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Committee for Medicinal Products for Human Use (CHMP)

Assessment report

ELMISOL

International non-proprietary name: levamisole

Procedure No. EMEA/H/C/004330/0000

Note

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

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List of abbreviations

Abbreviation	Definition
AMC	Academic Medical Center
AD	Alternate Day
ALT	Alanine aminotransferase
ANCA	Antineutrophil cytoplasmic antibody
AST	Aspartate aminotransferase
ATC	Anatomic therapeutic class
AUC	Area under the plasma concentration-time curve
BCS	Biopharmaceutics Classification System
CI	Confidence Interval
CL	Clearance
Cmax	Maximal concentration
ECG	Electrocardiogram
FOCE	First-order conditional estimation
GFR	Glomerular filtration rate
h	Hour
HPLC	High performance liquid chromatography
INR	International Normalization Ratio
kg	Kilogram
l	Litre
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LRT	Likelihood ratio test
mg	Milligram
mm	Millimeter
MMF	Mycophenolate mofetil
OFV	Objective function value
PD	Pharmacodynamic
popPK	Population pharmacokinetics
PT	Prothrombin time
QC	Quality control
RCT	Randomized clinical trial
rpm	Rotations per minute
RR	Risk ratio
SAP	Statistical analysis plan
SD	Standard deviation
SSNS	Steroid Sensitive Nephrotic Syndrome
tmax	Time at which the maximal concentration is observed
µg	Microgram
Vd	Volume of distribution
WHO	World Health Organization

1. Recommendations

Based on the CHMP review of the data on quality, safety, efficacy and risk management plan, the CHMP considers that the application for Elmisol, an orphan medicinal product, in the treatment of Steroid Sensitive Nephrotic syndrome in children from 2 years up to 18 years,

is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Questions.

In addition, satisfactory answers must be given to the "other concerns" as detailed in the List of Questions.

The major objections precluding a recommendation of marketing authorisation, pertain to the following principal deficiencies:

- The visual distinguishability of the different tablet strengths, which may lead to medication errors
- The missing evidence about the content of levamisole in the tablets used in the pivotal study due to lack of data on validation of the previous UV method (used for content, uniformity of content and for dissolution of levamisole)
- The lack of cross validation data for the changed method for levamisole content (used for content, uniformity of dosage units and dissolution) and of stability data for content and dissolution.
- The lack of data that exclude the carcinogenic potential of levamisole
- The unreliability of the reported plasma concentrations after prolonged storage time due to instability of levamisole in plasma during the storage time.
- The absence of data on the interaction potential between levamisole and other medicinal products, on transporters and the metabolic pathways of levamisole and on the interference on other metabolic pathways.
- A negative PK or PD interaction between levamisole and corticosteroids cannot be excluded. The appropriate time point of initiation of levamisole therapy after remission is unclear.
- The GCP compliance of the pivotal study is questioned
- The proposed indication does not appropriately reflect the patient population included in the clinical study

Inspection issues

GCP inspection(s)

A request for GCP inspection has been adopted for the following clinical study ACE080503 (EudraCT: 2005-005745-18). The outcome of this inspection and the satisfactory responses to its findings are part of the responses to the LoQ and will be needed by Day 121.

2. Executive summary

2.1. Problem statement

2.1.1. Disease or condition

ELMISOL is indicated for the treatment of Steroid Sensitive Nephrotic syndrome (SSNS) in children and adolescents from 2 years up to 18 years.

2.1.2. Epidemiology

Nephrotic syndrome is a condition in which the glomeruli of the kidney leak protein from the blood into the urine. Nephrotic syndrome results in hypoproteinaemia and generalised oedema. The incidence of nephrotic syndrome in Europe, North America and New Zealand is about 2/100,000 in children younger than 18 years, and the prevalence is 16 per 100,000 children. There is clear geographical variation, with a higher incidence reported in South Asian populations (3–9 per 100,000 children). In the Netherlands (16 million people) NS is diagnosed yearly in approximately 57 paediatric patients (http://www.zorgstandaarden.net/zza/media/zorgstandaard/20140123-001/index.html#16).

2.1.3. Aetiology and pathogenesis

Nephrotic syndrome is characterised by a triad of heavy proteinuria (urine protein/creatinine ratio ≥200 mg/mmol or ≥3+ proteinuria on urine dip- stick), hypoalbuminaemia (<25 g/l) and oedema. Most children have minimal change disease, in which changes on light microscopy are minor or absent. The cause of minimal change nephrotic syndrome is unknown. Although the immunological mechanisms involved in SSNS are not well understood, evidence is accumulating for the involvement of T and B cells in these immune mechanisms.

2.1.4. Clinical presentation, diagnosis

Since most children with nephrotic syndrome do not undergo a kidney biopsy (initially) their clinical classification is based upon their response to steroids (see definitions below). This approach has been widely used since the International Study for Kidney Disease in Children (ISKDC) trials in the 1960s demonstrated that 93% of children with SSNS have minimal change disease, which carries an excellent long term prognosis. Although 80%-90% of patients are initially steroid sensitive, a similar proportion will suffer one or more relapses. At some point in their disease course around 50% of children meet the diagnostic criteria for frequently relapsing nephrotic syndrome (FRNS) or steroid- dependent nephrotic syndrome (SDNS). Relapses occur most commonly during periods of increased immune activity, such as during an upper respiratory tract infection (URTI).

Definitions

- Remission - Three consecutive days of trace or negative proteinuria* on dipstick
- Relapse - Three consecutive days of ≥3+ proteinuria on dipstick
- Steroid sensitive - Remission with an initial 8-week course of oral steroids
- Steroid resistant - No remission after an 8-week course of oral steroids†
- Frequently relapsing - Four or more relapses in 12 months, or two or more in the initial 6 months of therapy

- Steroid dependent - Two consecutive relapses while on, or within 14 days of ceasing, corticosteroids

**Formal quantification of proteinuria may be performed using a spot urine protein: creatinine ratio; a value of<20 mg/mmol corresponds to remission and >200 mg/mmol to relapse.*

†As defined by International Study for Kidney Disease in Children (ISKDC) and Kidney Disease: Improving Global Outcomes (KDIGO); definitions used in other studies vary from 4 to 8 weeks.

2.1.5. Management

The long-term prognosis for most children with SSNS is for complete resolution of their disease over time and maintenance of normal kidney function. Therefore, limiting the long-term adverse effects of treatment is an important objective. The first-line treatment for children presenting with idiopathic nephrotic syndrome is oral corticosteroids. Of children who present with their first episode of nephrotic syndrome, 80% to 90% will achieve remission with corticosteroid therapy, and have steroid-sensitive nephrotic syndrome (SSNS). However, 80% of children experience a relapsing course with recurrent episodes of oedema and proteinuria, and of these children, half relapse frequently either a few weeks after ceasing corticosteroids (frequently relapsing SSNS) or while on reducing doses of corticosteroids (steroid-dependent SSNS) (Pravitsitthikul, The Cochrane Library, 2013). While corticosteroids lead to remission in most children with idiopathic nephrotic syndrome, most children relapse one or more times and many experience significant adverse effects, including impaired linear growth, behavioral changes, obesity, Cushing's syndrome, hypertension, ophthalmological disorders, impaired glucose tolerance, and reduced bone mineral density. Adverse effects may persist into adult life in young people, who continue to relapse after puberty.

Prior to treatment with corticosteroids there was a high mortality rate from associated sepsis and thrombosis. However, the capacity to induce remission with corticosteroids means that the majority of children now outgrow the condition without serious long-term sequelae. (Larkins, Arch Dis Child, 2016 p404-8). Before the use of corticosteroids and antibiotics, 40% of children died, with half of these deaths being from infection. A recent study reports only one death (0.7%) associated with nephrotic syndrome among 138 children with SSNS presenting between 1970 and 2003 (KDIGO).

Non-corticosteroid immunosuppressive medications are used to prolong periods of remission in these children. Recent guidelines recommend alkylating agents (cyclophosphamide and chlorambucil), levamisole, calcineurin inhibitors (cyclosporine, tacrolimus) and MMF in these children but there is no consensus as to the most appropriate second line agent in children who are steroid sensitive, but who continue to relapse. Clinical guidelines do no recommend the one agent over another. In the Netherlands cyclosporine is the only registered drug for steroid resistant (SRNS) or steroid dependent (SDNS) nephrotic syndrome. Adverse effects of alkylating agents include increased risk of infection and reduced fertility. For calcineurin inhibitors (cyclosporine, tacrolimus) these include kidney dysfunction and hypertension. Low-dose daily or alternate-day corticosteroids may still be required to maintain remission in SD SSNS, despite receiving corticosteroid-sparing agents.

According to the applicant, an advantage of levamisole over other treatments is that it has less severe side effects.

2.2. About the product

Levamisole is a product which has been used for more than 25 years in the indication of idiopathic nephrotic syndrome (INS). The first report in the literature was that of Tanphaichitr et al. in the Journal of Pediatrics (1980). Since the latter, 758 Levamisole-treated patients with INS are reported up to 28 June 2006 in 75 references obtained by combining the terms “Levamisole” and “nephrotic syndrome” in the data base PubMed.

The investigational product has been formulated as an immediate release tablet. For taste masking purposes the tablets are properly film-coated. A sincere consideration has been the choice of tablets for all ages in this study population. It has been proven to satisfaction of EMEA that each tablet size (5 mm, 6 mm, 7 mm and 8 mm diameter for resp. 5 mg, 10 mg, 25 mg and 50 mg Levamisole) has the same dissolution profile.

In addition, levamisole is the active lava-isomer of tetramisole hydrochloride and is used as a broad spectrum antihelmintic agent in cattle, sheep, poultry and pigs. Levamisole or levamisole hydrochloride is for this indication registered in all countries in the EU. It has, however, also been used in man, in particular for the treatment of ascariasis and hookworm infections (19). Levamisole is active against intestinal Nematode worms and appears to act by paralyzing susceptible worms which are subsequently eliminated from the intestines. Levamisole also affects the immune response. The term immunostimulant has often been used, which is appropriate only in cases of a depressed immunorespons. Stimulation above normal levels does not seem to occur. Levamisole has been tried in a wide variety of disorders involving the immune response, including bacterial and viral infections and rheumatic disorders (19). Levamisole has also been used as an adjunct in patients with malignant diseases. Recurrences of adenocarcinoma of the colon are inhibited by adjuvant treatment with Levamisole and Fluorouracil following resection of tumors, with regional lymph node involvement (19, 20).

2.3. The development programme/compliance with CHMP guidance/scientific advice

On 20 December 2005 the company ACE Pharmaceuticals BV requested protocol assistance for their product Levamisole. The company provided the EMEA with questions concerning the quality, preclinical and clinical development. During its meeting held on 20-23 March 2006, the CHMP adopted the advice to be given to the company. During its meeting held on 4-5 April 2006, the COMP adopted the advice to be given to the company on significant benefit. (Procedure No.: EMEA/H/SA/684/1/2006/PA/III).

On 18 August 2006 the company ACE Pharmaceuticals BV requested additional protocol assistance for their product Levamisole. This request is a follow-up to the advice provided by CHMP in March 2006. The company provided the EMEA with questions concerning the quality, preclinical and clinical development. During its meeting held on 16-18 October 2006, the CHMP adopted the advice to be given to the company. During its meeting held on 7-8 November 2006, the COMP adopted the advice to be given to the company on significant benefit. (Procedure No.: EMEA/H/SA/684/1/FU/1/2006/PA/III).

In the scientific advice for protocol assistance EMEA/CHMP/SAWP/94036/2006, it was indicated by the CHMP that bridging data will be needed to bridge the data to the marketed Ergamisol. A comparison of the pharmacokinetics of the 'old' formulation used to support data in adults with the new formulation to be used in children should be carried out. This advice concerned dispersible tablets. After this

advice, the applicant changed its formulation. In an additional scientific advice (EMEA/COMP/SAWP/396311/2006) the CHMP agreed that levamisol can be considered a BCS Class I drug and that a bioequivalence study can be waived using biowaiver principles. In vitro dissolution data could support the bridging between the new and previous formulations. However this advice could not be followed up, as in this application, the applicant indicated that the Ergamisol tablets have been withdrawn from the market in April 2004, so direct comparison is not possible anymore. This application will thus be assessed on its own merits. Moreover, although the CHMP concluded that levamisole could be considered a BCS Class I drug, based on the provided data in this submission this could not be concluded.

In procedure No.: EMEA/H/SA/684/1/FU/1/2006/PA/III, the CHMP indicated that juvenile toxicity studies, in which safety pharmacology endpoints could be incorporated, should be conducted. This was later considered not needed by the PDCO, and juvenile toxicity studies were not initiated by the Applicant. The pharmacokinetic programme was not considered to be sufficient by the CHMP but recommendations to identify substrates of levamisole metabolites in order to evaluate potential drug-drug interactions have, to date, not been followed up by the Applicant. Finally, the CHMP considered that the toxicological programme was not sufficient because of disparate results in in vivo clastogenicity studies and the absence of updated genotoxicity studies. The recommendation to update the genotoxicity programme and justify the equivocal data has not been adopted by the Applicant. A carcinogenicity study was also considered by the CHMP, but has not been initiated by the Applicant because recent peer reviewed publications were available and were submitted instead.

2.4. General comments on compliance with GMP, GLP, GCP

Issues have been raised on GCP.

This includes an uncertainty in the validity of the primary analysis. The average quantity of prednisone intake during the study was not evaluated due to incomplete recordings. This questions the validity of this primary analysis, in particular during the period when patients were still down-titrated with prednisone. Non-compliance to prednisone intake could initiate a relapse event and thus highly interfere with the primary analysis of the study. While this also seriously questions whether the study has been performed according to GCP recommendations.

Further, there appear some inconsistencies regarding the defined objectives, defined endpoints and endpoints evaluated across the SAP, clinical study protocol and study report. These should be clarified as these question whether the study was GCP compliant. These inconsistencies pertain to the following:

- There is discrepancy between the Clinical Study Report and the Statistical Analysis Plan with regard to the secondary objectives. While the SAP indicates that the only secondary objective is to evaluate whether the effect of Levamisole varies with the following subject characteristics: underlying disease process (steroid dependency); prior use of cyclophosphamide; age group; ethnicity; site group, the CSR indicates that the steroid-sparing effect of levamisole treatment as well as the sparing effect of other immunosuppressive drugs will be assessed, for which no data have been presented.
- There is a discrepancy between the Clinical Study Report and the Clinical Study Protocol (version 6.0) with regard to the secondary endpoints. While relapse after prednisone cessation was described as the only secondary outcome in the study report, the secondary endpoints that have been described in the study protocol are evaluation the average quantity in

milligrams of steroids administered per month during the study, an evaluation whether treatment effect differs with underlying disease process (steroid-dependency yes/no) and prior use of disease modifying agents (yes/no), and maintenance dose prednisone at time of relapse.

Consequently, there is some discrepancy between the description of the secondary *objectives* and the description of the secondary *endpoints*.

