM (37.3%), cyclophosphamide (12.0%) and lenalidomide (10.8%).

Table 46 Time to first subsequent therapy or death: All Randomised Patients (APL-C-001-09 study)

	Arm A (P+DXM)	Arm B (DXM)	Parameter	p-value
n	171	84	-	-
Number of events	151 (88.3%)	82 (97.6%)	-	-
Censored	20 (11.7%)	2 (2.4%)	-	-
Median (95% CI)	4.4 (3.1-5.8)	2.1 (1.9-2.6)	Log-rank:28.174 HR [a]: 0.479 95%CI (0.362-0.633)	LR: <0.0001 HR [a]: <0.0001
Time to first subsequent therapy or death at 6 months (95% CI)	40.7% (33.0-48.4%)	11.2% (4.3-18.1%)	Diff: 29.5	<0.0001
Time to first subsequent therapy or death at 12 months (95% CI)	22.4% (15.7-29.0%)	5.0% (0.2-9.7%)	Diff: 17.4	<0.0001

Data shown are n (%) of randomised patients except for median (months).
[a] HR: Arm A compared to Arm B. HR and p-value as determined by Cox regression.
CI, confidence interval; Diff, difference between Arm A and Arm B; DXM, dexamethasone; HR, hazard ratio; LR, unstratified log-rank test; P, plitidepsin.



Number of patients at risk

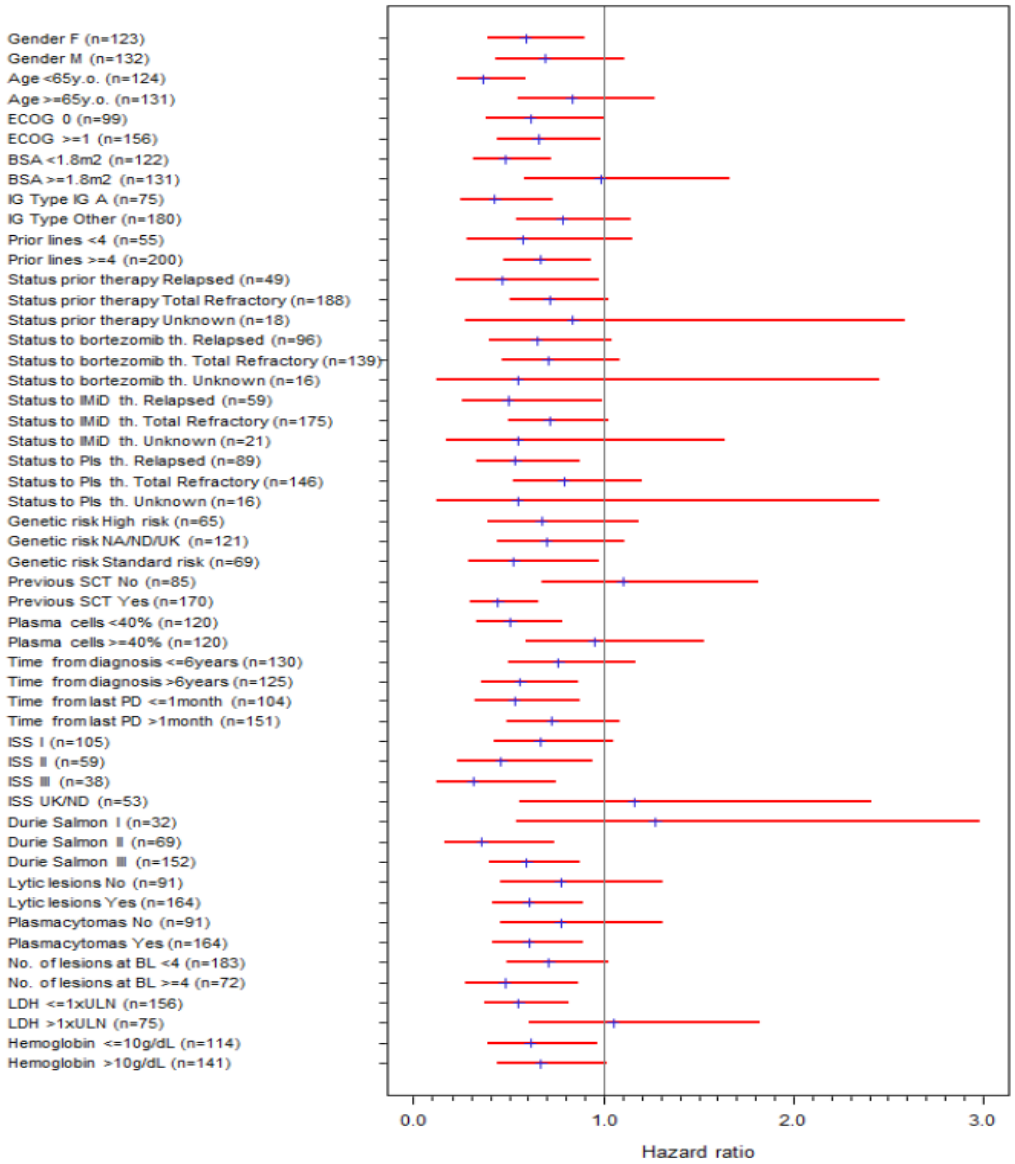
A	171	121	83	60	53	42	33	27	26	24	14	11	9	9	8	7	6	6	4	3	2	2	2	2	2	1	1
B	84	46	15	9	5	5	4	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1					

Figure 20 Kaplan-Meier plot of time to first subsequent therapy or death: All Randomised Patients (APL-C-001-09)

Response and PFS to Combination Treatment in Patients with Crossover from Arm B to Arm A

Thirty-seven patients (44.0%) initially randomized to Arm B (DXM) crossed over to Arm A (plitidepsin plus DXM) after progression of their disease, a choice allowed by the protocol. Four PRs and one MR were observed in this group of patients (ORR in this group=13.5%). Median PFS was 2.5 months (95% CI, 1.0-4.7 months) after crossover and 1.9 months (95% CI, 1.1-2.0 months) before crossover. PFS at 6 months was 24.2% after crossover and 5.6% before crossover (data not shown).

Ancillary analyses



The central vertical line denotes a HR of 1. Each black mark denotes an individual HR and the horizontal red lines their 95% CI. HR <1 indicates lower risk of the event in Arm A (plitidepsin plus DXM) relative to Arm B (DXM). Results reach statistical significance (at a 5% significance level) if the red line does not cross the vertical line.

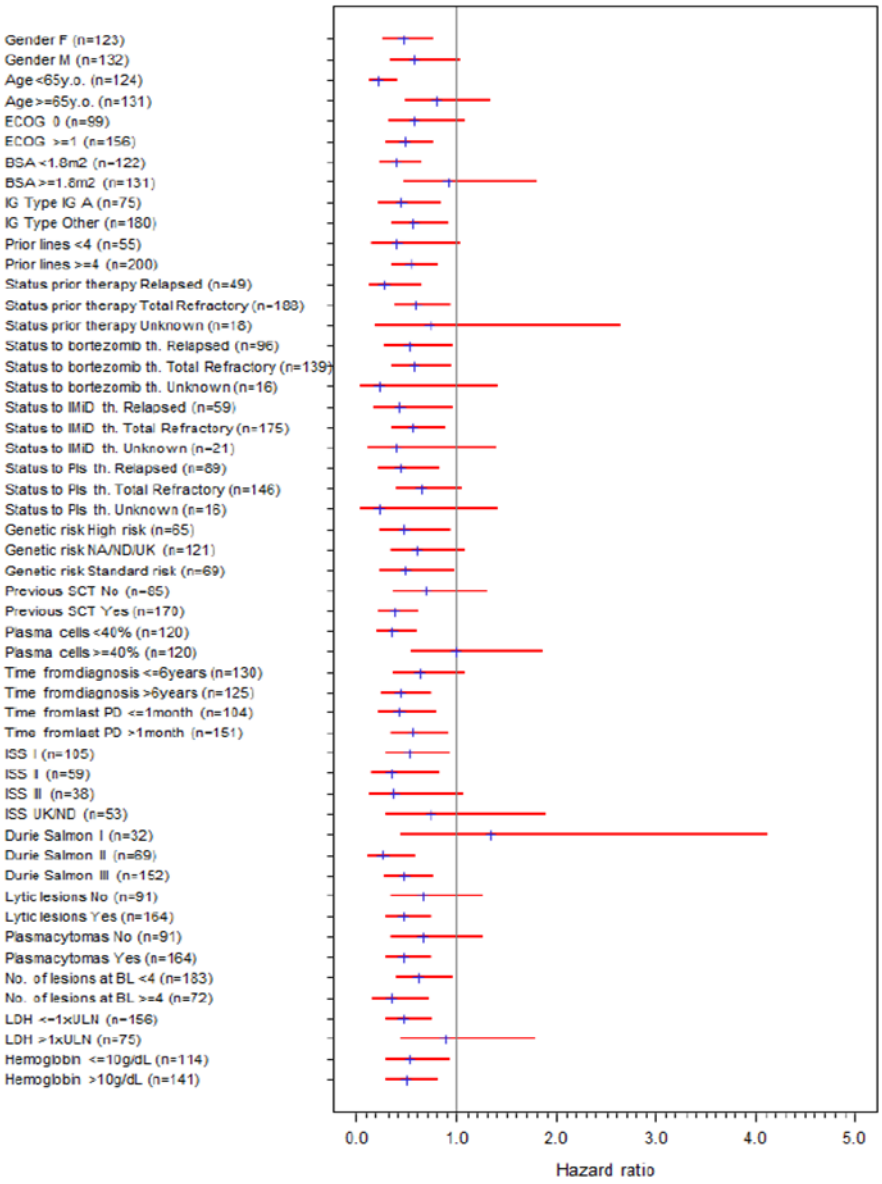
BL, baseline; BSA, body surface area; ECOG, Eastern Cooperative Oncology Group; F, female; IMiD, immunomodulatory drugs; ISS, International Staging System; LDH, lactate dehydrogenase; M, male; NA, not available; ND, not done; PD; progressive disease; PI, proteasome inhibitors; SCT, stem cell transplantation; th., therapy; UK, unknown; y.o., years old.

Figure 21 Univariate analysis of progression-free survival: IRC-All Randomized Patients (APL-C-001-09 study)

Table 47 Univariate analysis of progression-free survival: IRC-All Randomized Patients (APL-C-001-09 study)

		Arm A (P+DXM)	Arm B (DXM)	Hazard Ratio		
		n	n	HR	CI 95%	p-value [a]
Gender	**Female**	74	49	0.589	0.386-0.9	**0.0143**
	Male	97	35	0.689	0.427-1.112	0.1276
Age	**< 65 years**	88	36	0.364	0.226-0.587	**<.0001**
	≥ 65 years	83	48	0.831	0.544-1.271	0.3940
ECOG	**0**	68	31	0.614	0.376-1.001	0.0505
	≥ 1	103	53	0.657	0.439-0.981	**0.0401**
BSA	**< 1.8m^2**	73	49	0.477	0.314-0.723	**0.0005**
	≥ 1.8m^2	98	33	0.981	0.579-1.663	0.9430
IG Type	**IG A**	45	30	0.423	0.246-0.728	**0.0019**
	Other	126	54	0.782	0.534-1.145	0.2057
Prior lines	**< 4**	50	24	0.462	0.254-0.838	**0.0110**
	≥ 4	121	60	0.707	0.488-1.024	0.0668
Status prior therapy	**Relapsed**	34	15	0.460	0.218-0.971	**0.0417**
	Total refractory	126	62	0.714	0.5-1.021	0.0646
	Unknown	11	7	0.834	0.268-2.591	0.7537
Status to bortezomib therapy	**Relapsed**	64	32	0.642	0.397-1.039	0.0710
	Total refractory	92	47	0.703	0.458-1.08	0.1079
	Unknown	11	5	0.545	0.121-2.456	0.4294
Status to thalidomide/lenalidomide	**Relapsed**	44	19	0.526	0.269-1.029	0.0604
	Total refractory	112	59	0.700	0.484-1.013	0.0583
	Unknown	15	6	0.542	0.179-1.639	0.2779
Status to IMiD therapy	**Relapsed**	42	17	0.495	0.247-0.993	**0.0477**
	Total refractory	114	61	0.714	0.496-1.028	0.0701
	Unknown	15	6	0.542	0.179-1.639	0.2779
Status to PIs therapy	**Relapsed**	58	31	0.533	0.325-0.872	**0.0122**
	Total refractory	98	48	0.788	0.518-1.201	0.2678
	Unknown	11	5	0.545	0.121-2.456	0.4294
Genetic risk	**High risk**	45	20	0.673	0.382-1.186	0.1710
	NA/ND/UK	79	42	0.694	0.435-1.108	0.1256
	Standard risk	47	22	0.524	0.282-0.973	**0.0407**
Previous SCT	**No**	56	29	1.102	0.669-1.813	0.7038
	Yes	115	55	0.439	0.293-0.656	**<.0001**
Plasma cells	**<40%**	77	43	0.505	0.326-0.783	**0.0023**
	≥ 40%	85	35	0.950	0.59-1.532	0.8346
Time from diagnosis	**≤ 6years**	86	44	0.759	0.494-1.166	0.2078
	> 6years	85	40	0.556	0.356-0.869	**0.0100**
Time from last PD	**≤ 1month**	68	36	0.528	0.32-0.873	**0.0129**
	> 1month	103	48	0.726	0.487-1.082	0.1156
ISS	**I**	72	33	0.662	0.417-1.052	0.0808
	II	41	18	0.457	0.222-0.937	**0.0326**
	III	23	15	0.308	0.127-0.743	**0.0088**
	UK/ND	35	18	1.158	0.555-2.415	0.6956
Durie Salmon	**I**	21	11	1.271	0.541-2.987	0.5828
	II	48	21	0.355	0.171-0.739	**0.0056**
	III	101	51	0.591	0.398-0.877	**0.0090**
	NA	1	1	0.000	0-.	1.0000
Lytic lesions	**No**	58	33	0.771	0.455-1.307	0.3341
	Yes	113	51	0.605	0.413-0.886	**0.0098**
Plasmacytomas	**No**	58	33	0.771	0.455-1.307	0.3341
	Yes	113	51	0.605	0.413-0.886	**0.0098**
No. of lesions at baseline	**< 4 lesions**	122	61	0.708	0.49-1.022	0.0654
	> 4 lesions	49	23	0.480	0.265-0.868	0.0152
LDH	**≤ 1 x ULN**	107	49	0.549	0.369-0.817	**0.0031**
	> 1 x ULN	48	27	1.048	0.602-1.825	0.8684
Hemoglobin	**≤ 10 g/dL**	74	40	0.613	0.389-0.968	**0.0358**
	> 10 g/dL	97	44	0.667	0.438-1.015	0.0588

Significant p-values are shown in **bold**.
BSA, body surface area; CI, confidence interval; DXM, dexamethasone; ECOG, Eastern Cooperative Oncology Group; IMiD immunomodulatory drugs; ISS; International Staging System; LDH, lactate dehydrogenase; NA; not available; ND, not done; P plitidepsin; PD, progressive disease; PIs, proteasome inhibitors; SCT, stem cell transplantation; UK, unknown; ULN, upper limit o normal.



The central vertical line denotes a HR of 1. Each black mark denotes an individual HR and the horizontal red lines their 95% CI. HR <1 indicates lower risk of the event in Arm A (plitidepsin plus DXM) relative to Arm B (DXM). Results reach statistical significance (at a 5% significance level) if the red line does not cross the vertical line.
BL, baseline; BSA, body surface area; ECOG, Eastern Cooperative Oncology Group; F, female; IMiD, immunomodulatory drugs; ISS, International Staging System; LDH, lactate dehydrogenase; M, male; NA, not available; ND, not done; PD; progressive disease; PI, proteasome inhibitors; SCT, stem cell transplantation; th., therapy; UK, unknown; y.o., years old.

Figure 22 Forest plot of progression-free survival with confirmation of PD by subgroup, based on the Independent Review Committee assessment in All Randomised Patients (APL-C-001-09, ADMYRE, study)

Multivariate Analysis of Progression-free Survival by IRC

HR for the treatment effect after adjustment was 0.616 (95% CI, 0.434-0.875; p value=0.0067). Other patient characteristics that appeared to influence PFS at the p<0.05 level were age (≥65 years better than <65 years); haemoglobin (>10 g/dL better than ≤10 g/dL) and plasma cells (<40% cells better than ≥ 40%).

Multiple variate analysis performed according to PFS with confirmation of PD by IRC assessment showed that HR for the treatment effect after adjustment was 0.641 (95% CI, 0.422-0.975; p value=0.0375).

Most patients (70%) were recruited in Europe, but there were also patients recruited worldwide. To test a potential impact of geographical region on the observed results for PFS by IRC, different approaches were performed. The variable "region" was created with four categories (Europe, Asia, Oceania, and USA) and then three Cox regressions were performed.

1) PFS by IRC with arm, region and interaction term was evaluated: none of these variables was significant and, therefore, interaction was not relevant.

2) PFS by IRC with arm and region as main effects: region was not significant, while treatment arm maintained significance (HR=0.67; p=0.0107).

3) Whole multivariate analysis of PFS by IRC, including region as variable: the model obtained was the same as that previously shown.

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 48 Summary of Efficacy for trial (APL-C-001-09)

Title: Randomized, multicenter, open-label, phase III study of plitidepsin in combination with dexamethasone vs. dexamethasone alone in patients with relapsed/refractory multiple myeloma (ADMYRE Study)		
Study identifier	APL-C-001-09	
Design	Randomized, multicenter, open label	
	Duration of main phase:	Patients have received the study drugs while it was considered to be in their best interest (specifically, treatment has continued until e.g. disease progression, unacceptable toxicity).
	Duration of Run-in phase:	At least two-week washout period since the end of last therapy (six weeks if previous nitrosoureas-containing regimen).
	Duration of Extension phase:	Not applicable
Hypothesis	Superiority	

Treatments groups	Plitidepsin in combination with dexamethasone (DXM)		DXM: 40 mg orally on Day 1, 8, 15 and 22 every four weeks (q4wk). Plitidepsin: 5 mg/m^2 i.v. on Day 1 and 15 q4wk. 171 patients
	DXM		DXM: 40 mg orally on Day 1, 8, 15 and 22 every four weeks (q4wk). 84 patients
Endpoints and definitions	Primary endpoint	Progression free survival (PFS)	time from the date of randomization to the date of documented progressive disease (PD) by IMWG criteria or death (regardless of the cause of death)
	Secondary	Overall survival (OS)	time from the date of randomization to the date of death or last contact
	Secondary	Objective response rate (ORR)	Objective response defined as having minor response (MR) or better as best overall response based on the IMWG criteria
Database lock	20 November 2015		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat		
Descriptive statistics and estimate variability	Treatment group	Plitidepsin + DXM	DXM
	Number of subject	171	84
	PFS (median; months)	2.6	1.7
	95% CI	1.9, 3.0	1.1, 2.0
	OS (median, months)	11.6	8.9
	95% CI	9.2-16.1	6.0-15.4
	ORR (%) 95%CI	9.9 5.9-15.4	1.2 0.03-6.5
Effect estimate per comparison	Primary endpoint PFS	Comparison groups	Plitidepsin + DXM vs DXM
		HR	0.65
		95%CI	0.477-0.885
		P-value	0.0062
	Secondary OS	Comparison groups	Plitidepsin + DXM vs DXM
		HR	0.797
		95%CI	0.596-1.067
		P-value	0.1273
	Secondary ORR	Comparison groups	Plitidepsin + DXM vs DXM
		Fisher exact test (p-value)	0.0085
Notes	Eligible patients were stratified according to their ECOG PS score (0 and 1 vs. 2) and Durie-Salmon stage (I/II vs. III).		

