European Medicines Agency
Evaluation of Medicines for Human Use

Doc. Ref.: EMEA/793638/2009

ASSESSMENT REPORT

FOR

Zenas

International Nonproprietary Name: **amifampridine**

Procedure No.: EMEA/H/C/001032

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

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant EUSA Pharma SAS submitted on 5 June 2008 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Zenas, which was designated as an orphan medicinal product EU/3/02/124 on 18 December 2002. Zenas was designated as an orphan medicinal product in the following indication: Treatment of Lambert-Eaton myasthenic syndrome. The calculated prevalence of this condition was about 1 per 100,000 EU population.

The applicant applied for the following indication: Treatment of Lambert-Eaton Myasthenic Syndrome.

The legal basis for this application refers to:

A – Centralised / Article 8(3) of Directive 2001/83/EC

The application submitted is a complete dossier composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and bibliographic literature substituting/supporting certain tests or studies.

Protocol Assistance:

The applicant did not seek Protocol Assistance at the CHMP.

Licensing status:

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

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

Rapporteur: Ian Hudson Co-Rapporteur: Bengt Ljungberg

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 5 June 2008.
- The procedure started on 25 June 2008.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 12 September 2008. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 19 September 2008.
- During the meeting on 20-23 October 2008, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 24 October 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 24 April 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 5 June 2009.
- During the CHMP meeting on 22-25 June 2009, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of outstanding issues on 21 September 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 6 October 2009.

- During the meeting on 19-22 October 2009 the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation under exceptional circumstances to Zenas on 22 October 2009. The applicant provided the letter of undertaking on the specific obligations and follow-up measures to be fulfilled post-authorisation on 20 October 2009.
- The CHMP opinions were forwarded in all official languages of the European Union, to the European Commission, which adopted the corresponding Decision on 23 December 2009.

2. SCIENTIFIC DISCUSSION

2.1 Introduction

In 1956, Lambert Eaton and Rooke, and in 1957, Eaton and Lambert described the essential clinical and EMG features of a syndrome which has subsequently been referred to as the LEMS. Given a prevalence of LEMS as estimated the European Committee for Orphan Medicinal Products of 1 per 100,000, approximately 4,000 individuals in the European Union can be assumed to be currently suffering from LEMS.

LEMS is considered to be a neuromuscular transmission defect. Typically in LEMS, too few synaptic vesicles are released in response to nerve stimulation resulting in an abnormally low end-plate potential. The impairment of acetylcholine release is a consequence of the production of antibodies directed against voltage-gated calcium channels (VGCC), type P/Q. These auto-antibodies are found in 75% to 95% of cases.

LEMS is clinically characterised by muscle weakness and fatigability (mainly of the legs and trunk); ptosis and dysartria are also frequently detected. The increase of muscle strength immediately after exercise and the elicitation of tendon reflexes after brief muscle contraction are characteristic. The syndrome also includes a sensory neuropathy presenting e.g. as numbness or tingling in hands and feet. Autonomic disturbances, due to both parasympathetic and sympathetic dysfunction, are very common, causing dry eyes, dry mouth, constipation, impaired sweating, orthostatic hypotension and impotence. The onset of symptoms is usually gradual and insidious. In rare cases, weakness of the respiratory muscles can lead to a life-threatening condition necessitating artificial respiration.
LEMS occurs in two forms, neoplastic and non-neoplastic LEMS. LEMS is a paraneoplastic syndrome in at least 60 % of patients. The neoplastic LEMS is linked to small lung cell carcinoma (SCLC); 3 % of patients with SCLC are estimated to have LEMS. Conversely, 42 % of those with LEMS have SLCL and 5 % have other carcinomas. LEMS occurs also in the absence of a neoplasm and such cases are often associated with an autoimmune disorder.
Electrophysiological tests are useful for diagnosis as well as for monitoring the course of illness.
The typical abnormalities are:

- a reduced amplitude of resting compound muscle action potential (CMAP).
- a decrement in CMAP amplitude at low rates of repetitive nerve stimulation (3 Hz).
- an increment in CMAP amplitude, typically over 100%, after either high rates of nerve stimulation or 10 to 15 seconds maximal voluntary contraction.

3,4-DAP has been found to block voltage-dependent potassium channels, thereby prolonging pre-synaptic cell membrane depolarisation. Prolonging the action potential enhances the transport of calcium into the nerve ending. The resulting increase in intra-cellular calcium concentrations facilitates the exocytosis of acetylcholine-containing vesicles, which in turn enhances neuromuscular transmission.

3,4-DAP has been widely used as an *ad-hoc* hospital preparation in the treatment of LEMS for over twenty years. It currently has orphan drug status within the EU and has been used in compassionate use programmes for the treatment of LEMS in several Member States. It is recognised as a first-line symptomatic treatment for this disease as reflected in the recommendations of the European Federation of Neurological Societies (EFNS) Task Force (European Journal of Neurology 2006, 13: 682–690).

On-demand preparations of 3,4-DAP base may exhibit variability and a lack of reliability in the quality of the drug product. Moreover, the supply of 3, 4-DAP under this specific access scheme does not allow the medical community and regulatory bodies to collect safety data according to current pharmacovigilance procedures. Therefore, a new formulation has been developed to try and overcome these issues.

2.2 Quality aspects

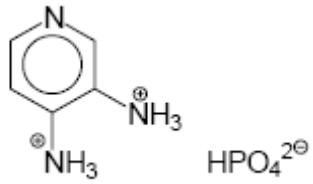
Introduction

Zenas is presented as tablets containing 10 mg of amifampridine (active substance) in the form of a phosphate salt. Excipients used in the preparation of Zenas are well known substances typically used in the tablet formulations, such as microcrystalline cellulose, anhydrous colloidal silica and calcium stearate.

Tablets are white, round, flat-faced and scored on one face so each tablet can be divided into equal halves. Zenas is supplied in perforated unit dose thermoformed blisters composed of aluminium-PVC/PVDC laminate sheets.

Active Substance

The active substance is chemically designated as 3,4-diaminopyridine phosphate (DAPP) or 3,4-pyridinediamine, phosphate salt and has the following structure:



Amifampridine phosphate is a white, crystalline powder, soluble in water, slightly soluble in dimethylsulfoxide, glacial acetic acid and methanol and very slightly soluble in ethanol and dimethylformamide. Its melting point is 229 ± 2 ^{o}C. pH of 1% solution in water at 25 ^{o}C is 4.6 ± 0.2

The molecule does not contain chiral centres, therefore stereoisomerism does not occur.

The substance does not exhibit polymorphism and only one crystal form is known. The same crystalline form is consistently produced using the commercially utilised manufacturing process.

- Manufacture

The synthesis of the active substance has been described in sufficient detail, and adequate evidence has been provided that the process is reproducible, and sufficiently controlled.

3,4-diaminopyridine phosphate (DAPP) is manufactured through a multi-step process to obtain the active substance in form of a phosphate salt.

The proposed manufacturer of the active substance has more than 10 years of experience in manufacturing this compound. During the development program first studies dealt with selection of salt and different ways of synthesis. Different salts were investigated but problems with *e.g.* yield and impurity profiles led to the final choice of the phosphate salt.

A detailed description of the process (including equipment, materials, amounts, conditions, parameters, process controls and yields) is provided as are also synthesis schemes and process flow diagrams. Process controls are specified. Appropriate specifications are in place for the starting material, intermediates and reagents, solvents and process aids used in synthesis.

The information provided in terms of process validation and manufacturing process development is satisfactory. Preliminary validation of the synthetic process has been performed, and was described for

9 batches of the active substance. Results of the full-scale process validation were performed on 3 further batches of the active substance with process parameters, in-process control results and yields tabulated for each step of the process. These data constitute satisfactory evidence that the synthesis is suitably controlled and capable of consistently yielding active substance of the desired quality.

The structure of 3,4-diaminopyridine phosphate has been confirmed by means of spectroscopic methods as UV, IR, MS and ^{1}H- and ^{13}C-NMR. Solid state investigations have been performed by X-ray powder diffraction. Studies confirmed the presence of only one crystal form.

Potential impurities have been listed and well discussed in relation to their origin and potential carry-over into the final drug substance.

- Specification

The drug substance specification includes tests for appearance, clearness and coloration of solution, solubility, pH, assay (potentiometric titration), identification (FT-IR and melting range), identification of phosphates, water content (Karl Fisher), residual solvents (GC), heavy metals, palladium (ICP-AES), related substances (HPLC), microbial purity.

A detailed description for analytical methods was provided. Standard pharmacopoeial methodology is largely employed. Validation reports are presented for the critical non compendial analytical procedures.

Residual solvents are determined using in-house GC method. The method has been validated satisfactorily with regard to specificity, precision (repeatability and intermediate precision), linearity and accuracy. Limits of detection and quantification have been determined based on signal/ noise ratios of 3:1 and 10:1.

An in-house HPLC method is employed for the determination of related substances. The method has been validated satisfactorily with regard to specificity, precision (repeatability and intermediate precision), linearity and accuracy, for the named impurity. Limits of detection and quantification have been determined based on signal/noise ratios of 3:1 and 10:1, respectively. The robustness of the method has also been demonstrated.

An in-house potentiometric endpoint titration method is employed for the determination of assay. The method has been validated satisfactorily with regard to specificity, linearity, precision and accuracy.

Palladium content is determined by atomic emission spectroscopy. The method has also been validated with regard to determination of limits of detection and quantification.

In general analytical methods proposed are suitable to control the quality of the drug substance however some minor issues need to be further addressed as post approval commitments.

Data on 3 batches of active substance obtained by the proposed drug substance manufacturer and on 6 batches from the manufacturer of the finished product have been provided. Full compliance with proposed specification has been demonstrated.

- Stability

The active substance manufacturer has initiated stability studies on three commercial scale batches of the active substance. Samples were stored in containers representative of the commercial packaging, under conditions in accordance with ICH recommendations (long-term: 25 ± 2 ^{o}C / 60 ± 5% RH; accelerated: 40 ± 2 ^{o}C / 75 ± 5% RH). Data from completed (12-month) accelerated studies and 18 months of ongoing long-term studies were included in the dossier.

Photostability studies on three batches of the active substance have been reported, with sample having been exposed to 13500 Lux / 1.96 W/m^2 at 25 ^{o}C for 102 hours. No significant increase in impurity

levels was apparent, but slight darkening of powder was observed and hence, it has been concluded that the active substance is light sensitive.

Additional, supportive stability data was provided from the finished product manufacturer; 3 batches entered in these studies have been prepared via an earlier manufacturing process, and stored in glass bottles. Data from up to 15 months of accelerated studies and 5 years of long-term studies were presented. Compliance with the specification was confirmed for the duration of the long-term studies.

In summary the stability data provided support the proposed shelf-life and storage conditions.

The active substance manufacturer has given a commitment to continue ongoing long-term stability studies for the duration of the proposed retest period. Their stability protocol also prescribes the inclusion of 1 further commercial scale batch per year in stability studies.

Medicinal Product

- Pharmaceutical Development

The aim of the pharmaceutical development programme was a straightforward, reproducible manufacturing process, yielding immediate-release tablets containing 10 mg of amifampridine (3,4-diaminopyridine) in the form of a phosphate salt (DAPP). Currently amifampridine is supplied (as base form) to hospital pharmacies where capsules containing 5 and 10 mg of the active substance are prepared extemporaneously. The focus has therefore been on the development of immediate release tablets allowing appropriate dosing. The stability of the product has also been in focus as the base form is not stable.
The chosen excipients are widely used for their respective proposed function in the current formulation in pharmaceutical products, and in direct compression manufacture. The use of lactose as a diluent was excluded on the basis of the potential for a Maillard reaction to occur with the active substance. Calcium stearate was chosen over magnesium stearate due to the potential effect of magnesium on neuromuscular transmission, hence it being considered unsuitable for use in a product for the treatment of Lambert-Eaton syndrome. No pre-formulation compatibility work was performed on the selected excipients, which are recognised as being chemically inert.

A secondary aim of the formulation development was the incorporation of tablet score-lines for posology adjustment. The proposed posology is: initially 5 mg three times daily, increasing by 5 mg increments up to maximum of 60 mg per day, with doses ≥ 20 mg/day divided into 4 separate doses. This means that standard posology will recommend the administration of half-tablets.

A series of trial batches were produced to study the effects of formulation on tablet pharmacotechnical properties. Having decided upon the final formulation, work on the optimisation of manufacturing process parameters was undertaken.

Comparative analyses (dissolution and impurity profiles) of the current product and the previously used extemporaneously prepared capsules containing active substance in form of the base were performed in order to demonstrate that the products can be considered pharmaceutically equivalent to a sufficient degree in support of the use of literature references for safety and efficacy data. Similar, rapid dissolution behaviour has been demonstrated for the tablets containing amifampridine phosphate (DAPP) and for the capsules containing amifampridine base (DAP). Based on the data provided it was concluded that dissolution behaviour of tablets formulated with DAPP and the range of extemporaneously prepared capsules formulated with DAP, will not differ significantly to impact upon the rate and extent of absorption of the active moiety and consequently safety and/or efficacy.

No pharmaceutical development work on the uniformity of subdivided tablets has been presented; however, this property is to be controlled in the finished product specification; and as an in-process control during tablet manufacture.

A simple and reliable method for the evaluation of dissolution of DAPP tablets has been developed, according to the current edition of the European Pharmacopoeia. The results of solubility studies in association with the determination of partition coefficient confirm that 3,4-diaminopyridine belongs to Category 3 (High Solubility – Low Permeability Drugs) of the Biopharmaceutics Classification System (BCS). In these conditions, dissolution and permeability are critical parameters affecting the drug absorption. Rapid dissolution is a criterion which maximise the contact time between the dissolved drug and absorption mucosa. Category 3 drugs exhibit a high variability in rate and extent of absorption. However, where dissolution is fast, such as is the case for DAPP tablets where it has been demonstrated that 85% of the drug dissolves in 15 minutes, variation may be attributed to gastro-intestinal transit, luminal contents, and membrane permeation, rather than dosage form factors. As drug permeation is rate controlling, limited or no *in vitro – in vivo* correlation is expected. Given the highly soluble nature of the active substance, dissolution testing is considered to be of limited use as a QC tool therefore, demonstration that the dissolution method is discriminatory was not considered to be critical. Rather than performing routine dissolution testing, the Applicant claimed that release of the active substance is adequately controlled by disintegration time. It has been demonstrated during the development program that the finished product has consistently rapid drug substance release characteristics as defined by the Decision Tree #7(1) of the guideline on test procedures and acceptance criteria for new substances and new drug products (CPMP/ICH/367/96), therefore the substitution of dissolution testing with disintegration testing for routine control of the finished product have been considered acceptable.

The development work resulted in the applied formulation and with a standard manufacturing process (tabletting) consisting of a series of steps of sieving and blending prior to compression.

- Adventitious Agents

None of the excipients used in the drug product are of animal origin. Calcium stearate used in the formulation is of vegetal origin.

- Manufacture of the Product

The manufacturing process is sufficiently described and a process flow diagram was provided. The process comprises a series of steps of sieving and mixing of ingredients, lubrication and finally compression of tablets followed by packaging. Critical steps have been identified and relate to the established in-process controls. The mixing steps are critical in respect of achieving blend uniformity (absence of agglomerates) and during the compression steps it has to be ensured that tablets with satisfactory properties are produced. This is ensured by in-process controls.

Process validation has been performed on 3 batches. Satisfactory results have been observed with regard to blend uniformity and compression. Uniformity of mass of half tablets was shown to be consistent. The validation satisfactorily demonstrates the manufacturing process to be consistently capable of yielding product of the desired quality.

- Product Specification

The product specifications (at release and during shelf-life) are standard for tablets and contain tests with suitable limits for appearance, identification (UV), identification of phosphates, divisibility of tablets, disintegration, dissolution, mean mass, resistance to crushing, control of dimensions, uniformity of dosage units, assay (HPLC or UV), related substances (HPLC), microbial contamination.

Methodology employed for monitoring of tablet characteristics is largely according to Ph Eur.

The active substance is identified in the product via UV spectrophotometry, with a further identification test confirming the presence of phosphate, as per the Ph Eur method. It is also stated that identification can be confirmed via HPLC retention times.

An in-house HPLC assay method has been developed and validated satisfactorily with regard to specificity, linearity and accuracy over the range 80 – 120% of theoretical sample concentration and precision (repeatability and intermediate precision).

The UV assay method and for determining uniformity of dosage units has been validated satisfactorily with regard to specificity, linearity and accuracy over the range 80 – 120% of theoretical sample concentration and precision (repeatability and intermediate precision).

An in-house HPLC method for evaluation of related substances has been developed and validated however further minor work is needed in order to complete the validation program for this method.

Batch analysis data have been presented for 3 production scale batches and as a supportive data further results for 3 smaller (non-commercial) scale batches, manufactured according to an earlier process, have also been provided. Compliance with the proposed specification was demonstrated. Results showed that tablets can be manufactured reproducibly according to the finished product specifications.