- The final SAP was drawn up after data collection was complete; the SAP is dated 19-12-2013, whilst the last patient completed the cohort on 15 October 2013. This questions whether there was insight in the (blinded) data at the time of drawing up the final SAP.
- Partial unblinding during the study was an issue of concern. Unblinding was permitted after completion of the RCT in order to make informed decisions about further treatment. It is understood that patients were unblinded at the latest after one year while the study was still ongoing. This has to be clarified by the applicant since this may have a relevant impact on the robustness of the data.
- For the measurements of PK as part of the pivotal study plasma samples were stored with a Median storage time at $\leq$-20^{o}C of 987 days (range 0-2101 days) (final pop-PK report, page 14). However, long term stability testing covered only a period of up to 486 days according to the POP-PK report. The validation report includes data up to 206 days (validation bioanalytical report page 15). Therefore, most of the plasma samples were measured after storage times without validation of stability.
- Patients with frequently relapsing disease were eligible according to the inclusion criteria but also patients with infrequently relapsing disease or without relapses during the past 6 – 12 months were included.
- There were concerns regarding handling of the urine samples. E.g. the urine sample at visit 5 of one subject was negative for levamisole, the urine sample of another subject receiving placebo contained levamisole.

Currently, the EMA has requested a for cause inspection to be started in early 2017.

2.5. Type of application and other comments on the submitted dossier

- Legal basis

The present application for Elmisol has been submitted through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004 (i.e. the mandatory scope). The application is a full application in accordance to Article 8(3) of Directive 2001/83/EC.

- Accelerated procedure

N/A

- Conditional approval

N/A

- Approval under exceptional circumstances

N/A

- Biosimilar application

N/A

- CHMP guidelines/Scientific Advice

The Applicant requested Protocol Assistance twice. First in December 2005, after which the CHMP/COMP advice was finalized during the plenary meeting in March 2006 (EMEA/H/SA/684/1/2006/PA/III). Follow-up assistance was requested in August 2006; The CHMP advice was discussed during the October 2006 meeting (EMEA/H/SA/684/1/FU/1/2006/PA/III). The following issues were addressed:

- Non-clinical: CHMP recommended that the applicant should conduct own non-clinical studies. Reference to published literature alone was not sufficient. The non-clinical programme should cover aspects of PD, safety pharmacology, PK and toxicology including a study in juvenile animals. This advice was not followed; only literature data were presented in this application to cover non-clinical aspects.

- **Biopharmaceutical issues:** The applicant did not follow the advice to develop orodispersable tablets due to the bitter taste of levamisole. Four strengths of film-coated tablets were used.

- **PK**: Agreement was reached on the design of a Pop PK study

- **PD/Safety**: The CHMP agreed on the proposal that no thorough QT study was necessary, provided that ECGs were assessed three times during the clinical study and ecg monitoring in dogs was included in the juvenile animal study.

- **Dose finding**: The CHMP agreed that it was not necessary to perform clinical dose-finding studies in patients with nephrotic syndrome since a dose of 2 – 2.5 mg/kg on alternate days has been used for many years by nephrologists in Europe.

- The CHMP agreed that Levamisole can be classified in the category of BCS class I “high solubility and high permeability” API and that formulations of Levamisole HCL can be approved based on strictly defined dissolution criteria as a surrogate for an in vivo BE test.

- **Pivotal study**: Two important hitherto unresolved questions were considered to be addressed by the pivotal trial: Whether levamisole has a significant effect on the clinical course of SSNS and to which extent a steroid sparing effect could be classified as clinically relevant.

The **CHMP Guideline** on the clinical investigation of medicinal products to prevent development/slow progression of chronic renal insufficiency EMA/CHMP/500825/2016 to be in effect by January 2017 addresses some of the issues relevant for this application.

- 1 year data exclusivity

N/A

- Significance of paediatric studies

On 18 March 2016 the European Medicines Agency (EMA) granted a waiver from the European Paediatric Regulation (Regulation [EC] Number 1901/2006) for Elmisol for the condition nephrotic syndrome for the age subset 0 years to 2 years, and agreed to the paediatric investigation plan for Elmisol for the condition nephrotic syndrome for the age subset 2 years to 18 years (procedure number EMEA-001885-PIP01-15; see Module 1.10).

On 8 July 2016 the European Medicines Agency (EMA) agreed to a modification of the agreed paediatric investigation plan for Elmisol for the condition nephrotic syndrome for the age subset 2 years to 18 years (procedure number EMEA-001885-PIP01-15-M01; see Module 1.10).

On 22 July 2016 the PDCO issued a positive opinion on the compliance with the agreed paediatric investigation plan for Elmisol for the condition nephrotic syndrome for the age subset 2 years to 18 years (procedure number EMEA-C-001885-PIP01-15-M01; see Module 1.10).

3. Scientific overview and discussion

3.1. Introduction

Elmisol film-coated tablets contain 5, 10, 25 or 50 mg levamisole (as levamisole hydrochloride) and is indicated for the treatment of Steroid Sensitive Nephrotic syndrome (SSNS) in children and adolescents from 2 years up to 18 years. Elmisol was designated as an orphan medicinal product (EU/3/05/324) in the European Union on 28 October 2005 for this indication.

3.2. Quality aspects

3.2.1. Introduction

Elmisol film-coated tablets contain 5, 10, 25 or 50 mg levamisole (as levamisole hydrochloride). Available pack sizes are 50 tablets for each strength, the 5 mg strength is presented in a bottle configuration, the other strengths in a blister configuration.

3.2.2. Active Substance

General Information

The active substance is levamisole hydrochloride, an established active substance described in the European Pharmacopoeia. The active substance is a white or almost white, crystalline powder and is freely soluble in water. Levamisole hydrochloride contains one stereogenic centre and is manufactured as a pure enantiomer. More information on polymorphism is requested. A CEP has been provided.

Manufacture, characterisation and process controls

The manufacturing process of the API is covered by the CEP. However, potentially genotoxic impurities from the synthesis should be discussed and controlled where relevant.

Specification

The applicant's drug substance specification is in general in line with the Ph.Eur. monograph on levamisole hydrochloride, with an additional tests for microbiological quality. The specification is not acceptable yet in view of the route of synthesis and the various European guidelines. The limit for unspecified impurities needs to be tightened, acceptance criteria for microbiological quality should be revised and particle size and polymorphic form of the API need to be controlled. Furthermore, some

issues on the validation of the UPLC method should be addressed and additional information on the reference standards is requested. Batch analytical data demonstrating compliance with the drug substance specification have been provided for two batches.

Stability

Reference is made to the CEP. However, as no retest period and storage conditions are stated on the currently valid version of the CEP, no retest period can be granted. Supportive stability data should be submitted.

Comparability exercise for Active Substance

N/A.

3.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

The drug product is a film-coated tablet. The tablets are round, biconvex film-coated tablets containing either 5 mg, 10 mg, 25 mg or 50 mg levamisole (as levamisole hydrochloride). The tablets have a diameter of 5, 6, 7 and 8 mm respectively. Tablets are film-coated in order to mask the bad taste of levamisole hydrochloride. The tablet core excipients are lactose monohydrate, maize starch, sodium starch glycolate type A, hydroxypropylcellulose, microcrystalline cellulose and sodium stearyl fumarate. The coating is composed of hypromellose, talc, titanium dioxide, polyethylene glycol 4000 and saccharin sodium. The 5 mg product is packed per 50 tablets in a HDPE container with a PP child-resistant closure in a carton box. The 10 mg, 25 mg and 50 mg tablets are packed in PVC-Al blisters in an outer carton. The excipients and packaging are usual for this type of dosage form. The visual distinguishability of the different tablet strengths is considered insufficient.

The development of the product has been described, the choice of excipients is justified and their functions explained. In general the pharmaceutical development has been adequately performed. The product is intended to be used in children from 2-18 years old. Although the applicant did not follow the scientific advice by the EMEA in 2006 to develop a liquid pharmaceutical form for oral use (or a dispersible or orodispersible tablet as alternative), an acceptable justification has been provided and in view of the recent knowledge, small tablets (also referred to as ‘mini-tablets’) could in principle be a suitable alternative dosage form also for very young children. Acceptability of the proposed formulation has been investigated as part of the clinical trials in children. In general no issues with acceptability were reported. However, some additional justification on the acceptability of the tablet formulation, especially in the youngest children is requested from a clinical point of view. The choice of the packaging and manufacturing process are justified. The batches of drug product that have been used in the pivotal clinical trial were manufactured according to the finalized composition. Only the manufacturing process has been further optimized in 2015. The changes made to the manufacturing process are considered minor changes which was in principle supported by comparative dissolution data. Batch analysis data, including dissolution dissolution results for all clinical batches need to be provided to fully support the bridging of the clinical batches to the finalized product and manufacturing process.

A major objection has been raised on the following. Based on the missing evidence, that the previous UV method for content of levamisole (used for content, uniformity of content and for dissolution) is

specific, precise, linear, accurate and robust, the informative value of the provided results of the development and the clinical batches is questionable. Therefore, for the UV method acceptable validation data should be submitted or all the results before 2014 cannot be assessed.

Manufacture of the product and process controls

The main steps of the manufacturing process are wet granulation, mixing of the granules with the extragranular components, final mixing and lubrication, tabletting, film-coating and packing. In general the manufacturing process has been described in sufficient detail. However, the in-process acceptance criteria are not fully justified yet.

The manufacturing process has been adequately validated according to relevant European guidelines. Process validation data on the product has been presented for insert three full scaled batches per strength.

Product specification

The product specification includes tests for appearance, identity, average mass, dissolution, assay, uniformity of dosage units, related substances and microbiological quality. The specification is not acceptable yet.

The analytical methods have not been adequately described and validated yet. The method for assay and related substances should be described in more detail.

Batch analytical data from the proposed production site have been provided on three full scaled batches per strength in the dossier, demonstrating compliance with the release specification.

Stability of the product

Stability data on the product has been provided on batches of each strength. The batch sizes and manufacturing dates of these batches have not been given and are asked for. The batches were stored at 5°C, 25°C/60% RH, 30°C/65% RH and 40°C/75% RH. The conditions used in the stability studies are according to the ICH stability guideline. The batches were stored in HDPE bottles (5 mg strength) or PVC-Al blisters (10, 25 and 50 mg strengths).

Out-of-specification results for impurities were reported. No clear trends or changes in the tested parameters were observed for the batches stored in a refrigerator (2-8°C) up to 60 months. Based on the available stability data the claimed shelf-life is not justified. Therefore, no shelf-life can be granted at this point and additional stability data need to be submitted. This issue is raised as major objection.

Results of a photostability study in accordance with ICH Q1B have been submitted showing that the drug product is not sensitive to light exposure.

The proposed temperature storage precaution needs to be reviewed.

In-use stability results were provided. Additional results of the in-use study are awaited to fully justify the proposed in-use shelf life and storage conditions.

Comparability exercise for Finished Medicinal Drug Product

N/A.

Adventitious agents

Only the lactose monohydrate used in the product is of animal origin. Compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via medicinal products has been satisfactorily confirmed.

GMO

N/A

3.2.4. Discussion on chemical, pharmaceutical and biological aspects

The active substance is well known, described in the Ph.Eur. and the quality of the drug substance is covered by the CEP. Only some other concerns on the control of the actives substance have been raised. In general the finished product is relatively straightforward and the dossier contains sufficient information on the relevant aspects. Although some further confirmation is asked for on the acceptability of the proposed tablet formulation in children, especially the youngest children, in general the product seems to be age-appropriate.

The main issues that have been identified for the drug product are the visual distinguishability of the different tablet strengths, which is considered insufficient, uncertainty whether a method previously used for levamisole content determination was validated and the incomplete stability data (especially regarding dissolution results).

3.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Major objections on the visual distinguishability of the different tablet strengths, on the validation of a method previously used for levamisole content determination and the incomplete stability data (especially regarding dissolution results) were raised as well as several other concerns that need to be resolved before the drug product can be accepted.

3.3. Non clinical aspects

No GLP or non-GLP compliant studies have been conducted to support this application. No non-clinical study reports have been submitted as part of this application and the non-clinical data is purely bibliographic. For a known active substance, this is acceptable if the submitted literature is of quality and relevance for the application.

3.3.1. Pharmacology

The exact mechanism of action of levamisole in the treatment of children with steroid-sensitive nephrotic syndrome is not well elucidated. Two mechanisms have been proposed by the Applicant that could contribute to the effect of levamisole in steroid-sensitive nephrotic syndrome, each supported by one publication: direct action on kidney podocytes and immunomodulation. Levamisole is able to protect against podocyte injury in an in vitro model of minimal change nephropathy via modulation of the glucocorticoid pathway. The immune-regulatory effect of levamisole correlates with the clinical course of the steroid-sensitive nephrotic syndrome. A study in children suggests that T-cell activity is increased during levamisole administration (n=7), resulting in reduction or discontinuation of prednisolone. It was suggested that this was a result of increased cytotoxic/suppressor T-cells. How

levamisole interacts with T-cells was not investigated. Levamisole is a well-known anthelmintic and is on the WHO essential medicines list, but the Applicant does not provide any discussion on the secondary pharmacology.

Cardiovascular effects in rabbits after IV administration of levamisole included QTc prolongation and considerable bradycardia. QTc prolongation increased from 145 ms and 143 ms to 172 ms and 167 ms (2.5 mg/kg and 5 mg/kg, respectively). No data on ion channel inhibition has been presented. An action of levamisole on L-type calcium channels is conceivable as mechanism underlying these observations. In dogs, mainly ventricular extrasystoles were observed when higher doses were administered. This could be due to levamisole effects on ion channels, but again no further information is available. Relevant QT prolongation was not observed in the animal species tested. ECG recordings from humans taking levamisole should be analysed in more detail; if there are any alterations which cannot be explained further non-clinical studies may be necessary for clarifying the seriousness of levamisole's cardiac effects.

CNS effects were investigated in a series of experiments. Lesions were found in 43 of 58 dogs. These consisted of disseminated perivascular cuffing with mononuclear cells throughout the brain and meninges. The severity of the perivascular cuffing varied considerably and could not be correlated to the length of the drug administration period nor to any other variable. There was no damage to the neural tissues associated with the perivascular cuffing. There was evidence of regression of the lesions whether levamisole treatment was continued or not. Perivascular cuffing may be attributed to the immunomodulating effect of levamisole. It is difficult to fully interpret the relevance of these findings due to the experimental protocol, multiple interventions and progeny of the animals. The publication also states that changes in EEG patterns shortly after oral administration of levamisole suggest a direct effect on the CNS. This is possible, because it has been shown that levamisole is thought to potentiate nicotinic acetyl cholinergic effects. In addition, a metabolite of levamisole, aminorex, is a substrate for serotonin transporters.