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

N/A

Clinical studies in special populations

Elderly patients

Table 49 Patients included in plitidepsin clinical program (including those from ARM B ADMYRE)

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	92/255	37/255	2/255
Non Controlled Trials	193/857*	39/857*	2/857*

- Four patients with missing age at cutoff

Table 50 Patients treated in plitidepsin clinical program (including those treated in ARM B ADMYRE)

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	89/250	36/250	1/250
Non Controlled Trials	189/842	38/842	2/842

Table 51 Patients treated (SCS population) (527 patients, excluding DXM)

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	68/204	30/204	0/204
Non Controlled Trials	49/323	20/323	1/323

Table 52 Multivariate analysis of progression-free survival: IRC-All Randomized Patients (APL-C-001-09 study)

Variable	ClassVal0	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	HR	CI95%
Treatment Arm	Arm A (P+DXM) better than Arm B (DXM)	1	-0.48442	0.17878	7.3420	0.0067	0.616	0.434-0.875
Age	≥65 years better than < 65 years	1	-0.44315	0.16811	6.9489	0.0084	0.642	0.462-0.893
Hemoglobin	>10 g/dL better than ≤10 g/dL	1	-0.39922	0.16076	6.1667	0.0130	0.671	0.490-0.919
Plasma cells	<40% cells better than ≥40%	1	-0.33950	0.16493	4.2373	0.0395	0.712	0.515-0.984

CI, confidence interval; DF, degrees of freedom; HR, hazard ratio.

HR for the treatment effect after adjustment was 0.616 (95% CI, 0.434-0.875; p value=0.0067). Other patient characteristics that appeared to influence PFS at the p<0.05 level were age (⩾65 years better than <65 years); haemoglobin (>10 g/dL better than ⩽10 g/dL), and plasma cells (<40% cells better than ⩾40%).

Results for PFS in the subgroup of patients older than 65 were apparently dissimilar to those obtain in younger patients (<65) (HR 0.364 95%CI 0.23,0.59 vs HR 0.821 95%CI 054, 1.27 <65 vs >65 respectively). The applicant has not provided further analyses related to age for the rest of secondary variables.

2.5.4. Supportive studies

Study APL-B-014-03

This was an exploratory, multicentre, open-label, single-arm, phase II trial conducted to assess the antitumor activity and safety of plitidepsin alone given at 5 mg/m^2 as a 3-hour i.v. infusion q2wk to patients with relapsed/refractory MM. This phase II study used the plitidepsin dose and schedule previously evaluated in a phase I trial conducted in patients with solid tumours or NHL. Protocol of APL-B-014-03 was amended after 23 patients had been treated to allow the addition of low-dose DXM (20 mg daily on Days 1-4 every cycle, for a total monthly dose of 160 mg) in patients with suboptimal response to single-agent plitidepsin.

Of the 53 enrolled patients, 14 patients (26.4%) had previously received radiotherapy. Median number of lines of previous systemic therapy was 4 (range, 1-8). Disease status with respect to last prior therapy was refractory in 64.2% of patients and relapsed in 26.4% (unknown in 9.4%). A total of 30 patients (56.6%) had previously received SCT.

Of the 19 patients treated with plitidepsin plus DXM, three patients (15.8%) had previously received radiotherapy. Median number of lines of previous systemic therapy was 4 (range, 1-8). Disease status with respect to last prior therapy was refractory in 78.9% of patients and relapsed in 21.1%. A total of nine patients (47.4%) had previously received SCT

The primary efficacy endpoint was the ORR achieved with single-agent plitidepsin as well as after DXM addition in suboptimal responding patients, according to (24). Fifty-one patients were enrolled and

received single-agent plitidepsin. Two PRs and four MRs were found in 47 evaluable patients treated with single-agent plitidepsin (ORR=12.8%).

Table 53 Best response to treatment in all included patients (APL-B-014-03)

	Plitidepsin (n=53)		Plitidepsin + DXM (n=19)	
	n	%	n	%
PR	2	3.8	2	10.5
MR	4	7.5	2	10.5
SD	24	45.3	11	57.9
PD	16	30.2	3	15.8
NE	6	11.3	1	5.3
Treatment failure	1	1.9	.	.
ORR [a]	11.3% [b]		21.1%	
(95% CI)	(4.3-23.0%)		(6.1-45.6%)	
ORR (excluding MR) [c]	3.8%		10.5%	
(95% CI)	(0.5-13.0%)		(1.3-33.1%)	
Clinical benefit rate [d]	56.6%		78.9%	
(95% CI)	(42.3-70.2%)		(54.4-94.0%)	

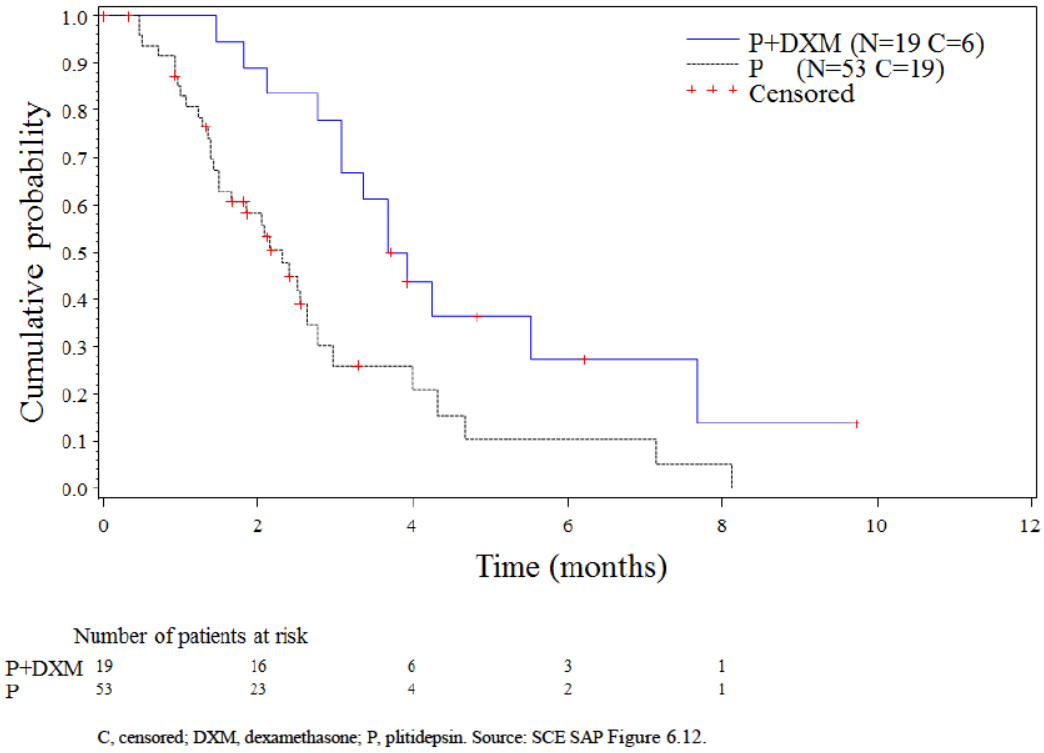
Data shown are n of all included patients (%) except for ORR and clinical benefit rate.
a Includes sCR, CR, VGPR, PR and MR.
b In the CSR of this study, ORR for all patients was calculated in an intention-to-treat over 51 patients, excluding the two patients untreated. Therefore, ORR shown in the CSR is 11.8% (6/51 patients).
c Includes sCR, CR, VGPR, and PR (excludes MR).
d Includes sCR, CR, VGPR, PR, MR and SD.
CI, confidence interval; CR, complete response; CSR, clinical study report; DXM, dexamethasone; MR, minor response; NE, not evaluable; ORR, overall response rate; P, plitidepsin; PD, progressive disease; PR, partial response; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

Twenty-four other patients had SD, which lasted for >3 months in four patients. Nineteen patients received DXM added to plitidepsin treatment. Two PRs and two MRs were found in 18 evaluable patients in this cohort (ORR=22.2%), while SD for >3 months occurred in eight patients. Addition of DXM improved the response rate (from 12.8 to 22.2%), and the type of response in patients previously showing suboptimal response (including two patients who showed stabilisation of their initial response in two subsequent cycles and two patients with initial SD); of note, some responses occurred in patients who were considered refractory to last prior therapy (42% of patients in this cohort), and one patient received salvage transplantation after nine courses following a > 90% M protein reduction. Furthermore, the plitidepsin and DXM combination resulted in longer median PFS values (3.8 vs. 2.1 months with plitidepsin alone).

Table 54 Progression-free survival in all included patients (APL-B-014-03)

	Plitidepsin	Plitidepsin + DXM
n	53	19
Number of events	34 (64.2%)	13 (68.4%)
Censored	19 (35.8%)	6 (31.6%)
Median	2.3 [a]	3.8
(95% CI)	(1.5-2.8)	(3.1-7.7)
PFS at 6 months	10.4%	27.3%
(95% CI)	(0.0-23.1%)	(4.0-50.7%)

Data shown are n of included patients (%) except for median (months) and PFS at 6 months.
[a] In the CSR of this study (Mod.5.3.5.2.2\APL-B-014-03\Sec.11.4.1.1.1\Table 22), PFS for all patients was calculated in an intention-to-treat over 51 patients, excluding the two patients untreated. Therefore, PFS shown in the CSR is 2.1 months.
CI, confidence interval; DXM, dexamethasone; PFS, progression-free survival.



C, censored; DXM, dexamethasone; P, plitidepsin. Source: SCE SAP Figure 6.12.

Figure 23 Kaplan-Meier plot of progression-free survival (all included patients) (APL-B-014-03)

Study APL-A-009-08

In APL-A-009-08, plitidepsin was administered as a 1-hour i.v. infusion on Day 1 and 8, every three weeks (q3wk); bortezomib was administered as a 3-5 sec i.v. bolus injection on Day 1, 4, 8 and 11, q3wk, and DXM was given orally on Day 1 and 2, 4 and 5, 8 and 9, 11 and 12, q3wk. The study was open for inclusion of patients from 12 December 2008 to 12 October 2009 and was closed after 12 months due to a Sponsor's decision, with a total of three included and treated patients. All three patients were treated at the first planned dose level: plitidepsin 2.0 mg/m^2+ bortezomib 1.0 mg/m^2, and DXM 20 mg, and received two, six and three cycles respectively. The RD was not determined due to the early termination of the trial. ORR according to Blade et al. Br J Haematol 1998; 102:1115-1123 was used as efficacy endpoint. One of the three treated patients, had a PR during plitidepsin/bortezomib/DXM treatment, with a TTP of 4.8 months; the other two patients had disease progression (data not shown).

Study APL-A-012-13

In APL-A-012-13, plitidepsin was administered as a 3-hour i.v. infusion on Day 1 and 15, q4wk; bortezomib was administered as a 3-5 sec bolus subcutaneous (s.c.) injection on Day 1, 4, 8 and 11, q4wk, and DXM was given orally on Day 1, 8, 15 and 22, q4wk. Patients were enrolled sequentially into three dose levels being the starting dose plitidepsin 4.0 mg/m^2 plus bortezomib 1.0 mg/m^2 and DXM 40 mg/m^2. A total of 22 patients were included at cut-off date for this analysis. Dose level III (plitidepsin 5.0 mg/m^2 plus bortezomib 1.3 mg/m^2 plus DXM 40 mg) was defined as the RD for further clinical studies. Dose intensity achieved with plitidepsin in this phase I study at the RD (1.8 mg/m^2/week) was similar to that obtained with plitidepsin plus DXM in APL-C-001-09 (ADMYRE) study (1.9 mg/m^2/week) and in APL-B-014-03 study (2.0 mg/m^2/week). A total of 18 of 22 patients were evaluable for efficacy. ORR according to the IMWG criteria (Rajkumar et al. Blood 2011: 117:4691-4695) was used as efficacy endpoint. ORR in patients evaluable for efficacy was 55.6% (95% CI, 30.8-78.5%). Two patients had

stringent complete response (sCR), one had CR, four had VGPR, and three had PR. Clinical benefit rate was 77.8% (95% CI, 52.4-93.6%). Median DR was not reached (95% CI, 1.8 months-not reached). Median PFS was 8.3 months (95% CI, 2.8-not reached) (data not shown).

2.5.5. Discussion on clinical efficacy

Design and conduct of clinical studies

The clinical package supporting the efficacy of plitidepsin plus DXM combination in the treatment of relapsed/refractory MM includes one primary phase III study (APL-C-001-09, ADMYRE), which is the pivotal trial supporting this application, and one supportive phase II study (APL-B-014-03).

The inclusion/exclusion criteria allowed the recruitment of patients with multiple myeloma with at least three but not more than six previous treatments, considering the induction and transplantation as only one treatment. Patients with moderate performance status were allowed into the study. Patients should have been treated with proteasome inhibitors (bortezomib) and immunomodulators (lenalidomide or thalidomide), which reflects a relapsed/refractory population.

Since the trial was conducted, new therapeutic options, mainly triplets (with carfilzomib and elotuzumab) are now available in the second line of the MM landscape. The previous exposure to these new alternatives could theoretically modify the setting/results where plitidepsin is intended to be authorised. To what extent that might impact on the benefit is unknown. However, this is inevitable in a field where a number of new agents have become available and does not constitute a major limitation as long as the patient characteristic are well described in the SmPC. Dexamethasone 40 mg on Day 1, 8, 15 and 22 q4wk, is the comparator of this study. This choice may not seem optimal according to current standards but it is acknowledged that the first patient was enrolled in the ADMYRE trial by June 2010, whereas the marketing authorisation for pomalidomide was granted in August 2013. Therefore, at the time of the design of the study, low dose dexamethasone might have been considered acceptable. Indeed, this was the CHMP advice given to the company about the use of low dose-dexamethasone as comparator. Thus, the choice of control arm does not constitute a major limitation.

The primary endpoint of the study was PFS according to IRC and by IWG criteria, which is acceptable. According to the definition of event, this was assigned as the first time a PD is reported without the need of confirmation, in accordance with updated guidelines of IWG (25).

HRQoL data were not collected. Even if such data would have been prone to bias due to the open-label design, they might still give a valuable supportive indication of the patients' condition and sense of well-being/daily functioning associated with a possible observed gain in PFS. If sufficiently robust, these data might have been useful to refine the understanding about the clinical impact of treatment on patient outcomes.

Efficacy data and additional analyses

According to the baseline characteristics, the population recruited has a median age of 64, with approximately half of patients below 65. Compared to the fact that median age for first diagnosis of MM in Europe is usually between 65 and 70 years, the included patient population in this study seems to be rather selected and of younger age than expected for a fourth line therapy setting. According to the latest ESMO guideline ("Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up", updated January 2017), the median age at diagnosis of MM in Europe is now as high as 72 years. The comparison of the included patient population in the ADMYRE study with other published clinical trials in regards to the older patients (i.e. ≥ 70 years) is characterised by very limited data. The

median time from first diagnosis of MM to randomisation was around 6 years in both groups with median ~ 6 months since last relapsed/PD. This means that the median age of the patients at the time of diagnosis has been around 59 years. This is quite young and below the median age for first diagnosis of MM in Europe. Furthermore, the patients have survived for 6 years before randomisation into the current study, which is longer than median survival time for MM (around 4-5 years). Around 15% had an ECOG 2 and the time from diagnosis to inclusion in the trial was 71 months, which suggests a population with few alternatives. Although patient selection may pose limitations in terms of generalisability of the results this should not hamper treatment comparisons as long as important prognostic factors are evenly distributed across treatment groups.

Overall, baseline demographics appear evenly balanced, apart from gender and body surface area. Cytogenetic risk (at baseline) was not available for almost half of the population but there were no reasons to expect major imbalances across treatment groups.

The median of number of lines of prior systemic therapy was 4. The most common previous agents were bortezomib (98.4% of patients in both arms), lenalidomide (97.6%), DXM (97.6%), melphalan (87.8%), cyclophosphamide (74.5%), thalidomide (65.1%), doxorubicin (47.1%), vincristine (32.5%), and prednisone (23.9%). Pomalidomide was received by 13% of the patients. In the combination arm, disease status with respect to last prior therapy was total refractory (including refractory and relapsed/refractory) in 73.7% of patients and relapsed in 19.9% (unknown in 6.4%). All but three patients had previously received bortezomib and lenalidomide.

Results from the primary analysis (PFS by IRC in ITT population) showed a HR 0.650 (95%CI 0.477-0.885), with a median PFS of 2.6 vs 1.7 months for plitidepsin plus DXM vs DXM respectively. These data can be considered mature enough according to the percentage of events (76% in the combination arm). The investigator assessment gave a similar result (HR 0.512, 95%CI 0.382-0.686) with medians of 2.9 and 1.1 months. Several sensitivity analyses were carried out so as to support the main analysis.

As planned per protocol, the final OS analysis was to be done when 80% of survival events had been reached or two years after the last patient had been randomised, whichever occurred first. Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on 195 death events (i.e., 76.5% of the 255 randomised patients). In the final primary OS analysis per intention-to-treat (all randomised patients), median OS was 11.6 months (95% CI, 9.2-16.1 months) in the plitidepsin + DXM arm and 8.9 months (95% CI, 6.0-15.4 months) in the DXM arm (HR=0.797, 95% CI: 0.596-1.067; p=0.1273). Overall, there seems not to be any detrimental effect on OS by adding plitidepsin to DXM. It is acknowledged that the lack of an effect on OS might be in part due to the number of patients crossing over to the plitidepsin + DXM arm from the DXM arm during the study (37 patients, 44%). In order to further explore the impact of the crossover, the applicant carried out a number of different analyses using different approaches to try to correct for the cross-over.

Best ORR (sCR+CR+VGPR+PR according to IMWG criteria; secondary endpoint; ITT; IRC) was 9.9% (95%CI 5.9%-15.4%) vs 1.2% (95%CI .03%-6,5%) in the DXM arm. Median DR was 3.7 months (95% CI, 2.7-10.5 months) in Arm A (plitidepsin plus DXM) and 1.8 months (95% CI, 1.8-5.5 months) in Arm B (DMX) as determined by the IRC in responder patients (IRC- All Responder Patients).

Additional expert consultation

The CHMP invited the experts of the biostatics working party (BSWP) to provide their views on the below:

The BSWP is asked about the acceptability and validity of the additional analyses (i.e. the two-stage method and RPSFT) that attempts to compensate for the bias introduced in the OS estimate due to this switch of control patients towards the active drug.

The BSWP discussed the acceptability and validity of the two-stage method and the rank-preserving structural failure time model (RPSFT) to estimate the counterfactual effect that would have been observed if no patient in the control group (dexamethasone, DMX) had switched to the experimental treatment (aplidin + DMX) at the time of patient's progression. As both are modeling approaches that try to estimate something that did not happen, both models need to make specific assumptions on speed of progression. The validity and therefore acceptability depend in principle on how reasonable the models' assumptions are in this particular case.

The main assumption of RPSFT is the 'common treatment effect' which can be understood as follows. Each patient has an 'internal clock' by which (s)he progresses through her/his disease course. The effect of receiving experimental treatment is then expressed as delaying this internal clock by a certain factor. The 'common treatment effect' assumption means that this delay factor is the same, regardless whether the patient receives the experimental treatment from the start of the trial or after his/her progression on control treatment. Therefore, RPSFT does not fully correct for differences in prognostic factors at baseline and at progression other than accounting for the fact that at progression the patient will have a less life span left. There is also some indication from analyzing the data that the delay factor is different at start and at progression. Therefore, it is likely that RPSFT provides a biased (more optimistic) estimate for the counterfactual effect.