- Stability of the Product

Stability studies have been initiated on 3 production scale batches, in the proposed commercial packaging. The studies have been undertaken in accordance with ICH recommended conditions: long-term studies at 25 ^{o}C/60% RH, intermediate studies at 30 ^{o}C/65% RH and accelerated studies at 40 ^{o}C/75% RH. 18 months for one batch and 12 months for two batches of long-term data were resented, along with completed accelerated (6-month) and intermediate (12-month) study results for all 3 batches.

The results from long-term studies (12 or 18 months) from the batches manufactured according to the proposed manufacturing process, follow similar trends, remaining within specification. Assay results are subject to fluctuation, but no significant downward trend is apparent. Completed studies under intermediate conditions on these batches showed the product to remain within specification for the duration of the testing. The proposed shelf-life and storage conditions were considered justified.

Supporting stability data have been provided for 6 batches manufactured according to a previous process. Between 24 and 48 months of data are presented for studies conducted at 25 ^{o}C/60% RH on these batches, with completed (6-month) accelerated studies at 40 ^{o}C/75% RH, as well as 7.5 months data from studies conducted at 30 ^{o}C/65% RH for 2 of the batches.

In addition photostability studies have been performed on the finished product, with three batches exposed to 13500 Lux/1.96 W/m^2 at 25 ^{o}C, for 102 hours (unprotected tablets and tablets in blisters). Some discolouration of tablets exposed directly to light was reported, but no change in quality was apparent for the blistered tablets.

Based on the stability data the proposed shelf-life and storage conditions as defined in the SmPC are acceptable.

Discussion on chemical, pharmaceutical and biological aspects

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

At the time of the CHMP opinion, there were minor unresolved quality issues, which have no impact on the Benefit/Risk ratio of the product. The applicant gave a Letter of Undertaking and committed to resolve them as Follow-up Measures after the opinion, within an agreed time-frame.

2.3 Non-clinical aspects

Introduction

3,4-diaminopirydine (3,4-DAP) blocks voltage-dependent potassium channels. This blockage causes depolarisation of the presynaptic membrane and slows down or inhibits repolarisation. Prolonged depolarisation induces the opening of slow voltage-gated calcium channels (VGCC) and the influx of calcium. The increased concentration of intracellular calcium induces exocytosis of the synaptic vesicles containing ACh. It has been proposed that treatment with 3,4-DAP counteracts the pathologically-reduced ACh release in patients with LEMS and hence provides symptomatic relief to the patient. These pharmacological actions support the proposed clinical indication.

All pivotal studies were conducted in compliance with Good Laboratory Practice as claimed by the applicant. The applicant refers to extensive bibliographic data available for the 3,4- DAP in the base form. The statement on the GLP status of these studies is not available.

Pharmacology

The pharmacology of 3,4-DAP phosphate is based on bibliographic data obtained from research mainly with 3,4-DAP base, and safety pharmacology studies conducted by the applicant with the 3,4-DAP phosphate salt.

- Primary pharmacodynamics

The primary pharmacology section was based on bibliographic data sourced from scientific literature published over the past four decades.

In vitro data

3,4-DAP effectively inhibits voltage-dependent potassium channels and by this mechanism triggers an increased release of ACh. In 1964, it was discovered that the direct stimulation of the smooth muscle tonus induced by 3,4-DAP can be partly antagonised by atropine (Vohra and Pradhan, 1964), in line with the proposed pharmacological effect of 3,4-DAP, i.e. the increased release of ACh via blockage of presynaptic potassium channels.

Potassium currents play an important role in resting and action potentials of neurons. In 1990, the mechanism of potassium channel-triggered neurotransmitter release was investigated in stellate ganglia of the squid Loligo opalescens and Loligo pealei (Augustine, 1990). The investigators described a pronounced effect of intracellular or extracellular 3,4-DAP on potassium currents. 3,4-diaminopyridine inhibited potassium currents with an effective dissociation constant of 7 μM. These findings are in line with results published in 1978 in the same species, reporting an apparent dissociation constant of 5.8 μM (external application) for blockage of potassium currents (Kirsh and Narashi, 1978). McGiverns et al. (1993) published results from a study that demonstrated that 3,4-DAP appears to have affinity for more than one potassium channel type, as the snake toxin α-dendrotoxin was only partially able to abolish the 3,4-DAP-sensitive potassium current and the residual potassium current could be effectively inhibited by 3,4-DAP.

Several papers published in the 1980's have shown that the inhibitory effects of botulinum toxin serotype A (but not of serotypes B, D or F; results on serotype E are equivocal) on spontaneous as well as evoked ACh release could be abolished by 3,4-DAP in vitro (Siegel et al. 1980; Simpson, 1986; Rowan and Harvey, 1988; Molgo et al. 1989). 3,4-diaminopyridine was ~ 10-fold more effective than the structural analogue, 4-aminopyridine. 3,4-diaminopyridine in the lower micromolar concentration range abolished the toxin-induced reduction in motor end plate potentials induced by botulinum toxin serotype A. A more recent study (Adler et al., 1996) using rat muscle preparations showed that the effects of botulinum toxin A (and partially for toxin E) could be antagonised by 3,4-DAP. In 1989 and 1998 it was reported that by diminishing potassium influx via blockage of fast voltage-dependent potassium channels, the presynaptic depolarisation is prolonged, resulting in an increase in calcium ion influx, which in turn increases the release of neurotransmitter (viz ACh) quanta (Huang et al. 1989; Maddison et al. 1998).

Thomsen et al. (1983) reported that in rat diaphragm neuromuscular junction preparations, a concentration of 100 μM 3,4-DAP, in vitro, prolonged the endplate potential from 6.1 ± 0.9 msec to 9.1 ± 1.1 msec. Furthermore, in 1990, Hong et al. reported a direct modulation of the function of the calcium channels involved by potassium channel function based on electrophysiological results obtained in mural nerve-hemidiaphragm preparations. Braga et al. in 1991 examined and confirmed the selective potassium channel blocking characteristics of 3,4-DAP in mural triangularis sterni muscle preparations by extracellular recording of potassium current wave form. At a concentration of 10 mM, 3,4-DAP has been found to be able to reverse a 70% reduction in twitch height induced by 1 to 5 mM tubocurarine, a competitive acetylcholine antagonist. In 1998, van Lunteren et al. published results from a study in young (3 - 4 months) and old (20 - 21 months) male Fisher rats that investigated the influence of age on the pharmacodynamic effect of 3,4-DAP. In tissue from young adult animals, a more pronounced effect was observed compared with muscle preparations obtained from old animals. The isometric twitch force was increased by 181 ± 12% in young adult animal muscle, while the increase was only 144 ± 24% in old animals ($p < 0.05$). The authors considered these differences to be of minor influence on the clinical efficacy of 3,4-DAP in elderly patients and fully recommended the use in this population.

Moreover, in papers published in 1990 and 1995, investigators reported that 3,4-DAP appears to activate calcium-dependent choline uptake, preventing membrane depletion from phosphatidylcholine and other major phospholipids (Hong and Chang, 1990; Buyukuysal et al. 1995).

In vivo data
Siegel (1986) showed that 3,4-DAP given intraperitoneally prolonged survival time in mice treated with botulotoxin type A. This study was performed with the phosphate salt.

- Secondary pharmacodynamics

The secondary pharmacology profile of 3,4-DAP, also presented as a bibliographic review, indicates that 3,4-DAP could potentially exert effects on the cardiovascular system, central nervous system and gastrointestinal tract.

It is well-documented that, *in vitro*, 3,4-DAP is able to modify cardiac conduction and, in particular, to induce phasic contractions in different arteries from several species (including humans).

Furthermore, 3,4-DAP has stimulatory effect on potassium-evoked dopamine release, noradrenaline release in rat hippocampal slices and upregulates ACh release in the brain.

Finally, 3,4-DAP base has been shown to potentiate adrenergic and cholinergic neuromuscular transmission in the gastrointestinal tract. These in vitro data are consistent with the moderate digestive adverse effects observed in the clinic.

Effects of 3,4-DAP on different organs and functions
3,4-DAP has been shown to dose-dependently increase blood pressure, induce salivation in the cat and induce full, dose-dependent myosis in anaesthetised rats.

- Safety pharmacology programme

Because of the well-documented fact that 3,4-DAP acts on ion channels involved in cardiac transmission and its effects on the CNS, the applicant has conducted safety pharmacology studies addressing these specific issues.

The safety pharmacology studies submitted are summarised in the table below.

Organ system evaluated/ Report No.	Species/ Sex/Number/ Group	Method of administration	Doses	Study design/ Main findings
CNS (Functional observatory battery)/ 20070139PGR	Wistar rats 8 males/group (4 treated groups, 1 negative control group and 1 positive control group (clonidine 3 mg/kg) and 9 supplementary males in each group for TK	Oral gavage single	5, 10, 20 or 40 mg/kg 3,4-DAP phosphate in 1% (w/v) CMC	Irwin standardised observation battery and body temperature evaluated. Measurements of the Irwin scores and body temperature were performed at 0.08, 0.5, 1, 2 and 4 hrs after administration. At 24 hrs post-dosing, only mortality was recorded. No effects on standard parameters. NOAEL: 40mg/kg
Cardiovascular/ 1710/AGE/03	Female Rabbit Purkinje fibres	Tissue bath	3,4-DAP phosphate 0.1, 1.0, 10, 30 or 100 µM	At 30 and 100 µM, the duration of the action potential at 90 and 50% repolarisation increased by 18 and 40% during bradycardia, indicating block of potassium channels (class III antiarrhythmic effects). The amplitude, the maximum upstroke velocity and the resting membrane potential were not modified by any tested concentration.
Cardiovascular/ 1711/AGE/03	hERG K+ currents in CHO-K1 hERG cells	Perfusion chamber	3,4-DAP phosphate 0.3, 3.0 or 30 µM	No effects. IC_{50} value for 3,4–diaminopyridine phosphate could not be determined, > 30 µM.

Cardiovascular system
Treatment-related increases in the action potential of rabbit Purkinje fibres were recorded at concentrations of 30 and 100 µM 3,4-DAP phosphate. In humans receiving the dose of 3,4-DAP phosphate equivalent to 20 mg of 3,4-DAP, C_{max} of 3,4-DAP was 51.8 ng/ml, i.e. ~ 0.5 µM. This concentration is 63 times lower than the lowest concentration shown to modify the action potential duration in isolated Purkinje fibres. 3,4-DAP phosphate did not produce a detectable inhibition of hERG current at all applied concentrations (please see discussion for more detail).

Central nervous system
Following the FOB in rats the NOAEL was observed at 40 mg/kg. Noteworthy physical signs included: decrease in motor activity and number of rearings and increase in finger approach.

Other systems
No pre-clinical studies specifically addressing respiratory or digestive functions were performed. However, information on the effects of 3,4-DAP on these systems has been gathered from the long-term clinical experience with 3,4-DAP in the treatment of LEMS and other conditions, such as multiple sclerosis (MS).

Respiratory system
Adverse effects related to the respiratory system are cough, bronchial hypersecretion and asthma attacks in asthmatic patients or in patients with a history of asthma. This is adequately reflected in the SmPC and the risk management plan for Zenas.

Gastro-intestinal tract
Because of its primary pharmacodynamic effect, viz. increasing the release of the neurotransmitter ACh, 3,4-DAP is known to produce nausea and adverse effects related to the gastrointestinal system, such as diarrhoea and abdominal pain. This is adequately reflected in the SmPC and the risk management plan for Zenas.

- Pharmacodynamic drug interactions

No specific pre-clinical studies investigating drug interactions have been performed. Please see clinical part of the report for more information.

Pharmacokinetics

Pharmacokinetic studies performed at the time of submission were bioavailability studies in rodent and non-rodent species (dog) to compare the pharmacokinetic profiles of 3,4-DAP base and its phosphate salt. These studies were performed in order to justify bridging to non-clinical bibliographic data available for 3,4-DAP in base form in support of the current application for 3,4-DAP phosphate salt.

In addition, a comparative metabolic clearance study was performed in isolated hepatocytes harvested from monkeys, rats, dogs and human subjects in order to justify the choice of the dog as the non-rodent species employed in toxicology studies.

Methods of analysis
The assays of 3,4-DAP in plasma were analysed by High Performance Liquid Chromatography (HPLC) with fetal calf serum used as a matrix.

Absorption
Male (n=12) and female (n=12) Sprague-Dawley rats received a single oral administration of 1 mg/kg of 3,4-DAP base either as free base or as phosphate salt (1.9 mg/kg). The same doses were orally administered in the dog study (one male and one female) in a cross-over design with a wash-out period of 14 days. Blood sampling was performed before dosing, 5, 15, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 12 and 24 hours after dosing, in both species. The results of the studies are presented below.

Table. MDS 266/009, pharmacokinetic indices after a single oral administration of 1 mg/kg 3,4-DAP base or 1.9 mg/kg phosphate salt to Sprague-Dawley rats.

Animal (Sprague-Dawley rats)	**F (n=12)**	**F (n=12)**	**M (n=12)**	**M (n=12)**
Formulation	Base	Salt	Base	Salt
$t_{1/2}$ (min)	62	105	164	134
T_{max} (min)	15	5	5	5
C_{max} (µg/L)	28.3	16.7	15.0	16.5
$AUC_{0.5\text{-}120\ min}$ (min*µg/L)	1609	1299	1186	1198

Table. MDS 266/008, pharmacokinetic indices after a single oral administration of 1 mg/kg 3,4-DAP base or 1.9 mg/kg phosphate salt to Beagle dogs.

Animal (Beagle dogs)	**Female (n=1)**	**Female (n=1)**	**Male (n=1)**	**Male (n=1)**
Formulation	Base	Salt	Salt	Base
Time Point	Day0	Day 14	Day0	Day14
$t_{1/2}$ (min)	148	135	104	136
T_{max} (min)	15	45	15	30
C_{max} (µg/L)	396	336	442	363
$AUC_{0\text{-}720\ min}$ (min*µg/L)	62960	73230	NA	76534

The data generated indicate that, in both species, all indices are of the same order of magnitude and the bioavailability of the base form of 3,4-DAP vs. the phosphate salt is similar. In addition, no significant gender differences were apparent. Hence cross-referring to the non-clinical profile for 3,4-DAP base in the rat and dog can be considered a valid approach.

Distribution
No new tissue distribution studies were performed during the development of Zenas. References to the two absorption studies described above (MDS 266/008 in dog and MDS 266/009 in rat) were provided. In the single-dose (1 mg/kg) rat oral bioavailability study, the apparent volume of distribution (Vd) ranged from 38.2 – 76.7 l/kg. In the dog oral bioavailability study, a Vd value of 2.71 l/kg was recorded. The clearance rate was 325 – 429 ml/min/kg in rats and 95 – 138 ml/min in dogs.
Moreover, an article describing penetrability of 3,4-DAP into cerebrospinal fluid in anesthetised rats (Lemeignan et al., 1984) was also presented. 3,4-DAP was less absorbed than 4-AP (4-amino-pyridine) suggesting that there might be more favourable safety profile for 3,4-DAP than for 4-aminopyridine with respect to CNS effects.

Metabolism
No new in vivo metabolism studies were performed during the development of Zenas. The applicant has initiated a new ADME programme to provide more data on the pharmacokinetic profile of 3,4-DAP and results from this programme will be submitted as a follow-up measure.

In vitro studies
A comparative clearance study (study number: PR6958/CC2206) was performed with hepatocytes from different species in order to compare the clearance of 3,4-DAP so that the choice of the non-rodent species for repeat-dose toxicity studies could be justified. Monolayer hepatocyte cultures were prepared from isolated cryopreserved hepatocytes obtained from a male Sprague-Dawley rat, a male Beagle dog, a male Cynomolgus monkey and three male human subjects. The test item at concentrations of 1, 10 or 100 µM (0.5 ml/well) was incubated in the cell cultures. After 0, 0.25, 0.5, 1, 2 and 4 hours, the 3,4-DAP concentration in culture was assayed using a MS/MS/HPLC method with the LLOQ of 0.05 µM. Hepatocyte activity was characterised by measuring one phase I enzymatic activity (ethoxyresorufin O-deethylase (CYP1A)) and one phase II enzymatic activity (paracetamol glucoronyltransferase) in animals, as well as two phase I enzymatic activities (phenacetin O deethylase (CYP1A2) and nifepidine oxidase (CYP3A4/5)) and one phase II enzymatic activity (paracetamol glucoronyltransferase (UGT1A6)) in humans.