Even though the non-clinical section of this MAA is based on literature, a thorough discussion on all pharmacological aspects is expected and required. The primary pharmacology and safety pharmacology has only been summarily described. The current overview is considered to be insufficient, in particular the section on secondary pharmacology, which did not contain any data. The Applicant should discuss the results and relevance of all papers in the IB pertaining to primary, secondary or safety pharmacology and pharmacodynamic drug-drug interactions. This should be followed by an exhaustive search of the available literature to date regarding immune system modulation and podocyte protection of levamisole and the pharmacodynamic data should be discussed in relation to the pathogenic mechanisms of the disease. In addition, information regarding potential binding of levamisole to other potential targets should be submitted.

3.3.2. Pharmacokinetics

Levamisole is rapidly absorbed in rat, rabbit, dog and pig. The bioavailability after oral administration ranged from 49 to 81%. The bioavailability of an oral dose was not significantly different in fasted or fed dogs. Levamisole is quickly distributed after oral administration in mice and rats and can penetrate into the brain of a rat. Binding to proteins is low in chicken, rabbit, sheep, cow, horse and pig. Levamisole was rapidly absorbed in rats. The maximum plasma concentration of 2.11 mg/L was reached after 0.75 hours. The AUC0-12 was 5.38 mg*h/L and the half-life was 1.12 hours. In rats and mice there is predominant and comparable distribution to the liver, kidney, urine, melanin containing organs and uveal tract. The liver concentrates the highest activity in both species as soon as 30 min after oral administration. The most important non-clinical pharmacokinetics publications should be

made available as an approved translation (LoQ). Levamisole is extensively metabolized. Similar metabolic pathways in several species were suggested, at least in a qualitative sense. Much of the data on the metabolism of levamisole is based on the WHO food additives series and contains abstracts of the study reports as submitted by the manufacturer of the innovator drug. While major metabolites have been identified, the main enzymes involved in the metabolism pathway have not been identified (see clinical PK LoQ). Large numbers of minor metabolites were noted in each species but remain unidentified. The major pathways seen in all animal species included scission of the thiazolidine ring followed by aliphatic oxidation and hydrolysis of the thiazolidine ring followed by S-methylation and sulphoxidation. The other major pathway in dogs, pigs, sheep and cattle was dehydrogenation in the imidazolidine ring followed by sulphoxidation. With human hepatocytes, other major metabolites were a result of dehydrogenation in the imidazolidine ring and aromatic hydroxylation. Apart from the latter formation of p-hydroxy levamisole, the major metabolic pathways in humans were also observed in other species in vitro. Enzymes involved in the metabolism of levamisole should be identified in order to rationalize potential drug-drug interactions, which was also requested in the Scientific Advice procedure in 2006. A low first-pass metabolism of levamisole was observed in rabbits. Levamisole is mainly excreted in urine as metabolites of levamisole. Unchanged levamisole is excreted in smaller fractions in rat urine (6.8%) and pig urine (18.1%). In rat, a larger fraction is excreted via faeces (33%). Levamisole is excreted in milk in cows and goats. It is likely that levamisole is also excreted in human milk. In a cirrhosis model of liver insufficiency in rats, levamisole metabolism and biliary excretion was decreased.

3.3.3. Toxicology

Toxicology data is largely based on the WHO Food Additives series, which contains summarized data. Lethality after levamisole poisoning occurred at very high doses after oral administration in rats (≥ 225mg/kg) and mice (≥217 mg/kg). Levamisole is relatively well tolerated in rats receiving 50 mg/kg/d or 100 mg/kg/d levamisole for 30 days, with the only observed effects being increased kidney and liver weights and a moderate reduction in weight gain. When levamisole was administered in rats up to 160 mg/kg for 13 weeks there were no treatment related effects in rats administered a 10 mg/kg dose in feed during 13 weeks with exception of elevated urine pH and decreased weight gain in rats receiving either 40 mg/kg or 160mg/kg levamisole. It is unclear whether animals received the entire dose since it was administered together with feed and this was eaten completely or if food was given ad libitum and rats only ate a portion and therefore were only partly exposed. No remarkable adverse effects occurred in groups up to 160 mg/kg. However, the relevance of this study is questionable since the methodology is not in accordance with the current standards.

In a subacute toxicity study in dogs, animals received an oral levamisole dose of 25 mg/kg followed by an SC dose of atropine one hour later. Levamisole was poorly tolerated in dogs not receiving atropine and 4/6 animals died. Prior to death, animals developed severe neurological signs, gastric haemorrhage, bloody vomiting, colic, anaemia and four dogs died. In the group of dogs with atropine these signs were mild and only one dog died. Levamisole poisoning was characterised by a significant reduction in red blood cells, haemoglobin and packed cell volume, and by anaemia. Peripheral blood pH, HCO3, base excess, pO2 and saturated O2 increased in both groups of animals and these dogs developed metabolic alkalosis 48 hours after the first administration of levamisole. Levamisole poisoning in dogs caused a significant increase in the activity of serum alanine aminotransferase and alkaline phosphatase and in the concentration of urea. In the group receiving atropine, adverse signs were milder although one dog died. Clearly this dose is far above the MTD and without the availability of toxicokinetic data or attempts by the Applicant to extrapolate the dose to human exposure, the relevance of the data is limited. A year long levamisole toxicity study was conducted in dogs, which

received oral levamisole up to 20mg/kg six times per week. All dogs receiving 20 mg/kg levamisole developed severe haemolytic anaemia after 8 weeks accompanied by decreases in haematocrit, haemoglobin and RBC-count with increases in erythroblasts and immature granulocytes. Blood is clearly a target organ of toxicity although the quality of the reporting of the studies is extremely limited. Toxicity studies are old, in adult animals and difficult to interpret due to the unknown provenance of test animals, unconventional experimental methods and lack of GLP. Nevertheless, since there is clinical experience with levamisole, including in steroid sparing therapy, the presented studies may be sufficient to evaluate prolonged exposure to levamisole. With exception of a chromosomal aberration and sister chromatid exchange study in human lymphocytes, a bacterial Ames test, mouse micronucleus test, sperm-head abnormality test, dominant lethal test and a proprietary chromosomal aberration test in human lymphocytes were all negative. But in contrast to the WHO report, that positively identifies genotoxicity in human lymphocytes in vitro and in vivo, the Applicant has labelled these results as inconclusive. As a justification, the Applicant claims that the results were small and statistically significant but questionable without further justification. The basis for this assumption is not satisfactorily explained. It is not agreed that the study is inconclusive and should be labelled as positive by the Applicant or a valid justification should be given. The results from the publication by Berger et al are the only genotoxicity study that can be assessed. All other studies are proprietary and only include summary conclusions of which the underlying data cannot be assessed. Without access to the data, the genotoxic potential of levamisole cannot be fully assessed.

A publication by Hibi et al. was considered the only relevant source to assess the carcinogenic potential of levamisole, as the methodology or increased mortality reported in older publications resulted in limited relevance of the study results or in difficulties in interpret the data. In this study, F344 rats were exposed to levamisole up to 12.9 mg/kg in males or 14.1 mg/kg in females for 2 years. The incidence of large granular lymphoma leukaemia appears to be increased in both male and female groups treated with levamisole, with a dose dependent trend in female rats. In males there was an increase in mammary gland adenoma and subcutis fibroma and thoracic mesothelioma. In females there was a minimal increase in mammary gland fibro adenoma. These findings are known to occur spontaneously in F344 rats. Therefore, the published study by Hibi et al, 2010, indicates that levamisole has no carcinogenic potential in the dose range tested (up to 12.9 and 14.1 mg/kg/day in males and females, respectively). However, some limitations of the study (missing toxicokinetic data, justification of the high dose) do not allow the exclusion of carcinogenicity without proper genotoxicity data. In order to exclude a carcinogenic potential of levamisole, the Applicant should first provide robust mutagenicity data in line with ICH S2 (R1), which was previously also suggested in the Scientific Advice meeting in 2006 (question C3, EMEA/CHMP/SAWP/94036/2006). Depending on the outcome, a further carcinogenicity study has to be considered or the usefulness of the study by Hibi et al, 2010, needs to be thoroughly discussed and the absence of further carcinogenicity testing needs to be justified.

Levamisole did not adversely influence male or female fertility in rats, nor did levamisole result in reproductive toxicity in sows. Levamisole administered before and during the entire pregnancy did not result in congenital abnormalities although a decrease in foetal implantations was observed in both 120 mg/kg/d or 240 mg/kg/d groups. Foetal growth retardation was observed at 120 mg/kg. In New Zealand rabbits, increased resorption and foetal death was correlated with maternal toxicity in dams receiving 40 mg/kg/day on GD 6-18, which is claimed to be below historical control ranges. No congenital anomalies were reported in Dutch rabbits receiving up to 90 mg/kg/day.. The cited publication by Kazy et al. reporting the rabbit data is a short review and does not include original research. Because this application is based on a bibliographic overview, and in particular because reproductive toxicity data is only derived from animal studies, the publication of Oghuro et al (1982)

which is discussed by Kazy et al. should be included in the dossier. The results should be integrated and discussed in the existing overview. Where relevant, validated translations should be submitted. Pre- and postnatal development of rats was negatively affected by levamisole in presence of maternal toxicity only. Maternal toxicity in the form of weight loss was observed in pregnant rats receiving 80 mg/kg levamisole. This was correlated with increased incidence of stillborn pups, reduced birthweight, reduced weight gain during lactation and decreased survival of the pups born to these dams. There is no reported data on the other groups. No juvenile toxicity studies were performed since it is already used in the paediatric population for a considerable time. During the review of the PIP it was stated that given the results of paediatric clinical experience in recent years the need for further non-clinical data may be obviated.

Aminorex is an active human metabolite of levamisole, and a substituted oxazoline derivative of amphetamine. Aminorex was marketed as an anorexic before being withdrawn from the European market in 1972 because of pulmonary hypertension. The Applicant considers that there is no risk for adverse reaction as result of exposure to aminorex because publications attempting to measure plasma aminorex could not do so above the LOQ. However, these studies are often based on a single dose application in adults, but not in children and not after repeated chronic or sub-chronic use. It is imaginable that in children receiving repeated doses, this will lead to higher exposure in plasma. Furthermore, levamisole is used as an adulterant in cocaine, possibly because conversion of levamisole to aminorex causes a synergistic effect. Indeed, aminorex was detected in one cocaine user at a plasma concentration of 2.4ng/ml (Eiden C et al. Prevalence of levamisole and aminorex in patients tested positive for cocaine in a French University Hospital. Clin Toxicol (Phila). 2015;53(7):604-8). Therefore, the potential for addiction to active metabolites of levamisole and levamisole should be discussed considering that these compounds have known CNS activity, and where needed should be further investigated according to the guideline on the non-clinical investigation of the dependence potential of medicinal products (EMEA/CHMP/SWP/94227/2004). In light of this discussion, the Applicant should consider including these risks as an important potential risk in the RMP.

3.3.4. Ecotoxicity/environmental risk assessment

The reported logKow of levamisole is 1.84 and is below the threshold of 4.5, which would trigger the requirement for a PBT analysis. The PEC was calculated using a refined Fpen based on the prevalence of idiopathic nephrotic syndrome, and was 0.003375µg/L. This is below the trigger value for a step-wise assessment for environmental risk. A review of the available literature to evaluate aquatic toxicity showed that levamisole is only toxic to eels at very high concentrations. Levamisole is extensively used in livestock. The potential increase in levamisole exposure to the environment in the case of this orphan indication is considered negligible. This data is considered sufficient to establish that levamisole is not a PBT and is not expected to pose a risk to the environment.

3.3.5. Discussion on non-clinical aspects

No GLP or non-GLP compliant studies have been conducted to support this application. No non-clinical study reports have been submitted as part of this application and the non-clinical data is purely bibliographic. For a known active substance this is acceptable, even though lack of toxicokinetics data impede a thorough assessment. The paucity of literature presented in the pharmacology section is striking and not sufficient to satisfactorily discuss the primary, secondary or safety pharmacology of levamisole. Of note, the Investigator's Brochure contains references suggesting further investigations into the mode of action of levamisole, which should be presented and discussed in the non-clinical overview. The granularity of the literature presented in the PD section is illustrative for the rest of the

non-clinical application, as other sections are largely based on the WHO Food Additives series, which is a summary of proprietary data and not original data. For the section of genotoxicity and carcinogenicity this is particularly problematic because the sought indication is for prolonged use in a paediatric population.

Levamisole is very poorly tolerated in animals after IV or SC administration. After IV or SC administration of levamisole, QTc prolongation or ventricular premature complexes, ventricular tachycardia, ventricular bigeminy and trigemini, suggesting that neither IV or SC are safe routes of administration and should not be used in the clinic. There is no assessment of binding of levamisole to cardiac ion channels (LoQ). Perivascular cuffing in the brain and meninges of dogs was reported in a published study. Perivascular cuffing may be attributed to the immunomodulating effect of levamisole. The difficulty in assessing the findings discussed in the publication is that it is a compilation of several separate experiments, some without within-experiment control groups to correlate findings. Several animals were purposely infected with Dirifilaria immitis, or were also previously vaccinated with live attenuated distemper and rabies virus, which may have masked or confounded the observed effects. Finally, there was no dose- or time dependent trend in the observed effects, which did not result in other tissue changes. Other CNS effects, such as changes in EEG patterns have been observed in animals but have not been discussed by the Applicant even though levamisole is thought to potentiate nicotinic acetyl cholinergic effects. In addition, a metabolite of levamisole, aminorex, is a substrate for serotonin transporters. This has not been discussed by the Applicant. The potential for addiction to active metabolites of levamisole and levamisole should be discussed considering that these compounds have known CNS activity and when considering the paediatric population after repeated exposure. Where needed it may be required to further investigate this according to the guideline on the non-clinical investigation of the dependence potential of medicinal products (EMEA/CHMP/SWP/94227/2004). The pharmacokinetic profile of levamisole is based on literature, of which one of the most frequently used and cited publications besides the WHO Food Additives Series is in Japanese. This is not acceptable and a translated version should be made available. Furthermore, while major metabolites have been identified, the enzymes involved in the metabolism have not. This is a major objection in this application, because human substrates have also not been identified in the clinic. This also leads to an inability to rationalize potential (pharmacodynamic) drug-drug interactions.