The two stage model estimates the effect of switching by comparing survival of those in the control arm that do and do not switch at the time of their progression (secondary baseline) and estimates from that by which factor switching delays the internal clock. In principle, those switching and not switching in the control group may be prognostically different at progression and the method allows to adjust for these confounders at progression, provided these are measured. In this application, factors at baseline and some at time of progression were adjusted for and the main assumption of this method, 'no unmeasured confounders at secondary baseline" (i.e. progression), is likely only partially met. Because it seems that the prognostically better patient in the control group tend to switch more, the report argues that the two-stage will likely provide a too favorable estimate of the counterfactual effect.

Conclusion for this application: both methods are likely providing biased (too optimistic) point estimates of the counterfactual effect of survival if no cross-over had occurred. In contrast to the two discussed methods, the ITT estimate will likely be a too pessimistic estimate for the counterfactual effect on survival. Yet, the results can only be interpreted in the context of the results provided for the ITT analysis. The true magnitude of the OS effect cannot be concluded from these analyses but taken together they delimit the range in which the survival effect in absence of switch would be.

Following the BSWP consultation the CHMP concluded that a number of statistical approaches were used in an attempt to correct for the impact of cross-over. In conclusion, although the approaches used are generally considered appropriate to explore the effect of cross-over, the results of such analyses did not allow concluding that plitidepsin plus DXM is associated with a statistically significant difference in terms of OS compared to DXM.

2.5.6. Conclusions on the clinical efficacy

The effect observed in terms of the primary endpoint PFS was modest and needs to be weighed against the unfavourable effects (see Benefit-Risk Assessment).

2.6. Clinical safety

The safety profile of plitidepsin plus DXM for the proposed indication is mainly characterised on the basis of safety data from patients with relapsed/refractory MM treated in the randomised phase III study APL-C-001-09 (ADMYRE). These safety data are supported by the results of a phase II study in patients with relapsed/refractory MM (APL-B-014-03) along with the safety data from two phase I studies (APL-A-009-08 and APL-A-012-13) evaluating plitidepsin combined with bortezomib and DXM in patients with relapsed and/or refractory MM.

Apart from these primary safety data, secondary safety data include a pooled Integrated Safety Analysis dataset, which contains not only APL-C-001-09 (ADMYRE) data but also data from 14 completed phase II studies in different indications. This analysis comprises 3 groups based on the infusion times/administration schedules used: group A with results of a total of 204 patients (167 Arm A plus 37 who crossed from Arm B to A) treated in the phase III trial with the proposed schedule of plitidepsin + DXM; group B comprise a total of 195 patients (from 9 phase 2 CT, 2 hematological (1MM + 1 myelofibrosis) and 7 solid tumours) treated with plitidepsin at the proposed dose regimen but as a single agent; Group 3 comprise information from 128 patients treated with a weekly 1h infusion of 3.2mg/m^2 plitidepsin in a total of 5 phase 2 CT (3 hematological (NHL/leukemia) plus 2 solid tumours).

Patient exposure

Primary safety data

Overview of studies contributing to primary safety data for plitidepsin plus DXM as treatment in relapsed/refractory MM is presented in **Table 55**.

Primary safety data come from the completed phase III study (excluding the cross-over patient data) in relapsed/refractory MM (APL-C-001-09, ADMYRE), in which 167 patients were treated with plitidepsin+DXM (Arm A) while 83 patients received only DXM (Arm B).

In the pivotal study APL-C-001-09, patients in the control arm with documented PD according to the Investigator's criteria after a minimum of 8 weeks from randomisation were offered crossover to combination arm. For Integrated Safety Analysis, patients treated with P+DXM and patients after crossing over from the control arm to the P+DXM arm were included in Group A. Patients were evaluable for safety if they received at least one dose of the study treatment.

Table 55. Overview of studies contributing to primary safety data for plitidepsin plus DXM as treatment in relapsed/refractory multiple myeloma

Study	Study design and objectives	Drug Dose (Administration way) Schedule	Treated patients	Status
Main primary safety data				
APL-C-001-09 (Mod.5.3.5.1.1)	Phase III, randomised, multicentre, open-label, clinical study of plitidepsin in combination with DXM *vs.* DXM alone in patients with relapsed/refractory MM.	**Arm A:** Plitidepsin 5 mg/m^2 (3-hour i.v.) D1, 15 q4wk + DXM 40 mg (orally) D1, 8, 15, 22 q4wk **Arm B:** DXM 40 mg (orally) D1, 8, 15, 22 q4wk	**Arm A:** 167 **Arm B:** 83	Completed
Supportive primary safety data				
APL-B-014-03 (Mod.5.3.5.2.2)	Phase II, multicentre, open-label, clinical and PK study of plitidepsin as a 3-hour i.v. infusion every two weeks alone or in combination with DXM, in pre-treated patients with relapsing or refractory MM	Plitidepsin 5 mg/m^2 (3-hour i.v.) D1 q2wk	51	Completed
		Plitidepsin 5 mg/m^2 (3-hour i.v.) D1 q2wk + DXM 20 mg (orally) D1-4 q2wk	19/51	
APL-A-009-08 (Mod.5.3.5.2.1)	Phase I, dose-finding study in patients with relapsed or refractory MM	Plitidepsin [a] (1-hour i.v.) D1, 8, q3wk + Bortezomib [a] (3-5 sec i.v. bolus injection)	3 [b]	Completed
		D1, 4, 8 and 11, q3wk + DXM [a] (orally) D1 and 2, 4 and 5, 8 and 9, 11 and 12, q3wk		
APL-A-012-13 (Mod.5.3.5.2.3)	Phase I, dose-finding study in patients with relapsed and/or refractory MM.	Plitidepsin [c] (3-hour i.v.) D1 and 15, q4wk + Bortezomib [c] (3-5 sec bolus s.c. injection) D1, 4, 8 and 11, q4wk + DXM [c] (orally) D1, 8, 15 and 22, q4wk	22	Completed [d]

[a] Starting dose level: plitidepsin 2.0 mg/m^2 plus bortezomib 1.0 mg/m^2 plus DXM 20 mg/m^2.
[b] This study was early closed due to a Sponsor's decision with a total of three patients, all of them treated at the starting dose level.
[c] RD: plitidepsin 5.0 mg/m^2 plus bortezomib 1.3 mg/m^2 plus DXM 40 mg/m^2.
[d] Dose escalation completed; RD expansion cohort ongoing.
D, day; DXM, dexamethasone; i.v., intravenous; MM, multiple myeloma; q2wk, every two weeks; q3wk, every three weeks; q4wk, every four weeks; RD, recommended dose.

Treatment duration and dosage (pivotal study)

Overall duration of exposure to plitidepsin in combination with DXM in patients with relapsed/refractory MM in the pivotal study is presented in

Table 56. Overall duration of exposure to plitidepsin alone or in combination by study in patients with relapsed/refractory MM (Primary safety data from phase III study)

	Primary safety data (APL-C-001-09)	
	Arm A (P+DXM) (n= 167)	**Arm B (DXM) (n=83)**
Time on treatment, months Median (range)	2.8 months (0.3-31.5)	1.9 months (0.3-19.6)
Cycles per patient Median (range)	3 (1-33)	2 (1-21)
Median cumulative dose, plitidepsin	24.8 mg/m2	-
Dose intensity, plitidepsin	1.9 (1.1-2.7)	-
Cycle delays, n/N (%) patients treated Total number of cycle delays Median length of delay	67/167 (40.1%) [a] 107 cycles 12 days	5/83 (6%) [b] 8 cycles 8.5. days
Dose omissions, n/N (%) patients treated Total number of cycles with dose omissions	91/167 (54.5%) 119 cycles [c] (14% of 837 cycles)	13/83 (15.7%) 22 cycles
Dose reduction, n/N (%) patients treated Total cycle number with dose reduction	56/183 (33.5%) (64 cycles plitidepsin [d], 33 cycles DXM)	

[a] The 8.9% of cycles were delayed due to treatment-related reasons, mostly because of non-haematological toxicity (50 of 60 cycles).
[b] Due to reasons unrelated to the study treatment.
[c] Sixty-six of 119 cycles had plitidepsin dose omitted due to treatment-related reasons, being mostly non-haematological toxicity (50 cycles).
[d] The most common cause of plitidepsin dose reduction related to treatment was non-haematological toxicity (57 of 59 cycles).

Overall duration of exposure to plitidepsin alone or in combination with DXM or bortezomid (BTZ) and DXM in patients with relapsed/refractory MM in the Phase II studies is presented in Table 57.

Table 57. Overall duration of exposure to plitidepsin alone or in combination in patients with relapsed/refractory MM (Primary safety data from supportive Phase II studies)

	Supportive primary data		
	APL-B-014-03		APL-A-012-13 P+DXM+BTZ
	Total (n=51)	P + DXM (n=19)	(n=10)
Time on treatment (weeks)	15.0 (6.0-39.4)	NA	30.4 (5.7-81.3+)
Cycles per patient	2 (1-9)	3 (2-9)	3 (1-10+) [a]
Plitidepsin dose intensity (mg/m^2/week)	2.3 (1.3-2.6)	2.0 (1.6-2.5)	1.8 (1.1-2.1+) [a]

Data shown are median (range) for all treated patients.
Data for APL-A-009-08 (P+DXM+BTZ) were obtained from only three patients and cannot be summarised in this table. Patients received two, three and six cycles, respectively. Median dose intensity for plitidepsin was 1.1 (1.0-1.2) mg/m^2/week.
[a] Data at the recommended dose: plitidepsin 5.0 mg/m^2 plus BTZ 1.3 mg/m^2 plus DXM 40 mg/m^2.
+, patients ongoing treatment at cut-off; BTZ, bortezomib; DXM, dexamethasone; NA, not available; P, plitidepsin.

Secondary safety data

a) Integrated safety analysis

Integrated analysis was based on data from 527 patients [356 (67.6%) with haematological malignancies and 171 (32.4%) with solid tumours] from a total of 14 completed phase II studies and one completed phase III study (including cross-over patients). Most patients included in these studies were male with a median age ranging from 60 to 64 years.

In the pooled Integrated Safety Analysis, the phase II/III studies were grouped into three categories or dose groups (Table 58) according to the similarity in treatment dose and regimen:

• Group A: plitidepsin fortnightly schedule (Day 1 and 15, q4wk) in combination with DXM (Day 1, 8, 15 and 22, q4wk), supported by data from the pivotal study APL-C-001-09 (ADMYRE). A total of 204 patients with relapsed/refractory MM were treated with this dose regimen, including 167 patients randomised to Arm A (plitidepsin plus DXM) and 37 patients who crossed over from Arm B (DXM) to Arm A. Consequently, only data from cycles with the combination with DXM are included in this analysis.

• Group B: plitidepsin as single agent fortnightly schedule (Day 1 and 15, q4wk), supported by data from nine phase II studies (Two studies in patients with haematological malignancies, and 7 studies conducted in patients with solid tumours). A total of 195 patients were treated in phase II studies, including 63 patients (32.3%) with haematological malignancies and 132 patients (67.7%) with solid tumours. In one of these phase II studies (APL-B-014-03), 51 patients with relapsed/refractory MM were treated.

• Group C: plitidepsin as single agent weekly schedule (Day 1, 8 and 15, q4wk), supported data from five phase II studies. A total of 128 patients were treated in phase II studies, including 89 patients (69.5%) with haematological malignancies and 39 patients (30.5%) with solid tumours.

In the dosing group A and B, plitidepsin was administered using a fortnightly schedule, i.e. 3-hour i.v. infusion on Day 1, 15 q4wk, at the RD of 5 mg/m^2.

In the dosing group C, plitidepsin was administered using a weekly schedule, i.e. 1-hour i.v. infusion on Day 1, 8, 15 q4wk, at the RD of 3.2 mg/m^2.

Table 58. Phase III/II studies included for Integrated Safety Analysis of plitidepsin

Dose Group, regimen and study	Indication	Treated patients
Group A: plitidepsin plus DXM [a] (plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion D1 and 15 q4wk plus DXM 40 mg orally D1, 8, 15 and 22, q4wk)		
APL-C-001-09 [b]	Multiple myeloma	204
Group B: plitidepsin fortnightly schedule (D1 and 15, q4wk) [c]		
Haematological malignancies		63
APL-B-014-03	Multiple myeloma	51[d]
APL-B-020-10	Myelofibrosis	12
Solid tumours		132
APL-B-002-02	MTC	16
APL-B-003-02	Exocrine pancreas	19
APL-B-004-02	NSCLC	21
APL-B-005-02	Bladder	21
APL-B-007-02	Melanoma	37
APL-B-010-02	Head and neck	10
APL-B-011-02	Prostate	8
Total Group B		195
Group C: plitidepsin weekly schedule (D1, 8 and 15, q4wk)		
Haematological malignancies		89
APL-B-012-02	NHL (indolent)	8
APL-B-013-02	NHL (aggressive)	64
APL-B-015-04	Leukaemia	17
Solid tumours		39
APL-B-006-02	SCLC	19
APL-B-016-05 [e]	Melanoma	20
Total Group C		128
TOTAL		**527**

[a] Plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion on D1 and 15 q4wk plus DXM 40 mg orally on D1, 8, 15 and 22, q4wk.
[b] Patients treated with plitidepsin plus DXM and patients after crossing over from Arm B (DXM) to Arm A (plitidepsin plus DXM) were included in this group; 204 patients as the sum of 167 patients treated in Arm A plus 37 patients who had crossover.
[c] In Group B, all protocol studies except for APL-B-020-10 defined plitidepsin administration as D1 q2wk but to maintain simplicity and being this scheme equivalent to that used in the study APL-C-001-09 (ADMYRE) (i.e., D1 and 15, q4wk), it was decided to report them as the same scheme (D1 and 15, q4wk).
[d] Nineteen of these 51 patients were first given plitidepsin alone, and after showing suboptimal response their treatment was supplemented with DXM. In these 19 patients, cycles with DXM supplementation were excluded from the pooled Integrated Safety Analysis data.
[e] Thirty-six patients who were treated with plitidepsin combined with dacarbazine in APL-B-016-05 study were excluded from the pooled Integrated Safety Analysis data. Only 20 patients treated with plitidepsin alone were included.
D, day; DXM, dexamethasone; i.v., intravenous; MTC, medullary thyroid carcinoma; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; q4wk, every four weeks; SCLC, small cell lung cancer.

A comparable percentage of patients had cycle delays in dose Group A (42.2%) and in the haematological malignancies subset of Group B (plitidepsin fortnightly schedule; 39.7%). The most common cause of cycle delay both in Group A and in the overall phase II dataset (Group B+C) was non-haematological toxicity (22.5% and 17.6% of patients, respectively).

The proportion of patients with dose omissions was higher in dose Group A (46.6%) compared to single-agent phase II data (Group B: 1.0% overall, and 3.2% in patients with haematological disease; Group C: 10.9% overall, and 7.9% in patients with haematological disease. Most dose omissions were due to non-haematological toxicity (22.5% in Group A), or non-drug related causes.

The percentage of patients with dose reductions was slightly higher in dose Group A (27.9%) compared to single agent fortnightly schedule phase II data (20.0% overall, and 11.1% in patients with haematological malignancies). The vast majority of dose reductions were due to non-haematological toxicity in all three dose groups (26.0% in Group A).

Overall duration of exposure to plitidepsin alone or in combination with DXM or BTZ and DXM by group (A, B, C and overall) in patients with haematological malignancies is presented in Table 59

Table 59. Treatment duration and dosage (Integrated Safety Analysis, phase III/II data)

		Group A (P+DXM) APL-C-001-09		Group B (P) D1,15 q4wk		Group C (P) D1,8,15 q4wk		Overall phase II P single-agent (B+C)		Overall (A+B+C)	
		n	%	n	%	n	%	n	%	n	%
Treatment duration (months)											
Haematological	**n**	204		63		89		152		356	
	Median	2.7		1.9		1.3		1.9		1.9	
	Range	0.1-31.5		0.4-7.3		0.5-7.9		0.4-7.9		0.1-31.5	
Solid tumours	**n**	.		132		39		171		171	
	Median	.		1.9		1.0		1.6		1.6	
	Range	.		0.1-16.2		0.3-3.7		0.1-16.2		0.1-16.2	
Total	**n**	204		195		128		323		527	
	Median	2.7		1.9		1.0		1.9		1.9	
	Range	0.1-31.5		0.1-16.2		0.3-7.9		0.1-16.2		0.1-31.5	
Plitidepsin cumulative dose (mg/m^2)											
Haematological	**n**	203		63		89		152		355	
	Median	20.5		18.4		12.7		15.3		18.9	
	Range	0 [a] -258		5-84		0-74		0-84		0-258	
Solid tumours	**n**	.		132		39		171		171	
	Median	.		14.9		9.6		14.6		14.6	
	Range	.		5-156		3-27		3-156		3-156	
Total	**n**	203		195		128		323		526	
	Median	20.5		15.0		9.7		14.9		15.2	
	Range	0 [a] -258		5-156		0-74		0-156		0-258	
Plitidepsin dose intensity (mg/m^2/week)											
Haematological	**n**	203		63		89		152		355	
	Median	1.9		2.2		2.1		2.2		2.0	
	Range	0 [a] -3		1-3		0-2		0-3		0-3	
Solid tumours	**n**	.		131		39		170		170	
	Median	.		1.9		1.6		1.9		1.9	
	Range	.		1-3		1-3		1-3		1-3	
Total	**n**	203		194		128		322		525	
	Median	1.9		2.0		2.1		2.0		1.9	
	Range	0 [a] -3		1-3		0-3		0-3		0-3	
Haematological	**n**	204		63		89		152		356	
	Median	3.0		2.0		2.0		2.0		2.0	
	Range	1-33		1-8		1-8		1-8		1-33	
Solid tumours	**n**	.		132		39		171		171	
	Median	.		2.0		1.0		2.0		2.0	
	Range	.		1-16		1-4		1-16		1-16	
Total	**n**	204		195		128		323		527	
	Median	3.0		2.0		1.0		2.0		2.0	
	Range	1-33		1-16		1-8		1-16		1-33	
Number of cycles administered per patient (q4wk)											
Haematological	**n**	204		63		89		152		356	
	1	48	23.5	15	23.8	42	47.2	57	37.5	105	29.5
	2	49	24.0	34	54.0	26	29.2	60	39.5	109	30.6
	3	26	12.7	6	9.5	9	10.1	15	9.9	41	11.5
	4	16	7.8	3	4.8	5	5.6	8	5.3	24	6.7
	5-10	43	21.1	5	7.9	7	7.9	12	7.9	55	15.4
	>10	22	10.8	.	.	.	.	.	.	22	6.2
Solid tumours	**n**	.		132		39		171		171	
	1	.	.	50	37.9	24	61.5	74	43.3	74	43.3
	2	.	.	57	43.2	14	35.9	71	41.5	71	41.5
	3	.	.	8	6.1	.	.	8	4.7	8	4.7
	4	.	.	5	3.8	1	2.6	6	3.5	6	3.5
	5-10	.	.	10	7.6	.	.	10	5.8	10	5.8
	>10	.	.	2	1.5	.	.	2	1.2	2	1.2
Total	**n**	204		195		128		323		527	
	1	48	23.5	65	33.3	66	51.6	131	40.6	179	34.0
	2	49	24.0	91	46.7	40	31.3	131	40.6	180	34.2
	3	26	12.7	14	7.2	9	7.0	23	7.1	49	9.3
	4	16	7.8	8	4.1	6	4.7	14	4.3	30	5.7
	5-10	43	21.1	15	7.7	7	5.5	22	6.8	65	12.3
	>10	22	10.8	2	1.0	.	.	2	0.6	24	4.6

Data shown are n (%) of treated patients except for median (range).
[a] One patient in Arm A (plitidepsin plus DXM) of APL-C-001-09 (ADMYRE) study received only one plitidepsin infusion, which was interrupted, and the total dose administered (even in the re-administration) was unknown. Therefore, data to calculate the dose intensity for this patient was not available.
Group A: plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion on D1 and 15 q4wk plus DXM 40 mg orally on D1, 8, 15 and 22, q4wk. Includes analysis from 167 patients who were treated in Arm A, and 37 patients who crossed over from Arm B (DXM) to Arm A (plitidepsin plus DXM) (only data from cycles with the combination are included in this analysis). All 204 patients had relapsed/refractory MM.
Group B: plitidepsin fortnightly schedule (D1 and 15, q4wk). Haematological malignancies included relapsed/refractory MM (n=51) and myelofibrosis (n=12). Solid tumours included bladder (n=21), exocrine pancreas (n=19), head and neck (n=10), melanoma (n=37), MTC (n=16), NSCLC (n=21), and prostate (n=8).
Group C: plitidepsin weekly schedule (D1, 8 and 15, q4wk). Haematological malignancies included leukaemia (n=17), NHL (indolent, n=8), and NHL (aggressive, n=64). Solid tumours included melanoma (n=20) and SCLC (n=19).
D, day; DXM, dexamethasone; MM, multiple myeloma; MTC, medullary thyroid carcinoma; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; P, plitidepsin; q4wk, every four weeks; SCLC, small cell lung cancer.

b) Phase I studies

A total of six completed single-agent phase I studies explored five different infusion times and three administration schedules in 252 patients (38 of them, paediatric patients).