In rat and monkey hepatocytes, there was a time-dependent decrease in the test item at every concentration tested. In dog hepatocytes, the concentration of the test item remained stable, indicating that the test item is poorly metabolised by hepatocytes in this species. In humans, the results were variable from one donor to another: in the first subject, the concentration of 3,4-DAP remained stable during the incubation period, in the second subject, there was a decrease in the test item concentration at 1 and 10 µM and in the third subject, a slight decrease in 3,4-DAP content was observed at every concentration. The metabolic intrinsic clearance was only calculated for the species where 3,4-DAP biotransformation was observed.

	Dose of 3,4- DAP		
	1µM	**10µM**	**100µM**
Species	**Metabolic intrinsic clearance of 3,4-DAP phosphate (ul/min/10^6 hepatocytes)**		
Rat	20.2	17.4	10.7
Monkey	17.1	14.4	10.0
Human HEP187045	12.1	10.7	8.07
Human HEP187053	7.57	7.62	8.28

The study did not investigate the metabolic pathway and elimination route of 3,4-DAP phosphate and did not provide information on 3,4-DAP metabolites. The comprehensive ADME programme has been started with the aim of providing new data to give reassurance that the species chosen for the toxicity studies metabolise 3,4-DAP in a similar manner to humans, and hence were a valid choice.

Excretion
No new data have been submitted. A comprehensive ADME programme has been started by the applicant including also excretion studies and results from this programme will be submitted as a follow-up measure.

- Pharmacokinetic drug interactions

No new preclinical data have been submitted. Please refer to the clinical part of the report for further information.

Toxicology

Single-dose toxicity studies were performed in the mouse and the rat while repeat-dose toxicology was studied in the rat and the dog. Additionally, the toxicology programme was supplemented with bibliographic data on toxicology of 3,4-DAP base.

- Single-dose toxicity

Acute studies include: single-dose administration of 3,4-DAP phosphate in mice and rats, either by the oral route (intended clinical route) or by the intravenous route. One single-dose rat study was performed with 3,4-DAP base in order to compare the toxicity of the free base and the phosphate salt.

In general, the observed CNS-related toxicities were similar in all dose groups in both mice and rats regardless of the route of administration. These findings were also reported in published scientific literature and included subdued behaviour, convulsions, irregular breathing, hypersalivation, agitated paw movements, excessive grooming, unsteady gait, and piloerection. In rats, 3,4-DAP base exhibited similar noteworthy findings to those observed with the phosphate salt and the reported NOEL and minimal lethal doses were also comparable. The approximate oral LD_{50} was >25mg/kg in rats and 100 mg/kg in mice. The approximate IV LD_{50} was 25 mg/kg in both rats and mice.

- Repeat-dose toxicity (with toxicokinetics)

Non-pivotal rat and dog repeat-dose toxicity studies
Data from two four-week non-pivotal studies (3,4-DAP phosphate) were presented in addition to two pivotal studies of the same duration in the same species (rat and dog). The first set of studies was repeated because there were discrepancies in the extent of exposure to the test item, an invalid HPLC method was used for analyses and a deficient blood sampling schedule was adopted. Therefore, these studies are considered as supportive.

The table below summarises the non-pivotal repeat dose toxicity studies in rats.

Study ID	Species/ Sex/ Number/ Group	Product	Dose (mg/kg/day) /Route /Duration	NOEL/ NOAEL (mg/kg/day)
MDS 266/013 Non-pivotal	Rat/ Sprague-Dawley/10 F and 10 M	3,4-DAP phosphate in 1% aqueous solution of CMC	0, 7.5, 24 and 75 PO (gavage) 4 weeks + 2 weeks follow-up period for 5F and 5M in control and high-dose group respectively Test item divided into 3 daily doses	7.5/24 mg/kg
Main findings				
CNS toxicity from 24 mg/kg (hypersalivation). Dose-dependent increase in urine volume (6.2 ml in controls, 9.2 ml at 75 mg/kg) and decreased urine gravity in F. Increased liver and kidney weights for F at 75 mg/kg. Dark areas/colour in mandibular lymph nodes in M and F from all dose groups and in one F control. These changes correlate with congestion seen microscopically.				

The table below summarises the non-pivotal repeat-dose toxicity studies in dogs.

Study ID	Species/ Sex/ Number/ Group	Product	Dose (mg/kg/day) /Route /Duration	NOEL/ NOAEL (mg/kg/day)
MDS 266/014 Non pivotal	Dog/Beagle/4 M and 4F	3,4-DAP phosphate in 1% aqueous solution of CMC	0, 1.9, 5.7 and 7.5 PO (gavage) 4 weeks Test item divided into 3 daily doses.	NOAEL: 1.9 mg/kg
Main findings				
Two males dead on Day 0 at 7.5 mg/kg, convulsions. Clinical signs: CNS toxicity from 1.9 mg/kg (tremors, hyperaesthesia, panting, coughing, hypersalivation, muscular tension, severe loss of balance and apparent changes in body temperature (hot or cold to touch). Conjunctivitis, lacrimation, pale or red mucous membranes (ocular, buccal or both), red ears and changes in muscular tone e.g., outstretched limbs and stiffness of hind limbs. Ophthalmology: increase in the incidence of white filaments in the posterior lens capsule and within the vitreous body in F at 5.7 and 7.5 mg/kg at W4, not seen pretest. Pinpoint opacity posterior lens capsule and brown spot in the tapetum in one F at 5.7 mg/kg at W4, not seen pre-test. Haematology: decrease in red blood cell counts and haemoglobin in 2/4 F at 7.5 mg/kg. Clinical chemistry: decrease in urine volume of 1 M and of females (-65%) at 7.5 mg/kg/day. Macroscopic findings: Dark and/or raised areas in the stomach and duodenum in all dose groups. Microscopic findings: Myodegeneration in the tongue and skeletal muscle and focal/ multifocal alveolar macrophages in the lungs from 1.9 mg/kg. No effect of treatment on arterial blood pressure, heart rate, cardiac conduction, cardiac rhythm or wave forms.				

Pivotal studies

Study MDS AA40848 - 4-week subchronic repeat-dose oral rat study without recovery

Female and male Sprague Dawley rats (14/sex/control group; 21/sex/treatment group) were given vehicle control (1.0% CMC in water for injection), or 7.5 or 24 or 75 mg/kg/day of 3,4-DAP phosphate split into 3 administrations (5 ml/kg approximately 6 hourly) for 4 weeks. At appropriate intervals, full clinical examinations were performed and blood samples were taken on day 0 and day 27 at:

• 0 (before dosing), 5, 15, 30, 45 minutes and 1, 2, 3 and 6 hours after the 1st daily dosing
• 5, 30 minutes and 6 hours after the 2nd daily dosing
• 5, 15, 30, 45 minutes and 1, 2, 3, 6 and 12 hours after the 3rd daily dosing

This study was performed in order to verify the exposure of animals to 3,4-DAP phosphate and hence validate results obtained in the previous 4-week rat repeat-dose study (MDS 266/013; please see table above). The blood sampling schedule was designed based on 3,4-DAP's pharmacokinetic profile in the rat to give a precise picture of the animals' systemic exposure in relation to the dose, gender and 3 daily administrations (intended clinical therapeutic regimen is 4 times daily for doses >20 mg and 3 times daily otherwise).

A dose-related increased incidence of hypersalivation was noted in treated animals. A dose-related increase in the body weight gain was observed in treated females; this increase was statistically significant for the two highest dose groups (24 or 75 mg/kg/day). No biological chemistry, necropsy or microscopic examinations were included in the study design.

The NOAEL was not determined.

Study MDS AA40847 - 4-week subchronic repeat-dose oral dog study with 2-week recovery

This study was performed in order to establish the extent of exposure of animals to 3,4-DAP phosphate and determine a NOAEL. Female and male Beagle dogs (3-5/sex/group) were given vehicle control (1.0% CMC in water for injection), or 1.0, 2.5 or 6.5 mg/kg/day of 3,4-DAP phosphate split in 3 administrations (5 ml/kg approximately 6 hourly) for 4 weeks followed by a 2-week recovery period. At appropriate intervals, full physical examinations, including cardiovascular examination in the pre-test period and on days 1, 22 and 40, were performed. Appropriate blood and urine chemistry indices were recorded in the pre-test period and on days 21, 26 and 41. Blood samples were taken on day 0 and day 26 according to the following schedule:

• Pre-dosing, then 0.25, 0.5, 0.75, 2, 4 and 6 hours after the first daily dosing
• 0.25, 2 and 6 hours after the second daily dosing
• 0.25, 0.5, 0.75, 2, 4 and 6 hours after the third daily dosing
• 24 hours after the first daily dosing.

There were no deaths, treatment-related changes in urine analysis, treatment-related macroscopic haematological or ophthalmology findings. A reduction in bodyweight gain was observed in all treatment groups. These changes were statistically significant in high-dose females. No effect on bodyweight was noted throughout the treatment-free period.

Treatment-related toxicity observed after low doses included tremors, subdued behaviour and decreased activity in single females. In the intermediate-dose group, both males and females had tremors. Moreover, one female had subdued behaviour, laboured breathing, paresis and stiff hind limbs. In the high-dose group, treatment-related toxicity was observed in most animals. These findings included subdued behaviour, increased muscle tone, tremors, anxious behaviour, panting hypersalivation, prostration, paresis, loss of balance, aggressiveness, laboured breathing, poor motor coordination, stiff hind limb, liquid faeces and tonic convulsions.

Tremors, decreased activity and hypersalivation persisted in a few animals throughout the treatment period while other signs decreased over time. Physical signs were reversible as there were no treatment-related signs during the recovery period.

3,4-DAP treatment did not modify heart rate, rhythm and cardiac conduction. There was no effect on PR or PQ intervals and QRS complex duration. Second degree atrioventricular blocks were observed in 1 intermediate-dose female. However, since this finding was isolated and is known to occur in the young dog, it was considered not to be treatment-related.

Treatment-related increases in the relative liver weight in high-dose females were recorded. Increases in male absolute and relative weights were not statistically significant. No correlation with blood chemistry or histological examination was apparent and, therefore, these findings were not attributed by the applicant to the test item. No other changes in organ weights related to treatment were recorded. After recovery, liver organ weights were similar to those of the controls.

Because of the marked neurotoxicity in the low-dose animals, the NOAEL was not determined.

In conclusion, findings in repeated-dose studies in rats and dogs were generally related to the pharmacological effect of 3,4-DAP phosphate and consisted of CNS-related toxicity and adverse effects on muscles (necrosis). These toxicities have been demonstrated to be either partially or completely reversible. There were no obvious target organs in either species; however, liver and kidney organ weights were seen to increase in treatment groups, although no corresponding changes in haematological indices or corresponding microscopic observations were noted.

In the non-pivotal four-week rat study, the dose levels of 24 mg/kg/day and 7.5 mg/kg were established as the NOAEL and NOEL, respectively. No NOAEL could be established in the four-week repeat-dose oral toxicity studies in dogs because of marked neurotoxicity in the low-dose animals. Although adverse physical signs appeared to be reversible in the rat and dog studies and not associated with chemical pathology and histopathology changes, no clinical safety margin for the treatment-related findings could be established.

Please refer to the toxicokinetic section below for an assessment of the PK indices.

- Genotoxicity

The genotoxicity of 3,4-DAP was assessed in a standard battery of tests.

Type of test/study ID	Study Number	Test system	Concentrations/ Concentration range/ Metabolising system	Results Positive/ negative/ equivocal
Gene mutations in bacteria	IPL-R 010310	Strains: *Salmonella typhimurium* (TA 1535, TA 1537, TA 98, TA 102 and TA 100)	0, 50, 150, 500, 1500, and 5000 µg/plate, +/- S9 (rat)	negative
	IPL-R 010506	Strains: *Salmonella typhimurium* (TA1538 and TA98)	0, 50, 150, 500, 1500, and 5000 µg/plate, +/- S9 (hamster)	negative
Gene mutations in mammalian cells	IPL-R 010515	L5178Y mouse lymphoma cells	3 hours incubation: 700.3, 910.3, 1183.4, 1538.5 or 2000 µg/ml +/- S9 24 hours incubation: (592.6, 888.9, 1333.3, 2000 or 3000 µg/ml) -S9 (rat)	No mutagenic activity was noted after 3 hours treatment, however after 24 hour treatment a weak mutagenic activity was reported without metabolic activation at 2000 and 3000 µg/ml.
Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes in vivo	IPL-R 020404	Fischer Rat hepatocytes; males only. **NB no toxicokinetics**	Single oral dose of 40 or 20 mg/kg (10 ml/kg) administered to 3 rats/group (2-4 hours exposure or 12-16 hours exposure)	negative
Chromosomal aberrations in vivo	IPL-R 010417	Rat, micronuclei in bone marrow. **NB no toxicokinetics**	Repeat oral dose of 80, 40 or 20 mg/kg/day (5/sex/group) administered for 3 days.	negative

In the performed standard package of genetic toxicology studies, a positive in vitro finding was seen in the Mouse Lymphoma L5178Y locus assay at and above 2000 µg/ml (≥9.7 mM) with 24 h treatment in the absence of the rat liver activation system (S9). Both small and large colony inductions were observed. These dose levels equate to several orders of magnitude greater than the clinical C_{max} and therefore this finding is not expected to be clinically significant. No genotoxic potential was evident in any other test system when tested up to appropriate concentrations and dose levels according to guidelines. Due to the presence of an aromatic amine, the compound was further tested in an Ames test with metabolic activation by a microsomal hamster liver fraction with strains TA98 and TA1538. No genotoxic effects were observed. Overall, 3,4-DAP was considered to lack genotoxic potential.

- Carcinogenicity

No carcinogenicity studies have been conducted. The appropriate information has been included in the SmPC. The carcinogenicity study will be performed as a specific obligation.

- Reproduction Toxicity

No reprotoxicity studies have been conducted. The applicant has committed to conduct Segment II and pre- and post-natal studies, according to current guidelines, as a follow-up measure to determine the potential for teratogenic effects of 3,4-DAP phosphate. In addition to this commitment, the present lack of reproductive toxicity data is dealt with by an inclusion of strict wording in Section 4.6 of the SmPC to warn against exposure during pregnancy and / or lactation in W(o)CBP.

- Toxicokinetic data

Toxicokinetic indices were evaluated during the 4-week bioavailability study MDS AA40848 performed with 3,4-DAP phosphate in Sprague-Dawley rats to support the 4-week toxicity study MDS 266/013 conducted under the same experimental conditions.

Time point	Dose (mg/kg/day)	Sex	T1			T2		T3			AUC_{0-15h} (ng*h/mL)
			C_{max} (ng/mL)	T_{max} (h)	AUC_{0-6h} (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-6h} (ng*h/mL)	
Day 0	7.5	Male/ Female	NA/5.68	NA/ 0.5	NA	7.55/ 8.64	0.083 /0.5	10.5/ 13.2	0.5/0. 5	NA	NA
	24	Male/ Female	18.3/ 30.9	0.25 /0.5	NA	32.4/ 43.8	0.50/ 0.5	29.3/ 82.9	0.08/ 0.75	NA	NA
	75	Male/ Female	131/227	0.5/ 0.5	187/31 2	743/ 219	0.5/ 0.5	232/ 285	0.25/ 0.75	166/ 385	2558/1320
Wk 4	7.5	Male/ Female	6.64/ 12.6	0.5/ 0.25	NA	16.9/ 12.9	0.083 /0.08 3	10.5/ 15.7	0.25/ 0.25	NA	NA
	24	Male/ Female	32.9/ 64.6	0.5/ 1.0	NA	41.3/ 55.6	0.5/0. 5	51.9/ 80.2	0.25/ 0.75	NA	NA
	75	Male/ Female	243/413	0.5/ 0.5	397/47 9	556/8 35	0.5/0. 5	268/ 516	0.25/ 0.5	368/ 629	2411/3565

Moreover, toxicokinetic indices were also evaluated during the 4-week toxicity study with 2-week recovery period, MDS AA40847, performed with 3,4-DAP phosphate in Beagle dogs.

Time point	Dose (mg/kg/day)	Sex	T1			T2			T3			AUC_{0-15h} (ng*h/mL)
			C_{max} (ng/mL)	T_{max} (h)	AUC_{0-6h} (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-6h} (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-6h} (ng*h/mL)	
Day 0	1	Male/ Female	46/59	0.75/ 0.25	136/ 143	37/31	2.0/ 0.25	158/ 120	57/57	0.75/ 0.75	186/ 151	513/438
	2.5	Male/ Female	145/ 188	0.75/ 0.75	391/ 421	106/ 96	2.0/ 2.0	442/ 369	176/ 120	0.75/ 0.75	473/3 89	1375/ 1243
	6.25	Male/ Female	352/ 345	0.75/ 0.75	918/ 912	271/ 326	2.0/ 0.25	1072/ 1074	329/ 377	0.75/ 0.75	1047/ 956	3211/ 3059
Wk 4	1	Male/Female	51/56	0.25/ 0.25	136/ 139	37/36	2.0/ 2.0	154/ 156	46/54	0.75/ 0.75	170/ 185	500/523
	2.5	Male/Female	95/ 146	0.75/ 0.75	303/ 396	112/ 96	2.0/ 2.0	480/ 399	166/ 136	0.75/ 0.75	514/ 441	1414/ 1314
	6.25	Male/Female	287/ 261	0.75/ 0.75	833/ 741	241/ 221	2.0/ 2.0	997/ 922	367/ 324	0.75/ 0.75	1094/ 984	3146/ 2841

The ratios between the human (at the maximum dose of 20 mg at one dose interval: 0.4 mg/kg based on a 50 kg human) and rat pharmacokinetic and dog indices are tabulated below.