Blood is a target organ for toxicity in the dog, which is the most sensitive toxicological species. Severe anaemia, which is reversible but reverts after a rechallenged, is considered a levamisole-related effect. The mechanisms via which this occurs should be discussed by the applicant. A major objection over the absence of assessable genotoxicity data was raised. Studies cited from a WHO report (WHO food additive series 1991) cannot be considered as guideline-conform and no study reports of these mutagenicity tests are available. One positive test result was reported after exposure to levamisole in vivo in humans. The Applicant considerers this to be inconclusive data but does not provide acceptable arguments for this position. The Applicant is asked to discuss whether it is possible that a human genotoxic metabolite has been formed in vivo or to propose a valid alternative mechanism to explain the damage to the lymphocytes in this study and to discuss the relevance for the clinic considering the intended repeated exposure to levamisole in children. Together with the fact that insufficient data from carcinogenicity studies are available for levamisole, the Applicant should provide valid mutagenicity testing in line with ICH S2 (R1). Three carcinogenicity studies performed in rodents are available for levamisole. Two old studies from 1980 conducted in Albino Swiss mice and Albino Wistar rats by Janssen Pharmaceutica already have been considered as not-valid in the 2006 Scientific Advice (EMEA/CHMP/SAWP/94036/2006 and CPMP/SWP/2877/00). A published study by Hibi et al, 2010 indicates that levamisole has no carcinogenic potential in the dose range tested (up to 12.9 and 14.1 mg/kg/day in males and females, respectively). However, some limitations of the study (missing

toxicokinetic data, justification of the high dose) do not allow the exclusion of carcinogenicity without proper genotoxicity data. In order to exclude a carcinogenic potential of levamisole, the Applicant should first provide robust mutagenicity data in line with ICH S2 (R1). Depending on the outcome, a further carcinogenicity study has to be considered or the usefulness of the study by Hibi et al, 2010 needs to be thoroughly discussed and the absence of further carcinogenicity testing needs to be justified.

The cited publication by Kazy et al. reporting the rabbit reproductive data is a short review and does not include original research. Because this application is based on a bibliographic overview, and in particular because reproductive toxicity data is only derived from animal studies, the publication of Oghuro et al (1982) which is discussed by Kazy et al. should be included in the dossier. The results should be integrated and discussed in the existing overview. Where relevant, validated translations should be submitted.

3.3.6. Conclusion on non-clinical aspects

There are major non-clinical objections to granting a marketing authorisation of levamisole for the proposed indication. There are also other concerns which preclude the granting of a marketing authorisation at this time.

3.4. Clinical aspects

• *Tabular overview of clinical studies*

A tabular overview of clinical pharmacology studies provided by the applicant is shown below.

Type of study	Study identifier	Location of study report	Objective(s) of the study	Study design and type of control	Test product(s); dosage regimen; route of administration	Number of subjects	Healthy subjects or diagnosis of patients	Duration of treatment	Study status; type of report
Acceptability	Kreeftmeijer et al., 2013	5.3.1.2	Test acceptability	Randomized placebo-controlled	ELMISOL; 2.5 mg/kg q.o.d.; oral	99	FR SSNS	1 year	Complete; article
Biowaiver	Wegman, 2006	5.3.1.1		In vitro	ELMISOL; in vitro; in vitro	NA	In vitro	NA	Complete; report
Healthy adult PK	Adams, 1978	5.4	Define PK	Open label	50 mg tablets and 5 mg/mL syrup; 150 mg; oral	3	Healthy subjects	Single dose	Complete; article
Healthy adult PK	Kouassi et al., 1986	5.4	Define PK	Open label	Solaskil 150 mg tablets, Specia (France); 150 mg; oral	10	Healthy subjects	Single dose	Complete; article
Healthy adult PK	Luyckx et al., 1982	5.4	Define PK	Open label	30 mg tablet; 2.5 mg/kg; oral	22	Healthy subjects	Single dose	Complete; article
Pop-PK	ACE080503PK	5.3.3.2; 5.3.3.5	Define PK	Randomized placebo-controlled	ELMISOL; 2.5 mg/kg q.o.d.; oral	38	FR SSNS		Complete; full report
PK/PD	PK/PD	5.3.4.2	Define PK/PD	Randomized placebo-controlled	ELMISOL; 2.5 mg/kg q.o.d.; oral	38	FR SSNS		Complete; report
Drug interaction	Awadzi et al., 2004	5.4	Explore drug interaction	Randomized placebo-controlled	50 mg tablets (Janssen Pharmaceutica, Belgium); 2.5 mg/kg; oral	42	Healthy subjects	Single dose	Complete; article
Drug interaction	Scarfe et al., 1994	5.4	Describe case	Case report	?; ?; ?	1	Colon cancer, atrial fibrillation and prosthetic valve replacements		Complete; case report
Drug interaction	Wehbe et al., 1996	5.4	Describe case	Case report	?; 150 mg 3 consecutive days/ 2 weeks; intravenous	1	Rectal carcinoma, lower DVT and pulmonary embolus	6 weeks	Complete; case report

The studies listed in Table 2.7.3.6.1 are relevant in relation to efficacy as part of the application. The data from the pivotal study are submitted as an original study report, the other studies are available in form of an abstract (Abeyagunaward et al-. 2006; Weiss et al., 1993; Basu et al. 2013) only or as published papers in scientific journals. In addition to the pivotal trial only one additional study was a

placebo controlled, randomized, double blind study that had no major drawbacks regarding study design or conduct of the study and that included patients from Europe (BAPN, 1991). It is considered the key supportive trial.

Table 2.7.3.6.1 *Description of clinical efficacy and safety studies in children*

Study	Study design	# Study centres, location, start-end study	Treatment arm	Treatment duration	# Subjects (per gender M/F)	Age at enrolment (range)	Diagnosis & inclusion criteria	Objective (E: efficacy, S: safety): primary endpoint
Pivotal clinical trial	Randomised placebo controlled clinical trial	12, NL, BE, IT, PO, FR and India 2007-2012	1: L 2.5 mg/kg qod +Pred 2: Placebo +Pred	1: 8 d - > 1 y 2: 12 d - 1 y	1: 50 (36/14) 2: 49 (34/15)	1: 5.7 y 2: 6.0 y	FR SSNS > 2 y - < 18 y	E: Patients with relapses S: Adverse events
Abeyagunawardena et al., 2003	Retrospective cohort study	1, UK 1980-2000	1: L 2.5 mg/kg qod + Pred	1: 1-7 y	1: 113 (NS)	1: NS	FR or SD NS Age at onset 0.5-16 y Follow-up period > 1 y	S: Adverse events
Abeyagunawardena et al., 2006	Randomised controlled trial	1, Sri Lanka 2002-2005	1: L 2.5 mg/kg qod 2: No treatment	1: 1 y 2: 1 y	1: 42 (NS) 2: 34 (NS)	1: 8.4 y 2: 7.2 y	SDNS Stable remission for 2 years on L + Pred	E: Patients with relapses S: Adverse events
Alsaran et al., 2001	Retrospective cohort study	1, Canada 1992-1997	1: L 2.5 mg/kg qod + Pred	1: ≥ 6 m	1: 24 (12/12)	1: 2.7 y	SRNS 1-18 y	S: Adverse events
Al-Saran et al., 2006	Controlled prospective study	1, Saudi Arabia 2001-2003	1: L 2.5 mg/kg qod + Pred 2: Pred	1: 1 y 2: 1 y	1: 32 (20/12) 2: 24 (15/9)	1: NS	FR or SD SRNS < 14 y	E: reduction in relapses S: Adverse events
Alshaya et al., 2002	Retrospective cohort study	1, Saudi Arabia 1997-2001	1: L 3 mg/kg qod + Pred	1: 6-24 m	1: 9 (6/3)	1: 6.5 y (3.5-10)	FR or SD SSNS	S: Adverse events
Bagga et al., 1997	Prospective cohort study	1, India	1: L 2.5 mg/kg qod + Pred	1: 6-31 m	1: 43 (30/13)	1: 4 y (1.3-10)	SDNS	S: Adverse events
BAPN, 1991	Placebo controlled randomised trial	12, UK	1: L 2.5 mg/kg qod + Pred 2: Placebo + Pred	1: 112 d 2: 112 d	1: 31 (21/10) 2: 30 (20/10)	1: 8.3 y 2: 8.8 y	FR and SD SRNS	E: Patients in remission S: Adverse events

Boyer et al., 2008	Retrospective cohort study	1, USA 1999-2004	1: L 2.5 mg/kg tiw + Pred	1: 12 m	1: 10 (6/4)	1: 10.9 y (4.2-14.9)	SDNS 1-18 y	S: Adverse events
Chen et al., 2010	Retrospective cohort study	1, Taiwan 1994-2006	1: L 2-3.3 mg/kg qd + Pred	1: 3-20 m	1: 15 (NS)	1: NS	SDNS Age at onset < 16 y	S: Adverse events
Couderc et al., 2015	Retrospective cohort study	France	1: L (dose not defined) + Mofetil + Pred	1: 277-205 d	1: 10 (5/5)	1: NS	FR or SD NS	S: Adverse events
Dayal et al., 1994	Randomised controlled trial	1, India 1988-1990	1: L 2-3 mg/kg biw 2: No treatment	1: 1 y	1: 33 (23/10) 2: 28 (19/9)	1: 5.0 y 2: 4.9 y	MCNS Remission with Pred	E: Patients in remission S: Adverse events
Donia et al., 2002	Retrospective cohort study	1, Egypt 1998	1: L 2.5 mg/kg qod + Pred	1: 6 m	1: 20 (16/4)	1: 7.4 y (3-15)	SD MCNS In relapse	S: Adverse events
Donia et al., 2005	Prospective randomised study	1, Egypt 1998	1: L 2.5 mg/kg qod + Pred 2: Cyclophosphamide 500 mg/m^2/month i.v. + Pred	1: 6 m 2: 6 m	1: 20 (16/4) 2: 20 (15/5)	1: 7.4 y 2: 7.38 y	SD MCNS	E: Remission S: Adverse events
Drachman et al., 1988	Retrospective cohort study	1, Israel	1: L 2.5-5 mg/kg biw + Pred	1: 4 - 18 m	L: 10 (7/3)	1: 9.1 y (4-15)	FR and SD NS	S: Adverse events
Esfahani et al., 2011	Retrospective cohort study	1, Iran 1978-2005	1: L 2-2.5 mg/kg qod + Pred	1: > 6 m	1: 124 (NS)	1: NS	SSNS Treated from start of disease Follow up period ≥ 5 y	S: Adverse events
Fu et al., 2000	Retrospective cohort study	1, Taiwan 1996-1998	1: L 2-3 mg/kg qod + Pred	1: 6 - 26 m	1: 27 (18/9)	1: 8.48 y (2-14.5)	FR or SD SSNS	S: Adverse events
Fu et al., 2004	Retrospective cohort study	1, Taiwan 1996-1999	1: L 2-3 mg/kg qod ± Pred 2: L 2-3 mg/kg qod < 3 months, then 2-3 mg/kg qd ± Pred	4 - 36 m	1: 20 (13/7) 2: 16 (12/4)	1: 8.02 y 2: 8.82 y	FR or SD SSNS	S: Adverse events
Hafeez et al. 2006	Retrospective cohort study	1, Pakistan 2000-2004	1: L 2.5 mg/kg qod + Pred	1: 1 y	1: 70 (56/14)	1: 5.5 y (1.25-12)	FR or SD NS 1 - 15 y	S: Adverse events
Jayaweera et al., 2015	Retrospective cohort study	1, Sri Lanka 2010-2015	1: L 2.5 mg/kg qd + Pred	1: 1 y	1: 58 (33/25)	1: 7.9 y	SDNS > 2 relapses/y Previous L qod	S: Adverse events
Kemper et al., 1998	Retrospective cohort study	1, Germany	1: L 2 mg/kg qod + Pred	1: 3 - 24 m	1: 25 (15/10)	1: 10 y (3.5-22)	FR SSNS	S: Adverse events
Ksiazek et al., 1995	Retrospective cohort study	1, Poland	1: L 2.5 mg/kg biw + Pred	1: 12 m	1: 27 (18/9)	1: NS	FR or SD NS	S: Adverse events
La Manna et al., 1988	Retrospective cohort study	1, Italia	1: L 2.5 mg/ kg biw + Pred	1: 2 - 16 m	1: 13 (11/2)	1: 5.3 y (2.3-11.5)	FR and/or SD NS	S: Adverse events
Madani et al., 2010	Retrospective cohort study	1, Iran 1986-2006	1: L 2.5 mg/kg qod + Pred	1: 18 ± 11 m	1: 304 (208/96)	1: NS	FR or SD SSNS Age at onset 0.5-16 y Follow up period > 6 m	S: Adverse events
Meregalli et al., 1994	Retrospective cohort study	2, Germany	1: L 5 mg/kg qw + Pred	1: 7-45 w	1: 10 (8/2)	1: NS	FR NS	S: Adverse events
Mongeau et al., 1988.	Retrospective cohort study	1, Canada	1: L 2.5 mg/kg/ qod + Pred	1: 1 y	1: 16 (11/5)	1: (2-15)	FR SS MCNS	S: Adverse events
Muorah et al., 2015	Retrospective cohort study	1, UK	1: L 2.5 mg/kg qod	1: NS	1: 73 (45/28)	1: 6.1 (2-14.1)	FR SSNS No previous SSA Follow up > 6 m	S: Adverse events
Neuhaus et al., 1994	Retrospective study	1, UK 1980-1994	1: L 2.5 mg/kg qod + Pred	1: 6 - 18 m	1: 58 (NS)	1: NS	SSNS	S: Adverse events

Niaudet et al., 1984	Retrospective cohort study	1, France	1: L 2.5 mg/ kg biw	1: 3 - 18 m	1: 30 (23/7)	1: NS	FR NS	S: Adverse events
Rashid et al., 1996	Prospective controlled randomised trial	1, Bangladesh	1: L 2.5 mg/kg qod + Pred 2: Pred	1: 6 m 2: 6 m	1: 20 (13/7) 2: 20 (14/6)	1: 8 y 2: 6/7 y	FR or SD SRNS	E: Patients in remission S: Adverse events
Sumegi et al., 2004	Retrospective study	1, Hungary 1988-2003	1: L 2 mg/kg qd + Pred	1: 5 - 36 m	1: 34 (25/9)	1: 5.03 ± 3.4 y	FR, SD or SR NS Age at onset > 1 - < 16 y	S: Adverse events
Tanphaichitr et al., 1980	Retrospective cohort study	1, Thailand	1: L 1.5 -3.9 mg/kg biw	1: 1 - 6 m	1: 7 (6/1)	1: NS	MCNS	S: Adverse events
Tenbrock et al., 1998	Retrospective cohort study	1, Germany 1992-1997	1: L 2.5 mg/kg qod + Pred	1: 2 - 18 m	1: 11 (7/4)	1: 12.5 y (4-18)	SS or SR NS	S: Adverse events
Weiss et al., 1993	Randomised placebo controlled clinical trial	USA	1: L 2.5 mg/kg biw + Pred 2: Placebo + Pred	1: 6 m 2: 6 m	1: 21 (NS) 2: 20 (NS)	1: NS 2: NS	FR or SD NS	E: Relapses/month

In addition, there are some reports in the public domain that were not discussed by the applicant.

Basu et al., 2013, a controlled study comparing levamisole 2.5 mg/kg AD with mycophenolate mofetile in 112 (56 in each group) children with frequently relapsing or steroid dependent nephrotic syndrome over 12 months. The relapse rate was similar after 6 months between both groups but lower after 12 months on mycophenolate mofetile.

Elmas et al. 2013, a retrospective analysis in 29 children with nephrotic syndrome with steroid sensitive frequently relapsing or steroid dependent nephrotic syndrome that received levamisole 2.5 mg/kg AD for 12 months. The annual steroid burden was significantly lower on levamisole. 18 patients remained relapse free. No specific adverse events were reported.