A total of five completed studies (four phase I, and one phase II with a dose-finding stage) explored plitidepsin combined with other antineoplastic drugs in 114 patients. Two of these studies, which evaluated plitidepsin combined with cytarabine or docetaxel, were terminated early following a decision by the Sponsor.

Adverse events

Adverse Events regardless of Relationship with the Study Treatment

A summary of AEs regardless of relationship with the study treatment in patients treated in the pivotal study is presented in Table 60.

Table 60 Summary of adverse events regardless of relationship with the study treatment (APL-C-001-09)

	Arm A (P+DXM)		Arm B (DXM)	
	n	%	n	%
n	167		83	
AEs regardless of relationship	166	99.4	81	97.6
Grade ≥3 AEs regardless of relationship	139	83.2	53	63.9
AEs regardless of relationship leading to treatment discontinuation	42	25.1	12	14.5
AEs regardless of relationship leading to death	22	13.2	5	6.0

Data shown are n (%) of treated patients.
AE, adverse event; DXM, dexamethasone; P, plitidepsin; q4wk, every four weeks.

A summary of AEs regardless of relationship with the study treatment by system organ class and preferred term occurring in ≥10% of patients, worst grade per patient) in patients treated in the pivotal study is presented in Table 61.

Table 61 Adverse events regardless of relationship with the study treatment by System Organ Class and Preferred Term occurring in ≥10% of patients, worst grade per patient (APL-C-001-09)

MedDRA SOC/PT	Arm A (P+DXM)										Arm B (DXM)									
	NCI-CTCAE grade								Total (n=167)		NCI-CTCAE grade								Total (n=83)	
	1		2		3		4				1		2		3		4			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Blood and lymphatic system disorders																				
Anemia	1	0.6	14	8.4	59	35.3	.	.	74	44.3	.	.	1	1.2	35	42.2	.	.	36	43.4
Thrombocytopenia	.	.	1	0.6	11	6.6	6	3.6	18	10.8	.	.	.	.	4	4.8	4	4.8	8	9.6
Gastrointestinal disorders																				
Constipation	9	5.4	14	8.4	.	.	.	.	23	13.8	3	3.6	3	3.6	.	.	.	.	6	7.2
Diarrhea	30	18.0	25	15.0	5	3.0	.	.	60	35.9	6	7.2	2	2.4	.	.	.	.	8	9.6
Nausea	44	26.3	31	18.6	7	4.2	.	.	82	49.1	13	15.7	5	6.0	1	1.2	.	.	19	22.9
Vomiting	22	13.2	18	10.8	4	2.4	.	.	44	26.3	3	3.6	.	.	1	1.2	.	.	4	4.8
General disorders and administration site conditions																				
Edema peripheral	21	12.5	12	7.1	2	1.2	.	.	34	20.4	4	4.8	3	3.6	.	.	.	.	7	8.4
Fatigue	32	19.2	38	22.8	19	11.4	1	0.6	90	53.9	10	12.0	12	14.5	9	10.8	.	.	31	37.3
Pyrexia	21	12.6	15	9.0	1	0.6	.	.	37	22.2	8	9.6	4	4.8	.	.	.	.	12	14.5
Investigations																				
ALT increased	1	0.6	9	5.4	14	8.4	3	1.8	27	16.2	.	.	.	.	.	.	.	.	.	.
Blood CPK increased	1	0.6	2	1.2	14	8.4	12	7.2	29	17.4	.	.	.	.	.	.	.	.	.	.
Metabolism and nutrition disorders																				
Decreased appetite	21	12.6	13	7.8	2	1.2	.	.	36	21.6	5	6.0	1	1.2	.	.	.	.	6	7.2
Hypercalcemia	.	.	5	3.0	4	2.4	3													
Hyperkalemia																				
Musculoskeletal and connective tissue disorders																				
Back pain	11	6.6	8	4.8	4	2.4	.	.	23	13.8	4	4.8	7	8.4	6	7.2	.	.	17	20.5
Bone pain	1	0.6	12	7.2	3	1.8	.	.	16	9.6	6	7.2	14	16.9	2	2.4	1	1.2	23	27.7
Muscular weakness	6	3.6	8	4.8	6	3.6	1	0.6	21	12.6	1	1.2	2	2.4	1	1.2	.	.	4	4.8
Myalgia	9	5.4	12	7.2	7	4.2	2	1.2	30	18.0	4	4.8	.	.	.	.	.	.	4	4.8
Nervous system disorders																				
Headache	14	8.4	6	3.6	1	0.6	.	.	21	12.6	3	3.6	2	2.4	.	.	.	.	5	6.0
Neuropathy peripheral	11	6.6	6	3.6	.	.	.	.	17	10.2	1	1.2	.	.	.	.	.	.	1	1.2
Psychiatric disorders																				
Insomnia	13	7.8	5	3.0	1	0.6	.	.	19	11.4	10	12.0	2	2.4	.	.	.	.	12	14.5
Respiratory, thoracic and mediastinal disorders																				
Cough	21	12.6	12	7.2	.	.	.	.	33	19.8	6	7.2	6	7.2	.	.	.	.	12	14.5
Dyspnea	6	3.6	13	7.8	3	1.8	2	1.2	24	14.4	2	2.4	.	.	.	.	1	1.2	3	3.6
Epistaxis	7	4.2	1	0.6	2	1.2	.	.	10	6.0	5	6.0	1	1.2	3	3.6	.	.	9	10.8
Vascular disorders																				
Hypertension	8	4.8	9	5.4	.	.	.	.	17	10.2	4	4.8	1	1.2	.	.	.	.	5	6.0
Hypotension	5	3.0	7	4.2	4	2.4	1	0.6	17	10.2	1	1.2	1	1.2	.	.	.	.	2	2.4

Adverse events that occurred after crossover have been excluded from this table.
ALT, alanine aminotransferase; CPK, creatine phosphokinase; DXM, dexamethasone; MedDRA, Medical Dictionary for Regulatory Activities v.16.0; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; P, plitidepsin; PT, preferred term; SOC, System Organ Class.

Treatment-related Adverse Events (including AEs with unknown relationship)

A summary of all treatment-related AEs (including AEs with unknown relationship) in patients treated in the pivotal study APL-C-001-09 is presented in Table 62.

Table 62. Summary of treatment-related adverse events (including adverse events with unknown relationship) (APL-C-001-09)

	Arm A (P+DXM)		Arm B (DXM)	
	n	%	n	%
n	167		83	
Treatment-related AEs [a]	144	86.2	38	45.8
Grade ≥3 treatment-related AEs [a]	86	51.5	9	10.8
Treatment-related AEs leading to treatment discontinuation [a]	16	9.6	3	3.6
Treatment-related AEs leading to death [a]	3 [b]	1.8	1	1.2 [c]

Data shown are n (%) of treated patients.
[a] Including AEs with unknown relationship.
[b] One of these three patients with AEs leading to death had treatment-related AEs: grade 4 myopathy in patient (Arm A; APL-C-001-09, ADMYRE). The other two patients had AEs with unknown causality. The first case is further explained in Section 3.1.5.2 and Section 3.2.
[c] Grade 4 respiratory infection in patient (Arm B; APL-C-001-09, ADMYRE).
AE, adverse event; DXM, dexamethasone; P, plitidepsin; q4wk, every four weeks.

Table 63 summarises the most frequently reported (≥10% of patients) AEs in Arm A vs. in Arm B of study APL-C-001-09. In Arm A (plitidepsin plus DXM), the most common AEs (all grades) were gastrointestinal disorders (nausea, 37.1% of patients; vomiting, 16.8%; diarrhoea, 14.4%), general disorders (fatigue, 36.5%; oedema peripheral, 12.0%), metabolic/nutritional disorders (decreased appetite, 12.6%), and musculoskeletal disorders (myalgia, 14.4%; muscular weakness, 9.6%). The most common grade ≥3 AEs observed in plitidepsin-treated patients (Arm A) were fatigue (10.8%), myalgia (5.4%), muscular weakness (3.6%) and nausea (3.6%).

Table 63. Treatment-related adverse events (including adverse events with unknown relationship) by SOC and PT occurring in ⩾10% of patients, worst grade per patient (APL-C-001-09)

MedDRA SOC/PT [a]	Arm A (P+DXM)										Arm B (DXM)							
	NCI-CTCAE grade								Total (n=167)		NCI-CTCAE grade						Total (n=83)	
	1		2		3		4				1		2		3			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Gastrointestinal disorders																		
Diarrhea	11	6.6	11	6.6	2	1.2	.	.	24	14.4	2	2.4	.	.	.	.	2	2.4
Nausea	34	20.4	22	13.2	6	3.6	.	.	62	37.1	7	8.4	1	1.2	1	1.2	9	10.8
Vomiting	14	8.4	11	6.6	3	1.8	.	.	28	16.8	1	1.2	.	.	1	1.2	2	2.4
General disorders and administration site conditions																		
Edema peripheral	11	6.6	7	4.2	2	1.2	.	.	20	12.0	2	2.4	.	.	.	.	2	2.4
Fatigue	16	9.6	27	16.2	17	10.2	1	0.6	61	36.5	3	3.6	3	3.6	1	1.2	7	8.4
Metabolism and nutrition disorders																		
Decreased appetite	13	7.8	7	4.2	1	0.6	.	.	21	12.6	2	2.4	.	.	.	.	2	2.4
Musculoskeletal and connective tissue disorders																		
Myalgia	7	4.2	8	4.8	7	4.2	2	1.2	24	14.4	2	2.4	.	.	.	.	2	2.4

[a] Adverse events related to laboratory values are shown in Section 12.4.
Adverse events that occurred after crossover have been excluded from this table.
DXM, dexamethasone; MedDRA, Medical Dictionary for Regulatory Activities v.16.0; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; P, plitidepsin; PT, preferred term; SOC, System Organ Class.

Serious adverse event/deaths/other significant events

Serious adverse events regardless of relationship with the study treatment

In APL-C-001-09, overall SAEs regardless of relationship with the study treatment occurred more frequently in Arm A (plitidepsin plus DXM; 58.1%) than in Arm B (DXM; 33.7%) (Table 64). SAE leading to treatment discontinuation were 18.0 (Arm A) vs. 8.4% (Arm B) of patients.

The rate of patients with SAEs regardless of relationship with the study treatment that led to patient's death was 13.2% in Arm A and 6.0% in Arm B. Of the 22 patients from Arm A with death due to SAEs regardless of relationship with the study treatment, only one case was due to a treatment-related event (grade 4 myopathy). Then, the rate of treatment-related deaths was 0.6% in Arm A (1/167 patients) and 1.2% in Arm B (1/83 patients).

Table 64 Summary of serious adverse events regardless of relationship with the study treatment (APL-C-001-09)

	Arm A (P+DXM)		Arm B (DXM)	
	n	%	n	%
n	167		83	
SAEs regardless of relationship	97	58.1	28	33.7
Grade ≥3 SAEs regardless of relationship	80	47.9	24	28.9
SAEs regardless of relationship leading to treatment discontinuation	30	18.0	7	8.4
SAEs regardless of relationship leading to death	22	13.2	5	6.0

Data shown are n (%) of treated patients.
DXM, dexamethasone; P, plitidepsin; q4wk, every four weeks; SAE, serious adverse event.

In Arm A (plitidepsin plus DXM), the most frequent SAEs regardless of relationship with the study treatment were pneumonia (n=19; 11.4%), AST increased (n=12; 7.2%), sepsis (n=11; 6.6%), ALT increased (n=10; 6.0%), blood CPK increase (n=9; 5.4%), vomiting (n=8; 4.8%), pyrexia (n=8; 4.8%), fatigue (n=7; 4.2%), hypercalcaemia (n=6; 3.6%), and anaemia (n=5; 3.0%).

In Arm B (DXM), the most frequent SAEs regardless of relationship with the study treatment were anaemia (n=4; 4.8%), pneumonia (n=3; 3.6%), hypercalcaemia (n=3; 3.6%), and acute renal failure (n=3; 3.6%).

Treatment-related Serious Adverse Events (including SAEs with unknown relationship)

In APL-C-001-09, overall treatment-related (or with unknown relationship) SAEs occurred more frequently in Arm A (plitidepsin plus DXM; 28.1%) than in Arm B (DXM; 7.2%) (Table 65). Arm A also showed more frequent grade ≥ 3 treatment related (or with unknown relationship) SAEs (20.4% vs. 7.2% in Arm B), with treatment-related (or with unknown relationship) SAEs leading to treatment discontinuation in 6.0 vs. 2.4% of patients.

The rate of patients with treatment-related (or with unknown relationship) SAEs that led to patient's death was similar in both treatment arms: 1.8% in Arm A and 1.2% in Arm B. Of the three patients from Arm A with death due to SAEs, only one case was due to a treatment-related event (grade 4 myopathy), and the other two patients had SAEs with unknown relationship, consisting of grade 3 escherichia sepsis, and grade 5 cardiac arrest.

Table 65 Summary of treatment-related serious adverse events (including serious adverse events with unknown relationship) (APL-C-001-09)

	Arm A (P+DXM)		Arm B (DXM)	
	n	%	n	%
n	167		83	
Treatment-related SAEs [a]	47	28.1	6	7.2
Grade ≥3 treatment-related SAEs [a]	34	20.4	6	7.2
Treatment-related SAEs leading to treatment discontinuation [a]	10	6.0	2	2.4
Treatment-related SAEs leading to death [a]	3 [b]	1.8	1 [c]	1.2

Data shown are n (%) of treated patients.
[a] Including AEs with unknown relationship.
[b] One of these three patients with SAEs leading to death had treatment-related AEs: grade 4 myopathy in patient #0 rm A; APL-C-001-09, ADMYRE). The other two patients had SAEs with unknown causality (*Escherichia* sepsis, and grade 5 cardiac arrest). The first case is further explained in Section 3.1.5.2 and Section 3.2.
[c] Grade 4 respiratory infection in patient #1 rm B; APL-C-001-09, ADMYRE).
DXM, dexamethasone; P, plitidepsin; q4wk, every four weeks; SAE, serious adverse event.

In Arm A (plitidepsin plus DXM), 47 patients (28.1%) had a total of 96 SAEs related to the study treatment and 24 SAEs with unknown causality. The most frequent SAEs were ALT increase (n=7; 4.2%), pneumonia (n=7; 4.2%), AST increase (n=6; 3.6%), and CPK increase (n=5; 3.0%). Most of these SAEs (85.0%) resolved. Three patients with SAEs (1.9%) had a fatal outcome: grade 4 myopathy related to the study treatment, grade 3 Escherichia sepsis with unknown causality, and grade 5 cardiac arrest with unknown causality.

In Arm B (DXM), six patients (7.2%) had a total of four SAEs related to the study treatment and seven SAEs with unknown causality. Most of these SAEs (90.9%) resolved. One patient with a SAE had a fatal outcome due to grade 4 respiratory tract infection related to DXM.

Deaths

In Arm A (plitidepsin plus DXM), 99 of 167 treated patients (59.3%) died during treatment or follow-up. Most of these deaths were due to progression of the patient's underlying malignant disease: 73 of 99 deaths (73.7%). One patient (1/167, 0.6%) died due to a treatment-related AE: grade 4 myopathy after having received one cycle. Furthermore, 25 patients (25.3% of total deaths in this arm) died due to AEs unrelated with the study treatment, but related to complications from the malignant disease (e.g., multi-organ failure, sepsis, pneumonia or renal failure).

Three patients with treatment-related (or with unknown causality) SAEs (1.9%) had a fatal outcome; these consisted of the above mentioned case of grade 4 myopathy related to the study treatment; grade 3 Escherichia sepsis with unknown causality, and grade 5 cardiac arrest with unknown causality.

In Arm B (DXM), 57 of 83 treated patients (68.7%) died during treatment or follow-up. Most of these deaths were due to progression of the patient's underlying malignant disease: 42 of 57 deaths (73.7%). One patient (1/83; 1.2%) died due to a treatment-related AE: grade 4 respiratory tract infection after having received two cycles. Furthermore, 10 patients (17.5% of total deaths in this arm) died due to AEs unrelated with the study treatment, but related to complications from the malignant disease (e.g., multi-organ failure, sepsis, pneumonia or renal failure), and four patients due other causes.

Table 66 Cause of deaths occurred during the study APL-C-001-09

Reason [a]	Arm A (P+DXM)		Arm B (DXM)	
	n	%	n	%
Malignant disease	73	73.7	42	73.7
Adverse event (non-treatment-related)	25 [b]	25.3	10	17.5
Adverse event (treatment-related)	1	1.0	1	1.8
Other	.	.	4	7.0
Total	99	100.0	57	100.0

A listing of deaths reported during the study is provided in Section 14.3.2, Table 14.74.
[a] Percentage based on the number of patients who died.
[b] Patient had cardiac arrest reported as a SAE with unknown causality and fatal outcome (see details in Section 12.3.1.2, Table 59 and narrative in Appendix 16.5).
DXM, dexamethasone; P, plitidepsin.

A total of 26/167 (15.6%) deaths due to AEs were registered in the intervention arm and 11/83 (13%) were registered in the control arm.

Regarding the 4 patients who died due to other causes in arm B, the cause of death was unknown for 3 of them.

Other significant events

Musculoskeletal Disorders, CPK Increases and Rhabdomyolysis

The most common musculoskeletal disorders reported as adverse reactions were myalgia (14.4%) and muscular weakness (9.6%). CPK increase regardless of grade was reported in 44.5% of patients; this parameter was the most common grade 3/4 biochemical abnormality, reported in 20.0% of patients. The median onset of grade 3/4 CPK increase (day from first dose) was 48 days; the median number of cycles during which the patients experienced grade 3/4 CPK increase was 1 cycle, and the median time to recovery was 14.5 days. The treatment was withdrawn only in 0.5% of patients who experienced grade 3/4 CPK increase, while dose delay, dose reduction, dose omission and dose interruption occurred in 8.8%, 11.3%, 5.9% and 0.5%, respectively. The vast majority of patients (83.9%) who experienced CPK increase recovered. Rhabdomyolysis of grade 3/4 was reported in 1.2% of patients.