		Dose (mg/kg/day)	Cmax (ng/mL)	Ratio Animal/Human	AUC0-24hrs (ng*h/mL)	Ratio Animal/Human
Human		0.4	64.8 ng/mL		115.8	
Male rat	D0	7.5 24 75	0.0 18.3 131.0	NA 0.3 2.0	NA NA 187.0	NA NA 1.6
	W4	7.5 24 75	6.6 32.9 243.0	0.1 0.5 3.8	NA NA 397.0	NA NA 3.4
Female rat	D0	7.5 24 75	5.7 30.9 227.0	0.1 0.5 3.5	NA NA 312.0	NA NA 2.7
	W4	7.5 24 75	12.6 64.6 413.0	0.2 1.0 6.4	NA NA 479.0	NA NA 4.1
Male dog	D0	1 2.5 6.25	46.0 145.0 188.0	0.7 2.2 2.9	136.0 391.0 918.0	1.2 3.4 7.9
	W4	1 2.5 6.25	51.0 95.0 287.0	0.8 1.5 4.4	136.0 303.0 833.0	1.2 2.6 7.2
Female dog	D0	1 2.5 6.25	56.0 146.0 261.0	0.9 2.3 4.0	143.0 421.0 912.0	1.2 3.6 7.9
	W4	1 2.5 6.25	12.6 64.6 413.0	0.2 1.0 6.4	139.0 396.0 741.0	1.2 3.4 6.4

The toxicokinetic data were generally unremarkable. In the rat absorption was rapid (T_{max} was between 5 min and 1 h) and not dose-related. At the highest dose values recorded for C_{max} and AUC were higher in females than in males and there was evidence of only slight accumulation of 3,4-DAP phosphate. In the dog, absorption was slow (T_{max} was between 0.75 min and 2 h) and not dose-related. There were no gender-specific differences in pharmacokinetic indices determined and no accumulation was evident.

- Local tolerance

Specific local tolerance studies are not required for orally administered formulation.

- Other toxicity studies

The impurities in 3,4-DAP phosphate drug substance include:

- 4-aminopyridine (4-AP) - controlled to <0.05% at release and shelf life
- 4-ethoxycarnonylaminopyridine (ECAP)
- 4-amino-3-nitropyridine (ANPB) - controlled to <0.05% at release and shelf life.

In silico tests have been performed (DEREK) for all three impurities. No alerts were triggered for 4 AP and ECAP; however alerts for mutagenicity and carcinogenicity have been triggered for ANPB. Therefore, an Ames test was conducted on 4-amino-3-nitropyridine. Results showed that 4-amino-3-nitropyridine did not have mutagenic activity in the five Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and TA102 tested, either in the absence or in the presence of metabolic activation.

The listed impurities have been controlled in accordance with the ICH Guidance for Industry "Impurities in New Drug Substances" Q3A(R) as claimed by the applicant. It was unknown whether these might be genotoxic and hence the proposed limits of <0.05% at release and at shelf life (for 4-AP and ANPB) have been justified by toxicology data.

Moreover, the carcinogenicity studies will investigate further the carcinogenic potential of the drug substance and its impurities.

Ecotoxicity/environmental risk assessment

An environmental risk assessment concerning 3,4-DAP ending in Phase I was provided. The log Kow was calculated to -1.442, and it was concluded that screening for persistence, bioaccumulation, and toxicity was not necessary. Considering the orphan medicinal product status of 3,4-DAP, it is reasonable to calculate the Fpen according to the prevalence of LEMS in Europe (i.e. 1:100,000 inhabitants). The PECsurfacewater was calculated to 0.0004 µg/L, using a refined Fpen (0.00001), the maximum daily dose (80 mg), and the default values provided in the guideline (EMEA/CHMP/SWP/4447/00). Therefore 3,4-DAP is not expected to pose a risk to the environment.

Discussion on the non-clinical aspects

Limited information is currently available on the non-clinical pharmacology, pharmacokinetics and toxicology of 3,4-DAP.

The mechanism of action of amifapridine (increase in calcium influx into the nerve and prolongation of the action potential duration leading to the acetylcholine release) supports its use in the proposed clinical indication. The pharmacodynamic properties of 3,4-DAP contribute also to some of the findings observed in the safety studies or reported in the literature including convulsions and possible QTc prolongation. Therefore, as a precautionary measure, Zenas is contraindicated in patients with epilepsy. The risk of convulsions is dose-dependent and is increased for patients with risk factors that are capable of lowering the epileptic threshold, including concomitant use of other medicinal products liable to lower the epileptic threshold. Relevant guidance has been included in the SmPC.

Furthermore, due to a theoretical potential for QTc prolongation a thorough QT/QTc study according to ICH E14 guideline will be performed as a specific obligation, and relevant contraindications, warnings and guidance have been included in the SmPC.

The available pharmacokinetic data deriving from the bioequivalence study indicate similar bioavailability of the base form of 3,4-DAP and the phosphate salt thus allowing to cross-refer to the non-clinical profile for 3,4-DAP base. A number of ADME investigations are scheduled to complete the information relating to the pharmacokinetic (PK) profile of the toxicology species and results from this programme will be submitted as a follow-up measure.

The 4-week repeat-dose toxicity studies in rats and dogs revealed 3,4-DAP effects on the central nervous system, increased liver and kidney weight, muscle necrosis and second degree atrio-ventricular block. As the duration of the available toxicology studies was limited to 1 month the applicant has committed to performing a three-month repeat dose toxicology study in rats as a follow-up measure in order to provide chronic toxicology data to support long-term use of the product.

Amifampridine was not genotoxic in a standard battery of in vitro and in vivo tests.

As no specific reproductive toxicity or carcinogenicity studies have been performed the applicant has committed to conducting a carcinogenicity study in the rat as a specific obligation and a reprotoxicity study as a follow-up measure. The CHMP considered these commitments together with appropriate labelling and other risk minimisation measures including the patient registry as an adequate approach. On this basis, it is accepted that the proposed indication, including patients with the non-paraneoplastic form of LEMS, can be supported.

2.4 Clinical aspects

Introduction

3, 4- DAP phosphate has been developed for the treatment of Lambert-Eaton Myasthenic Syndrome.
3, 4-DAP has been found to block voltage-dependent potassium channels, thereby prolonging pre-synaptic cell membrane depolarisation. Prolonging the action potential enhances the transport of calcium into the nerve ending. The resulting increase in intra-cellular calcium concentrations

facilitates the exocytosis of acetylcholine-containing vesicles, which in turn enhances neuromuscular transmission supporting the proposed indication.

According to the conclusion of the COMP (opinion dated 15/11/02) the prevalence of the "condition" Lambert-Eaton Myaesthenic Syndrome is 0.1 per 10 000 individuals in the EU. According to Regulation (EC) No 141/2000 of 16 December 1999, the Committee for Orphan Medicinal Products (COMP) adopted on 15 November 2002 a positive opinion and on 18 December 2002, orphan designation (EU/3/02/124) was granted by the European Commission for 3,4-diaminopyridine phosphate for the treatment of Lambert-Eaton Myasthenic Syndrome.

The applicant received scientific advice from FR, SE and the UK during 2007.

The clinical evaluation of the product is based on published literature which mainly refers to clinical experience with the free base form of 3,4-DAP.

In order to demonstrate that 3,4-DAP phosphate scored tablets are an appropriate alternative to capsules containing 3,4-DAP as base, the applicant conducted a bioequivalence trial comparing the two different formulations (DAPSEL Study). This study has provided some safety data also. In addition, clinical and safety information has been gathered through Named Patient Basis Experience in France from December 2006 to March 2008, in which 82 patients have been treated with 3,4-DAP phosphate to date (March 2008).

The claimed indication is Treatment of Lambert-Eaton Myasthenic Syndrome.

The approved indication is Symptomatic treatment of Lambert-Eaton Myasthenic Syndrome (LEMS) in adults.

There is no paediatric development programme. According to the European legislation valid at the time of submission there was no requirement to submit a paediatric investigation plan before July 2008.

3,4-DAP has been widely used as an ad-hoc hospital preparation in the treatment of LEMS for over twenty years including compassionate use programmes in DE, FR, PT and the UK. As mentioned earlier on-demand preparations of 3,4-DAP base might exhibit variability and a lack of reliability in the quality of the drug product. Moreover, the supply of 3, 4-DAP under this specific access scheme does not allow the medical community and regulatory bodies to collect safety data according to current pharmacovigilance procedures. Therefore, this product has been developed to address these issues.

GCP
To support this application the applicant has performed a bioequivalence study of crossover design. This trial was conducted according to current ICH E6 GCP guidelines. The application makes reference to published literature. The information on the GCP status of these trials is not available.

Pharmacokinetics
The applicant provides a comparative bioequivalence study (DAPSEL) between capsules with amifampridine base, which have been used clinically as extempore product, and a tablet with amifampridine phosphate, the formulation applied for in this application.

Two literature references have also been provided: one to an indirect clinical comparison between the oral route and intravenous route of administration for 3,4-DAP base, and another examining basic pharmacokinetics of 3,4-DAP in patients with multiple sclerosis.

- Absorption

Bioequivalence

DAPSEL Study: Relative bioavailability of 3,4-DAP administered as a salt or a base.
Study Centre: Clinical Investigation Centre, St. Antoine University Hospital, Paris, France

27 healthy adult males, aged 18-45 years, were included in this study. One subject was withdrawn from the study due to an elevation of liver enzymes during Phase I, and 26 completed the study.
For the first 'phase' of this study, five subjects received a 10 mg dose of the test 3,4-DAP phosphate formulation to assess tolerability. Thereafter, each of the 27 subjects were randomized to receive a single 20 mg dose of either the 3,4-DAP salt or base formulations. A randomization scheme was submitted with the study report. According to the study report this phase of the trial was 'double-blind'. The following formulations were administered:

Reference: 3,4-DAP Base 10 mg x 2 Capsules (Mfg by Pharmacy Department of the Henri Mondor University Hospital, Creteil, France, Batch no. N°03M152)

Test: 3,4-DAP Phosphate 10mg x 2 Tablets (Mfg by Etablissement Pharmaceutique des Hopitaux de Paris, Batch no. N°03M153).

In the first phase subjects were fasted since midnight the day before, in the second phase – for at least 12 hours. Blood samples were taken at pre-dose and followed with 16 samples over the following 24 hours after administration of the products. There followed a washout period of at least 72 hours, thereafter the subjects crossed over to the second 3,4-DAP base or salt formulation and the process repeated as above. The half-life of 3,4-DAP is reported as 2 hours. Therefore, 72 hours is considerably longer than 5 half-lives and should represent a sufficiently long wash-out period. This was confirmed with a pre-dose plasma 3,4-DAP concentration <LLOQ in all subjects at the start of period II.

Plasma samples were analysed for 3,4-DAP concentration by the HPLC method. The lower limit of quantification was 5 ng/ml for 3,4-DAP with an upper limit of 150 ng/ml. The method was validated and the validation report was provided.

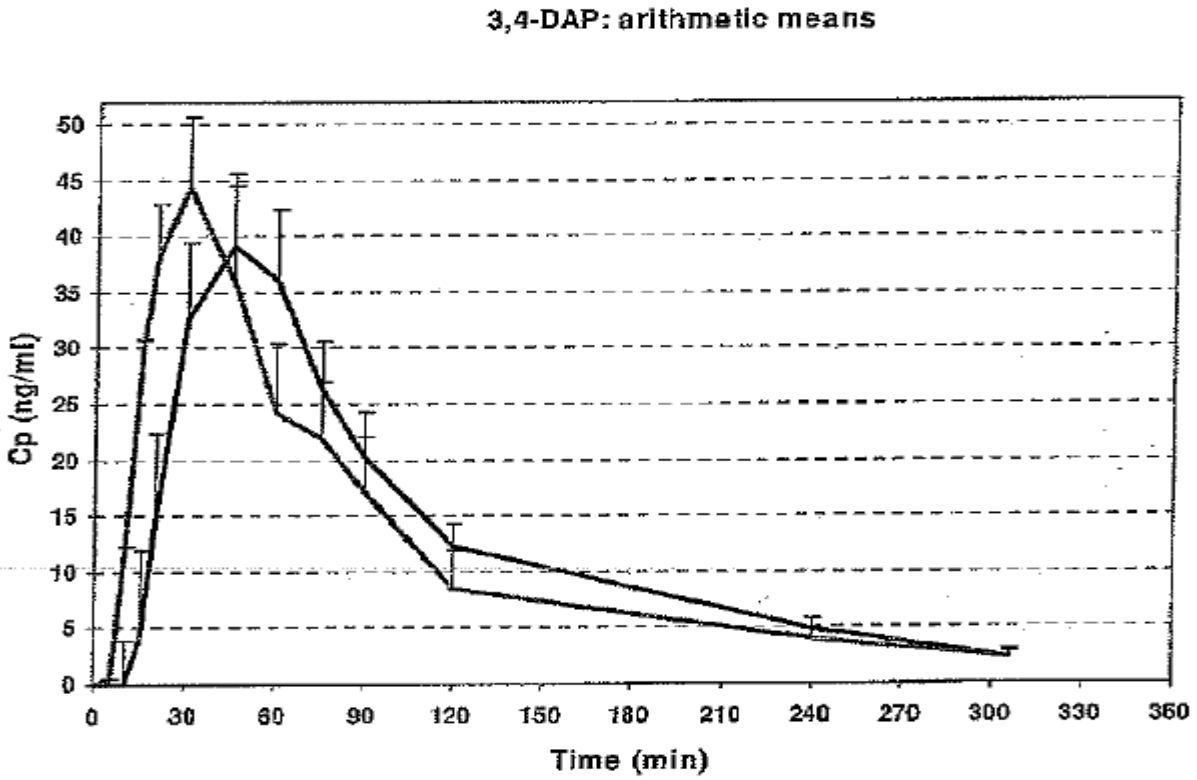
$AUC_{(0-t)}$, C_{max}, t_{max} and t½ were calculated according normal standard procedures.

Statistical evaluation was performed for $AUC_{(0-t)}$ and C_{max} with ANOVA and the 90% confidence intervals (CI) for the ratio of reference formulation over the test formulation were calculated.

Table. Mean reference/test ratios and 90% confidence intervals for AUC and C_{max} comparing tablets (salt) and capsules (base); log-transformed data

PARAMETER	RATIO R/T (%)	90% CI
$AUC_{0-\infty}$	102.7	93.1-113.3
C_{max}	86.3	73.7-100.8

Figure. Plasma concentrations of 3,4-DAP after administration of salt in tablets and compared to base in capsules (the curve with the highest C_{max} corresponds to salt administration)



In conclusion, AUCs ratio fell within the pre-specified bioequivalence limits (80-125%). For C_{max} the observed inferior limit exceeded the 80.0% bound and was near the 75% bound proposed for highly variable drugs. T_{max} was significantly shorter for the salt formulation as compared to the base (0.6 h + 0.3 vs. 0.9 h + 0.4, respectively; $p \leq 0.001$). With regards to the oral bioavailability of 3,4-DAP phosphate, it has been demonstrated that following a 20 mg dose, a plasma C_{max} of approximately 44 ng/ml can be expected to be achieved at 30 minutes post-dosing.

Bioavailability

Bever C, Leslie J, Camenga D, Panitch H, Johnson K Preliminary trial of 3,4-diaminopyridine in patients with multiple sclerosis. Ann Neurol 1990;27:421-427.

Ten male patients, aged 38-61 years, with multiple sclerosis were enrolled in a preliminary trial of 3,4-DAP to evaluate drug toxicity and pharmacokinetics. No patients with any complicating illness, including history or EEG evidence of epilepsy, were included.
After informed consent was obtained, patients entered a two-phase, open label treatment protocol. In the first phase, patients were challenged with single oral doses of 3,4-DAP. Patients 1-3 received doses on alternate days, starting with 20 mg and increasing by 10 mg until a maximum tolerated dose or 100 mg was reached, whichever was the lower. Blood samples were taken to measure 3,4-DAP levels. As these results showed that the serum half-life was <2 hours and that no DAP was detectable after 24 hours, patients 4-10 were treated daily, increasing in 20 mg increments. Phase II of the trial consisted of treating with the maximum tolerated dose or 100 mg in divided dosages for 5 to 21 days. Toxic side effects and neurological status were monitored throughout and blood samples were obtained for peak and trough DAP concentrations.