3.4.1. Pharmacokinetics

Pharmaceutical development

The applicant has developed a film coated taste masking tablet for children at a size of 5 – 8 mm for the different strengths. Development an orodispersable tablet as proposed by the EMA in a scientific advice procedure was not considered appropriate due to the unpleasant taste of levamisole.

When holding the tablet in the mouth the mean time to bitter taste was 31.4 ± 7 s in adults.

Bioequivalence

The applicant has provided data supporting a biowaiver for bioequivalence testing. The report has been evaluated by the CHMP within the frame of an Scientific Advice. In that procedure the CHMP agreed that Levamisole can be classified in the category of BCS class I “high solubility and high permeability” API and that formulations of levamisole HCl can be approved based on strictly defined dissolution criteria as a surrogate for an in vivo BE test but there are issues to be addressed.

Regarding the BCS classification, solubility data, and dissolution data were submitted. Solubility was not fully supported and a concern is raised to further support this (see Quality assessment report).

Regarding permeability or absorption, the applicant indicated that according WHO, Working document QAS/04.093/Rev.4, Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability (2005): 1-39, levamisole HCl can be considered a BSC Class III/I drug, based on its high solubility and borderline permeability. However, the applicant is of opinion that levamisole should be considered a BCS Class I drug, as:

- literature on pharmacokinetic studies of levamisole HCl report that most of the levamisole HCl is excreted as unchanged drug or as metabolite in urine (total 70% of the administered dose)

- less than 5% of the drug is found unchanged in faeces, and it is therefore most likely that the remainder of the drug (95%) was absorbed.

This is not agreed. Lack of recovery data in faeces does not proof that the remainder is absorbed. Faeces data only indicate that minimal 5% is not absorbed. The urinary data showed that 70% is absorbed. Of the remaining 25% it cannot be positively confirmed that it is absorbed. As such, as at least 70% is absorbed, levamisole should be considered a BCS Class III drug, in line with the WHO, Working document QAS/04.093/Rev.4.

PK

The dossier includes literature data and 1 pivotal in vivo clinical study in children receiving levamisole treatment in a dose of 2.5 mg/kg on alternate days in the prevention of relapses and prolonging the time to relapse after cessation of corticosteroids treatment in children with idiopathic SSNS. Pharmacokinetic data obtained in this study were used in a population pharmacokinetic analysis (report EudraCT no.: 2005-005745-18 (internal code: ACE080503PK).

The applicant has provided and discussed some data on PK in adults from the public area addressing absorption, distribution, elimination and PK interactions.

A summary of the results is provided in table 2.7.2.3.1.

Table 2.7.2.3.1 *Pharmacokinetic parameters in healthy adults and children with steroid-sensitive nephrotic syndrome*

	Adams [a]	Kouassi et al. [a]	Luyckx et al. [b]	Pivotal clinical trial [a]
Population	Healthy adults	Healthy adults	Healthy adults	Children with SSNS
Dose	150 mg (tablets)	150 mg	2.5 mg/kg	2.5 mg/kg
t_{max} (hours)	1.27 ± 0.12	1.52 ± 0.49	1.94 ± 0.16	1.81 ± 0.71
C_{max} (mg/L)	0.76 ± 0.12	0.72 ± 0.22	0.71 ± 0.05	0.53 ± 0.27
AUC (mg*h/L)	4.15 ± 1.04 (0-24)	3.07 ± 1.32 (0-∞)	6.83 ± 1.10 (0-∞)	3.54 ± 2.90 (0-∞)
F (%)		62 ± 10	68 ± 3	
$T_{1/2}$ (hours)	4.4 ± 0.7	5.56 ± 2.46	5.06 ± 0.88	3.07 ± 1.47
Cl (L/h)		33.8 ± 9.0	17.8 ± 1.9	44 (8.5%) [c]
V (L)		266.3 ± 120.3	109.7 ± 10.2	236 (13.3%) [c]

[a] Data presented as mean (standard deviation) unless otherwise mentioned; [b] Data presented as mean (standard error); [c] data presented as estimate (RSE%) based on a standard body weight (70 kg).

The applicant referred to WHO Food Additives Series, No. 33, 1994 Toxicological evaluation of certain veterinary drug residues in food, no's 804-817 on INCHEM. 805., to support the pharmacokinetics of levamisole. However, this publication is an overview and refers to additional literature references, which are not included. As such, it cannot be evaluated of the data in this overview are supported by adequate data. Therefore, the applicant should use the original data, discuss the data in more detail to support the pharmacokinetics, in those cases were a reference is made to WHO Food Additives Series, No. 33, 1994. Furthermore there is additional literature in the public area concerning pharmacokinetics of levamisole that is neither discussed nor is provided. A comprehensive update of the summary of clinical pharmacology studies (2.7.2) should be provided. (OC)

Levamisole is a chiral compound with a stereo genic centre at the C-6 position of the imidazole(2,1-b)thiazole moiety. S-configured levamisole hydrochloride was used for the clinical studies and for the final product intended for marketing authorization. The possible interconversion in vitro or in vivo of the S-configured levamisole hydrochloride should be discussed.

Analytical methods:

The LC-MS/MS analytical method is precise and accurate for the analysis of levamisole in plasma. Stability of levamisole in plasma has been proven over 206 days at -18 and -70ºC. However in the popPK report the following is indicated: "Short-term stability of levamisole under several conditions (e.g. bench-top, freeze/thaw, stock solution) was found acceptable. Long-term stability at ≤-70°C was validated up to 486 days. At ≤-18°C, long-term stability was valid for 206 days. The medium QC samples showed a deviation in bias of -22% after 351 days, while all QC samples showed unacceptable bias after 486 days of storage at ≤-18°C. The overall stability of the samples could not be validated, because samples were stored for longer periods."

The validation data on storage conditions longer than 206 days could not retrieved from the dossier. The applicant should submit these data.

The median storage time of the patient samples at ≤-20°C was 987 days (range 0-2101 days). Stability data showed that levamisole is not stable over this time period. The pharmacokinetic data were used in a popPK analysis (see below). Storage time had a linear effect on the stability of the samples, with an average decrease in stability of 0.05% per day in storage (validated up to 486 days). Storage duration (defined as the time between sample collection and bioanalysis) was included in the model as a fixed effect (-0.0005 × days in storage at -20°C) directly on the observed concentrations, and the model was evaluated using the storage time–corrected observed concentrations. However this is considered not acceptable. Levamisole is not stable during the storage period; correction of the instability by modelling is not acceptable, as the major number of samples were stored over a longer time period than the period the stability was evaluated (extrapolation to an unknown). The accuracy and precision of the plasma concentrations are unknown and as such the data are not reliable.

Regarding analysis of urine samples, because of stability issues, urine samples have been analysed semi-quantitatively using the same method as used for plasma samples. No metabolites (p-hydroxy-levamisole and its glucuronic acid in urine) were determined. As indicated by the applicant, there was insufficient information about the degradation pathways of levamisole and these metabolites in plasma and urine samples. Considering the variations in storage time of the samples, determinations of metabolites were not performed. This indicates that overall no pharmacokinetic data are available in the evaluated patient group.

The complete analytical report regarding the analysis of the patient's samples should be submitted, as it could not be retrieved from the dossier.

Absorption:

No absolute bioavailability data is available. Limited literature data indicate that the bioavailability of levamisole after oral administration is at least 70%.

After oral administration to healthy adults, tmax values are observed after 1.27 and 1.94. In the popPK analysis in children with SNNS, a mean tmax was estimated of 1.81h (median 1.65 h)).

It is unclear which transport pathway is involved in the absorption of levamisole. In addition, the applicant should elucidate whether levamisole is a substrate for P-gp (MDR1) or BCRP.

No information is submitted regarding the effect of food on the pharmacokinetics of levamisole. According to the SmPC, the film-coated tablets should not be chewed because of bad taste. Tablets should be swallowed as a whole with a glass of water or, if necessary, another appropriate liquid or some custard. This indicates that the tablets may be taken with food. As indicated in the popPK report, an effect of amount and type of food intake is expected on levamisole absorption and metabolism. The applicant should elucidate the effect of food on the absorption of levamisole.

Regarding linear pharmacokinetics, reference is made to WHO Food Additive Series, No. 33, 1994) stating that over a range of 50 to 150 mg levamisole, systemic exposure was proportional to dose. The study by Luyckx indicated dose proportionality in adults between 2.5 and 5.0 mg/kg . As indicated before, there are important drawbacks related to pharmacokinetic data in children due to instability issues of levamisole in plasma. So clear dose proportionality in pharmacokinetics in the paediatric population is lacking.

Exposure was about 25 – 33% lower in the paediatric population than described for the adult population. Due to the methodological issues described above, the data have to be assessed with caution.

No data is submitted regarding time dependency in pharmacokinetics. The applicant should discuss whether levamisole is subject to time dependent pharmacokinetics.

The proposed, 5, 10, 25 and 50 mg tablets are not dose proportional. Waiving the bioavailability of levamisole from these tablets would be acceptable based upon the very rapidly dissolution, and if levamisole would be considered a BCS Class I drug. As it can only be confirmed that levamisole is a BCS Class III drug, this would hamper the waiving, as the formulations are not dose proportional. But keeping in mind that the tablets are very rapidly dissolving, the core excipients used are not critical and all the formulations were used in the clinical trial, waiving is considered not a concern.

Distribution:

Very limited data is submitted regarding the distribution of levamisole. Levamisole seems to have a low to moderate protein binding (51%), however this is minimal supported (any information was given regarding study conditions). The protein binding should be further supported. (OC)

Based upon the limited pharmacokinetic data in healthy volunteers, after a 150 mg dose as tablets the volume of distribution (Vd) was 266 ± 120 l (Kouassi et al., 1986) and 99 - 109 l (Luyckx et al., (1981). In the popPK analysis in children a Vd was estimated of 236 l.

Preclinical data indicated that levamisole may be excreted with mother milk. In addition levamisole can penetrate into the brain of rat. No information from placental transfer studies is available.

It is not clear whether levamisole in humans crosses the blood brain barrier or whether levamisole is a substrate for transporters involved. The applicant should further elucidate this, also taken into account

possible interactions which may increase distribution into the brain (see also section Interactions). (part MO)

Metabolism:

A review of published literature indicated that levamisole is extensively metabolised and only 4.5% was excreted in urine unchanged. The p-OH-levamisole metabolite R9313 and its glucuronide conjugate accounted up to 17% of the administered dose. Other metabolites were not identified.

Adams et al (1978) showed after administration of labelled levamisole (n=3; 150 mg), at tmax the parent drug only accounted for 30% of drug plasma concentrations. The plasma elimination half-life of levamisole was about 4h, while that of total radioactivity about 16h. Urinary recovery over 72h was about 70% (60% within 24h); 6% was excreted as parent (intact) drug. 4% of the administered dose could be recovered in feces. The data indicate that levamisole is extensively metabolised based upon the low urinary and faecal excretion of intact drug (10% or less of the administered dose). The suggested in vitro metabolism pathways were not confirmed in vivo (no data shown) and as such no data are available regarding plasma exposure to main metabolites. This should be elucidated. (part MO)

A metabolite (Aminorex) has been identified in urine of subjects after intake of levamisole. (Bertol E, et al., J Pharm Biomed Anal 2011; 55: 1186-1189; Hess et al., Analytical and Bioanalytical Chemistry May 2013, Volume 405, Issue 12, pp 4077–4088). Aminorex is known as a stimulant that has been taken off market because of the development of lethal pulmonary hypertension. Plasma levels remained below LLOQ after single dose administration and the issue is most likely not considered clinically relevant. Aminorex is detectable up to 54 hours in urine after intake of levamisole. In addition, five isomers of aminorex and 4 hydroxy-metabolites of aminorex or its isomers were found. Furthermore, levamisole is also hydroxylated and eliminated free or conjugated with sulfate or glucuronide into urine. These data have not been discussed by the applicant.

From the in vitro and in vivo data it is not clear for which enzymes levamisole is a substrate. The applicant should elucidate this, as it is important to know whether other medicinal products may influence the exposure of levamisole.

Excretion:

In adults mean t1/2 was between 4.4 and 5.06 h. In the paediatric patients mean/median half-life was shorter (mean: 3.07 ± 1.47 h / median: 2.6 h (2.06 – 3.65 interquartile range)).

CL/F in adults after oral administration ranged from 18 - 34 l/h and in children with SSNS 44 l/h/70 kg. Age (between 2 – 13 years) and body weight had a significant effect on clearance. With increasing age, clearance decreased. As indicated before, due to stability problems of levamisole in plasma, the data from the study with SNNS children are not reliable.

Special patient groups:

Levamisole is indicated in a special patient group, i.e., children aged 2 - 18 years of age with SSNS. The patients are considered to have severe renal impairment. No further information is provided regarding the effect of a . The lack of such data should be justified.

It can not be excluded that the intended patient group will have normal liver function. It may be even anticipated that they may have different stages of hepatic impairment due to their disease. As such, the effect of hepatic impairment on the pharmacokinetics of levamisole should be elucidated/discussed.

The population pharmacokinetic analysis did not identify a gender effect on pharmacokinetics in children, and did identify a significant effect of age on Cl/F (-10.1%/year), however the data are not reliable due to instability issues with levamisole in plasma. In adults the data did not indicate a clinically relevant gender effect. The effect of body weight on the pharmacokinetics of levamisole is not addressed.

Interactions:

Limited data on the interaction potential of levamisole is submitted. These data indicate that there may be potential for interaction between levamisole and other medicinal products e.g. at the level of P-gp and CYP450 enzymes. No data is presented confirming the metabolic pathways for elimination of levamisole and its metabolites. Furthermore, no data is presented whether levamisole and its metabolites may interfere with other metabolic pathways (inhibition and/or induction) at CYP enzyme level and transporter level. Considering that the patient group will likely receive concomitant treatment and medications, the interaction potential of levamisole and its metabolites should be elucidated. The applicant should follow the Guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1 Corr.*). In particular, unless justified, a drug-drug interaction study investigating the potential for PK interactions between prednisone and levamisol is required. Both drugs were administered concomitantly during the pivotal study and a negative interaction cannot be ruled out.

Overall conclusion regarding pharmacokinetics: Major objections have been identified relating to the incompleteness of the dossier and the lack of pharmacokinetic data in the intended patient group due to instability issues of levamisole in plasma samples of the patients.

3.4.2. Pharmacodynamics

Primary pharmacology

No formal description on the mechanism of action has been provided by the applicant. However, some information on the mechanism of action have been described in other sections of the submitted dossier.

The exact mechanism of action of levamisole is unknown according to the applicant. According to the applicant, two mechanisms are expected to contribute to the effect of levamisole in steroid sensitive nephrotic syndrome, immunomodulation and the direct action on kidney podocytes. The immunoregulatory effect of levamisole correlated with the clinical course of the steroid responsive nephrotic syndrome. Remissions were associated with correction of the suppressed T-lymphocyte activity and the OKT4+/OKT8+ ratio. Patients without immunoregulatory abnormalities did not respond to levamisole. Levamisole is able to protect against podocyte injury in an in vitro model of minimal change nephropathy. It is suggested that the effects of levamisole on podocytes are mediated by the glucocorticoid signalling pathway.