In APL-C-001-09 study, the most common musculoskeletal disorders reported as treatment-related AEs (or with unknown causality) were myalgia (24/167 patients, 14.4%) and muscular weakness (16/167 patients, 9.6%) (Table 67).

Table 67 Musculoskeletal and connective tissue disorders reported as treatment-related adverse events (including adverse events with unknown relationship) (APL-C-001-09)

MedDRA PT	Arm A (P+DXM)										Arm B (DXM)									
	NCI-CTCAE grade								Total (n=167)		NCI-CTCAE grade								Total (n=83)	
	1		2		3		4				1		2		3		4			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Arthralgia	.	.	1	0.6	1	0.6	.	.	2	1.2	.	.	.	.	.	.	.	.	.	.
Back pain	1	0.6	.	.	1	0.6	.	.	2	1.2	.	.	1	1.2	.	.	.	.	1	1.2
Bone pain	.	.	1	0.6	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Muscle atrophy	.	.	.	.	.	.	.	.	.	.	.	.	1	1.2	.	.	.	.	1	1.2
Muscle contracture	1	0.6	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Muscle spasms	3	1.8	1	0.6	.	.	.	.	4	2.4	.	.	1	1.2	.	.	.	.	1	1.2
Muscular weakness	4	2.4	6	3.6	5	3.0	1	0.6	16	9.6	1	1.2	1	1.2	.	.	.	.	2	2.4
Musculoskeletal pain	2	1.2	1	0.6	1	0.6	.	.	4	2.4	.	.	.	.	.	.	.	.	.	.
Myalgia	7	4.2	8	4.8	7	4.2	2	1.2	24	14.4	2	2.4	.	.	.	.	.	.	2	2.4
Myopathy	.	.	2	1.2	1	0.6	1	0.6	4	2.4	1	1.2	.	.	1	1.2	.	.	2	2.4
Myopathy toxic	.	.	.	.	.	.	1	0.6	1	0.6	.	.	.	.	.	.	.	.	.	.
Myositis	.	.	.	.	.	.	1	0.6	1	0.6	.	.	.	.	.	.	.	.	.	.
Osteopenia	.	.	.	.	.	.	.	.	.	.	1	1.2	.	.	.	.	.	.	1	1.2
Pain in extremity	1	0.6	2	1.2	1	0.6	.	.	4	2.4	.	.	.	.	1	1.2	.	.	1	1.2
Rhabdomyolysis	.	.	.	.	1	0.6	1	0.6	2	1.2	.	.	.	.	.	.	.	.	.	.

Adverse events that occurred after crossover have been excluded from this table.
DXM, dexamethasone; MedDRA, Medical Dictionary for Regulatory Activities v.16.0; NA, not available; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; P, plitidepsin; PT, preferred term; SOC, System Organ Class.

During treatment, 44.5% of patients had CPK increase, being grade 3/4 in 20.0% of patients. The median values for peak counts of CPK from all patients, over 32 cycles, showed no evidence of cumulative toxicity. Grade 4 CPK increase was reported as treatment-related SAE in five patients (3.0%) from Arm A (plitidepsin plus DXM). One of these cases resulted in treatment discontinuation and patient's death; the cause of death was related by the Investigator to grade 4 myopathy but, indeed, it was derived from complications of grade 4 rhabdomyolysis.

Apart from the SAEs above mentioned, other effects on treatment of grade 3/4 CPK increases were cycle delay in 13/167 patients (1.8%), dose omission in 10/167 patients (6.0%), and dose reduction in 18/167 patients (10.8%).

Two events of grade 3/4 rhabdomyolysis (2/167=1.2%) were reported as AEs (one treatment-related and the other with unknown causality) in Arm A (plitidepsin plus DXM) of APL-C-001-09 (ADMYRE) study. Apart from patient mentioned above, another patient had grade 3 rhabdomyolysis concomitant with grade 1 renal failure, grade 3 bilirubin increase and grade 3 increase in hepatic enzymes, that required hospitalisation, were reported as a treatment-related SAEs, and led to cycle delay and dose reduction of plitidepsin, as well as omission of DXM dose.

In the Integrated Safety Analysis (phase III and II data), during treatment in patients with haematological malignancies, CPK increase was found in 84/185 patients (45.4%) in Group A, 28/62 (45.2%) in Group B, and 18/80 (22.5%) in Group C. Grade 3/4 CPK increase was observed in 39/185 patients (21.1%) in Group A, 7/62 (11.3%) in Group B, and 6/80 (7.5%) in Group C.

Musculoskeletal disorders are commonly reported with plitidepsin or with plitidepsin plus DXM. The most frequent of these disorders are myalgia (grade ≥3 in 4.4% of patients in Group A; 4.1% of patients in Group B; and 1.6% in Group C), muscular weakness (grade ≥3 in 4.4% of patients in Group A; 2.1% of patients in Group B; and 4.7% in Group C), as well as the above mentioned increased blood CPK levels. Most of these events are grade ≤ 2 and reversible, as they often resolved after treatment discontinuation or dose reduction without requiring additional therapeutic measures.

Patient accrual into clinical studies with plitidepsin is currently restricted to patients with grade < 2 myopathy and no clinical situations that may cause significant and persistent CPK increase. In addition,

the clinical study protocols include guidelines for adjusting plitidepsin dose given to the patients depending on the severity of CPK increase and/or muscular AEs.

Cardiac Disorders

In APL-C-001-09 study, cardiac disorders reported as treatment-related AEs (or with unknown causality) were uncommon, with atrial fibrillation (4/167 patients, 2.4%) as the most frequent event (Table 68). Three events of atrial fibrillation, one cardiac arrest, one cardiac failure, one cardiac failure congestive, one myocardial infarction, and one systolic dysfunction were reported as treatment-related SAEs.

Table 68 Cardiac disorders reported as treatment-related adverse events (including adverse events with unknown relationship) (APL-C-001-09)

MedDRA PT	Arm A (P+DXM)												Arm B (DXM)									
	NCI-CTCAE grade										Total (n=167)		NCI-CTCAE grade								Total (n=83)	
	1		2		3		4		5				1		2		3		4			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Angina pectoris	.	.	1	0.6	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Atrial fibrillation	.	.	2	1.2	2	1.2	.	.	.	.	4	2.4	.	.	.	.	1	1.2	.	.	1	1.2
Atrial tachycardia	.	.	1	0.6	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Cardiac arrest	.	.	.	.	.	.	.	.	1	0.6	1	0.6	.	.	.	.	.	.	.	.	.	.
Cardiac failure	.	.	1	0.6	2	1.2	.	.	.	.	3	1.8	.	.	.	.	.	.	.	.	.	.
Cardiac failure chronic	.	.	1	0.6	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Cardiac failure congestive	.	.	.	.	1	0.6	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Left ventricular dysfunction	1	0.6	1	0.6	.	.	.	.	.	.	2	1.2	.	.	.	.	.	.	.	.	.	.
Left ventricular failure	1	0.6	.	.	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Mitral valve incompetence	1	0.6	.	.	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Myocardial infarction	.	.	1	0.6	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Palpitations	1	0.6	.	.	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Sinus tachycardia	1	0.6	1	0.6	.	.	.	.	.	.	2	1.2	1	1.2	.	.	.	.	.	.	1	1.2
Supraventricular tachycardia	1	0.6	.	.	.	.	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Systolic dysfunction	.	.	.	.	1	0.6	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Tachycardia	2	1.2	.	.	.	.	.	.	.	.	2	1.2	.	.	.	.	.	.	.	.	.	.
Ventricular extrasystoles	.	.	.	.	.	.	.	.	.	.	.	.	1	1.2	.	.	.	.	.	.	1	1.2

Adverse events that occurred after crossover have been excluded from this table.
DXM, dexamethasone; MedDRA, Medical Dictionary for Regulatory Activities v.16.0; NA, not available; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; P, plitidepsin; PT, preferred term; SOC, System Organ Class.

Patients with cardiac events, regardless of their relationship with the study treatment, were 44/167 (26.3%) in Arm A (plitidepsin plus DXM), 9/83 (10.8%) in Arm B (DXM), and 13/37 (35.1%) patients in the crossover population. Nineteen (11.4%) patients experienced Grade ≥ 3 cardiac events in Arm A, 2 (2.4%) patients in Arm B, and 5 (13.5%) in the crossover population.

The number of cardiac events, regardless of their treatment relationship with the study treatment were 100 (0.60 cardiac events per plitidepsin plus DXM-treated patient) in Arm A, 13 (0.16 cardiac events per DXM-treated patient) in Arm B, and 25 (0.68 cardiac events per cross over patient) in the cross over population. Patients with cardiac events, treatment-related or with unknown relationship, were 30/167 (18.0%) in Arm A (plitidepsin plus DXM), 4/83 (4.8%) in Arm B (DXM), and 9/37 (24.3%) patients in the crossover population.

The number of cardiac events, treatment-related or with unknown relationship, were 54 (0.32 cardiac events per plitidepsin plus DXM-treated patient) in Arm A, 5 (0.06 cardiac events per DXM-treated patient) in Arm B, and 13 (0.35 cardiac events per cross over patient) in the cross over population.

Twenty-three of the 100 total cardiac events occurred in Arm A (plitidepsin plus DXM) led to any treatment modification in 17/167 patients (10.2%). The most reported treatment modification were treatment discontinuation (n=8 events in seven patients) and skipped infusions (n=7 events in six patients). Four patients died during the study period. 71 of 100 cardiac events regardless of relationship reported in Arm A (plitidepsin plus DXM) were recovered.

The most common cardiac events in Arm A (plitidepsin plus DXM) were 41/100 rhythm abnormalities/QT prolongation (41.0%) described in 30 patients, followed by 34/100 cardiac insufficiency events (including asymptomatic decrease of LVEF) (34.0%) described in 17 patients. Other events reported were 15/100 myocardial ischaemia (including asymptomatic ECG changes or troponin increase) (15.0%), and 10 cases described as "other" (10.0%) that occurred in 12 and 8 patients, respectively.

No deaths were reported in the crossover population as associated to cardiac events. Twelve of 25 cardiac events reported regardless of relationship were not resolved.

The most common cardiac events in the crossover population were ten cardiac insufficiency events (40.0%) described in seven patients, followed by seven rhythm abnormalities/QT prolongation (28.0%) described in six patients. Other events reported were six myocardial ischaemia (24.0%), and two cases described as "other" (8.0%) that occurred in six and two patients, respectively.

In the Integrated Safety Analysis (phase III and II data), the cardiac disorders most reported as treatment-related AEs (or with unknown relationship) were atrial fibrillation (7/527 patients, 1.3%), tachycardia (6/527 patients, 1.1%), and palpitations (5/527, 0.9%). The only grade 5 AE reported was the event of cardiac arrest which occurred in APL-C-001-09 study. Additionally, one patient treated in study APL-B-014-03 had sudden death due to cardiorespiratory arrest, with unknown relationship, and reported from the general disorder and administration site condition SOC.

Preclinical data already showed changes in heart rate, namely tachycardia and/or occasional bradycardia, recorded in dogs, without relation either to the dose level or the number of doses administered. Furthermore, ST-segment depressions (unrelated with the dose and with no association with other ECG abnormalities) were recorded in most of the dogs (both genders) after single or repeated plitidepsin administration. Similar results were found in the safety pharmacology studies performed in telemetered dogs. These findings may be explained by an initial peripheral vasodilation that gives rise to hypotension, leading to tachycardia, as well as heart hypoperfusion, sub-endocardial ischaemia and thus, ST-segment depression.

Hypersensitivity

In APL-C-001-09 study, hypersensitivity reactions were reported as treatment-related AE in a low number of patients and without consequences on the study treatment: grade 1/2 was observed in five patients, and grade 3 in three patients (6.1%). One patient had grade 4 anaphylactic shock in Cycle 16 concomitant with grade 4 cardiac arrest that were reported as SAEs and caused treatment discontinuation. Another patient had grade 3 infusion-related reaction with grade 3 hypoxia, required hospitalisation, and led to treatment discontinuation. With respect to skin and subcutaneous reactions, one patient in Arm A had grade 3 rash in the first cycle that required reduction of plitidepsin dose.

In the Integrated Safety Analysis (phase III and II data), hypersensitivity reactions were mostly grade 1/2, and without associated complications; 11 patients (11/527=2.1%) required hospitalisation, and these events were reported as treatment-related SAEs.

With respect to skin and subcutaneous reactions, the only case that reached grade 3 was one patient that had rash in Arm A of APL-C-001-98 study.

Infusion-related events

In APL-C-001-09 study, infusion-related events were reported as treatment-related AEs in a low number of patients, and the vast majority of them were ≤ grade 2 and without effects on the study treatment. They consisted of grade 1 catheter site pain (1/167 patients= 0.6%); grade 1/2 catheter site phlebitis (2/167 patients=1.2%), grade 1 infusion site reaction (1/167 patients= 0.6%); and grade 2 injection site

extravasation (1/167 patients= 0.6%). One event of grade 3 thrombosis in device was reported (1/167 patients=0.6%). The latter event was the only reported as treatment-related SAE, as the patient required hospitalisation and the study treatment was discontinued.In the Integrated Safety Analysis (phase III and II data), most of the infusion-related events reported were grade 1/2, and mostly without clinically relevant consequences. Three of them were reported as SAEs.

Transaminases Elevations and Hepatobiliary Disorders

In APL-C-001-09 study, during treatment, ALT was increased in 84.9% of patients, and AST in 66.0% of patients, being grade 3/4 in 14.5% and 9.0% of patients, respectively. Grade 3/4 ALT increase appeared on Day 14 (range, 1-35 days) after dosing and most cases (58.3%) returned to values ≤ 2.5 x ULN before Day 28, with a median duration of 6 days (range, 1-16 days). Grade 3/4 AST appeared on Day 18 (range, 1-35 days) after dosing; half of episodes returned to values ≤ 2.5 x ULN before Day 28, with a median duration of 6.5 days (range, 1-28 days). The median values for peak counts of ALT or AST from all patients, over 32 cycles, showed no evidence of cumulative toxicity.

ALT increase was reported as a treatment-related SAE in seven patients (4.2%), being grade 3 in three patients and grade 4 in one patient. AST increase was reported as a treatment-related SAE in six patients (3.6%), being grade 3 in five patients.

Apart from the SAEs mentioned above, other effects on treatment of ALT/AST increases were treatment discontinuation in 2/167 patients (1.2%), cycle delay in 11/167 patients (6.6%), dose omission in 12/167 patients (7.2%), and dose reduction in 8/167 patients (4.8%).

The frequency of shifts for ALT and AST increases from grade 0-1 to grade ≥3 is considered particularly high and represents a concern. It is agreed with the applicant that an improvement is observed from cycle 3 onwards and few patients require treatment discontinuation that would be explained, at least partially, by the dose adjustments. However, the effect of any potential mechanism of tolerance cannot be disentangled from the dose adjustment effect with the data provided.

Hepatobiliary disorders were rarely reported as treatment-related AEs (or with unknown causality) in Arm A of APL-C-001-09study, and consisted of grade 3 cholestasis, grade 3 portal vein thrombosis, grade 2 hepatocellular injury, and grade 2 hepatomegaly (one patient each, 0.6%) (Table 69). The event of grade 3 cholestasis and three events of hyperbilirubinemia (grade 1, n=1; grade 3, n=2) were reported as treatment-related SAEs. The event of grade 3 cholestasis occurred in one patient who had several serious events with a fatal outcome.

Table 69 Hepatobiliary disorders reported as treatment-related AEs (including adverse events with unknown relationship) (APL-C-001-09)

MedDRA PT	Arm A (P+DXM)										Arm B (DXM)									
	NCI-CTCAE grade								Total (n=167)		NCI-CTCAE grade								Total (n=83)	
	1		2		3		4				1		2		3		4			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cholestasis	.	.	.	.	1	0.6	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Hepatocellular injury	.	.	1	0.6	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Hepatomegaly	.	.	1	0.6	.	.	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.
Portal vein thrombosis	.	.	.	.	1	0.6	.	.	1	0.6	.	.	.	.	.	.	.	.	.	.

Adverse events that occurred after crossover have been excluded from this table.
DXM, dexamethasone; MedDRA, Medical Dictionary for Regulatory Activities v.16.0; NA, not available; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; P, plitidepsin; PT, preferred term; SOC, System Organ Class.

In the Integrated Safety Analysis (phase III and II data), incidence of grade 3/4 AST or ALT was similar among groups: 9.1% (Group A), 9.5% (Group B) and 14.1% (Group C) for AST; 15.2% (Group A), 22.2% (Group B) and 24.7% (Group C) for AST. According to the Applicant, despite the common occurrence of grade 3/4 transaminases increases, severe elevations in bilirubin were infrequent (1.6% in Group A, 2.1% in Group B, and 2.5% in Group C), and none of them reached grade 4. The analysis of NCI-CTCAE grade shifts from grade 0-2 at baseline to grade 3-4 during treatment showed that patients with haematological malignancies with grade 3/4 ALT during treatment that had grade ≤2 at baseline were as follows in each dose group: n=29/204 treated patients (14.2%) in Group A (plitidepsin plus DXM); n=14/63 (22.2%) in Group B (plitidepsin fortnightly schedule), and 15/89 (16.9%) in Group C (plitidepsin weekly schedule). Patients with haematological malignancies with grade 3/4 AST during treatment that had grade ≤2 at baseline were as follows in each dose group: n=17/204 treated patients (8.3%) in Group A (plitidepsin plus DXM); n=6/63 (9.5%) in Group B (plitidepsin fortnightly schedule), and 11/89 (12.4%) in Group C (plitidepsin weekly schedule).

Neutropenia and Selected Infections/Septic Shock

In APL-C-001-09 study, neutropenia was found as haematological abnormality at baseline in 39.2% of patients in Arm A (plitidepsin plus DXM), being grade 3/4 in 4.7% of patients. During treatment, neutropenia occurred as haematological abnormality in 47.5% of patients, being grade 3/4 in 15.6% of patients. Grade 3/4 neutropenia was slightly more frequent in the first treatment cycle (13/160 cycles; 8.1%) than in subsequent cycles (31/641 cycles; 4.8%). Only one case of grade 3 febrile neutropenia was reported as a treatment-related AE/SAE with plitidepsin plus DXM in APL-C-001-09 study. Neutropenia was reported as AE regardless of relationship with the study treatment in 13/167 patients (7.8%): 12 patients with grade 3 and one patient with grade 4. Of these 13 patients, two had grade 1, nine had grade 2, and two had grade 3 neutropenia at baseline. Grade 3/4 neutrophil count decreased was also reported verbatim as AE in four patients (2.4%).

Neutropenia was mostly not associated to infections or septic shock; none of the 13 cases of neutropenia were reported as a SAE. The only effects on treatment of grade 3/4 neutropenia were discontinuation of treatment in Cycle 2 in one patient due to grade 3 neutropenia (concomitant with grade 3 fatigue); cycle delay in 6/167 patients (3.6%); dose omission in 4/167 patients (2.4%), and dose reduction in 1/167 patients (0.6%).

The median values for nadir counts of neutrophils from all treated patients, over 32 cycles, showed no evidence of cumulative toxicity.