Serial serum DAP levels were available from 39 treatment days in 9 patients, with doses ranging between 20-100 mg. Time to peak serum level varied from <20 minutes to 1 hour after oral dosing. For each patient, DAP pharmacokinetics were consistent and levels correlated closely with the dose administered. It was noted that there was considerable patient-to-patient variability in dose response magnitude, with one patient achieving a plasma concentration of 80 ng/ml following a 50 mg dose, and another patient achieving a level of 40 ng/ml following a 100 mg dose. There was a similar degree of variability seen in the peak to trough levels between patients. Lumbar punctures were performed on 2 patients during the Phase II treatment and DAP concentrations assayed. The suggestion is that CSF levels reached around 10% of serum levels.

Lundh H, Nilsson O, Rosen I. Treatment of Lambert-Eaton syndrome: 3,4-DAP and pyridostigmine. Neurology 1984;34:1324-1330.

In this small study, the investigators compared the clinical effects of either oral, rectal or intravenous administration of 3,4-DAP base in 5 subjects with an EMG confirmed diagnosis of LEMS. Muscle power, before and after treatment, was quantified by counting the number of repeated identical movements or the time of isometric contraction of several affected muscle groups following electrical stimulation of a motor nerve. One patient suffered with dysarthria which was evaluated blindly by logopedists from tape recordings.

All patients demonstrated transient improvements in the measured parameters following both IV and oral administrations although no statistical analysis was performed on these data. No plasma concentrations of 3,4-DAP were assessed. The doses given varied between the five patients and are summarised as follows, in relation to the change from baseline seen in compound action potential (CMAP) after electro-stimulation of the median nerve:

		Oral Administration			IV Administration	
Patient	CMAP, thenar; rest/2s after max vol contr (mV)	Dose (mg)	CMAP, rest (mV)	Latency to max effect (h)	Dose (mg)	CMAP, rest (mV)
1	1.5/5	12	3.5	3.5	18	8.0
2	2.5/8.5	30	5.0	1.5	8	5.0
3	0.35/1.6	30	1.5	3	8	2.0
4	2.7/4.7	12	5.0	1	Not given	Not given
5	5.0/7.0	18	7.0	2.5	8	8

The investigators stated that in sufficient doses, 3,4-DAP was successful in treating LEMS when administered by oral, rectal and IV routes, however no suggestion as to what a sufficient dose might be was made.

- Distribution and Elimination

At present there are no clinical data known for 3,4-DAP as regards protein binding, distribution, metabolism and route of excretion. Based on literature data (Bever et al., 1990) 3,4-DAP is rapidly cleared and would appear to have a serum half-life between 20 minutes and 2 hours. It has been also suggested that the main pathway of elimination may be renal excretion due to hydrophilic properties of 3,4-DAP.

Metabolism

The metabolism of amifampridine has not been investigated in vitro or in vivo. The applicant performed a comparative clearance study PR6958/CC2206 which included incubation of amifampridine with human hepatocytes (please see non-clinical part of the report). The hepatocytes were from 3 donors, all with a cancer diagnosis. It is unknown, but not excluded, that the tissue was from metastases; hence, the protein expression could have been altered.
The activity of CYP1A2 and CYP3A as well as UGT1A6 was measured to assess enzymatic activity. The enzyme activities were within the range of historical data from the laboratory. There was no metabolism in the hepatocytes from one of the donors. The CLint values determined were in line with the values reported for rat and monkey hepatocytes in the same study. The study did not include metabolite detection. The CHMP considered this study as inconclusive.
Further in vitro metabolism studies will be performed as a follow-up measure (please see discussion on PK).

- Dose proportionality and time dependencies

Presented literature data did not allow detailed assessment of dose-dependency in the studied population. No studies investigating time dependency or multiple dose pharmacokinetics were submitted. The dose has been determined on the basis of clinical experience.

- Intra- and inter-individual variability

The inter-individual variability is reported to be quite high (CV about 50%). The intra-individual variability is unknown. The CHMP concluded that doses should be titrated individually in a stepwise manner with particular care in patients with renal and hepatic impairment (see SmPC sections 4.2 and 4.4).

- Special populations

There are no data on the use of 3,4-DAP in any special populations, no information on its use in hepatically or renally impaired subjects. As discussed above, the appropriate information is included in the SmPC.

- Pharmacokinetic interaction studies

There are no data on PK interactions. The appropriate information is included in Section 4.5 of the SmPC.
No food interaction studies have been performed. Due to the fact that it is not known whether standardisation of administration of drug in relation to food intake will reduce the variability of drug exposure, and because Zenas tablets are to be taken with food, a food interaction study will be performed as a specific obligation.

- Pharmacokinetics using human biomaterials

Please see metabolism section above.

Discussion on pharmacokinetics
There are few data available on bioavailability of 3,4-DAP. The applicant provides a comparative bioequivalence study (DAPSEL) between oral base and an example of the salt formulation, which displays a difference between the salt and base formulations with respect to C_{max}. Given the results of the DAPSEL study, i.e. that the proposed formulation was more rapidly dissolved and more completely absorbed, leading to a higher C_{max} and shorter T_{max} than the reference formulation, and the fact that the submitted dossier depended entirely on the demonstration that the proposed formulation is suitably similar to those referenced in the published efficacy studies, CHMP expressed concern whether literature data provided for the base formulation can be extrapolated to the salt.
In further discussion it was acknowledged that the PK profiles of the formulations used in the published literature were not known, therefore a comparison could not be made. Moreover, the extent of absorption, as measured by AUC, was similar between products, hence it was accepted that the extent of exposure to the active moiety would be similar, irrespective of the formulation difference. It was suggested that this profile would not impact on efficacy, but may have an effect on safety. Consequently, a reduction in the maximum daily dose from 80 mg to 60 mg was proposed and endorsed.

The applicant also provided two literature references: one to basic pharmacokinetics of 3,4-DAP in patients with multiple sclerosis. These data would suggest that 3,4-DAP base is rapidly absorbed following oral dose, with peak plasma concentrations being reached within 1 hour. There would appear to be a significant level of inter-individual variability in the plasma concentrations reached. 3,4-DAP is rapidly cleared and would appear to have a serum half-life between 20 minutes and 2 hours. Another study examined an indirect clinical comparison between the oral route and intravenous route of administration for 3,4-DAP base. However, no conclusion could be made from this study with regards to the oral bioavailability of 3,4-DAP.

The applicant has committed to perform in vitro studies to assess the potential of 3,4-DAP to inhibit human hepatic cytochrome P450, to assess the effect of 3,4-DAP on NAT and Pgp and to assess which enzymes are involved in the metabolism of 3,4 DAP, within a prespecified time frame. Similarly, the potential for enzyme induction is to be investigated as well as an attempt to identify human 3,4-DAP metabolites *in vitro* is to be made as at present there are no human data known for 3,4-DAP as regards protein binding, distribution, metabolism and route of excretion or information about potential PK or PD interactions.

There are no data on the use of 3,4-DAP in any special populations, including hepatically or renally impaired subjects. This is adequately reflected in the SmPC. No food interaction study has been performed. The CHMP has requested that the applicant performs this study as one of the specific obligations which form the basis for the marketing authorisation under exceptional circumstances.

Pharmacodynamics
As discussed earlier, in patients with LEMS, a reduced number of synaptic vesicles are released in response to nerve stimulation so that the quantal content of the end-plate potential is abnormally low. The relationship between the number of vesicles released in response to nerve stimulation and extracellular calcium is disturbed in LEMS, indicating that calcium entry into the nerve terminations is compromised. The auto-antibodies found in LEMS induce a decrease in the release of neurotransmitters. This decrease in neurotransmitters is thought to cause the reduced action potential of the muscle in those patients.

- Mechanism of action

Animal models have shown that 3,4-DAP blocks voltage dependent potassium channels. This blockade sustains depolarisation of the presynaptic membrane and slows or inhibits repolarisation. The resulting prolonged action potential increases calcium influx to the nerve terminal. The increased concentration of intracellular calcium promotes exocytosis of acetylcholine-containing synaptic vesicles. This hypothesis implies that in man 3,4-DAP not only improves resting muscle responses by increasing the inward flow of calcium but also increases the rate of calcium efflux from the pre-synaptic terminal after nerve stimulation.

- Primary and Secondary pharmacology

The proposed primary pharmacology has been outlined in animal models, whereby 3,4-DAP has been shown to block voltage dependent potassium channels thereby sustaining depolarisation of the pre-synaptic membrane and slowing repolarisation. No specific studies in man have been performed. There are a number of literature references which report the effect of 3,4-DAP on increasing the amplitude of the compound muscle action potential, an example of which is outlined below:

Effect of 3,4-Diaminopyridine on the time course of decay of compound muscle action potential augmentation in the Lambert-Eaton Myasthenic Syndrome. Maddison P, Newsom-Davis J, Mills K. Muscle Nerve 21: 1196-1198, 1998.

6 patients with LEMS, all positive for autoantibodies to voltage gated calcium channels, were studied. Measurements of peak-to-peak compound muscle action potential (CMAP) amplitudes were made from abductor digiti minimi using standard bipolar surface electrodes. A single supra-maximal stimulus was delivered at rest prior to a 10 second maximal voluntary contraction, which was immediately followed by thirty 1Hz stimuli. An identical series of recordings was made exactly 1 hour after ingestion of 10 mg oral 3,4-DAP. All patients had abnormally small resting CMAP amplitudes prior to the ingestion of DAP. They all demonstrated characteristic augmentation of the CMAP amplitude after 10 s voluntary contraction. After ingestion of DAP, 4 out of 6 patients had an increase in resting CMAP amplitude. The time constant for decay in CMAP amplitude was significantly reduced after treatment indicating an effect on the efflux of calcium ions from the presynaptic nerve terminal.

No studies have been provided regarding the secondary pharmacology. Based on the mechanism of action and literature data it is believed that 3,4-DAP may cause cholinergic effects. For more detail please refer to the non-clinical part of the report.

- Relationship between plasma concentration and effect

As discussed under pharmacokinetics, there would appear to be a high degree of inter-individual variability in the plasma concentration reached following a given oral dose. It is not known, therefore, what plasma concentration can be expected to be reached from a given dose of 3,4-DAP and the dose has to be individually titrated.

- Pharmacodynamic interactions with other medicinal products or substances

The applicant has provided no formal PD interaction studies. However, there are data available from published literature relating to DAP which would suggest a number of areas for consideration.

3,4-DAP has been associated with reports of seizures, especially at high doses. It is reasonable to hypothesise that concomitant administration of 3,4-DAP with other agents lowering the epileptogenic threshold may give rise to an increased risk of convulsions. This potential interaction is identified as a precaution for use in the SmPC.

Through the suggested pharmacodynamic effect on K^+ channel blockade, and resultant prolongation of the action potential, an effect on cardiac repolarisation cannot be fully excluded, as discussed earlier. No specific impact on ECG was observed in the DAPSEL study. However, there are at least two reports of potential effect on QTc in the literature, including one ventricular tachycardia confirmed on a Holter and one QTc prolongation. This potential interaction with other QTc prolonging drugs including sultopride is identified as a contraindication in the SmPC.

It is also reasonable to assume, based on theoretical considerations of the pharmacodynamic properties of 3,4-DAP, that it may cause cholinergic effects. Although clear clinical evidence is lacking, interactions between 3,4-DAP and cholinergic substances including acetylcholine esterase inhibitors would seem to be possible. In addition, caution should be exercised with drugs having an anti-cholinergic effect (such as phenothiazine, neuroleptics, antispasmodics, H1-blockers) since 3,4-DAP might mitigate their effectiveness or a cholinergic attack might occur on discontinuation. The acetylcholine releasing properties of 3,4-DAP may reduce the action of depolarising and non-depolarising muscle relaxants. These potential interactions are identified as combinations to be taken into consideration in the SmPC.

Discussion on pharmacodynamics

In conclusion, the pharmacodynamic studies provided as literature references for 3,4-DAP base, considered to be studies of efficacy in small populations, suggest that 3,4-DAP almost certainly has an impact on the amplitude of compound muscle action potential (CMAP), which in turn improves clinical motor function in patients. In terms of defining a specific receptor, its occupancy, the agonist vs. antagonist effect no studies have been provided. Due to limited data a dose-response relationship remains not clarified. Consequently, a number of precautions have been advised in the SmPC on potential pharmacodynamic interactions.

Clinical efficacy

This was a mixed application, and the pivotal efficacy data are based on published studies.

The results from four randomised placebo-controlled studies and one active controlled study have been published. Two of the placebo-controlled studies, McEvoy et al. , 1989 and Sanders et al., 2000, were published as journal articles and both were included in a recent Cochrane review. These two studies are considered as the pivotal studies.

The other controlled trials were published in abstract form [Murray et al., 1984, Wirtz et al., 2002] or as a short book article [Sanders et al., 1993] and they are considered as supportive. In addition, there are reports of uncontrolled investigations and case reports which provide supportive information on efficacy.

Moreover, the available data relating directly to the use of 3,4-DAP phosphate from the Named Patient Basis experience in France is also presented – 82 patients have been treated with 3,4-DAP phosphate 10 mg tablets between December 2006 and March 2008.

- Main study(ies)

Study by McEvoy at al, 1989.[1]
This was a prospective, double blind, cross-over, placebo controlled trial of 3,4-DAP in 12 patients with LEMS.

METHODS

Study participants
Twelve (12) patients were randomized and had a diagnosis of LEMS based on accepted criteria. Eligible patients had electrophysiologic confirmed LEMS with stable or progressive weakness. One patient was screened but not included due to failure to meet those criteria. The electrophysiologic criteria used for the diagnosis were a decrement of more than 10 % during repetitive stimulation of 2 Hz and a facilitation of more than 200% after 10 seconds of exercise, in two different nerve-muscle combinations, without evidence of other nerve or muscle disease. Seven patients had cancer, and five patients had autoimmune disorders, reflecting the LEMS population. All 12 randomised patients completed both periods of the study.

Treatments
For the first 8 days of treatment (Days 2 – 9), patients were administered DAP in an open label fashion. The oral dose was titrated up to the individual's maximum tolerable dose or a maximum of 25 mg four times daily. Thereafter, patients entered a double blind phase of crossover design. Patients were randomised to receive their maximum tolerated dose, as per the open label phase, or placebo for three days before crossover and repeat. Ten patients tolerated the full dosage of 25 mg four times a day. One patient was maintained on 15 mg and another on 10 mg due to side effects.

Objectives
The objectives were not clearly stated in the publication.

Outcomes/endpoints
The primary or secondary efficacy outcome measures are not clearly defined in the publication. The efficacy variables used were the neurological disability score (NDS) (see Table below), the isometric strength and electrophysiological measurements. The NDS score reflects a number of symptoms relevant to patients with LEMS.

Baseline electrophysiological measurements were taken of standard electromyography and nerve conduction studies of the upper and lower extremities, with 2Hz repetitive stimulation of the ulnar, median, musculocutaneous, peroneal and tibial nerves both at rest and after exercise. The nerve-muscle combination (CMAP) showing the greatest facilitation after exercise in one upper and one lower extremity were used for subsequent repetitive-stimulation testing on days 3, 5, 12 and 15. Isometric strength testing was performed in Days 1, 3, 5, 9, 12 and 15. This included measurement of the force generated by bilateral elbow and wrist flexion, knee extension and ankle dorsiflexion. Neurologic disability scores were determined at the same intervals. This comprised of a strength score for a unilateral set of muscles and reflexes as follows:

[1] McEvoy K, Windembank A, Daube J, Low P. 3,4 Diaminopyridine in the Treatment of Lambert-Eaton Myasthenic Syndrome. The New England Journal of Medicine. 1989; 321: 1567-71.

Table. The Neurologic Disability Score Sheet.*

Strength score+	**Reflex score++**
Muscle	**Reflex**
Extraocular	Biceps
Facial	Triceps
Jaw	Brachioradialis
Palate	Knee
Tongue	Ankle
Respiratory	
Shoulder abduction	Subtotal
Shoulder external rotation	**Total score**
Biceps	
Triceps	
Brachioradialis	
Wrist extensioin	
Wrist flexion	
Finger extension	
Finger flexion	
Interossei	
Hip flexion	
Hip adduction	
Hip abduction	
Quadriceps	
Hamstrings	
Ankle dorsiflexors	
Ankle plantar flexors	
Toe dorsiflexors	
Toe plantar flexors	
Subtotal	

*Each index is scored on both the right and left sides. A strength subtotal of at least 20 was required for entry into the study. +A score of 0 denotes no deficit, 1 mild weakness, 2 moderate weakness, 3 severe weakness, and 4 no movement. ++A score of 0 denotes normal activity, 1 reduce activity, and 2 no activity.