A thorough QT study was not submitted. This was agreed on with the CHMP in a scientific advice procedure provided that sufficient ECG data are available form the pivotal study

3.4.3. Discussion on clinical pharmacology

There are relevant methodological issues regarding the in study validation of the PK analysis. Levamisole is not stable during the storage period of the patient samples. Correction of the instability by modelling is not acceptable, as the major number of samples were stored over a longer time period than the period the stability was evaluated. The accuracy and precision of the plasma concentrations are unknown and as such the data are not reliable. A sensitivity analysis comparing PK for storage values times up to day 206 vs. thereafter may provide more insight in the robustness of the data and should be provided.

Limited data on the interaction potential of levamisole is submitted. These data indicate that there may be potential for interaction between levamisole and other medicinal products. No data is presented confirming the metabolic pathways for elimination of levamisole and its metabolites. Furthermore, no data is presented whether levamisole and its metabolites may interfere with other metabolic pathways (inhibition and/or induction) at CYP enzyme level and transporter level. Considering that the patient group will likely receive concomitant treatment and medications, the interaction potential of levamisole and its metabolites should be elucidated.

No data is submitted regarding the effect of food and the time dependency of PK. Relevant data regarding protein binding was not discussed. It may be anticipated that some patients may have different stages of hepatic impairment due to their disease. The effect of hepatic impairment on PK should be discussed. In addition, the effect of an impaired renal function on the pharmacokinetics of levamisole has not been discussed and the lack of a pharmacokinetic study to evaluate the effect of a different degree of impaired renal function requires further justification.

In addition, the applicant has not provided an overview addressing primary and secondary pharmacology and possible PD interactions in human subjects. The applicant should provide such a review and update Module 2 accordingly. The applicant has poorly described the mechanism of action. It is believed that levamisole has some immunomodulating effect and a direct effect on kidney podocytes. However, more effort should be made to further elucidate on the mechanism of action and update the SmPC section 5.1 accordingly.

As an immunomodulatory agent levamisole may affect efficacy and safety of vaccines. As this is a relevant issue in a paediatric population, a thorough review is expected discussing efficacy and safety of coadministration of attenuated and inactivated vaccines that are usually recommended in the paediatric population and to indicate in which case treatment with levamisole should be discontinued or may be maintained.

3.4.4. Conclusions on clinical pharmacology

Pharmacokinetics is insufficient supported to make a reliable evaluation of the disposition of levamisole in the intended patient group. Moreover, no reliable pharmacokinetic data are available in the intended patient group, and as such safety exposure data. Limited literature data indicate that there may be potential for interaction between levamisole and other medicinal products. However, no data is presented confirming the metabolic pathways for elimination of levamisole and its metabolites and no data is presented whether levamisole and its metabolites may interfere with other metabolic pathways (inhibition and/or induction) at CYP enzyme level and transporter level. Considering that the patient group will likely receive concomitant treatment and medications, the lack of these data is of concern.

3.4.5. Clinical efficacy

Dose-response studies and main clinical studies

Dose response studies

No dose response studies were submitted. In the study report of the pivotal study dose selection was justified based on the dose used for short term anthelmintic therapy and on doses used in clinical practice since the 1980s in paediatric patients with nephrotic syndrome. Relevant for dose selection in published studies were dose related adverse events.

Overall, dose finding is unsatisfactorily but the choice of a 2.5 mg/kg AD dosing regimen for the pivotal study is acceptable based on current clinical practice and on published data.

Pivotal study

Study protocol number: EudraCT: 2005-005745-18 Study medication code: ACE080503.

Levamisole treatment for children with steroid-sensitive nephrotic syndrome. A multi-centre, double-blind, placebo-controlled, randomised trial.

Data presented are based on clinical study report (CSR) dated 27 October 2015, Study Protocol version 6.0 and Statistical Analysis Plan dated 29-12-2013.

The **general design** of the main study ACE080503 was a double-blind, randomised, placebo-controlled trial. Subjects were randomly allocated to levamisole 2.5 mg/kg/AD or matching placebo. Only one dosage strength was allowed per supply period (i.e. 5 mg, 10 mg, 25 mg or 50 mg). Tablets were **taste masked** for covering the bitter taste of levamisole.

Key aspects are summarized in Figure 9.1.1

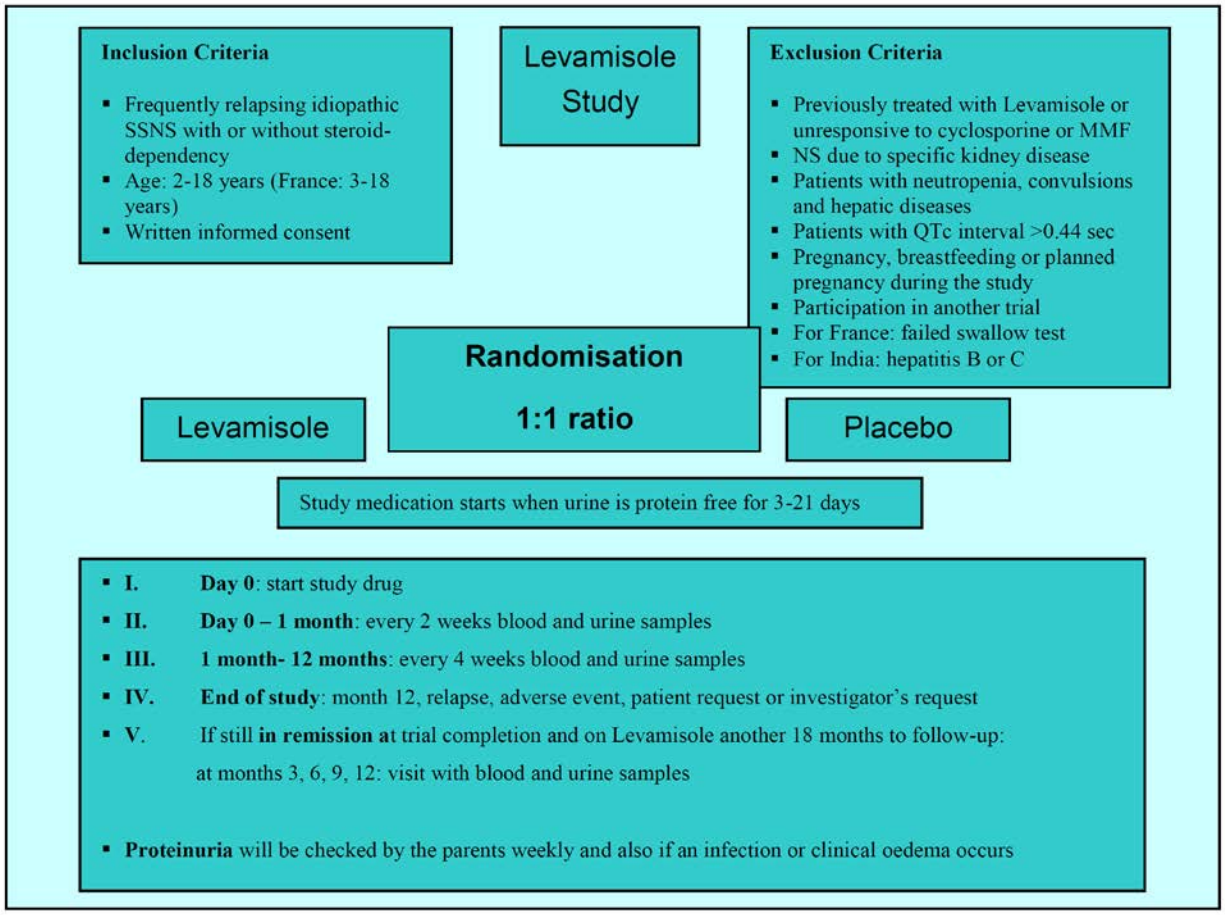


Figure 9.1.1: Flow-chart of the Levamisole Study

The **first study visit** took place at the time of relapse. **Corticosteroid background treatment** was started according to the protocol of the French Society of Nephrology (start prednisone 60 mg daily (during run-in after relapse) until protein-free for 6-8 days; then subsequently 60 mg AD 4 weeks, 45 mg AD 4 weeks, 30 mg AD 4 weeks, 15 mg AD 4 weeks). **Study treatment** was started during steroid treatment, after the subject's urine had been protein-free for 3 to 21 days. This was considered day 0 of the trial. Study visits were scheduled every 2 weeks in the first month and every 4 weeks in the remaining months. Treatment was continued until a relapse necessitating corticosteroid treatment occurred or until 12 months of treatment (i.e. normal completion). In case treatment was withdrawn for any other reason, this was specified (i.e. adverse event (AE), investigator's request or patient's request).

The primary objective of this study was to assess the efficacy of one year of treatment with levamisole in a dose of 2.5 mg/kg on alternate days (AD) in the prevention of relapses and prolonging time to relapse after cessation of corticosteroids treatment in children with idiopathic SSNS.

Primary endpoint: Time to relapse, defined as the time from start of study treatment to the time of relapse necessitating corticosteroids treatment, or, in case of no relapse, time of censoring (i.e. 12 months after start of trial medication). It was considered a relapse when proteinuria (albustix 3+ or albumin in urine of >200 mg/mmol creatinine) recurred after remission for at least 3 consecutive days.

Treatment was started immediately in case of oedema, hypoalbuminaemia or when isolated proteinuria (>110 mg/mmol creatinine without hypoalbuminaemia or clinical signs of NS) lasted more than 3 weeks. Only a relapse necessitating corticosteroid treatment was considered a primary endpoint relapse.

Secondary objectives were to evaluate whether the effect of levamisole varied with disease status (steroid-dependent versus frequently relapsing and low- versus high-dose steroid-dependency), to evaluate whether the effect of levamisole varied with prior treatment with cyclophosphamide, to assess the steroid-sparing effect of levamisole treatment as well as the sparing effect of other immunosuppressive drugs (i.e. average amount administered per patient), to determine whether the effectiveness of levamisole remained constant or waned over time, to assess the pharmacokinetics (PK) of levamisole in the target population, and to assess the safety of treatment with levamisole.

Eligible subjects were patients between 2 and 18 years of age (for France: 3 to 18 years) with a primary diagnosis of frequently relapsing idiopathic SSNS with or without steroid-dependency.

Subjects were excluded if they had previously been treated with levamisole, or been unresponsive to cyclosporine or mycophenolate mofetil (MMF), nephrotic syndrome due to a specific kidney disease, in case of neutropenia, convulsions or with hepatic diseases, QTc prolongation, a failed swallow test in France, and hepatitis B or C in India.

Subjects who were still in remission after one year of treatment with levamisole were eligible for the **cohort study**. Five levamisole-treated subjects were included in this cohort. Patients could be treated with levamisole for another 18 months, but was limited to 12 months when the subject had more than one relapse in 6 months. Subjects who received placebo and were in remission after one year of treatment were eligible for **follow-up** for a maximum of 12 months without taking placebo. Follow-up could be performed through a phone-call by the investigator every 6 months. Subjects who relapsed within one year of the study were **followed** according to the visit schedule up to 12 months, regardless of treatment assignment. Follow-up visits took place at least every 3 months. The one year visit after start treatment was assigned as the end of study visit in each scenario.

Randomisation was **stratified** by steroid-dependency (yes/no), prior use of cyclophosphamide (yes/no), and country (India, France or other European countries).

Steroid-dependency was **stratified** according to 3 classifications: non-steroid-dependent versus steroid-dependent, low versus high steroid-dependent using 15 mg/m2/AD as a cut-off value for steroid-dependency. For the third classification (low versus high), the cut-off value was dependent on the median value of the prednisone maintenance doses for the safety population.

Pre-specified **withdrawal** criteria have been used including neutropenia (<1500 10^6/L), vasculitis, hepatitis, convulsions or levamisole side effects, for which the investigator judged it necessary to stop or when serious adverse events (SAEs) occurred, patient's request to stop, and serious non-compliance of the patient with the protocol.

According to the (updated) SAP (statistical analysis plan) the average quantity of steroids administered during the study, which was to be used as a secondary efficacy endpoint according to the study protocol, was not evaluated due **to incomplete recording of steroid intake**. The quantitative assessment of the steroid-sparing effect of levamisole treatment and that of other immunosuppressive drugs could not be performed (i.e. average amount administered per patient).

The **study population** was 5.9 years old (mean, SD-2.88 years, 2 to 16 years). All but one subject were under the age of 14 years. One 16-year old male was allocated to placebo. More males (70.7%) than females (29.3%) were included, mainly Caucasian (50.5%; all recruited in European sites) and

Asian (44.4%; mainly India), with a large percentage (69.7%) of steroid-dependency, especially among Caucasian (92.0%). The use of cyclophosphamide prior to the study was low (6.0%). 72% in the levamisole and 67% in placebo group were steroid dependent with a 19 mg/m^2/ad and 14 mg/m^2/ad, respectively, as lowest prednisone dose to prevent relapse in last year. Patients had a mean of 3 relapses in last 6-12 months. Demographics were comparable between the levamisole and placebo groups.

Statistical analysis: A total sample size of 100 patients was planned. Statistical efficacy analyses were performed for all endpoints in the ITT and PP populations. Kaplan Meier survival curves and the log-rank test for confirmatory testing were used to compare the time to relapse in both treatment groups. The relative efficacy of Levamisole compared to placebo was expressed as relative risk of relapse with the corresponding 95% confidence interval, estimated as hazard ratio from a Cox proportional hazards regression analysis. As a secondary (landmark) analysis, the primary efficacy analysis was repeated using the time to relapse after prednisone cessation, approximately 100-120 days after first dose administration. This endpoint was added, while the average quantity of steroid medication administered during the study was not used due to incomplete recording of steroid intake. An unblinded safety analysis was planned after entry of 50 subjects.