Consistent results were observed in the integrated safety summary. The analysis of NCI-CTCAE grade shifts from grade 0-2 at baseline to grade 3-4 during treatment showed that a low percentage of patients worsened their haematological status during treatment with the plitidepsin plus DXM combination (Group A): i.e., 12.7% of treated patients had severe worsening during treatment of nadir values for neutrophils. Patients with haematological malignancies with grade 3/4 neutropenia during treatment that had grade ≤ 2 at baseline were as follows in each dose group: n=26/204 treated patients (12.7%) in Group A (plitidepsin plus DXM); n=8/63 (12.7%) in Group B (plitidepsin fortnightly schedule), and 8/89 (9.0%) in Group C (plitidepsin weekly schedule).

Nausea, Vomiting and Fatigue

In APL-C-001-09 study, nausea, vomiting and fatigue were common treatment-related AEs with plitidepsin plus DXM, occurring in 37.1%, 16.8% and 36.5% of patients, respectively. Few events were severe: grade 3 nausea was found in 3.6% of patients; grade 3 vomiting in 1.8%, and grade 3/4 fatigue in 10.2% (grade 4, n=1; 0.6%). Three events of vomiting (1.8%), two events of fatigue (1.2%), and one event of nausea (0.6%) were reported as treatment-related SAEs. Grade 3 fatigue was reported as cause

of treatment discontinuation in three patients, two of them associated with other AEs. Consistent results were observed in the integrated safety summary.

Laboratory findings

Haematological Abnormalities

The haematological abnormalities during treatment are presented in Table 70. In Arm A (plitidepsin plus DXM), the most common haematological abnormality at baseline (all grades) was anaemia (85.4% of patients), followed by thrombocytopenia (53.2%), leukopenia (52.6%), lymphopenia (50.3%), and neutropenia (39.2%). In Arm B (DXM), the most common haematological abnormality (all grades) during treatment was anaemia (97.5% of patients), followed by lymphopenia (69.2%), thrombocytopenia (67.1%), leukopenia (46.8%), and neutropenia (42.3%).

Table 70 Haematological abnormalities during treatment. Worst grade per patient (APL-C-001-09)

	Arm A (P+DXM)											Arm B (DXM)									
	n[a]	NCI-CTCAE grade								Total		n[a]	NCI-CTCAE grade							Total	
		1		2		3		4					1		2		3		4		
		n	%	n	%	n	%	n	%	n	%		n	%	N	%	n	%	n	%	n	%
Anaemia	160	34	21.3	73	45.6	48	30.0	2	1.3	157	98.1	79	19	24.1	30	38.0	26	32.9	2	2.5	77	97.5
Leukopenia	160	24	15.0	44	27.5	14	8.8	2	1.3	84	52.5	79	14	17.7	21	26.6	2	2.5	.	.	37	46.8
Lymphocytosis	160	.	.	10	6.3	3	1.9	.	.	13	8.1	78	.	.	1	1.3	.	.	.	.	1	1.3
Lymphopenia	160	37	23.1	36	22.5	32	20.0	5	3.1	110	68.8	78	22	28.2	19	24.4	11	14.1	2	2.6	54	69.2
Neutropenia	160	19	11.9	32	20.0	22	13.8	3	1.9	76	47.5	78	10	12.8	19	24.4	3	3.8	1	1.3	33	42.3
Thrombocytopenia	160	40	25.0	20	12.5	21	13.1	14	8.8	95	59.4	79	23	29.1	8	10.1	9	11.4	13	16.5	53	67.1

Abnormalities that occurred after crossover have been excluded from this table. Denominator for percentages was patients with available laboratory tests.
[a] Patients with available laboratory tests.
DXM, dexamethasone; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; P, plitidepsin.

Biochemical Abnormalities

During the treatment with the combination plitidepsin plus dexamethasone, 84.9% and 66% of patients respectively reported ALT and AST increase (Table 71). Grade 3/4 ALT and AST increase was respectively reported in 14.5% and 9.0% of patients who received plitidepsin plus dexamethasone. Grade 3/4 ALT increase occurred on Day 14 (range, 1 - 35 days) after dosing, with a median duration of 6 days (range, 1 - 16 days). Grade 3/4 AST appeared on Day 18 (range, 1 - 35 days) after dosing with a median duration of 6.5 days (range, 1 - 28 days). Grade 3/4 AST or ALT were more frequent in the first treatment cycle than in subsequent cycles. The median values for peak counts of ALT or AST from all patients, over 32 cycles, showed no evidence of cumulative toxicity.

Table 71. Biochemical abnormalities during treatment. Worst grade per patient (APL-C-001-09)

	Arm A (P+DXM)											Arm B (DXM)									
	n[a]	NCI-CTCAE grade								Total		n[a]	NCI-CTCAE grade							Total	
		1		2		3		4					1		2		3		4		
		n	%	n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%	n	%
ALP increased	158	40	25.3	6	3.8	2	1.3	1	0.6	49	31.0	77	9	11.7	1	1.3	.	.	.	.	10	13.0
ALT increased	159	72	45.3	40	25.2	20	12.6	3	1.9	135	84.9	79	16	20.3	.	.	.	.	.	.	16	20.3
AST increased	156	73	46.8	16	10.3	13	8.3	1	0.6	103	66.0	78	18	23.1	1	1.3	.	.	.	.	19	24.4
Bilirubin increased	159	11	6.9	4	2.5	3	1.9	.	.	18	11.3	79	4	5.1	3	3.8	.	.	.	.	7	8.9
CPK increased	155	18	11.6	20	12.9	13	8.4	18	11.6	69	44.5	70	1	1.4	2	2.9	.	.	.	.	3	4.3
Creatinine increased	160	97	60.6	32	20.0	2	1.3	1	0.6	132	82.5	79	46	58.2	21	26.6	2	2.5	1	1.3	70	88.6

Abnormalities that occurred after crossover have been excluded from this table. Denominator for percentages was patients with available laboratory tests.
[a] Patients with available laboratory tests.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; DXM, dexamethasone; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; P, plitidepsin.

Vital Signs and Physical findings

Vital signs, including body weight, blood pressure, pulse rate, and temperature, were assessed in all clinical studies before administration of the first dose of study treatment and periodically during treatment. No clinically relevant changes from baseline were identified in body weight or pulse rate, blood pressure, respiration rate, or body temperature in clinical studies. Information provided in this SCS is focused on LVEF and ECG changes for both the primary study APL-C-001-09 (ADMYRE) and the Integrated Safety Analysis (phase III/II data).

- APL-C-001-09 (ADMYRE)

Left Ventricular Ejection Fraction

Abnormal LVEF was an exclusion criterion in ADMYRE CT. LVEF was systematically evaluated during the ADMYRE CT by ECHO or MUGA (at baseline and every 12 weeks and end of treatment). From the available information, it is reported that a total of 7.5% of patients exposed to plitidepsin + dexa had LVEF values below normal limit during treatment together with an absolute decrease ≥15% in LVEF from baseline. The frequency was even higher in patients with haematological malignancies exposed to plitidepsin in monotherapy (group B and C). By contrary, few cases were observed in patients with solid tumours. Ejection fraction decrease was reported as AE with unknown relationship in three patients in treatment Arm A (n=3/167; 1.8%), being grade 3 in two patients and grade 2 in one patient. One patient had grade 1 ejection fraction abnormal reported as treatment-related AE after crossover from Arm B to Arm A. In Arm B (DXM), no abnormal values or AEs related to LVEF data were reported.

The most relevant information for the assessment of the effect on LVEF comes from the ADMYRE trial, however, despite being planned in the protocol, not all patients underwent a systematic assessment of LVEF.

Electrocardiogram

In Arm A (plitidepsin plus DXM), ECG QT prolongation was reported as a treatment-related AE in nine patients (n=9/167 treated patients=5.4%), reaching grade 3 in one patient and one cycle. Two of these cases were reported as SAEs. One patient had grade 3 ECG QT prolonged concomitantly with grade 3 atrial fibrillation of unknown cause that required hospitalisation and treatment discontinuation after four cycles. This patient received concomitant medication (levofloxacin and venlafaxine) that can prolong QT. Other patient had grade 2 ECG QT prolonged in the first cycle that required hospitalisation and dose omission, delay and reduction. Concomitant medication (propafenone and amiodarone) was considered as the primary cause of QTc prolongation that extended patient hospitalisation. In addition, two other patients required dose omission (one of them also required cycle delay) due to grade 1 and grade 2 ECG QT prolonged, respectively. These two patients had QT prolongation already present at baseline.

ECG QT prolonged was reported as treatment-related AE in three patients after crossover (reaching grade 1 in two patients and grade 2 in one patient). The patient with grade 2 required treatment discontinuation and the other patient required cycle delay.

A QTc substudy was conducted at some of the sites participating in study APL-C-001-09. The potential effects of a 5 mg/m^2 plitidepsin dose given as a 3-hour i.v. infusion on the QTc interval duration was assessed based on ECG evaluation when patients were treated in Arm A with plitidepsin for the first time (Day 1 of Cycle 1). In order to detect any delayed drug effect on the QTc interval, these investigations were repeated at the second infusion of plitidepsin (Day 15 of Cycle 1), when the drug was expected to reach the steady. In addition, a 10-mL blood sample was collected from each patient to conduct a genetic evaluation of mutations associated with ion channelopathies if marked plitidepsin-related changes of the QTc interval were observed.

The QTc substudy was started with its implementation through substantial amendment No.2 in 12 April 2013 (i.e., three years after beginning of APL-C-001-09 study) and was not mandatory for all patients (i.e., to be enrolled in this substudy, patients had to meet all inclusion and exclusion criteria for the substudy and sign a separate Informed Consent Form). Furthermore, the QTc substudy was not conducted in some countries. Then, finally only seven patients participated: six of them were evaluable for Day 1 measurements and four for Day 15 measurements. According to the applicant, available data do not show any relevant effect of plitidepsin on the QT interval or any other ECG parameter, although it is recognised that the limited sample size precludes ruling out an effect of plitidepsin on QT interval and. In Arm B (DXM), no abnormal values or AEs related to ECG data were reported.

Safety in special populations

Subgroup analyses by age (<18-64 years vs. 65-74 years vs. 75-86 years) were performed for the Integrated Safety Analysis (phase III and II data). A higher incidence of SAE and severe AEs is reported in patients >65 years old treated with plitidepsin both used in combination with DXM and as single agent. Also, there appears to be a trend for a higher rate of treatment discontinuation due to treatment related AE and treatment related AE leading to death, although numbers are too low to draw firm conclusions.

Table 72 Adverse events by age group in Arm A (plitidepsin plus DXM) of APL-C-001-09 (ADMYRE) study

		Arm A (plitidepsin plus DXM)		
		Age < 65 (n=88)	Age 65-75 (n=59)	Age > 75 (n=20)
		n (%)	n (%)	n (%)
Total AEs	Regardless of relationship	87 (98.9%)	59 (100%)	20 (100%)
	Related or unknown	79 (89.8%)	49 (83.1%)	16 (80.0%)
Grade ≥3 AEs	Regardless of relationship	67 (76.1%)	51 (86.4%)	20 (100%)
	Related or unknown	43 (48.9%)	30 (50.8%)	13 (65.0%)
SAEs	Regardless of relationship	46 (52.3%)	36 (61.0%)	15 (75.0%)
	Related or unknown	18 (20.5%)	22 (37.3%)	7 (35.0%)
AE leading to discontinuation	Regardless of relationship	19 (21.6%)	16 (27.1%)	7 (35.0%)
	Related or unknown	10 (11.4%)	5 (8.5%)	1 (5.0%)
AEs leading to death	Regardless of relationship	9 (10.2%)	9 (15.3%)	4 (20.0%)
	Related or unknown	.	3 (5.1%)	.

AE, adverse event; DXM, dexamethasone; SAE, serious adverse event.

The most frequent treatment-related AEs in older patients were hyperglycaemia, muscular weakness, musculoskeletal pain, myalgia, and renal failure. None of the reported AEs led to death and only one case of renal failure led to treatment discontinuation. However, the information in the elderly population (age >75 years) is limited.

Table 73 Adverse events by age group in Arm A (plitidepsin plus DXM) of APL-C-001-09 (ADMYRE) study

	Arm A (plitidepsin plus DXM) (n=167)					
	AEs regardless of relationship			Treatment-related (or with unknown relationship) AEs		
	Age <65 (n=88)	Age 65-74 (n=55)	Age 75-84 [a] (n=24)	Age <65 (n=88)	Age 65-74 (n=55)	Age 75-84 [a] (n=24)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total AEs	87 (98.9%)	55 (100%)	24 (100%)	79 (89.8%)	45 (81.8%)	20 (83.3%)
SAEs - Total	46 (52.3%)	33 (60.0%)	18 (75.0%)	14 (15.9%)	14 (25.5%)	6 (25.0%)
• Fatal	9 (10.2%)	7 (12.7%)	6 (25.0%)	.	3 (5.5%)	.
• Hospitalisation/prolong existing hospitalisation	43 (48.9%)	32 (58.2%)	15 (62.5%)	18 (20.5%)	19 (34.5%)	7 (29.2%)
• Life-threatening	3 (3.4%)	2 (3.6%)	3 (12.5%)	1 (1.1%)	2 (3.6%)	1 (4.2%)
• Disability/incapacity	.	3 (5.5%)	.	.	1 (1.8%)	.
• Other (medically significant)	6 (6.8%)	4 (7.3%)	3 (12.5%)	2 (2.3%)	3 (5.5%)	3 (12.5%)
AEs leading to drop-out	19 (21.6%)	14 (25.5%)	9 (37.5%)	10 (11.4%)	5 (9.1%)	1 (4.2%)
Psychiatric disorders	21 (23.9%)	10 (18.2%)	6 (25.0%)	13 (14.8%)	6 (10.9%)	3 (12.5%)
Nervous system disorders	31 (35.2%)	16 (29.1%)	8 (33.3%)	9 (10.2%)	5 (9.1%)	3 (12.5%)
Accidents and injuries	9 (10.2%)	10 (18.2%)	2 (8.3%)	1 (1.1%)	3 (5.5%)	1 (4.2%)
Cardiac disorders	16 (18.2%)	15 (27.3%)	4 (16.7%)	6 (6.8%)	10 (18.2%)	2 (8.3%)
Vascular disorders	27 (30.7%)	19 (34.5%)	3 (12.5%)	17 (19.3%)	8 (14.5%)	1 (4.2%)
Cerebrovascular disorders [b]	.	(3.6%)	1 (4.2%)	.	.	.
Infections and infestations	55 (62.5%)	28 (50.9%)	15 (62.5%)	19 (21.6%)	10 (18.2%)	4 (16.7%)
Anticholinergic syndrome	.	.	.	.	.	.
Quality of life decreased	.	.	.	.	.	.
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	11 (12.5%)	10 (18.2%)	1 (4.2%)	2 (2.3%)	2 (3.6%)	.
Other AEs appearing more frequently in older patients						
• Cardiac failure	.	3 (5.5%)	1 (4.2%)	.	.	.
• Electrocardiogram QT prolonged	5 (5.7%)	11 (20.0%)	.	.	.	.
• Hyperglycaemia	2 (2.3%)	6 (10.9%)	4 (16.7%)	2 (2.3%)	5 (9.1%)	3 (12.5%)
• Muscular weakness	10 (11.4%)	8 (14.5%)	3 (12.5%)	6 (6.8%)	8 (14.5%)	2 (8.3%)
• Musculoskeletal pain	4 (4.5%)	7 (12.7%)	1 (4.2%)	1 (1.1%)	3 (5.5%)	.
• Myalgia	13 (14.8%)	13 (23.6%)	4 (16.7%)	9 (10.2%)	11 (20.0%)	4 (16.7%)
• Renal failure	1 (1.1%)	3 (5.5%)	3 (12.5%)	.	1 (1.8%)	3 (12.5%)
• Urinary tract infection	4 (4.5%)	6 (10.9%)	4 (16.7%)	.	.	.
• Vertigo	1 (1.1%)	5 (9.1%)	.	.	.	.
• Weight decreased	3 (3.4%)	7 (12.7%)	3 (12.5%)	.	.	.

[a] No patients ≥ 85-years-old were treated in Arm A (plitidepsin plus DXM).
[b] Cerebrovascular disorders category includes the following MedDRA preferred terms in the search: cerebrovascular disorder, carotid arteriosclerosis, cerebrovascular accident, and cerebral ischaemia.
AEs, adverse events; DXM, dexamethasone; MedDRA, Medical Dictionary for Regulatory Activities; SAEs, serious adverse events.

Table 74 Treatment related adverse events (including those with unknown relationship by age (Integrated Safety Analysis, phase III and II data, Groups A, B and C)

	Group A (P+DXM) APL-C-001-09						Group B (P) D1,15 q4wk						Group C (P) D1,8,15 q4wk					
	18-64[b]		65-74		75-85[c]		18-64[b]		65-74		75-86[c]		18-64[b]		65-74		75-85[c]	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Haematological																		
Treated patients	106	100	68	100	30	100	30	100	19	100	14	100	52	100	30	100	7	100
Treatment-related AEs [a]	94	88.7	56	82.4	25	83.3	27	90.0	16	84.2	12	85.7	40	76.9	20	66.7	4	57.1
Grade ≥3 treatment-related AEs [a]	53	50.0	37	54.4	19	63.3	13	43.3	7	36.8	6	42.9	12	23.1	7	23.3	3	42.9
Treatment-related SAEs [a]	21	19.8	26	38.2	10	33.3	3	10.0	3	15.8	3	21.4	4	7.7	2	6.7	1	14.3
Treatment-related grade ≥3 SAEs [a]	16	15.1	19	27.9	8	26.7	3	10.0	3	15.8	3	21.4	4	7.7	2	6.7	1	14.3
Treatment-related AEs leading to treatment discontinuation [a]	11	10.4	9	13.2	2	6.7	3	10.0	2	10.5	3	21.4	4	7.7	3	10.0	1	14.3
Treatment-related AEs leading to death [a]	.	.	3	4.4	.	.	.	.	1	5.3	1	7.1	2	3.8	.	.	.	.
Treatment-related AEs leading to death within 30 days of last dose [a]	.	.	2	2.9	.	.	.	.	1	5.3	1	7.1	2	3.8	.	.	.	.
Treatment-related AEs leading to death within 60 days of first plitidepsin dose [a]	.	.	3	4.4	.	.	.	.	1	5.3	.	.	2	3.8	.	.	.	.
Solid tumours																		
Treated patients	.	.	.	.	.	.	99	100	30	100	3	100	29	100	8	100	2	100
Treatment-related AEs [a]	.	.	.	.	.	.	81	81.8	24	80.0	2	66.7	26	89.7	8	100	1	50.0
Grade ≥3 treatment-related AEs [a]	.	.	.	.	.	.	31	31.3	12	40.0	1	33.3	13	44.8	4	50.0	.	.
Treatment-related SAEs [a]	.	.	.	.	.	.	17	17.2	8	26.7	1	33.3	6	20.7	2	25.0	.	.
Treatment-related grade ≥3 SAEs [a]	.	.	.	.	.	.	12	12.1	6	20.0	1	33.3	5	17.2	2	25.0	.	.
Treatment-related AEs leading to treatment discontinuation [a]	.	.	.	.	.	.	17	17.2	8	26.7	1	33.3	11	37.9	2	25.0	.	.
Treatment-related AEs leading to death [a]	.	.	.	.	.	.	2	2.0	.	.	.	.	.	.	1	12.5	.	.
Treatment-related AEs leading to death within 30 days of last dose [a]	.	.	.	.	.	.	1	1.0	.	.	.	.	.	.	1	12.5	.	.
Treatment-related AEs leading to death within 60 days of first plitidepsin dose [a]	.	.	.	.	.	.	1	1.0	.	.	.	.	.	.	1	12.5	.	.
Total																		
Treated patients	106	100	68	100	30	100	129	100	49	100	17	100	81	100	38	100	9	100
Treatment-related AEs [a]	94	88.7	56	82.4	25	83.3	108	83.7	40	81.6	14	82.4	66	81.5	28	73.7	5	55.6
Grade ≥3 treatment-related AEs [a]	53	50.0	37	54.4	19	63.3	44	34.1	19	38.8	7	41.2	25	30.9	11	28.9	3	33.3
Treatment-related SAEs [a]	21	19.8	26	38.2	10	33.3	20	15.5	11	22.4	4	23.5	10	12.3	4	10.5	1	11.1
Treatment-related grade ≥3 SAEs [a]	16	15.1	19	27.9	8	26.7	15	11.6	9	18.4	4	23.5	9	11.1	4	10.5	1	11.1
Treatment-related AEs leading to treatment discontinuation [a]	11	10.4	9	13.2	2	6.7	20	15.5	10	20.4	4	23.5	15	18.5	5	13.2	1	11.1
Treatment-related AEs leading to death [a]	.	.	3	4.4	.	.	2	1.6	1	2.0	1	5.9	2	2.5	1	2.6	.	.
Treatment-related AEs leading to death within 30 days of last dose [a]	.	.	2	2.9	.	.	1	0.8	1	2.0	1	5.9	2	2.5	1	2.6	.	.
Treatment-related AEs leading to death within 60 days of first plitidepsin dose [a]	.	.	3	4.4	.	.	1	0.8	1	2.0	.	.	2	2.5	1	2.6	.	.