Measures of autonomic function were also made. These included sweat production (sudomotor axon reflex test), salivation, tilt table orthostatic blood pressure measurement, and heart rate responses to deep breathing and the Valsalva manoeuvre, corrected for age. Electroencephalography and electrocardiography were performed at baseline and at Days 3, 5, 8 and 15.

The CHMP considered these outcome variables as adequate, although a clinically meaningful effect was not predefined.

Randomisation
It is stated in the publication that a random number table was used, which would be appropriate.

Blinding
It is only stated in the publication that the cross-over phase was double blind. There is no description of the appearance of the placebo compared to the active drug.

Statistical methods
The quantative measures of severity before therapy and during maximal doses of 3,4-DAP were compared by paired t-test.

RESULTS
Recruitment
There is no detailed description of how the patients were recruited.

Conduct of the study
There is no discussion about deviations from the protocol.

Baseline data:
The 12 patients consisted of 8 women and 4 men aged between 34 and 75 years. Seven of the patients had cancers and five auto-immune disorders. Ten of the patients had been previously treated with anticholinesterase inhibitors. Four had been treated with prednisolone, four with guanidine, three with plasmapharesis and two with azathioprine. None had received any previous aminopyridines. Any anticholinesterases were stopped 4 days before the trial drugs were commenced and patients receiving immunosuppressive agents were held constant for at least 5 months before entry into the study.

Numbers analysed
12 patients were included and all 12 patients completed the cross-over period.

Outcomes and estimation
The neurologic disability scores decreased from a base-line mean of 40 to 22 on active treatment, and 35 on placebo ($p<0.05$).
Isometric strength also showed significant improvement with treatment. Upper extremity strength at baseline averaged 70% of normal (sex matched for healthy subjects) and improved to 81% on treatment. Lower extremity strength improved from 45% to 65%. Both results were significantly better than that for placebo ($p<0.005$ and $p<0.001$, respectively).
Electrophysiological improvement occurred as well, with increase in the resting areas and amplitudes of CMAPs nearly doubling on treatment. An increase in sweating of the foot was seen in ten out of the twelve patients ($p<0.05$) although this was not significant in the case of the forearm. Even though slight increases in heart rate were seen for both deep breathing and Valsalva manoeuvres, neither result was significant, nor were any changes for orthostatic blood pressure.

After the trial period, patients had the option of continuing on open label DAP treatment if they chose. All twelve did and three month data were available for all. Nine month data were available for 11 as one patient died from their pre-existing cancer during the intermittent period. Nine of the remaining 11 returned at 15 months.

No significant decline in the efficacy of the treatment was noted; the average resting amplitude of the CMAP at nine months were 5.1±1.0 mV in the arm, and 3.1±0.7 mV in the leg, and at 15 months they were 5.5±1.2 mV in the arm, and 3.2±0.8 mV in the leg. It has however to be noted that after the three month period, four patients had pyridostigmine added to their treatment regimes which may have contributed to the overall effect.

Study by Sanders D, et al, 2000.[2]
This was a prospective, double blind, placebo controlled efficacy trial of 3,4-DAP in 26 patients with LEMS.

METHODS
Study participants
Patients older than 18 years of age with a confirmed diagnosis of LEMS were candidates for screening. The diagnosis of LEMS was made if there was weakness that predominated in proximal limb muscles and electromyographic findings characteristic of LEMS. Patients who met the screening criteria were admitted to the General Clinical Research Center, Duke University Medical Center.

The 26 patients consisted of 15 women and 11 men aged between 41 and 68 years. Ten of the patients had cancers.

Treatments
Patients took one capsule containing lactose alone or 20 mg of DAP in lactose three times a day for 6 days.

[2] Sanders D, Massey J, Sanders L, Edwards L. A Randomised trial of 3,4-diaminopyridine in Lambert-Eaton Myasthenic Syndrome. Neurology 2000;54:603-607.

Objectives
To assess the effectiveness of 3,4-diaminopyridine (DAP) in patients with Lambert-Eaton myasthenic syndrome (LEMS) and to determine the acute and long-term side effects of DAP.

Outcomes/endpoints
A standardized, quantitative assessment of function of muscle groups typically involved in myasthenia gravis (MG) and LEMS, the quantitative myasthenia gravis (QMG) score, was used as the primary measure of efficacy.

In each patient, the average of the baseline QMG scores obtained on 2 consecutive days before beginning the study drug was compared with the average of postbaseline QMG scores obtained on the fifth and sixth days of the study drug administration. QMG scoring was performed by the same observer and at the same time interval after the most recent dose of study medication.

Changes in the amplitude of CMAPs elicited by nerve stimulation were used as a secondary measure of efficacy. The average of the summated CMAPs obtained on 2 consecutive days before beginning the study drug was used as the baseline CMAP amplitude, and the average of the summated CMAPs obtained on the fifth and sixth days of the study drug administration was used as the postbaseline CMAP amplitude. The change in summated CMAP amplitude between baseline and postbaseline values was calculated for each patient.

These were considered an appropriate clinical scale and an objective electrophysiological measurement.

Randomization and blinding
Patients were randomized to receive either DAP or placebo using a random allocation table, which was maintained by the Duke University Medical Center Hospital Pharmacy. Identical capsules were prepared by the pharmacy.

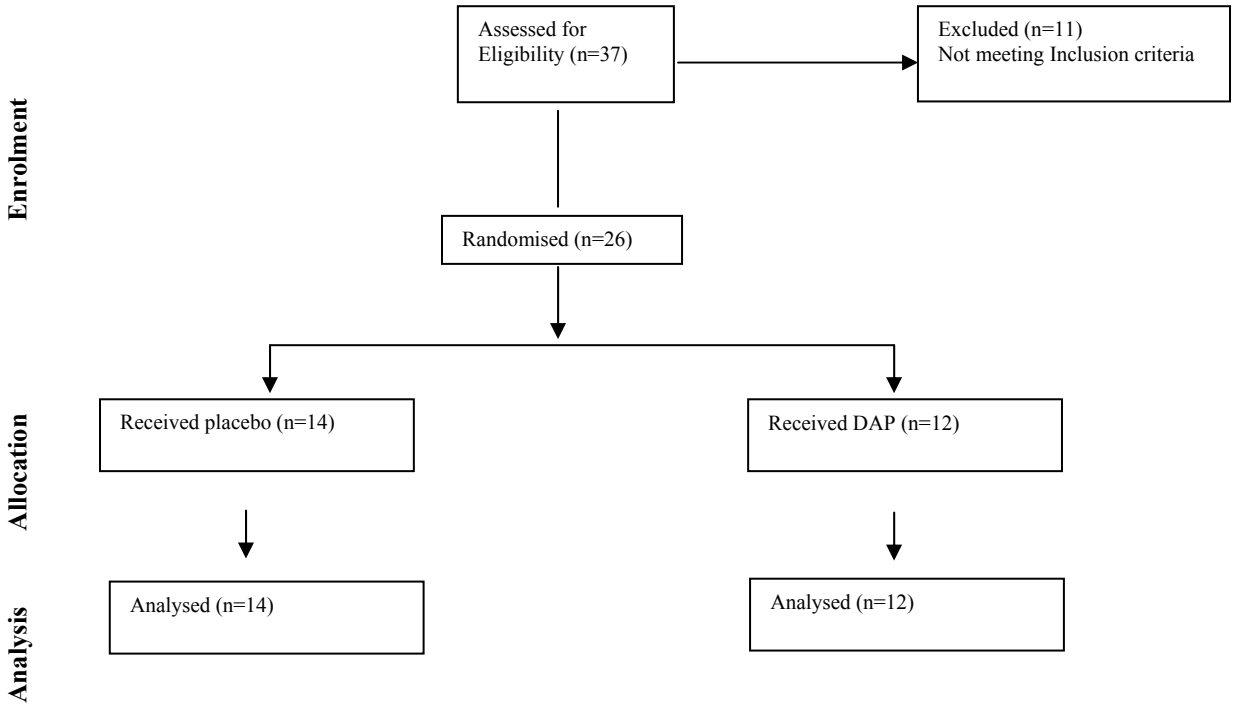
14 patients received placebo and 12 DAP. Patients took one 20 mg capsule of the study drug (or matching placebo) three times a day for 6 days.

Statistical methods
Results of preliminary studies had indicated that LEMS patients improve an average of two QMG points while taking DAP, compared with an average improvement of 0.7 points in patients receiving placebo. There was a power of 80% that a placebo-controlled parallel treatment study with 26 patients would demonstrate a clinically meaningful difference of two QMG points between patients randomly assigned to DAP and those randomly assigned to placebo that is significant at the 5% type I error level. The few patients with this rare disease that could be studied did not provide sufficient statistical power to perform any meaningful subgroup analysis.

The original study design specified that a t-test would be used for data analysis, assuming that the underlying distribution of data would be normal. However, subsequent analysis demonstrated that the distribution of most of the continuous variables was sufficiently skewed to warrant using a nonparametric statistical method. Thus, descriptive statistics for continuous variables were summarized in terms of medians and interquartile ranges, and the Wilcoxon's rank sum test was used to evaluate group differences. This included the evaluation of differences between placebo- and DAP-treated groups for baseline and postbaseline values for QMG, CMAP, as well as differences in the change of these variables from baseline. Categorical variables were reported in terms of the number and percentage of patients affected. Because of small sample sizes, Fisher's exact tests were used to evaluate group differences for gender and incidence of small cell lung cancer at baseline, as well as for symptomatic response to treatment. All analyses were completed on an intention-to-treat basis.

RESULTS

Participant flow
All randomized patients completed the study.



Recruitment
Patients with LEMS were identified and recruited from the Myasthenia Gravis Clinic at Duke University Medical Center, both by personal contact with physicians throughout the country and by notices on the Internet. This is appropriate.

Conduct of the study
There is no detailed description in the publication of the conduct of the study.

Baseline data
QMG score testing and nerve stimulation studies were performed at baseline and repeated on the fifth and sixth days of drug administration. On the sixth day ECG, EEG and screening blood tests were repeated. No statistically significant differences between the groups even though some smaller differences in number of patients with SCLC and the baseline values of QMG could be noted.

Numbers analysed
All randomized patients were analysed.

Outcomes and estimation
The QMG score improved by at least 2 points in seven of the twelve patients taking DAP, the maximum change being an improvement of 3.0 points. No patient who received placebo improved more than 1.5 points. The change from baseline reached statistical significance.

QMG (points)	**Placebo n=14**	**DAP n=12**	**p value**
Baseline	12.3 (9.0-13.5)	8.5 (7.3-17.0)	0.62
Post baseline	13.0 (9.0-13.5)	6.5 (5.0-14.3)	0.14
Change	0.25 (-1.0-1.0)	-2.0 (-3.0-0.0)	0.01

The median summated CMAP amplitude increased 1.30mV among patients who took DAP, compared with a median decrease of 0.1mV in those taking placebo, this change being a highly significant difference from placebo.

CMAP (mV)	Placebo n=14	DAP n=12	p value
Baseline	1.3 (0.8-2.2)	1.5 (0.6-2.6)	0.79
Post baseline	1.3 (1.1-2.9)	3.4 (1.5-5.1)	0.05
Change	-0.1 (-0.1-0.1)	1.3 (0.5-2.5)	<0.001

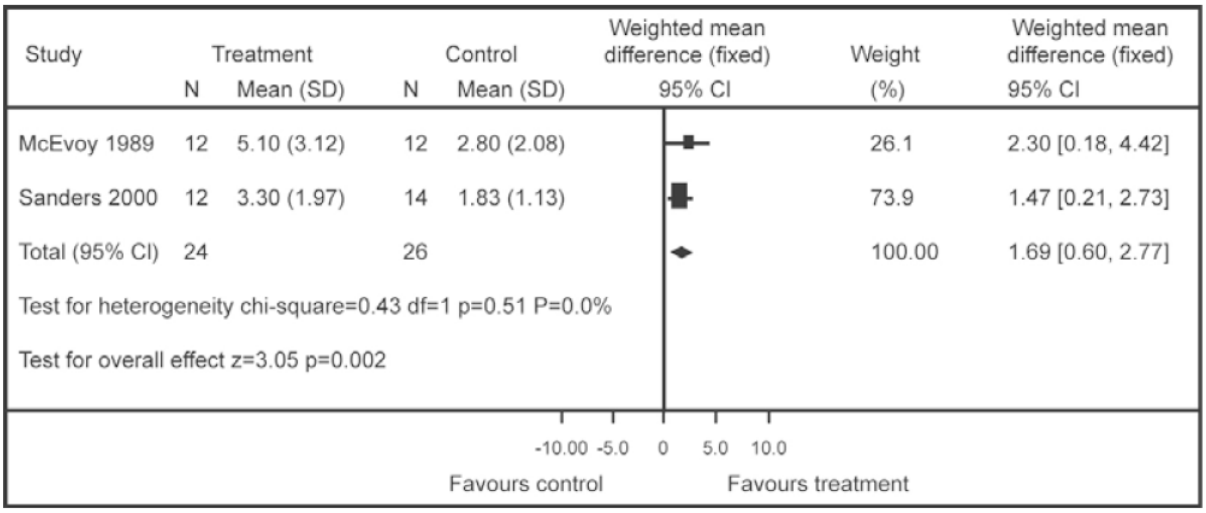
As a further stage, patients could continue with open label DAP after the trial period. All but three of the 25 patients continuing with open label treatment improved at least two QMG points during this extended treatment. Patients had pyridostigmine added to their treatment regimes at this time, which might have contributed to the overall effect. Only one patient had no symptomatic improvement while taking open-label DAP. Two of the three patients who did not improve symptomatically while taking blinded DAP subsequently had symptomatic improvement while taking open-label DAP. Conversely, four patients believed that their strength improved while they were taking placebo, but this was not corroborated by a significant change in their QMG score. All four of these patients had more marked symptomatic improvement while subsequently taking open-label DAP, and all of this was corroborated by an improved QMG score. In more than half of the patients, the optimum clinical response was obtained with 30 or 40 mg of DAP per day. The results of this open label phase seem to corroborate the results of the blinded phase.

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

Maddison P, Newsom-Davis J . Treatment for Lambert Eaton Myasthenic Syndrome. 2005 Cochrane Review.

The above two studies were included in a Cochrane review for the treatment of LEMS in 2005. The authors did not perform a meta-analysis on the primary (clinical) end-points due to marked differences in primary outcome measures used in the studies. Instead, the authors carried out a meta-analysis using CMAP as an end-point, which was a secondary end-point in both studies. They stated that improvements in CMAP were an ideal, objective, and reproducible secondary end-point for trials investigating treatments of LEMS.

The overall weighted mean difference was 1.80 mV (95% confidence interval 0.82 to 2.78), favouring treatment (see below).



The Cochrane review concluded that these trials showed that 3,4-DAP improved muscle strength scores and CMAP amplitudes in patients with LEMS. The results were considered in line with other reports showing that 3,4-DAP is beneficial in the treatment of LEMS and mirror the current practice of using 3,4-DAP as symptomatic first-line treatment in LEMS patients.

- Clinical studies in special populations

No studies in special populations have been conducted. Appropriate information is included in the SmPC.

- Supportive study(ies)

The applicant refers to other controlled trials that have been published in abstract form or as a short book article: these are considered as supportive studies. In addition, there is a plethora of reports of uncontrolled investigations and case reports. Although many of them may be classified as anecdotal, they provide some supportive information on efficacy, in view of the rarity of the condition.

Sanders et al., 1993
This was a randomised, double-blind, placebo-controlled, crossover study. After an uptitration period to identify the optimal dose, 10 patients with LEMS received 3,4-DAP 5-15 mg three times a day or matching placebo for 1 week. In addition, 8 patients with MG were enrolled.
Seven of 10 patients with LEMS improved more than 3 QMG points on 3,4-DAP ($p<0.1$) A therapeutic response was seen with doses of 3,4-DAP 5 to 15 mg three times a day. After the blinded, crossover trial, pyridostigmine was added and was reported to produce greater symptomatic improvement than 3,4-DAP alone and permitted reduction of 3,4-DAP dose in most patients.
Sixteen patients took 3,4-DAP (15 to 50 mg/day) in divided doses for 4 to 45 months (average 22 months). The authors concluded that 3,4-DAP was an effective symptomatic treatment in patients with LEMS, and in some patients with acquired MG.