Table E1: Baseline data (ITT population)

	Levamisole (n = 50)	Placebo (n = 49)
Gender		
- Male	36 (72%)	34 (69%)
- Female	14 (28%)	15 (31%)
Weight (kg)	23.5 (10.52)	23.2 (9.17)
Length (cm)	114.2 (17.35)	114.4 (17.84)
Age (years)	5.7 (2.64)	6.0 (3.12)
- 2-5 years	23 (46%)	24 (49%)
- 6-10 years	24 (48%)	20 (41%)
- 11-18 years	3 (6%)	5 (10%)
Ethnicity		
- Caucasian	25 (50%)	25 (51%)
- Non-Caucasian	25 (50%)	24 (49%)
Country		
- France	5 (10%)	4 (8%)
- Other European countries	23 (46%)	24 (49%)
- India	22 (44%)	21 (43%)
Steroid dependent?		
- Yes	36 (72%)	33 (67%)
- No	14 (28%)	16 (33%)
Prior cyclophosphamide use?		
- Yes	3 (6%)	3 (6%)
- No	47 (94%)	46 (94%)
Relapses in past 6-12 months	3.2 (1.20)	3.4 (1.21)
Cumulative steroid dosage in past year (mg/m^2/ad)	4.074 (1.9583)	4.372 (2.3489)
Lowest prednisone dose to prevent relapse in past year (mg/m^2/ad)	19.0 (18.31)	14.2 (14.31)

Data are presented as n (%) or mean (standard deviation)

Table E1(cont): Medical and disease history - status at baseline - Randomized clinical trial - Safety Population

	Statistic	Levamisole N = 50	Placebo N = 50	Total N = 100
History of allergic symptoms related or not with renal symptoms				
unknown	% (n/N)	4.0% (2/50)	4.0% (2/50)	4.0% (4/100)
yes	% (n/N)	6.0% (3/50)	4.0% (2/50)	5.0% (5/100)
no	% (n/N)	90.0% (45/50)	92.0% (46/50)	91.0% (91/100)
History of infectious symptoms(within 4 weeks before relapse) related or not with renal symptoms				
unknown	% (n/N)	6.0% (3/50)	6.0% (3/50)	6.0% (6/100)
yes	% (n/N)	36.0% (18/50)	38.0% (19/50)	37.0% (37/100)
no	% (n/N)	58.0% (29/50)	56.0% (28/50)	57.0% (57/100)
Number of relapses in past 6-12 months				
	n	50	49	99
	mean (SD)	3.2 (1.20)	3.4 (1.21)	3.3 (1.20)
	min - max	0 - 5	1 - 6	0 - 6
Familial history of renal disease				
unknown	% (n/N)	8.0% (4/50)	6.0% (3/50)	7.0% (7/100)
yes	% (n/N)	4.0% (2/50)	8.0% (4/50)	6.0% (6/100)
no	% (n/N)	88.0% (44/50)	86.0% (43/50)	87.0% (87/100)
Prior use of other immunosuppressive drugs				
unknown	% (n/N)	6.0% (3/50)	4.0% (2/50)	5.0% (5/100)
yes	% (n/N)	2.0% (1/50)	8.0% (4/50)	5.0% (5/100)
no	% (n/N)	92.0% (46/50)	88.0% (44/50)	90.0% (90/100)

Program name:T_MH_V02.SAS, Programmer: sofiec, Output created: 09APR2015

Table E2 : Patient disposition including description of run-in medication

	Statistic	Levamisole N = 51	Placebo N = 52	Total N = 103
Randomized				
yes	% (n/N)	100% (51/51)	100% (52/52)	100% (103/103)
Treated (at least one dose taken)				
no	% (n/N)	2.0% (1/51)	3.8% (2/52)	2.9% (3/103)
yes	% (n/N)	98.0% (50/51)	96.2% (50/52)	97.1% (100/103)
Reason stop trial medication				
Normal completion	% (n/N)	24.0% (12/50)	6.0% (3/50)	15.0% (15/100)
Adverse event	% (n/N)	2.0% (1/50)	2.0% (1/50)	2.0% (2/100)
Patient request	% (n/N)	2.0% (1/50)	0.0% (0/50)	1.0% (1/100)
Investigator's request	% (n/N)	4.0% (2/50)	6.0% (3/50)	5.0% (5/100)
Relapse	% (n/N)	68.0% (34/50)	86.0% (43/50)	77.0% (77/100)

Summary of main efficacy results

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

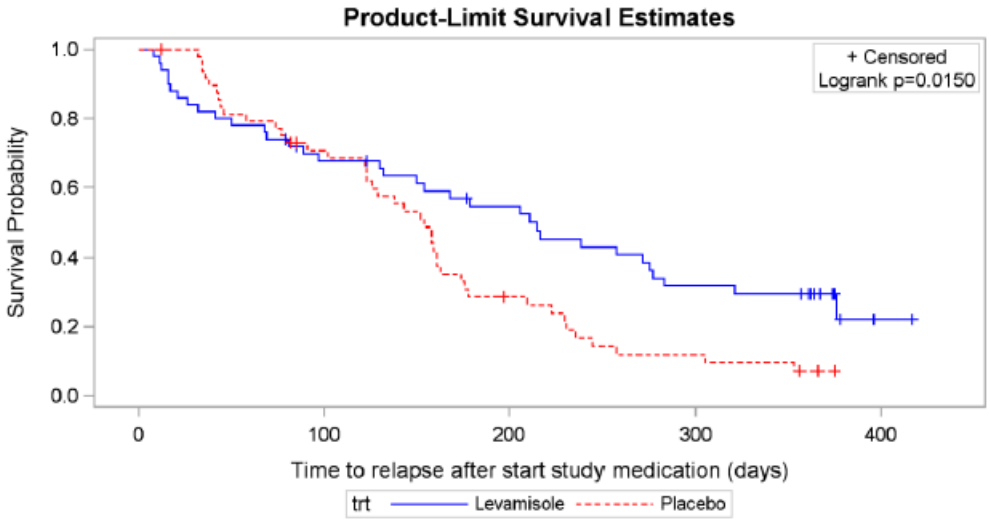
Table E3: Summary of efficacy for trial ACE080503

Title: Levamisole treatment for children with steroid sensitive nephrotic syndrome (SSNS)			
Study identifier	ACE080503		
Design	A multi-centre, double-blind, placebo-controlled, randomised trial.		
	Duration of main phase:		Treatment of study drug was started during steroid treatment for relapse when the patient's urine was protein-free for 3-21 days. It was continued until a relapse necessitating steroid treatment occurred or until 12 months of treatment.
	Duration of Run-in phase:		Start prednisone 60 mg daily until protein-free for 6-8 days
	Duration of Extension phase:		12 to 18 months
Hypothesis	Superiority		
Treatments groups	Levamisole		2.5 mg/kg on alternate days (rounded to the dosage available in the appropriate tablet strength (5, 10, 25 or 50 mg)), until a relapse necessitating steroid treatment occurred or until 12 months of treatment (tablets masked for bitter taste; diameter 5-8 mm)
	Placebo		Placebo tablets
	Cessation of corticosteroid background therapy		Start prednisone 60 mg daily (during run-in after relapse according to French Society of Nephrology protocol) until protein-free for 6-8 days; then subsequently 60 mg AD 4 weeks, 45 mg AD 4 weeks, 30 mg AD 4 weeks, 15 mg AD 4 weeks
Endpoints and definitions	Primary endpoint		Time to relapse necessitating corticosteroid treatment It was considered a relapse when proteinuria (albustix 3+ or albumin in urine of >200 mg/mmol creatinine) recurred after remission for at least 3 consecutive days. Treatment was started immediately in case of oedema, hypoalbuminaemia or when isolated proteinuria (>110 mg/mmol creatinine without hypoalbuminaemia or clinical signs of NS) lasted more than 3 weeks.
	Secondary	*Not defined as endpoint in study protocol*	A landmark analysis of time to relapse after prednisone cessation
Database lock	December 2012 (RCT) – October 2013 (cohort)		

Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat		
Descriptive statistics and estimate variability	Treatment group	Levamisole	Placebo
	Number of subject	51	50
	Numbers of relapses	34	42
	Mean time to relapse (days) (relapsers only)	138.7 ±107.5 (8-376)	138.5 ±79.24 (32-353)
Effect estimate per comparison	Primary endpoint	Comparison groups	levamisole versus placebo
		Cox proportional hazard	HR 0.564 K-M curve crossed at approximately 100-120 days
		variability statistic	95% CI: 0.354-0.899
		P-value	0.016 logrank test was 0.0150
	Secondary	Comparison groups	**Table 1.** Levamisole (n=32) versus placebo (n=29)
		Cox proportional hazard	HR 0.373
		variability statistic	95% CI: 0.200-0.699
		P-value	0.0021 logrank test was 0.0150

The **primary endpoint** occurred in 34 patients in levamisole and 42 patients in the placebo group with a HR after 12 months of HR 0.564 (95% CI: 0.354-0.899), though K-M lines crossed and about 100-120 days (see figure below).

Figure E1: K-M curve of primary endpoint (occurrence of relapse over time)



The landmark analysis of time to relapse after prednisone cessation was used as a **secondary outcome**. The analyses contained subjects who were still in the trial after the prednisone cessation scheme had been finalised. This concerned 32 subjects with levamisole and 29 subjects with placebo in the ITT population. A significant longer time to relapse after prednisone cessation was observed in the levamisole group compared to the placebo group (p=0.0014). The HR was 0.373 (95% CI: 0.200-0.699 and p=0.0021).

Subgroup analyses for age, ethnicity, region, steroid dependency, and prior cyclophosphamide use were comparable to the main analysis (including crossing K-M curves) with a *significant* beneficial effect found in 6-10 years, Non-Caucasians, India, steroid dependency, high steroid-dependency (>15 mg/m2) and no prior cyclophosphamide use.

Table E4: Statistical analysis results for prespecified subgroup analyses of time to relapse

		Log rank test p-value	Hazard ratio	p-value	Crossing of K-M curves (yes/no)
Age	2-5 years	0.2468	0.686 (0.361-1.302)	0.2487	Yes
	6-10 years	0.0117	0.380 (0.174-0.827)	0.0148	Yes
	11-18 years	0.5318	0.491 (0.050-4.788)	0.5406	No
Ethnicity	Caucasian	0.3578	0.734 (0.381-1.416)	0.3562	Yes
	Non-Caucasian	0.0142	0.440 (0.225-0.859)	0.0161	Yes
Site group	France	0.1211	0.280 (0.050-1.555)	0.1455	No
	Other EU countries	0.5042	0.796 (0.405-1.562)	0.5064	Yes
	India	0.0282	0.453 (0.221-0.930)	0.0309	Yes
Steroid dependency	Yes	0.0461	0.567 (0.323-0.995)	0.0479	Yes
	No	0.0593	0.421 (0.168-1.059)	0.0661	Yes

Prior cyclophosphamide use	Yes		3495017 (0,-)	0.9871	No
	No		0.498 (0.307, 0.809)	0.0049	Yes

Table E5: Exploratory subgroup analyses based on mean steroid dose of the safety population

Steroid dependency	High (>20 mg/m^2)	0.0371	0.571 (0.272-1.197)	0.1376	Yes
	Low ((<20 mg/m^2)	0.4482	0.380 (0.138-1.049)	0.0618	Yes
	No	0.0593	0.421 (0.168-1.059)	0.0661	Yes
Steroid dependency	High (>15 mg/m^2)	0.0588	0.483 (0.242-0.964)	0.0390	Yes
	Low (<15 mg/m^2)	0.0043	0.669 (0.234-1.915)	0.4539	Yes
	No		0.421 (0.168-1.059)	0.0661	Yes

Additional analyses showed that the **prednisone dose** at time of relapse had a mean of 28.2 ±37.4 (0-120) mg/m2/AD in the levamisole group (n=34) and a mean of 16.1 ±25.53 (0-120) mg/m2/AD in the placebo group (n=42). The median dose was 0 in both treatment groups. The two-sided Wilcoxon Rank Sum test found no difference in the prednisone dose at time of relapse between the levamisole and placebo groups (p=0.2225).

Two additional comparisons were made for the prednisone dose *at* time of relapse using the two-sided Wilcoxon Signed Rank test. No differences were found between the prednisone dose at time of relapse and the lowest prednisone dose to prevent relapse prior to the study. P-values were 0.3068 for the levamisole group and 0.8159 for the placebo group. The comparison between the prednisone dose administered *prior* to the dose at time of relapse and the lowest prednisone dose to prevent relapse prior to the study showed significant p-values for both treatment groups. Obtained p-values were 0.0154 and 0.0349, respectively.

Usability of the tablets was tested. In some cases, placebo tablets were provided prior to start of study medication to check if the child was able to swallow tablets. If it is necessary, according to the opinion of the investigator, to train children to swallow the study medication in whole, a container with pills especially for training pill swallowing will be provided during the initiation visit. No swallowing problems were encountered, also not in the youngest children.

Clinical studies in special populations

No studies addressing special populations (as e.g. patients with renal dysfunction or hepatic dysfunction) have been presented and no studies are available in the public domain.

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

Supportive study(ies)

In addition to the pivotal study, seven other literature references have been provided to further support clinical efficacy data (see table below). Further details of the studies have been provided below the table.

In the studies where levamisole 2.5 mg/kg every other day was compared with placebo or no treatment a positive effect of levamisole was observed (pivotal clinical trial; Abeyagunawardena et al., 2006; Al-Saran et al., 2006; BAPN, 1991; Donia et al., 2005; Rashid et al., 1996). In the two studies were levamisole was dosed two times a week no significant effect was detected (Dayal et al., 1994; Weiss et al., 1993). The effect of levamisole treatment was comparable with cyclophosphamide treatment in the study of Donia et al. (2005).