Data shown are n (%) of treated patients.

[a] Including adverse events with unknown relationship.

[b] A 17-year-old patient is included in the 18-64 subgroup.

[c] A 86-year-old patients is included in the 75-85 subgroup.

Group A: plitidepsin 5 mg/m^2 as a 3-hour i.v. infusion on D1 and 15 q4wk plus DXM 40 mg orally on D1, 8, 15 and 22, q4wk. Includes analysis from 167 patients who were treated in Arm A, and 37 patients who crossed over from Arm B (DXM) to Arm A (plitidepsin plus DXM) (only data from cycles with the combination are included in this analysis). All 204 patients had relapsed/refractory MM.

Group B: plitidepsin fortnightly schedule (D1 and 15, q4wk). Haematological malignancies included relapsed/refractory MM (n=51) and myelofibrosis (n=12). Solid tumours included bladder (n=21), exocrine pancreas (n=19), head and neck (n=10), melanoma (n=37), MTC (n=16), NSCLC (n=21), and prostate (n=8).

Group C: plitidepsin weekly schedule (D1, 8 and 15, q4wk). Haematological malignancies included leukaemia (n=17), NHL (indolent, n=8), and NHL (aggressive, n=64). Solid tumours included melanoma (n=20) and SCLC (n=19).

AE, adverse event; D, day; DXM, dexamethasone; MM, multiple myeloma; MTC, medullary thyroid carcinoma; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; P, plitidepsin; q4wk, every four weeks; SAE, serious adverse event; SCLC, small cell lung cancer.

Safety related to drug-drug interactions and other interactions

Plitidepsin co-administration is unlikely to affect the plasma protein binding of other highly plasma protein bound drugs. This is inferred from the fact that plitidepsin plasma levels are extremely low (typically less than 10 ng/mL or 13 nM) compared to the physiological levels of albumin (~43 mg/mL) and α1-acid-glycoprotein (~0.5-1.0 mg/mL).

The potential of plitidepsin to inhibit major CYP isoforms has been evaluated in vitro using human liver microsomes. Results demonstrated that plitidepsin exhibits direct inhibitory effects on CYP3A4-dependent midazolam 1'-hydroxylation (IC50 value of 1.1μM; 1210 ng/mL) and testosterone 6'-hydroxylation (IC50 value of 1.5μM; 1650 ng/mL). However, taking into account the mean (standard deviation) blood maximum plasma concentration (Cmax) 91.6 (76.6) ng/mL in MM patients at the pivotal study, these data suggest that plitidepsin has a limited potential for cytochrome P450 enzymatic inhibition. In vitro data generated in primary human hepatocytes demonstrated that plitidepsin at concentrations up to 1000 ng/mL (1.3 μM) showed no induction potential for CYP1A2 and CYP3A4 activities, suggesting that plitidepsin has a limited potential for cytochrome P450 enzymatic induction. The in vitro results were confirmed by the concomitant use with either CYP inducers or inhibitors that resulted in no effect on plitidepsin pharmacokinetics in patients.

The effect of concomitant administration of CYP inhibitors and inducers on the PK of plitidepsin on a pooled database containing 303 patients was evaluated by means of population methodology. No effect of concomitant co-medication on plitidepsin PK was observed.

In spite of this, co-administration of potent inhibitors or inducers of cytochromes with plitidepsin should be used cautiously.

In vitro studies on transport and intracellular accumulation experiments demonstrated that plitidepsin is a substrate for MDR1, which plays a key role in biliary excretion. Moreover, the majority of the administered dose of plitidepsin is eliminated by biliary excretion, as shown in an in vivo study of patients receiving a C14-plitidepsin dose (2.2 mg), in which 70% of total radioactivity was recuperated in faeces. Therefore, potential in vivo effects on the distribution of plitidepsin upon co-administration with MDR1 modulating compounds cannot be excluded at this time.

Also in vitro, plitidepsin may inhibit some efflux (MDR1, MRP2 and BSEP) and uptake (OATP1B1, OATP1B3) human transporters; however, its IC50 calculated values considered in the context of the pharmacokinetics properties of plitidepsin do not indicate potential for clinically relevant interactions.

Discontinuation due to adverse events

In study APL-C-001-09 the most common reason for treatment discontinuation was progressive disease (PD): 50.9% of patients in Arm A (plitidepsin plus DXM) and 53.0% in Arm B (DXM).

Table 75 Reasons for treatment discontinuation (study APL-C-001-09)

	Arm A (P+DXM) (n=167)		Arm B (DXM) (n=83)		Total (n=250)	
	n	%	n	%	n	%
Treatment status						
End of treatment	161	96.4	80	96.4	241	96.4
Ongoing	6	3.6	3	3.6	9	3.6
Reason for treatment discontinuation [a,b]						
Progressive disease	85 [c]	50.9	44 [d]	53.0	129	51.6
Patient refusal	24	14.4	12	14.5	36	14.4
Death (non-related to the study treatment)	19	11.4	8	9.6	27	10.8
Adverse events (treatment-related) [e]	15	9.0	8 [f]	9.6	23	9.2
Investigator decision	9	5.4	6	7.2	15	6.0
Adverse events (unrelated to treatment)	9	5.4	1	1.2	10	4.0
Death (due to adverse events related to the study treatment)	. [g]	.	1 [h]	1.2	1	0.4

Data shown are n of patients (%).

In the Integrated Safety Analysis, comparable percentages of patients had treatment discontinuation due to AEs regardless of relationship with the study treatment across groups: 25.5% in Group A, 27.0% in Group B and 31.5% in Group C. Comparable percentages of patients had treatment discontinuation due to treatment-related AEs across groups: 10.8% in Group A, 12.7% in Group B and 9.0% in Group C.

Post marketing experience

N/A

2.6.1. Discussion on clinical safety

Primary safety data for the intended indication comprise the main data from the randomised phase III clinical study APL-C-001-09 (ADMYRE).

In addition to the limited number of patients exposed to plitidepsin, at the time of the initial assessment, long-term treatment data were also limited, which hampered someway a proper characterisation of the safety profile of this medicinal product: median time on treatment 2.8 months (range, 0.3-31.5 months) for plitidepsin + DXAa vs 1.9 months (range, 0.3-19.6 months) for DXA alone. Median plitidepsin relative dose intensity was 75.5% (93.2% in the first two cycles, and from cycle three onwards was 75.9% (in patients requiring dose reduction, this was done at the beginning of the treatment, and then dose was maintained in further cycles)) and for DXA, median relative dose intensity was 79.7%. Cycle delays (40% of patients Arm A vs 6% Arm B), dose omissions (55% arm A vs 16% Arm B) and dose reductions (34% Arm A vs 7,2% Arm B) were highly common in Arm A (plitidepsin + DXA) and mostly due to non-haematological toxicity. Therefore, information on the use of plitidepsin in the long-term was limited. Updated safety information has been submitted with a data cut off date 28 February 2017. At this date 43/167 (25.7%) and 18/167 (10.8%) were exposed to plitidepsin plus DXM longer than 6 and 12 months, respectively. At this more recent date the exposure to study treatment was 32 new cycles over 842 cycles in CSR with a maximum number of cycles of 39. The exposure to DXM was 6 cycles over 251 cycles in CSR data analysis with a maximum number of cycles of 27. The frequency of Grade $\geq$ 3 AEs, and SAEs (treatment-related and regardless of the relationship) appear to be higher in patients on treatment for 6-12 months than in patients on treatment for $\leq$6 months.

In the pivotal trial, the most common reason for treatment discontinuation was progressive disease: 50.9% of patients in arm A and 53.0% of patients in arm B, followed by AEs: 25.1% treatment A vs 14.5% Arm B. So, treatment with plitidepsin + DXM appears poorly tolerated by this heavily pretreated population.

The higher toxicity of the treatment combination vs dexametasone alone is shown by the consistent higher frequency of grade ≥ 3 AEs (overall: arm A 83.2% vs. 63.9% in arm B; treatment related: 51.3% arm A vs 10.8% arm B), SAEs (overall: arm A 58.1% vs arm B 33.7%; treatment related: arm A 28% vs arm B 7.2%), AEs leading to treatment discontinuation (overall: arm A 25.1% vs. 14.5% arm B, treatment related: arm A 9.6% vs arm B3.6%), SAE leading to treatment discontinuation were 18.0 (arm A) vs. 8.4% (arm B) of patients, the rate of patients with AEs that led to patient's death (overall: arm A 13.2% vs arm B 6.0%; treatment related: arm A 1.8% vs 1.2% in arm B).

The most common AEs (overall) in the experimental treatment arm were GI disorders (74.5% of patients), general disorders and administration site conditions (73.5%), musculoskeletal and connective tissue disorders (58.3%), infections and infestations (58.3%), investigations (54.9%), and blood and lymphatic system disorders SOCs (52.5%). In particular, a higher incidence for the following AEs were reported in the arm A compared to arm B: constipation (13.8% vs 7.2%), diarrhoea (35.9% vs 9.6%), nausea (49.1% vs 22.9%), fatigue (53.9% vs 37.3%), oedema peripheral (20.4% vs 8.4%), pyrexia (22% vs 14.5%), ALT increases (16.2% vs 0%), blood CPK increases (17% vs 0%), decreased appetite (21.6% vs 7.2%), headache (12.6% vs 6%), peripheral neuropathy (10.2% vs 1.2%), dyspnoea (14.4% vs 3.6%), hypotension (10.2% vs 2.4%). In Arm B (DXM), the most common AEs (all grades) regardless of relationship with the study treatment were blood and lymphatic system disorders (anaemia, 43.4%), general disorders (fatigue, 37.3%), musculoskeletal and connective tissue disorders (bone pain, 27.7%) and gastrointestinal disorders (nausea, 22.9%).

In Arm A, the most common AEs related to the study treatment (or with unknown causality) were GI disorders (nausea, 37.1% of patients; vomiting, 16.8%; diarrhoea, 14.4%), general disorders (fatigue, 36.5%; oedema peripheral, 12.0%), metabolic/nutritional disorders (decreased appetite, 12.6%), and musculoskeletal disorders (myalgia, 14.4%; muscular weakness, 9.6%). The most common grade ≥3 AEs related to the study treatment (or with unknown causality) were fatigue (n=18, 10.8%), myalgia (n=9, 5.4%), muscular weakness (n=6, 3.6%) and nausea (n=6, 3.6%). In Arm B, the most common AEs (all grades) related to the study treatment (or with unknown causality) were gastrointestinal disorders (nausea, 10.8% of patients), general disorders (fatigue, 8.4%), and psychiatric disorders (insomnia, 9.6%).

A similar pattern was observed in the integrated safety summary when looking at the studies on haematological malignancies. The frequency and the most commonly reported AEs do not change substantially when treating with plitidepsin as a single agent at the same dose level.

The most common musculoskeletal disorders reported as adverse reactions were myalgia (14.4%) and muscular weakness (9.6%). CPK increase regardless of grade was reported in 44.5% of patients; this parameter was the most common grade 3/4 biochemical abnormality, reported in 20.0% of patients. The median onset of grade 3/4 CPK increase (day from first dose) was 48 days; the median number of cycles during which the patients experienced grade 3/4 CPK increase was 1 cycle, and the median time to recovery was 14.5 days. The treatment was withdrawn only in 0.5% of patients who experienced grade 3/4 CPK increase, while dose delay, dose reduction, dose omission and dose interruption occurred in 8.8%, 11.3%, 5.9% and 0.5%, respectively. The vast majority of patients (83.9%) who experienced CPK increase recovered. Rhabdomyolysis of grade 3/4 was reported in 1.2% of patients. These musculoskeletal AEs, known also to be associated with several drugs (including anti-cancer drugs), may occur rather infrequently when appropriate measures are taken. Whereas rhabdomyolysis can be prevented to a certain extent by frequent monitoring of CPK levels.

Regarding the cardiovascular events, the most recent safety update submitted (cut-off date February 28th 2017) revealed 100 cardiac events in 44 patients (5.4% of the total number of AEs occurred in Arm A) in arm A vs 13 in 9 patients (2.5% of the total number of AEs occurred in Arm B) in arm B. Focusing on the combination arm, the most common were 41/100 rhythm abnormalities/QT prolongation (41.0%) described in 30 patients, followed by 34/100 cardiac insufficiency events (including asymptomatic decrease of LVEF) (34.0%) described in 17 patients. Other events reported were 15/100 myocardial ischaemia (including asymptomatic ECG changes or troponin increase) (15.0%), and 10 cases described as "other" (10.0%) that occurred in 12 and 8 patients, respectively.

There were three events considered grade 3 QT prolongation, all with prior cardiac history or confounding factors. Plitidepsin in combination with DXM increases the risk of cardiac events, with a higher percentage of cardiac events grade ≥3 (11.4% vs 2.4% respectively). This fact could be related to the mechanism of action of plitidepsin since its primary target eEF1A2, seems to antagonise the effect of acetylcholine on M3 receptors, which may impair vasodilation in endothelial cells as well as vasoconstriction caused in vascular smooth muscle cells, therefore altering this balance. This cardiotoxicity is even more evident when the cardiac events rate per patient is observed according to the group of treatment, with quite similar results in arm A and the cross over population vs arm B (0.60, 0.68 and 0.16 respectively). The latter is consistently seen in the cardiac events treatment-related or with unknown relationship (0.32, 0.35 and 0.06, arm A, cross over population and arm B respectively). Nevertheless, as above described, the majority of these cardiac events were recovered and in those where it was not possible, 84% were grade 1-2 with only four grade 3. It should be also noted the high presence of cardiovascular risk factors, prior cardiac history or confounding factors, which undoubtedly adds background noise, making challenging to weigh the actual risk.

The applicant committed to further investigate the arrhythmogenic potential of plitidepsin in a sufficient number of patients, which is acknowledged.

Infusion reactions have been reported in patients who received plitidepsin. Symptoms may include, pain, catheter site phlebitis, and infusion site reaction. These reactions can occur during or immediately after the administration of plitidepsin.

Treatment related (or with unknown relationship) hypersensitivity reactions were reported in the phase III study in 6.6% in Arm A (plitidepsin plus DXM), 5.4% in patients who crossed over from Arm B to Arm A. The frequency of grade ≥ 3 hypersensitivity reactions was 1.2% in Arm A and 2.7% in the crossover patients. Severe hypersensitivity reactions included an anaphylactic shock with grade 4 cardiac arrest, a grade 3 infusion related reaction with hypoxia that led to treatment discontinuation, and a grade 3 rash requiring dose reduction. The majority of hypersensitivity reactions occurred in the first two cycles and, no change in severity was observed over time. Hypersensitivity reactions usually occurred on the day of plitidepsin plus dexamethasone infusion (median was the infusion day, range: 0 - 13 days).

Transaminases increments were frequently observed in patients treated with plitidepsin +DXM. During treatment, ALT was increased in 84.9% of patients, and AST in 66.0% of patients, being grade 3/4 in 14.5% and 9.0% of patients, respectively. ALT increase was reported as a treatment-related SAE in seven patients (4.2%), including 1 grade 4. AST increase was reported as a treatment-related SAE in six patients (3.6%). Transaminases elevations appear mostly transient increments in relation to treatment administration, but full recovery to normal/baseline values was unlikely while on treatment. In most cases these were not associated to other hepatobiliary disorders, but some may occur, which may be severe (see proposed RMP).

During treatment, neutropenia occurred as haematological abnormality in 47.5% of patients, being grade 3/4 in 15.6% of patients, which was slightly more frequent in the first treatment cycle (13/160 cycles; 8.1%) than in subsequent cycles (31/641 cycles; 4.8%). Neutropenia was reported as AE regardless of relationship with the study treatment in 13/167 patients (7.8%), with only one case of grade 3 febrile

neutropenia reported as a treatment-related AE/SAE. Neutropenia was mostly not associated to infections or septic shock.

Finally, nausea, vomiting and fatigue were common treatment-related AEs with plitidepsin plus DXM, occurring in 37.1%, 16.8% and 36.5% of patients, respectively. Severe nauseas, vomiting and fatigue were found in 3.6%, 1.8%, and 10.2% of patients, respectively. Nausea and vomiting did not lead to treatment discontinuation or dose reduction, and grade ≥ 3 cases occurred only in the first four cycles. Therefore, it appears that a degree of tolerance was developed in patients who had nausea and vomiting. From current data, treatment-related fatigue appears not to be particularly related to an increased frequency of severe or life-threatening musculoskeletal or gastrointestinal AEs.

Fatigue was also common treatment-related AE with plitidepsin plus DXM, occurring in 36.5% of patients. Severe fatigue was found in 10.2% of patients.

Aplidin contains 15% vol ethanol (alcohol) up to 2.1 g per dose, or the equivalent to of 54 ml beer, or 22.5 ml wine per dose. Aplidin also contains macrogolglycerol ricinoleate (polyoxyl 35 castor oil), which may cause severe allergic reactions.

2.6.2. Conclusions on the clinical safety

The safety database for plitidepsin supporting its use in combination with DXM for the treatment of patients with MM (heavily pre-treated) is certainly limited. Moreover, the add-on of plitidepsin to DXM is associated with an overall rather substantial increase in toxicity relative to the comparator.