Wirtz et al., 2002
This was a double-blind placebo-controlled, randomised, four-period crossover study, which compared the effects of single doses of 3,4-DAP, pyridostigmine, their combination, and placebo in nine patients with LEMS. 3,4-DAP 10 mg was given as an intravenous infusion over 1 hour. Pyridostigmine was given as an intravenous bolus of 1 mg at 0 and 40 minutes.
Mean baseline muscle strength was 108 ± 61.2 N in the nine patients included in the trial. Mean baseline CMAP amplitude was 3.49 ± 2.41 mV.
Strength and CMAP amplitude increased significantly during treatment with 3,4-DAP compared with placebo (mean differences 23.0 N; 95% CI = 12.2-33.7 and 0.89 mV; 95% CI = 0.39-1.39). A similar significant improvement versus placebo was also seen with combination therapy (mean differences 26.2 N; 95% CI = 14.6-37.9 and 1.09 mV; 95% CI = 0.53-1.65 respectively). However, treatment with pyridostigmine alone did not result in significant improvement. Treatment with combination therapy did not significantly differ from treatment with 3,4-DAP alone (mean differences -1.9 N; 95% CI = 17.7-13.9 and -0.12 mV; 95% CI = -0.83-0.58)

Murray et al., 1984
This was a double-blind, active-controlled study which compared single doses of 3,4-DAP 20 mg and pyridostigmine 120 mg in patients with LEMS. Six patients were enrolled. Improvements after 3,4-DAP were more marked than after pyridostigmine in five patients, as shown by clinical and electrophysiological criteria. The peak effect occurred 1-2 hours after administration.

Named Patient Basis Experience
3,4-DAP base as ad-hoc hospital preparations was distributed by AGEPS in France between 1995 and mid-2006. During this period approximately 90 patients with LEMS were treated. No efficacy data were collected. Since December 2006, the distribution of 3,4-DAP phosphate has been authorised on a Named Patient Basis under the French ATU programme. Of the 82 patients treated in France from December 2006 until March 2008, 31 were de-novo patients. No formal efficacy data have been

recorded. However, it is reported that very few patients have described a lack of efficacy with 3,4-DAP phosphate.

- Discussion on clinical efficacy

The applicant has not conducted any pivotal efficacy study by themselves, and the efficacy is supported by published studies.

The CHMP discussed the following shortcomings of this application:

1) Most of the experience with 3,4-DAP in LEMS has been gained with the base formulation, however, majority of the clinical publications, with a few exceptions, do not explicitly specify 3-4-DAP as a free base. Zenas contains the drug substance 3,4-diaminopyridine (3,4-DAP) formulated as phosphate. This was discussed by the CHMP and the results of the DAPSEL study (see the section on Pharmacokinetics) addressed the CHMP questions on this aspect. As a consequence of the differences in C_{max} and T_{max} between the phosphate and the base, the maximum daily dose was adjusted from 80 to 60 mg.

2) The two published studies that the applicant refers to as pivotal are small. The small number of patients reflects the rarity of LEMS.
The published studies present methodological problems and the publications generally contain sparse data. The clinical efficacy is also supported by numerous publications of supportive studies/case reports, however these offer an even lower level of evidence due to methodology, detail of data and potential for bias. In particular, the study by Mc Evoy et al. presented the following weaknesses: there was no description of how the patients were recruited, the blinding was questionable due to pre-treatment of active drug and the drug/placebo appearance was unclear, the statistic analysis was not well defined in advance and there was no discussion around the multiple analyses. In the Sanders et al. study the statistical analyses were changed from the originally planned. Most importantly, there was no clear analysis of how to relate the measured effect to a clinical meaningful effect.
The applicant discussed in their responses the concerns of the CHMP regarding the methodological shortcomings of these publications, and the clinical relevance of the results. It was concluded that both studies show a clear and significant positive effect on all variables used, and additionally on an objective parameters such as electrophysiological measures (CMAP). The concordant effects shown on the neurological disability score, the myasthenia gravis score (QMG) and on the muscle strength (as also mirrored by the electrophysiology results) were considered by the CHMP as sufficient, albeit weak, demonstration of efficacy. The effects seem to be sustained over time as showed by CMAP data at long-term follow-up.

3) The applicant did not explain convincingly why a pivotal efficacy study with 3,4-DAP as a phosphate has not been conducted by themselves. The study would have been possible to conduct according to GCP and guidelines for confirmatory studies, and then more convincing data would have been available. In their responses, the applicant argued that the PK results showed that the available body of data, including the case reports, was applicable to the submitted formulation, therefore further placebo-controlled studies would not be warranted. The CHMP concluded that the point was resolved due to the concurrence of the available results, although a study even in a limited number of patients would have been preferable. To further substantiate this conclusion, the CHMP has requested that the patient registry established as a specific obligation should incorporate measures of efficacy, and that the marketing authorisation should therefore be granted under exceptional circumstances.

Clinical safety
The majority of the safety information relates to published documented exposure to DAP base. There are some data from the named patient database in France (French ATU programme), as well as that recorded during the DAPSEL bioequivalence study.

- Patient exposure

The clinical data in the application for Zenas are primarily taken from published clinical trials. In total, approximately 2300 patients have been exposed to 3,4-DAP. Of these, approximately 282-324 patients suffered from LEMS. Most of the patients were exposed to the 3,4-DAP base (please refer to the Risk Management Plan for clarification on total patient exposure).

The patient exposure is as follows:
- Approximately 150 patients with LEMS were exposed to treatment in the controlled, uncontrolled studies and the case reports according to the literature data included in the application.

- Approximately 90 patients with LEMS have been treated in France with 3,4-DAP provided by Agence Générale des Equipments et Produits de Santé (AGEPS) since 1996 and approximately 1,170 patients were treated with 3-4 DAP provided by AGEPS for other indications. In addition, since December 2006 till March 2007, 82 patients have been treated with 3,4-DAP under the Therapeutic Use Protocol in France (French ATU programme). Of these patients 28 suffered from LEMS, 19 from Congenital Myashenic Syndrome (CMS) and 35 patients from either LEMS or CMS (no definitive information available).

- Moreover, at a later stage during the procedure additional safety data became available from 32 new patients treated with 3,4-DAP under the ATU programme between 1 April 2008 and 31 December 2008. Of these 11 suffered from LEMS, 10 from CMS, 4 from nystagmus and for 7 patients the indication was not available.

- Adverse events

The seven placebo-controlled studies with 3,4-DAP (two in LEMS and five in other indications) reported adverse events. All the other reports on the safety and tolerability of 3,4-DAP report suspected “side-effects” or adverse reactions. Paraesthesias were the most commonly seen side-effect. They occurred most frequently as peri-oral or digital (peripheral) paraesthesias with daily doses of 3,4-DAP of between 10 and 40 mg, usually about 1 hour after ingestion. Paraesthesias did not always occur after every single dose and may be more marked when 3,4-DAP is taken together with pyridostigmine. They did not lead to treatment cessation. In some patients, transient dizziness, a sense of light-headedness, heavy-headedness or fatigue developed after 3-4 DAP intake. Subjective symptoms resolved with dose reduction. Some patients developed a subjective sense of weakness and malaise 3 or 4 days after dose escalation. This resolved with dose reduction and therapeutic benefit was maintained. Abdominal pain, epigastric distress or abdominal cramps are other frequent adverse reactions seen with 3,4-DAP.

In the DAPSEL study, which consisted of 26 healthy volunteers, there were 40 adverse events reported. Of the 40 reported events, 25 were paraesthesias, mainly peri-oral paraesthesias, which are documented AE’s in patients treated with 3,4-DAP. All were minor and were considered as possibly related to the administered treatment by the investigator. Other events were non-serious events usually occurring in trials with healthy subjects. No deaths were reported during this study. One adverse event, increase in liver enzymes, was classified as serious leading to premature withdrawal (please see below).

Over the period from 1995 to mid-2006 3 safety reports were collected from the compassionate use programme in France: paraesthesias, anxiety and visual disturbance; myoclonia; epigastric distress, nausea, diarrhoea, headache and dizziness. Most of the adverse events resolved after discontinuation of the treatment.

As mentioned earlier, since December 2006, the distribution of 3,4-DAP phosphate (10 mg scored tablets) has been authorised on a Named Patient basis (French ATU programme) by the AFSSAPS as part of a Therapeutic Use Protocol (TUP). During the period from 1 December 2006 to 17 March 2008, 13 case reports have been received which were observed in 11 patients and included 25 different AEs. Of the 13 reports, two were serious and 11 were non-serious. The most common treatment-

related AEs were those classified as gastro-intestinal disorders (n=12; 14.7%). The most common individual AEs were paraesthesia (n=4; 4.9%), nausea and vomiting (n=3; 3.6%).

Survey of the Hospital Centre of Rennes 2005
This study was a retrospective observational cohort safety study performed by the Centre Hospitalier Régional de Rennes (France). The main objectives of the study were to determine the reasons for early discontinuation of treatment with 3,4-DAP and to evaluate the safety of use profile of 3,4-DAP. A total of 669 patients were included, the vast majority with multiple sclerosis (n=665). Three patients had LEMS and 1 patient myotonic dystrophy. 3,4 DAP was administered in multiple oral doses. The daily dose was 20 or 30 mg administered in two or three daily divided doses in 77,6 % of patients. The three patients with LEMS received higher doses, 30, 50 and 80 mg, respectively. The mean duration of treatment was 6.2 months, median 2 months, and range 0-51 months. 53.8 % of patients received treatment for 1 or 2 months. There were 124 reports of adverse reactions over the survey period, corresponding to 122 patients (18.2% of 669 patients exposed). Of these, four cases were serious. There were 59 reported cases of paraesthesias occurring in 14 men and 45 women. Several types were distinguished, particularly peri-oral paraesthesias, which appears to be the most common form of paraesthesias related to 3,4-DAP. Seven cases of positive re-exposure were reported. Three cases of convulsions or exacerbation of epilepsy were reported following the administration of 3,4-DAP. 3,4-DAP was not considered to be a contributor in two cases, but could not be excluded in the third case. Five cases of worsening of Raynaud-like syndrome and peripheral coldness have been also reported.

The information on AEs has been included in the Section 4.8 of the SmPC.

- Serious adverse event/deaths/other significant events

From the information available through the French ATU programme, there would appear to have been one death in a patient exposed to DAP salt, although this was not found to be related to the drug as the patient died of their pre-existing small cell lung cancer. A further 3 patients have died whilst under treatment with the base formulation, although none of these have been attributed to the drug.
The most frequent serious adverse event is seizure. Based on safety data from controlled trials six patients have experienced single seizures: five after 3,4-DAP treatment at high doses (100 mg/day). Sanders et al. (2000) described one patient who continued to take 3,4-DAP at a lower dose for more than 10 years and has had no further seizures. The second patient who was taking 3,4-DAP 100 mg/day experienced seizures while receiving toxic doses of theophylline; seizures did not recur on the same dose of 3,4-DAP after theophylline was discontinued. In the same study a third patient with metastatic cancer in the brain had seizures while taking 3,4-DAP 60 mg/day. In a study by McEvoy et al. (1989) one patient had a seizure after 10 months of treatment. This occurred shortly after the total daily dose of 3,4-DAP was increased from 90 mg to 100 mg and the daily dose of pyridostigmine was doubled (from 120mg to 240 mg). Seizures did not recur with the patient taking a daily dose of 40 mg of 3,4-DAP. One case of grand mal seizure occurred in a double-blind study. Three other cases of seizures were reported from the Rennes study, two of them with unlikely causality. The risk of seizures appears to increase with the dose of 3,4-DAP; seizures appear to be rare below doses of 80 mg/day. As discussed earlier, the potential of 3,4-DAP to affect the seizure threshold has been addressed by including relevant contraindications, warnings and precautions in the SmPC and by the risk management plan.

Serious adverse events other than seizures included one episode each of chorea, and myoclonia. In the DAPSEL study an episode of elevated liver enzymes was seen. The increase in liver enzymes was reversible and laboratory variables returned to normal ranges 1 month later. The case was considered as “related” to study medication. In the French ATU programme a serious case of gastro-oesophageal reflux in a patient with CMS was reported. The causal relationship between the AE and 3,4-DAP administration was considered doubtful.

There are two reports of serious cardiovascular events. One non-fatal cardiac arrest was possibly due to iatrogenic intoxication and occurred in a 65-year-old woman who received 3,4-DAP 60 mg six times a day instead of 10 mg six times a day. The second case was a myocardial infarction that occurred a few weeks after starting 3,4-DAP. The authors considered this event as a possible "result of sudden increase of physical activity".

Effects on QTc
In the DAPSEL study, QTc levels were analysed at the subjects' individual C_{max}. No increase in QTc was observed at any time-point after administration of the study medication. However, single low doses of 10 mg or 20 mg of 3,4-DAP were administered in this study.
A case report from Japan which concerned a 57-year-old man with LEMS associated with euthyroid Hashimoto's disease revealed a slight prolongation of QT interval corrected by heart rate (0.46 ms; normal 0.36- 0.44 ms). The patient was treated with 3,4-DAP 90 mg/day and azathioprine 100 mg/day and remained well 2 years after the diagnosis.
The Rennes report included one case of ventricular arrhythmia confirmed on Holter.
Additional safety data from a multicentre randomised, double-blind, placebo-controlled study (MINOSEP) of 3,4- DAP in the treatment of fatigue in multiple sclerosis will be submitted when the clinical study report is available. From available raw data one case of prolonged QTc interval has been reported, which led to premature withdrawal of the patient from the study. This adverse event was considered to be of 'moderate severity' and 'probably related' to the 3,4-DAP treatment.
As mentioned before, the CHMP has requested a thorough QT/QTc study in healthy volunteers in line with ICH E14 guidelines to address this issue.

- Laboratory findings

Evidence from the literature shows that renal, haematological or endocrine function tests have not revealed changes during 3,4-DAP therapy.
There was one serious adverse event with elevations in ASAT and ALAT in the bioequivalence trial DAPSEL. For the subject in question, the last blood samples used for clinical laboratory testing were drawn approximately 2 weeks prior to exposure, and not immediately prior to drug exposure, as for all other subjects enrolled. Therefore, it is possible that the elevations in liver enzymes were present prior to drug exposure. Liver enzymes decreased after treatment cessation; however this cannot be interpreted as only related to the study drug, since a possible prior exposure to a liver affecting substance cannot be excluded. Insufficient documentation prevented complete assessment of this case.
Additionally, literature data report an asymptomatic rise of liver enzyme levels in 1 patient at a daily dose of 60 mg 3,4-DAP. Moreover, an isolated gamma GT increase was reported in a patient treated with 30 mg/day 3,4-DAP who already presented with a high level of gamma GT (3xULN) before 3,4-DAP treatment initiation.

- Safety in special populations

No studies have been conducted in patients with hepatic or renal impairment. The appropriate information is included in the SmPC.

Clinical data on exposure during pregnancy with 3,4-DAP are limited. Only two literature reports concern fetal exposure to 3,4-DAP. In both cases, the pregnancies were uneventful and the babies were healthy (Lecky 2006; Pelufo-Pellicer et al., 2006). Neither of these reports mention lactation when using 3,4-DAP. As discussed earlier the applicant has therefore committed to conduct reprotoxicity studies, according to current guidelines, as a follow-up measure to determine the potential for teratogenic effects of 3,4-DAP phosphate. Moreover, as mentioned previously, the lack of reproductive toxicity data is dealt with by an inclusion of strict wording in the 4.6 section of the SmPC to warn against exposure during pregnancy and / or lactation in W(o)CBP.

Limited data are available on paediatric patients. There are two studies where 3,4-DAP has been used to treat congenital myasthenia gravis and juvenile myasthenia gravis. In one double-blind placebo-controlled crossover study, 11 patients with congenital and five with juvenile MG, aged 5 to 24 years, were treated with 3,4-DAP. The daily dose varied between 10 and 80 mg. Maximum dose was 30 mg

in patients aged 5 to 8 years, 60 mg in patients 8 to 14 years and 80 mg in patients over 15 years. Side-effects were observed in six patients during treatment with 3,4-DAP, and consisted of headache (n=2), numbness around the mouth and fingers (n=6), nausea (n=4) and photophobia (n=1) (Anlar et al., 1996).
In an open label prospective trial, 17 patients with congenital myasthenia (aged 7 to 47 years) received 3,4-DAP at doses between 5 to 20 mg three or four times daily. Most of the patients experienced peri-oral or distal paraesthesia and some exhibited mild hyper-excitability during the initial period of dose adjustment. The publication also reported on a girl aged 5 years with severe congenital myasthenia, who was not included in the study. After receiving the drug without incident for several weeks, she developed a fatal pneumonia. The authors reported that the fatal pneumonia was not thought to be causally related to the treatment with 3,4-DAP (Palace et al. 1991). The limited clinical experience with 3,4-DAP in paediatric population is adequately reflected in the SmPC. Furthermore, the clinical indication for 3,4-DAP recommends its use in adults.

- Safety related to drug-drug interactions and other interactions

Intrinsic Factors

Patients with LEMS have an extreme sensitivity to depolarizing and non-depolarizing muscle relaxants (succcinylcholine, turbocurarine, etc.). Prolongation of neuromuscular blockade requires reduced doses of such drugs for anaesthesia. Indeed, drugs with neuromuscular blocking action should be avoided if possible. If their use is necessary, neuromuscular transmission should be closely monitored. 3,4-DAP should be continued until time of surgery and recommenced as soon as possible.