2.7. Risk Management Plan

Safety concerns

Table 76 Summary of Safety Concerns

Important identified risks	Myopathies, including rhabdomyolysis
	Severe hypersensitivity reactions
	Liver enzymes increased
Important potential risks	Cardiac effects and QT prolongation
	Off-label use in malignancies other than MM (in adults and children)
	Pancreatic toxicity (potential risk from non-clinical finding)
	Medication errors
Missing Information	Use in pregnancy/ Reproductive toxicity and development toxicities
	Long-term safety
	Use in very elderly patients
	Use in lactation
	Use in patients with hepatic impairment
	Use in patients with severe renal impairment and end-stage renal impairment (CrCL or GFR<15 mL/min)
	Drug interaction with potent CYP3A4 inhibitors

Pharmacovigilance plan

Table 77 Table of on-going and planned additional PhV studies/activities in the Pharmacovigilance Plan

Study/activity Type, title and category (1-3)	**Objectives**	**Safety concerns addressed**	**Status (planned, started)**	**Date for submission of interim or final reports (planned or actual)**
Phase 1 QT Study in patients with all types of advanced malignancies including solid tumours. (Category 3)	To formally investigate the effects of plitidepsin on cardiac repolarisation and QTc interval	Cardiac effects including QT prolongation	Planned	Final study report date to be confirmed
Feasibility assessment to explore the possibility of using Swedish and Austrian MM registries to assess and characterise cardiac events, use in hepatically impaired patients and long-term safety. (the registry study would be a Category 3 study, if feasible)	To explore whether it is feasible to utilise Swedish and Austrian MM registries to further assess and characterise cardiac events, use in hepatically impaired patients and long-term safety.	Cardiac effects including QT prolongation Use in hepatically impaired patients Long-term safety	Feasibility assessment planned	Results of feasibility assessment to be provided to EMA within 6 months of product launch. A study protocol will be submitted and updated RMP.
Drug-drug interaction study with plitidepsin plus dexamethasone (DXM) and a strong CYP3A4 inhibitor such as itraconazole (category 3)	To evaluate the pharmacokinetics and safety of plitidepsin when co administered with potent CYP3A4 inhibitors	Missing information: Drug interaction with potent CYP3A4 inhibitors	Planned	Final study report date to be confirmed

Risk minimisation measures

Table 78 Summary Table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Myopathies, including rhabdomyolysis	Warning in section 4.4 of the SmPC that CPK should be monitored prior to each infusion (Day 1 and Day 15) from Cycle 1 to Cycle 4 and treatment with plitidepsin should be not be started in patients with myopathy > grade 2 or any clinical condition that causes significant and persistent elevation of CPK. Recommendations for dose adjustment if myopathy occurs in section 4.2 of the SmPC. In addition, section 4.4 states that plitidepsin treatment should be stopped and supportive measures should be promptly established if rhabdomyolysis occurs. Caution is also advised if medicinal products associated with rhabdomyolysis (e.g. statins) are administered concomitantly with plitidepsin plus dexamethasone. Section 4.8 of the SmPC lists the following ADR: Very Common (≥1/10) Blood Creatine Phosphokinase Increased Myalgia, Muscular Weakness, Musculoskeletal pain, Common (≥1/100 to <1/10): Myopathy, Arthralgia, Muscle Contracture Uncommon (≥1/1000 to <1/100) Rhabdomyolysis, Myositis	None
Severe hypersensitivity reactions	Section 4.2 of the SmPC advises on premedication for the prevention of infusion reactions and the management of infusion reactions including the need to discontinue and not restart plitidepsin in the event of a severe/life threatening reaction. Instructions for stopping the drug, monitoring vital signs, administering premedication (if it was not given), giving additional diphenhydramine, restarting the infusion at the same or a reduced rate, as applicable, are provided in the event of mild to moderate/non-life threatening reactions. Section 4.3of the SmPC contraindicates use in patients who are hypersensitive to the active substance or to any of the excipients. A warning is provided in section 4.4 of the SmPC concerning hypersensitivity reactions and the need to administer premedication to reduce the incidence of these reactions. Section 4.8 of the SmPC lists the following ADR: Common (≥1/100 to <1/10) Hypersensitivity, Rash, Erythema Uncommon (≥1/1000 to <1/100) Anaphylactic Shock	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Liver enzymes increased	Section 4.2 of the SmPC details criteria for starting treatment (AST and ALT ≤ 3.0 x the upper limit of normal) criteria for treatment continuation, dose delay or interruption (AST and ALT ≤ 5.0 x ULN) and dose reduction criteria. Section 4.4 of the SmPC informs that transient and reversible Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increases were reported during treatment with plitidepsin. It also advises that patients must have total bilirubin ≤ 1.0 x ULN (or direct bilirubin ≤ 1.0 x ULN when total bilirubin is above the ULN) and AST/ALT ≤ 3.0 x ULN before plitdepsin plus dexamethasone administration. In addition, AST and ALT measurements should be performed before the administration of plitidepsin. Advises to closely monitor for potentially serious hepatobiliary adverse events in patients with grade ≥ 3 ALT and/or AST elevations. Section 4.8 provides a detailed description of the AST and ALT increases observed in patients treated with plitidepsin plus dexamethasone.	None
Cardiac effects including QT prolongation	Section 4.4 of the SmPC warns that cardiac effects have been observed in patients treated with the combination of plitidepsin plus dexamethasone but the causal relationship has not been established yet. It advises that patients with risk factors for or existing heart disease should be closely monitored including periodic ECG monitoring. This section of the SmPC also provides details of the cardiovascular conditions that were excluded from the clinical trials and recommends that caution should be taken when considering plitidepsin treatment in patients with these conditions. Recommends monitoring for signs or symptoms of cardiac abnormalities and to only treat patients with left ventricular ejection fraction (LVEF) by echocardiography above the lower limit of normal at baseline and to measure LVEF every 12 weeks. Section 4.8 of the SmPC lists the following ADR: <u>Very Common (≥1/10);</u> <u>Electrocardiogram QT Prolonged</u> <u>Common (≥1/100 to <1/10)</u> Cardiac Arrest, Cardiac Failure, Atrial Fibrillation, Tachycardia <u>Uncommon (≥1/1000 to <1/100)</u> Myocardial Infarction , Angina Pectoris, Left Ventricular Hypertrophy	None
Off-label use in malignancies other than MM (in adults and children)	Section 4.1 of the SmPC states that Aplidin is indicated in combination with dexamethasone for the treatment of adult patients with relapsed/refractory multiple myeloma (MM) who have received at least three prior regimens including bortezomib, and either lenalidomide or thalidomide.	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Pancreatic toxicity (potential risk from non-clinical finding)	Section 5.3 informs that the pancreas was a primary target for toxicity in the pre-clinical species.	None
Medication errors	Section 4.2 provides detailed instructions concerning posology, premedication, management of infusion reactions, criteria for starting treatment, criteria for treatment continuation, dose delay or interruption and dose reduction criteria.	Compliance cards for patients and Healthcare professionals
Use in pregnancy/ Reproductive toxicity and development toxicities	Section 4.6 of the SmPC warns that Aplidin should only be used during pregnancy if the expected benefits outweigh the potential risks to the foetus and that effective contraceptive measures during and up to 6 months after treatment must be used in women of childbearing potential and in males whose partner is pregnant or of childbearing potential. It also mentions that reproductive capacity was affected in animals. Section 5.3 states that embryo-foetal toxicity was observed in rats at the maximum tolerated dose.	None
Long-term safety	No specific measures.	None
Use in very elderly patients	No specific measures.	None
Use in lactation	Section 4.6 of the SmPC warns that it is unknown whether plitidepsin and/or its metabolites are excreted in human breast milk and a risk to newborns/infants cannot be excluded. It also recommends that breast-feeding should be discontinued during treatment with Aplidin.	None
Use in patients with hepatic impairment	Section 4.2 of the SmPC advises that plitidepsin has not been formally studied in patients with impaired hepatic function. Since most administered plitidepsin is eliminated by biliary excretion, patients with impaired hepatic function (AST >3xULN and/or bilirubin >1×ULN) should not be treated with plitidepsin. Information on population pharmacokinetic analyses in patients with mild hepatic impairment is also provided in section 5.2. There is not sufficient data available for patients with moderate (n=3) or severe (n=1) hepatic impairment.	None

Use in patients with severe renal impairment and end-stage renal impairment (CrCL or GFR<15 mL/min)	Section 4.2 details a Calculated creatinine clearance (CrCl) ≥ 30 ml/minute as a criterion for starting treatment. Also advises that data on patients with severe renal impairment is limited and that there are no efficacy or safety data in patients with end stage renal impairment. Section 5.2 provides additional information.	None
Drug interaction with potent CYP3A4 inhibitors	Section 4.5 of the SmPC warns that strong CYP3A4 inhibitors should be discontinued at least 1 week prior to starting treatment and while on treatment with plitidepsin and that moderate CYP3A4-enzyme inhibitors should be used cautiously. Section 5.2 informs that CYP3A4 is the main isoform involved in the phase I metabolism of plitidepsin.	None

Conclusion

The CHMP and PRAC, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application, the risk management plan cannot be agreed at this stage.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. New Active Substance

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.

2.10. Product information

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use. However, due to the aforementioned concerns a satisfactory package leaflet cannot be agreed at this stage.

2.10.2. Additional monitoring

Not applicable.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Aplidin (plitidepsin) is proposed for treatment in combination with dexamethasone for relapsed/refractory multiple myeloma (MM) in adult patients who have received at least three prior regimens including bortezomib, and either lenalidomide or thalidomide.

Relapsed myeloma was defined as relapse of disease in patients who received at least one prior regimen, and not meeting criteria for relapsed and refractory myeloma.

Relapsed and refractory myeloma was defined as relapse of disease in patients who have relapse of disease while on salvage therapy, or who have a progression within 60 days of most recent therapy.

3.1.2. Available therapies and unmet medical need

The standard treatment approach in younger patients or eligible older patients include an induction regimen, which typically consists of a combination of a proteasome inhibitor and/or an immunomodulator and DXM, to reduce the tumour load before consolidation with high-dose chemotherapy and stem cell transplantation (SCT) support. In patients non-eligible for transplantation consolidation due to age and/or comorbidities, similar induction regimens that may include alkylating agents are used and then, consolidation with more cycles of the same, or different regimens is typically undertaken.

At present, several treatment options are available: immunomodulatory and proteasome inhibitor agents, such as thalidomide, lenalidomide and bortezomib, respectively, and the most recent approved second generation agents pomalidomide, panobinostat, carfilzomib and ixazomib, and monoclonal antibodies, daratumumab and elotuzumab. In trials, in patients who have received prior therapy or relapsed/refractory disease, these agents given as a single agent or in combination with DXM or in triple combinations of an immunomodulatory plus a proteasome inhibitor plus DXM, have been associated with various improvements in terms of PFS or OS.

3.1.3. Main clinical studies

The main evidence of efficacy is based on the ADMYRE study, a randomized, multicenter, open-label, phase III study of plitidepsin in combination with dexamethasone vs. dexamethasone alone in patients with relapsed/refractory multiple myeloma.

3.2. Favourable effects

In the primary analysis of the main study, a statistically significant difference was observed in terms of the primary endpoint PFS by IRC (ITT population, logrank p=.0054) with a HR of 0.650 (95%CI 0.477-0.885) and a median PFS of 2.6 vs 1.7 months for plitidepsin plus DXM vs DXM respectively.

Median OS (secondary endpoint, ITT, cut-off 19 May 2017) was 11.6 months in the plitidepsin + DXM group v. 8.9 months in the DXM group (HR=0.797, 95% CI: 0.596-1.067).

Best ORR (sCR+CR+VGPR+PR according to IMWG criteria; secondary endpoint; ITT; IRC) was 9.9% (95%CI 5.9%-15.4%) vs 1.2% (95%CI .03%-6,5%) in the DXM arm. Median DR was 3.7 months (95% CI, 2.7-10.5 months) in Arm A (plitidepsin plus DXM) and 1.8 months (95% CI, 1.8-5.5 months) in Arm B (DMX) as determined by the IRC in responder patients (IRC- All Responder Patients).

3.3. Uncertainties and limitations about favourable effects

No statistically significant difference was observed in terms of OS between the two treatment groups (ITT). It is acknowledged that any potential effect on OS would be likely to be underestimated in the ITT analysis due to the number of patients crossing over to the plitidepsin + DXM arm from the DXM arm during the study (37 patients, 44%). Thus, the association between plitidepsin plus DXM and a favourable effect on OS is unknown. A number of statistical approaches were used in an attempt to correct for the impact of cross-over. Although the approaches used are generally considered appropriate to explore the effect of cross-over, the results of such analyses did not allow concluding that plitidepsin plus DXM is associated with a statistically significant difference in terms of OS compared to DXM (see discussion on clinical efficacy).

HRQoL data were not collected. Even if such data would have been prone to bias due to the open-label design, they might still give a valuable supportive indication of the patients' condition and sense of well-being/daily functioning associated with a possible observed gain in PFS. If sufficiently robust, these data might have been useful to refine the understanding about the clinical impact of treatment on patient outcomes.

3.4. Unfavourable effects

The high toxicity of the treatment combination vs dexamethasone alone is shown by the consistent higher frequency of grade ≥ 3 AEs (treatment-related: 51.3% Arm A vs 10.8% Arm B), SAEs (treatment related: Arm A 28% vs Arm B 7.2%), AEs leading to treatment discontinuation (treatment related: Arm A 9.6% vs Arm B 3.6%), SAE leading to treatment discontinuation were 18.0% (Arm A) vs. 8.4% (Arm B) of patients.

The most common treatment-related adverse events grade 3 observed in plitidepsin-treated patients (Arm A) were fatigue (10.2%), myalgia (4.2%), nausea (3.6%), vomiting (1.8%) and diarrhoea (1.2%).

The most common reason for treatment discontinuation was progressive disease: 50.9% of patients in Arm A and 53.0% of patients in Arm B, followed by AEs: 25.1% treatment in Arm A vs 14.5% in Arm B.

Plitidepsin in combination with DXM increases the risk of cardiac events, with a higher percentage of cardiac events grade ≥3 (11.4% vs 2.4% respectively).

3.5. Uncertainties and limitations about unfavourable effects

Due to the limited number of patients exposed to plitidepsin and the limited duration of exposure, there are uncertainties about the precise toxicity profile of this medicinal product, including incidence of rare ADRs and long-term toxicity. There are also missing information in terms of certain special populations and drug-drug interactions (see RMP). However, given the context of the disease, the small size of the safety database could be accepted despite the inherent limitations.

3.6. Effects Table

Table 79. Effects Table for Aplidin in the treatment of MM in R/R patients (data cut-off: 19 May 2017)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
PFS	Time from randomization to the first evidence of PD (IMWG criteria) or death due to any cause	Median (months)	2.6	1.7	Event was assigned as the first time a PD is reported without the necessity of its confirmation Median PFS :HR=0.650 (95%CI 0.477-0.885)	n/a
OS	Time from randomization to death due to any cause	Median (months)	11.6	8.9	No differences according to HR. 37/84 patients (44.0%) crossed over from Arm B (DXM) to Arm A (plitidepsin plus DXM) HR=0.797 (95%CI 0.596-1.067)	
Unfavourable Effects						
Diarrhoea	Grade 3 Treatment-related AEs	%	1.2	0		
Nausea	Grade 3 Treatment-related AEs	%	3.6	1.2		
Fatigue	Grade 3 Treatment-related AEs	%	10.2	1.2		
Myalgia	Grade 3 Treatment-related AEs	%	4.2	0		
Vomiting	Grade 3 Treatment-related AEs	%	1.8	1.2		

Abbreviations: AEs (adverse event), HR (Hazard Ratio), PFS (Progression free survival)

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The only beneficial effect that has been doubtless observed for plitidepsin+DXM is in terms of PFS (probably as a consequence of a rather limited antitumor activity). The observed effect in terms of PFS for the combination is considered small and of modest clinical significance. No effect has been robustly demonstrated in terms of other important supportive clinical endpoints such as OS or HRQoL.

Notwithstanding the uncertainties about the exact type and extent of toxicity due to the relatively small safety database, the available data allow concluding that plitidepsin plus DXM is associated with substantial toxicity. The toxicity, considering the high proportion of patients experiencing treatment-related severe and life-threatening adverse events was considered to be clinical important.

3.7.2. Balance of benefits and risks

Given the modest effect in terms of PFS and the substantial toxicity, considering the high proportion of patients experiencing treatment-related severe and life-threatening adverse events, it is not possible to conclude that the benefits outweigh the risks.

3.7.3. Additional considerations on the benefit-risk balance

Not applicable.

3.8. Conclusions

The overall B/R of Aplidin is negative.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy for Aplidin in combination with dexamethasone for the treatment of adult patients with relapsed/refractory multiple myeloma (MM) who have received at least three prior regimens including bortezomib, and either lenalidomide or thalidomide, the CHMP considers by majority decision that the efficacy and safety of the above mentioned medicinal product is not sufficiently demonstrated, and consequently that the benefits did not outweigh the risks. Therefore, the CHMP recommends the refusal of the granting of the marketing authorisation for the above mentioned medicinal product. The CHMP considers that:

- Results from the primary analysis (PFS by IRC in ITT population) showed a HR 0.650 (95%CI 0.477-0.885), with a median PFS of 2.6 vs 1.7 months for plitidepsin plus DXM vs DXM respectively. No statistically significant difference was observed in terms of OS between the two treatment groups (ITT). Similarly, no effect has been established in terms of patient-reported outcomes. Despite the difference in PFS being statistically significant, the clinical relevance of such small effect is limited. An effect on other relevant endpoints, in particular overall survival or quality of life, cannot be considered established.

- Furthermore, the use of Aplidin in combination with dexamethasone is associated with substantial toxicity, in particular considering the high proportion of patients experiencing treatment-related severe and life-threatening adverse events. The benefits observed cannot be considered to outweigh the observed risks.

Based on the above, the risk-benefit balance of Aplidin is considered negative.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and post-authorisation measures to address other concerns cannot be agreed at this stage.

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 and similarity at this time.

Divergent position to the majority recommendation is appended to this report.

Appendices

1. Divergent positions to the majority recommendation 14 December 2017

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APPENDIX 1

DIVERGENT POSITION DATED 14 December 2017

DIVERGENT POSITION DATED 14 December 2017

Product name EMEA/H/C/004354/0000

It is the opinion of the undersigned members:

The evidence available to date regarding efficacy and safety of Aplidin (plitidepsin) in combination with dexamethasone for the treatment of relapsed/refractory multiple myeloma (MM) in adult patients who have received at least three prior regimens including bortezomib, and either lenalidomide or thalidomide is considered sufficient to support a positive benefit/risk.

The main evidence of efficacy is based on the ADMYRE study, a randomized, multicenter, open-label, phase III study of plitidepsin in combination with dexamethasone (DXM) vs. dexamethasone alone in patients with relapsed/refractory multiple myeloma.

The combined treatment of Aplidin and dexamethasone met the primary endpoint in the pivotal clinical trial, showing a statistical superiority to dexamethasone in terms of PFS. However, the clinical relevance of this result is rather doubtful. The final OS ITT analysis did not show any differences and the response rate by itself cannot be considered enough in the context of this treatment (even though appears to reinforce the idea that there is a superior activity of this combination). However, the high crossover from DXM alone to the experimental arm cannot be disregarded, having a critical impact on the survival analysis. Furthermore, it seems plausible to assume that there is a real benefit in treating patients with this new combination but as there was a significant crossover, that effect was not captured with the survival analysis as specified in the protocol. Only after carrying out some methods to mitigate this confounding factor, a survival advantage could be intuited. The actual benefit is unknown, though. So, no further conclusion can be reached so far, apart from that the ITT analysis is probably underestimating the result and that the other methods (RPSFT, the two-stage) are likely overestimating the benefit.

Upon these uncertainties, there is the fact that the current treatments of multiple myeloma do not offer a true cure in this stage of the disease, all patients relapsing sooner or later. Therefore, the introduction of a new treatment modality with a different mechanism of action could pose another alternative where there are not so many available drugs. Taking into account that the safety profile of the combination is different and not worse than other treatments in the later lines of MM, and with a credible benefit in survival in mind, the rapporteurs consider that the benefit risk balance is positive

Signed by:

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Bart Van Der Schueren

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