Extrinsic Factors

3,4-DAP and anaesthesia
Relief of neuromuscular blockade (in particular with vecuronium) using neostigmine or atropine is usually ineffective in patients with LEMS. 3,4-DAP provides rapid relief in such cases. 3,4-DAP should be continued until time of surgery and recommenced as soon as possible.

Drugs compromising neuromuscular transmission
The following drugs compromise neuromuscular transmission and frequently exacerbate weakness in LEMS:

- d-tubocurarine and pancuronium
Competitive neuromuscular blocking agents such as d-turbocurarine and pancuronium have an exaggerated and prolonged effect in patients with LEMS.

- Antibiotics
Aminoglycosides, especially gentamycin, kanamycin, neomycin or streptomycin and fluoroquinolone have a significant neuromuscular effect.

- Cardiovascular drugs
Some anti-arrhythmics as quinine, quinidine and procainamide worsen myasthenic weakness, as do beta-adrenergic blocking agent and calcium channel blockers.

- Others
Magnesium and intravenous iodinated radiographic contrast media could worsen LEMS. In general patients with LEMS should be observed for clinical worsening after any new medication is begun.

Temperature
The weakness associated with LEMS may be worsened when the ambient temperature is elevated or when the patient is febrile. Patients should avoid hot showers or baths.

- Discontinuation due to adverse events

One participant was withdrawn from DAPSEL study due to elevation in liver enzymes.
Between December 2006 and 17 March 2008, five patients have discontinued the treatment under the French ATU program: two patients withdrew due to gastrointestinal disturbances and three – due to non-safety reasons. According to available raw data from the MINOSEP study one case of prolonged QTc interval led to premature withdrawal of one patient from the study.

- Post marketing experience

There is no post-marketing data for 3,4-DAP phosphate.

- Discussion on clinical safety

Based on the available data approximately 2300 patients have been exposed to 3,4-DAP and approximately 282-324 patients suffered from LEMS. Most of the patients were exposed to the 3,4-DAP base.

The extent of patient exposure to the proposed salt formulation is limited to the 26 healthy subjects involved in the DAPSEL study and the 82 patients treated under the therapeutic use protocol in France. The exposure to the base formulation is documented in the published literature rather than in sponsored clinical trials therefore these safety data could not be fully examined. However, the patient numbers, dosage and treatment duration have been provided both for the total number of patients who have been exposed to 3,4-DAP, and for all patients with the diagnosis of LEMS. Overall, the CHMP has considered that sufficient data on patient exposure has been presented. To provide additional data and to further monitor the exposure to 3,4-DAP, the patient registry will be established by the applicant as a specific obligation of the marketing authorisation under exceptional circumstances.

Paraesthesia is the most commonly seen side-effect with DAP. In some patients, transient dizziness, a sense of light-headedness, heavy-headedness or fatigue developed after 3-4 DAP intake. Abdominal pain, epigastric distress or abdominal cramps were other frequent adverse reactions seen with 3,4-DAP. Seizures are the most frequent serious adverse event, with a suggestion that this risk increases with increasing dose. This is reflected in the SmPC as a contraindication to use of 3,4-DAP in patients with epilepsy. Other serious adverse events observed included movement disorders. There were also reports on liver enzymes elevation after 3,4-DAP, including a patient in the DAPSEL study who was withdrawn from the treatment. Other treatment discontinuations were observed under the French ATU programme, where two patients were withdrawn due to gastrointestinal disturbances and the MINOSEP study – withdrawal due to a QTc prolongation. Information regarding adverse reactions is reflected in section 4.8 of the SmPC.

There is a potential for the 3,4- DAP to affect repolarisation, however the extent to which 3,4-DAP is associated with QT prolongation remains unclear. The QTc prolongation has been identified as an important potential risk in the RMP and 3,4-DAP cardiac effects will be monitored in the patient registry and through yearly ECG (SmPC Section 4.4). Furthermore, as discussed earlier, a thorough QT/QTc study according to ICH E14 guidelines will be conducted as a specific obligation to which this marketing authorisation is subject.

Due to limited information available warnings have been inserted in the SmPC concerning the unknown risks for pregnant and lactating women. As mentioned earlier, the applicant has also committed to performing a reprotoxicity study as a follow-up measure. The limited clinical experience with 3,4-DAP in the paediatric population is adequately reflected in the SmPC. Furthermore, the clinical indication for 3,4-DAP recommends its use in adults only.

As a final point, the LEMS population is at increased risk of cancer, and the carcinogenic potential of amifampridine is not yet elucidated. The RMP will include active monitoring of the treated population concerning the development of malignancies, and a carcinogenicity study will be performed as one of the specific obligations to which this marketing authorisation is subject.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan

Table Summary of the risk management plan

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Important identified risks		

▪ Seizures	▪ Routine pharmacovigilance
	▪ Cumulative review of topic in each PSUR
	▪ Zenas® Registry
		Labelling:
		▪ Contraindication for patients with epilepsy
		▪ Warning/precaution statement on seizure, including risk minimisation recommendations:
		  ▪ Description of risk groups and dose dependency
		  ▪ Recommendations for treatment discontinuation
		▪ Interactions section includes statement on seizure threshold lowering drugs
		▪ Listed as undesirable effect

Important potential risks		
▪ Movement disorders	▪ Routine pharmacovigilance ▪ Cumulative review of topic in each PSUR ▪ Zenas® Registry	▪ Chorea and myoclonia listed as undesirable effects

▪ Cardiac toxicity including QTc prolongation	▪ Routine pharmacovigilance
	▪ Cumulative review of topic in each PSUR
	▪ Zenas® Registry
	▪ Review of ECG data from MINOSEP study
		Labelling:
		▪ Contraindication for patients taking sultopride
		▪ Contraindication in patients with congenital QT syndromes of concomitant use of drugs with a known potential to cause QTc prolongation
		▪ Warning/precaution statement on:
		  ▪ concurrent use with drugs with QTc prolonging potential
		  ▪ clinical and ECG monitoring at initiation of the treatment, yearly thereafter, and immediately in case of signs and symptoms indicative of cardiac arrhythmias

		▪ Interactions section includes statement on drugs with QTc prolonging potential ▪ Cardiac rhythm disorders listed as undesirable effect
▪ Peripheral vascular disorders / Raynaud's phenomenon	▪ Routine pharmacovigilance ▪ Cumulative review of topic in each PSUR ▪ Zenas® Registry	Labelling: ▪ Listed undesirable effect
▪ Respiratory disorders incl. bronchospasm	▪ Routine pharmacovigilance ▪ Cumulative review of topic in each PSUR ▪ Zenas® Registry	Labelling: ▪ Contraindication for patients with uncontrolled asthma ▪ Warning/precaution that asthma patients should be closely monitored ▪ Listed undesirable effect
▪ Hepatotoxicity	▪ Routine pharmacovigilance ▪ Cumulative review of topic in each PSUR ▪ Zenas® Registry	Labelling: ▪ Elevated liver function tests listed as undesirable effect
▪ Serious gastrointestinal conditions	▪ Routine pharmacovigilance ▪ Cumulative review of topic in each PSUR ▪ Zenas® Registry	Labelling: ▪ Epigastralgia, diarrhoea, nausea and abdominal pain listed as undesirable effects ▪ No labelling regarding serious gastrointestinal conditions
Important missing information		
▪ Lack of non-clinical data on carcinogenicity, pharmacokinetics, and long-term toxicity	▪ Routine pharmacovigilance ▪ Cumulative analysis of ADR reports after long-term use of Zenas® to be included in each PSUR ▪ Cumulative analysis of all neoplasms reported from patients with non-paraneoplastic LEMS to be included in each PSUR ▪ Zenas® Registry will yield additional clinical information on long term use which will capture any potential adverse events indicating any carcinogenic events	Labelling: ▪ Pharmacological properties section emphasises lack of information with regard to these topics
▪ Lack of information on use in patients with hepatic disease or renal impairment	▪ Routine pharmacovigilance ▪ Cumulative review of ADR reports and other information on use in these populations in each PSUR ▪ Zenas® Registry may yield	Labelling: ▪ Warnings/precautions section: ▪ communicates lack of information regarding patients with renal or hepatic disease,

	additional information	▪ recommends close monitoring of patients with renal or hepatic impairment, and ▪ advises that dose adjustment may be needed in these patient groups
▪ Lack of clinical information on paediatric use	▪ Routine pharmacovigilance ▪ Cumulative review of ADR reports and other information on paediatric use in each PSUR ▪ Zenas® Registry may yield additional information (see Section 2.3)	Labelling: ▪ SmPC states that use in this patient group is not recommended due to lack of data on safety and efficacy (section 4.2)
▪ Lack of non-clinical data on reproductive toxicity and information on use during pregnancy and lactation	▪ Routine pharmacovigilance ▪ Cumulative review in each PSUR ▪ Zenas® Registry may yield additional information (see Section 2.3)	Labelling: ▪ Warning/precaution recommending effective contraception ▪ Pregnancy/lactation section communicates lack of information discourages use during pregnancy or breast feeding, and recommends effective contraception ▪ Pharmacological properties section emphasises lack of information with regard to reproductive toxicity
▪ Lack of information on potential drug-drug interactions (incl. torsades de pointes and/or QTc prolonging drugs, seizure threshold reducing drugs, atropinic and cholinergic drugs, depolarising and non-depolarising muscle relaxants, CYP-dependently metabolised drugs)	▪ Routine pharmacovigilance ▪ Cumulative review of reports of suspected drug-drug interactions in each PSUR ▪ Review of ADRs reported from patients concomitantly receiving CYP-dependently metabolised drugs, QTc prolonging drugs, and heart rate reducing drugs in each PSUR ▪ Zenas® Registry may yield additional information	Labelling: ▪ Interaction section communicates these potential interactions and the assumed clinical consequences ▪ Warning/precaution to carefully monitor when amifampridine is used concomitantly with other medicinal products ▪ Warning/precaution in addition regarding drugs with torsades de pointes and/or QTc prolonging potential and seizure threshold lowering drugs
▪ Lack of photosafety data	▪ Routine pharmacovigilance ▪ Cumulative review of reports of suspected drug-drug interactions in each PSUR ▪ Zenas® Registry may yield additional information	Labelling: ▪ Currently none

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

2.6 Overall conclusions, risk/benefit assessment and recommendation

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

Non-clinical pharmacology and toxicology
Limited information is currently available on the non-clinical pharmacology, pharmacokinetics and toxicology of 3,4-DAP.
The mechanism of action of amifapridine (increase in calcium influx into the nerve and prolongation of the action potential duration leading to the acetylcholine release) supports its use in the proposed clinical indication. The pharmacodynamic properties of 3,4-DAP contribute also to some of the findings observed in the safety studies or reported in the literature including convulsions and possible QTc prolongation. Therefore, as a precautionary measure, Zenas is contraindicated in patients with epilepsy. The risk of convulsions is dose-dependent and is increased for patients with risk factors that are capable of lowering the epileptic threshold, including concomitant use of other medicinal products liable to lower the epileptic threshold. Relevant guidance has been included in the SmPC. Furthermore, due to a theoretical potential for QTc prolongation a thorough QT/QTc study according to ICH E14 guideline will be performed as a specific obligation, and relevant contraindications, warnings and guidance have been included in the SmPC.
The available pharmacokinetic data deriving from the bioequivalence study indicate similar bioavailability of the base form of 3,4-DAP and the phosphate salt thus allowing to cross-refer to the non-clinical profile for 3,4-DAP base. A number of ADME investigations are scheduled to complete the information relating to the pharmacokinetic (PK) profile of the toxicology species and results from these studies will be submitted as a follow-up measure.
The 4-week repeat-dose toxicity studies in rats and dogs revealed 3,4-DAP effects on the central nervous system, increased liver and kidney weight, muscle necrosis and second degree atrio-ventricular block. As the duration of the available toxicology studies was limited to 1 month the applicant has committed to performing a three-month repeat dose toxicology study in rats as a follow-up measure in order to provide chronic toxicology data to support long-term use of the product.
Amifampridine was not genotoxic in a standard battery of in vitro and in vivo tests.
As no specific reproductive toxicity or carcinogenicity studies have been performed the applicant has committed to conducting a carcinogenicity study in the rat as a specific obligation and a reprotoxicity study as a follow-up measure. The CHMP considered these commitments together with appropriate labelling and other risk minimisation measures including the patient registry as an adequate approach.

Efficacy
The two pivotal studies supported efficacy of DAP base in the treatment of LEMS. The study by McEvoy et al. was a small study, however, it was placebo controlled, blinded and showed highly significant results in most measured parameters. As a published article and not a full study report, there are aspects of the study which could not be examined closely, such as the nature of the blinding, randomisation, or placebo control. However, a significant change in the electrophysiological component to the efficacy measurements (CMAP) was observed and this is considered an objective parameter for which blinding was irrelevant. This provided reassurance on the strength of the evidence. This study showed efficacy superior to placebo for 3,4-DAP base in the treatment of LEMS in measured parameters. Similarly, in Sanders et al. the improvement seen in CMAP amplitude reached high significance, again providing reassurance as CMAP amplitude is unlikely to be affected by bias and blinding.

Nevertheless, a number of concerns regarding methodology, endpoints and statistical analyses in the published studies were raised. Consequently, the CHMP had asked the applicant to address concerns over the magnitude of the effect observed by McEvoy et al., the methodology used to analyse data in Sanders et al. and how the measured effects translated into clinically meaningful outcomes. The applicant has discussed the clinical relevance of the treatment effect magnitude in their answers to the CHMP questions. Furthermore, the initial concerns over the trial design have been addressed. The link

between clinical effects and plasma concentrations has also been demonstrated. Therefore, it has been accepted that the statistical evidence to bridge the gap between PK, trial design and efficacy has been demonstrated and the shortcomings of the data used in support of the application were sufficiently well addressed to conclude on the efficacy of the treatment.

Safety

Based on the available data approximately 2300 patients have been exposed to 3,4-DAP and approximately 282-324 patients suffered from LEMS. Most of the patients were exposed to the 3,4-DAP base. The extent of patient exposure to the proposed salt formulation is limited to the 26 healthy subjects involved in the DAPSEL study and the 82 patients treated under the therapeutic use protocol in France. The extent of exposure was considered sufficient. It also allowed delineating identified and potential risks and missing information which will be addressed by the post-approval commitments including the patient registry.

Paraesthesia, dizziness, light-headedness, heavy-headedness or fatigue are the most commonly seen side-effect with DAP. Seizures are the most frequent serious adverse event, with a suggestion that this risk increases with increasing dose. Other serious adverse events include movement disorders.

3,4-DAP is associated with QT prolongation however, it remains unclear to what extent it may affect cardiac conduction. Consequently, QTc prolongation has been delineated as an important potential risk in the RMP and the cardiac effects of 3,4-DAP will be monitored in the patients registry and through yearly ECG. Furthermore, a thorough QT/QTc study according to ICH E14 guidelines will be conducted as a specific obligation.

Due to limited information available warnings have been inserted in the SmPC concerning the unknown risks for pregnant and lactating women. The applicant has also committed to performing a reprotoxicity study as a follow-up measure. The limited clinical experience with 3,4-DAP in the paediatric population is reflected in the SmPC. Furthermore, the clinical indication for 3,4-DAP recommends its use in adults only.

As a final note, the LEMS population is at increased risk of cancer, and the carcinogenic potential of amifampridine is not yet elucidated. The RMP will include active monitoring of the treated population concerning the development of malignancies, and a carcinogenicity study will be performed as a specific obligation.

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

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

The applicant submitted results of user testing of the package leaflet. The results 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. Subsequent amendments to the leaflet made at Day 180 of the procedure were considered to further simplify the leaflet and, hence, do not required further bridging studies. Overall the user testing was found acceptable.

Risk-benefit assessment

This application has been made with data principally derived from literature and case report studies. In spite of the methodological shortcomings, the CHMP was convinced, by the concordance of the limited results, that amifampridine is effective in the treatment of LEMS.

Some aspects need to be further clarified from the safety point of view, in particular concerning reproductive and cardiovascular safety, and the carcinogenic potential. These points are addressed in the SmPC, post-approval commitments, and are part of the Risk Management Plan for this application. A registry will also be established to monitor patients undergoing treatment.

Although further randomized controlled efficacy and safety data would have been preferable, The CHMP considers that there is a sufficient body of data to allow the granting of the marketing authorization under exceptional circumstances.

The CHMP recommends granting this marketing authorisation for Zenas under exceptional circumstances, because the indication for which this product is intended is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence. Therefore the applicant has agreed to provide further evidence as specific obligations relating in particular to the safety and efficacy of Zenas.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- routine pharmacovigilance was adequate to monitor the safety of the product
- no additional risk minimisation activities were required beyond those included in the product information.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus decision that the risk-benefit balance of Zenas in the symptomatic treatment of Lambert-Eaton myasthenic syndrome (LEMS) in adults was favourable and therefore recommended the granting of the marketing authorisation under exceptional circumstances.