Table E6: Results of efficacy studies

Study	Study design	Treatment Arm	# Enrolled/ completed	Primary Endpoint	Outcome primary endpoint	Statistical test/ P value	Secondary Endpoints	Outcome secondary endpoint	Statistical test/ P value
Pivotal clinical trial	Randomized placebo controlled	1: L 2.5 mg/kg qod +Pred	1: 50/50	Patients that relapsed within one year	1: 33/50 (66%)	Fisher's exact test P = 0.012	Duration of remission		Log-rank test/ P = 0.0150
		2: Placebo +Pred	2: 50/49		2: 42/49 (86%)				
Abeyaguna-wardena et al., 2006	Randomized controlled	1: L 2.5 mg/kg qod	1: 42/42	Patients that relapsed within one year	1: 8/42 (19%)	Comparison of 2 proportions P < 0.01			
		2: no treatment	2: 34/34		2: 26/34 (76%)				
Al-Saran et al., 2006	Controlled prospective	1: L 2.5 mg/kg qod + Pred	1: 32/32	Reduction in number of relapses/month (mean)	1: 0.29	One-tailed t-test/ P = 0.0001	Reduction in cumulative prednisolone dose ($mg/m^2/m$) (mean)	1: 293	One-tailed t-test/ P < 0.0001
		2: Pred	2: 24/24		2: 0.11			2: 102	
BAPN, 1991	Randomized placebo controlled	1: L 2.5 mg/kg qod + Pred	1: 31/31	Patients in remission after 112 days	1: 14/31 (45%)	X^2-test/ P = 0.008			
		2: Placebo + Pred	2: 30/30		2: 4/30 (13%)				
Dayal et al., 1994	Randomized controlled	1: L 2-3 mg/kg biw + Pred	1: 33/32	Patients in remission after 30 months	1: 21/33 [a] (64%)	X^2-test / P = 0.11	Duration of remission	1: 12 months	Survival analysis/ P = 0.10
		2: Pred	2: 28/27		2: 12/28 [a] (43%)			2: 10.5 months	
Donia et al., 2005	prospective randomized	1: L 2.5 mg/kg qod + Pred	1: 20/20	Patients in remission after 6 months	1: 10/20 (50%)	X^2-test / P = NS	Duration of steroid-free remission (months) (median)	1: 6.83	Mann-Whitney/ P = NS
		2: Cycloph iv 500 mg/m^2/ month + Pred	2: 20/20		2: 9/20 (45%)			2: 5.2	
Rashid et al., 1996	Prospective controlled randomized	1: L 2.5 mg/kg qod + Pred	1: 20/20	Patients in remission after 44 weeks	1: 9/20 (45%)	X^2-test/ P < 0.02			
		2: Pred	2: 20/20		2: 2/20 (10%)				
Weiss et al., 1993	Randomized placebo controlled	1: L 2.5 mg/kg biw + Pred	1: 21/21	Number of relapses/month (mean)	1: 0.7	/ P = NS	Daily prednisolone dose (mg/day) (mean)	1: 43.2	/ P = NS
		2: Placebo + Pred	2: 20/20		2: 0.6			2: 45.4	

[a] the patient that was lost to follow-up was included in this analysis

Abeyagunawardena et al. (2006)

Type of publication: Short summary (Pediatric Nephrology. 2006;21(10):1503.); Type of patients: SDNS – stable remission – low dose levamisole and low dose prednisolone; Number of patients: 76; Study location: Single centre, Sri Lanka; Study period: One year; Study outcome: Patient that relapsed within one year (8/42 vs 26/34; p<0.001)

Al-Saran et al. (2006)

Type of publication: Original article – clinical study (Pediatric Nephrol (2006) 21: 201-205.); Type of patients: FR- SDNS; Number of patients: 56; Study location: Single centre, Saudi Arabia; Study period: One year; Study outcome: Reduction of number of relapses/month (0.29 vs 0.11; p=0.0001 one sided)

British Association for Paediatric Nephrology (BAPN; 1991)

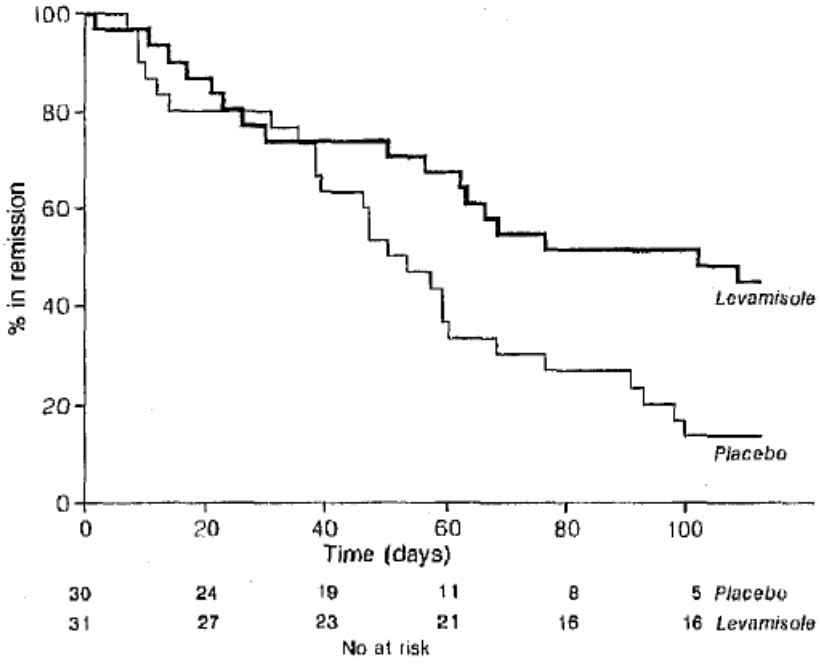
Type of publication: Original article – clinical study (Lancet. 1991;337(8757):1555-7.); Type of

patients: FRNS - high SDNS; Number of patients: 70; Study location: multiple centres, UK; Study period: 112 days; Study outcome: Patients in remission after 112 days (14/31 vs 4/30; p=0.008)

BASELINE CHARACTERISTICS

—	Levamisole (n=31)	Placebo (n=30)
Mean age (SD)	8·3 (3·6)	8·8 (3·7)
Mean weight/height (SD)	123 (19·3)	130 (27·3)
Mean height SDS (SD)	+0·2 (0·8)	−0·4 (0·79)
Sex F/M	10/21	10/20
Previous alkylating therapy	20	18
Mean time in years from completion of alkylating therapy until trial entry (SD)	3·6 (3·1)	2·6 (1·75)
Biopsy	4	8

SDS = standard deviation score.



Percentage in remission by time to relapse in days for levamisole and placebo groups.

Dayal et al. (1994)

Type of publication: Original article – clinical study (Nephron. 1994;66(4):408-12); Type of patients: SDNS in remission; Number of patients: 61; Study location: single centre, India; Study period: 30 months; Study outcome: Patients in remission after 30 months (21/33 vs 12/28; p=0.11)

Donia et al. (2005)

Type of publication: Original article – clinical study (Pediatr Nephrol. 2002;17(5):355-8.); Type of patients: SDNS; Number of patients: 40; Study location: Single centre, Egypt; Study period: 6 months; Study outcome: Patients in remission after 6 months (10/20 vs 9/20 in cyclophosphamide; p=ns)

Rashid et al. (1996)

Type of publication: Original article – clinical study (Bangladesh Renal Journal. 1996;15(1):6-8.); Type of patients: SDNS or FRNS; Number of patients: 40; Study location: Single centre, Bangladesh; Study

period: 44 weeks; Study outcome: Patients in remission after 44 weeks (9/20 vs 2/20; p<0.02)

Weiss et al. (1993)

Type of publication: Short summary (Clinical Nephrology. 1993:289.); Type of patients: SDNS or FRNS; Number of patients: 22; Study location: Two centres, US; Study period: 6 months; Study outcome: Number of relapses/month (0.7 vs 0.6; p=ns)

Not submitted by the applicant:

Basu B, Pandey R, Mishra OP. Randomized controlled trial to compare the efficacy & safety of Mycophenolate mofetile vs. Levamisole in children with frequent relapsing & steroid dependent nephrotic syndrome. Pediatr. Nephrol. 28, 1353 (2013).

The study indicated superior efficacy of Mycophenolate mofetile in the treatment of steroid dependent nephrotic syndrome. However, since the study is available as abstract only and no reliable conclusions can be drawn.

3.4.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The application was based on one pivotal trial and a number of supportive trials provided as bibliographical data in form of paper or abstracts. In addition, some reports were assessed from the public domain. The bibliographical data contain placebo controlled and active controlled trials, uncontrolled reports and retrospective chart reviews.

A multi-centre, double-blind, placebo-controlled, randomised trial has been submitted to demonstrate the efficacy and safety of levamisole with the objective to evaluate whether levamisole in combination with a down titration scheme of prednisone after relapse could prevent relapses in comparison with patients treated with levamisole after one year of treatment for children between 2 and 18 with SSNS. Another objective has also been formulated whether levamisole treatment could prolong time to relapse after cessation of corticosteroids treatment, which has been evaluated as a landmark analysis.

Further, the secondary objectives of evaluating treatment effect of levamisole according to steroid-dependent versus frequently relapsing and low- versus high-dose steroid-dependency, and prior treatment of cyclophosphamide have been presented. Patients were stratified according to these subgroups, expect for steroid-dependent versus frequently relapsing. The applicant has also formulated a secondary objective of assessing the steroid-sparing effect of levamisole treatment as well as the sparing effect of other immunosuppressive drugs (i.e. average amount administered per patient). However, the study design does not allow to evaluate these objectives as steroid cessation was performed according to a fixed regimen. Also, a sparing effect of other immunosuppressive drugs cannot be answered by this study protocol as these drugs were not allowed during this study.

Questioning these objectives, there also appears an inconsistency with the statistical analysis plan with regard to steroid intake. The applicant mentions that the average quantity of steroids administered during the study, was not evaluated due to incomplete recording of steroid intake. This is of major concern, as non-compliance to prednisone intake could initiate a relapse event and thus highly interfere with the main objective of the study. Further, this questions whether the other analysis related to steroid intake could be performed and can be considered reliable.

In addition, some other inconsistencies regarding the study conduct, objectives, and endpoints evaluated and presented have also been noticed:

- There is discrepancy between the Clinical Study Report and the Statistical Analysis Plan with regard to the secondary objectives. While the SAP indicates that the only secondary objective is to evaluate whether the effect of Levamisole varies with the following subject characteristics: underlying disease process (steroid dependency); prior use of cyclophosphamide; age group; ethnicity; site group, the CSR indicates that the steroid-sparing effect of levamisole treatment as well as the sparing effect of other immunosuppressive drugs will be assessed, for which no data have been presented.
- There is a discrepancy between the Clinical Study Report and the Clinical Study Protocol (version 6.0) with regard to the secondary endpoints. While relapse after prednisone cessation was described as the only secondary outcome in the study report, the secondary endpoints that have been described in the study protocol are evaluation of average quantity in milligrams of steroids administered per month during the study, evaluation whether treatment effect differs with underlying disease process (steroid-dependency yes/no) and prior use of disease modifying agents (yes/no), and maintenance dose prednisone at time of relapse. Consequently, there is some discrepancy between the description of the secondary objectives and the description of the secondary endpoints.
- The final SAP was drawn up after data collection was complete; the SAP is dated 19-12-2013, whilst the last patient completed the cohort on 15 October 2013. This questions whether there was insight in the (blinded) data at the time of drawing up the final SAP.
- Also, e.g. the time points of the amendments were not entirely clear. Documentation regarding the ethic committees was not provided and it is not entirely clear if approvals were available for all of the study centres in France.

In an application that is based mainly on one pivotal trial, robustness of the data should be particularly high. Since there are serious concerns in this regard affecting different aspects of the trial, the issue of conduct of the trial and robustness of the data is considered a major objection.

The inclusion criteria are considered acceptable and would allow to include a paediatric population with SNSS. Although, a different inclusion criteria of a lower limit of 3 years instead of 2 years applied in France is not considered ideal, the impact of this difference on the results is anticipated being marginal. The exclusion criteria are considered acceptable although the exclusion criterion of patients unresponsive to cyclosporine or mycophenolate mofetil is not applicable because the included patient population has not been previously treated with such medication.

Patients were included in the study at time of relapse. During this run-in phase prednisone was given at a dose of 60 mg/m^2 once daily until the urine was protein-free for at least 6-8 days. This was the first step of dose reduction to 60 mg/m^2/AD (every other day). It is not specified how and at what frequency protein in urine was measured at run-in. In theory, waiting until patients were protein-free could mean a large run-in difference between patients. Patients were started study medication when the patient was 3-21 days protein-free. This range appears to be wide, and introduces variability to the start of the study in relation to start of the prednisone down titration scheme between patients.

The prednisone scheme is according to the French guideline. This may be acceptable, although other schemes are also described in other clinical practice guidelines, albeit there appears no consensus on the optimal corticosteroid scheme to be applied. For instance, in the Netherlands a scheme of 60 mg/m^2 for 6 weeks and 40 mg/m^2 for 6 weeks is used (Teeninga, JASN, 2012). However, after the end of the forced down titration scheme patients were not on corticosteroid medication. This may not reflect clinical practice for these steroid sensitive patients who are steroid dependent, 67-72% of the included patients at baseline. Those patients may be in need for a less intensive down titration regimen

and in the need for a longer term stable low dose of corticosteroid treatment (e.g. 7.5 mg prednisone AD) before slowly tapering of corticosteroid treatment to prevent a relapse, and questions the clinical relevance of the current study design for those patients.

Other immunosuppressive medication was not allowed during study, except for cyclophosphamide. It is not exactly understood why patients who were unresponsive to cyclosporine or mycophenolate mofetil were not included into the study. Probably, the investigators expected that such patients may also be less likely to be responsive to levamisole which needs further discussion.

The primary efficacy variable proteinuria was measured weekly by stick and monthly by laboratory examinations in urine. The method used has been described in clinical guidelines (KDIGO) and can be acceptable. The primary endpoint is considered acceptable. The study protocol describes that if detection by stick is positive, the TP/ creatinine ratio will be determined if possible, which question the number of primary endpoints that will only be assessed by dipstick measurement. In addition, the following sentence *"Treatment was started immediately in case of oedema, hypoalbuminaemia or when isolated proteinuria (>110 mg/mmol creatinine without hypoalbuminaemia or clinical signs of NS) lasted more than 3 weeks"*, is not understood, considering that immediate action is done by treatment of corticosteroids, while this sentence suggests that nothing will be done at least for 3 weeks.

In general, the proposed statistical analysis appears appropriate considering the primary objective, although potential important co-variates, such as centre and steroid dependency, are not taken into account in the primary analyses. These are evaluated in planned subgroup analyses. The approach regarding the subgroup analyses is considered appropriate. It is noted that no attempts are being made to control the type I error nor that the study is powered for subgroup analyses therefore these, albeit informative, will not carry strong value for confirmatory purposes.

The randomisation procedure is considered acceptable. In particular, stratification according to region is important due to different inclusion criteria in France. Also, stratification according to steroid dependency and cyclophosphamide are important for a balanced treatment allocation. The blinding methods used are considered appropriate. The bitter taste of levamisole was masked, which is considered appropriate. Discontinuation criteria are considered appropriate.

Patients were still followed when unblinded, so these data should be interpreted with caution, as any bias may be likely. Furthermore, it is not clearly mentioned whether patients were still treated with study medication during follow-up. Finally, patients free of relapse during one year could be additionally treated with levamisole in the cohort extension for 18 months.

Efficacy data and additional analyses

Dose response studies

The applicant has conducted a pivotal trial using a dose of 2.5 mg levamisole AD. Since the mode of action is unclear, no PD parameters to support dose selection are available. No clinical dose response studies were provided. Dose selection in the pivotal trial is based mainly on the fact that this dose has been widely used over decades in paediatric patients with nephrotic syndrome. In particular, one placebo controlled clinical trial (BAPN, 1991) showed a reduction in relapse rate at this dose level.

The following considerations are of relevance:

In a study with children with rheumatic disease, a dose of 5 mg (kg QD was associated with severe AEs. Intermittent dosing is considered useful in reducing AEs of levamisole. Most studies in the public domain in this therapeutic field were conducted with the proposed dosing regimen. Overall, the dose of 2.5 mg/kg AD was reported to be well tolerated.

With a lower dosing regimen of 2.0 – 3.0 mg/kg twice a week no significant efficacy was observed, although it is unclear whether this was due to the dose (Dayal U et al., 1994). Slightly higher doses up to 3.0 mg/kg were investigated without reports of serious AEs. There were case reports that some patients not responding to a lower dose of 2.5 mg/kg twice a week to a three times a week application or to 5 mg/kg AD was associated with remission in some patients (Drachman et al. 1988). Similarly, a step wise approach starting with 2 -3 mg/kg AD and an increment to the same daily dose QD in patients not being in remission was investigated with success rates on each dose level. The totality of data indicated that a dose of 2.5 mg/kg was within a reasonable range

Taken together, it is unclear whether the dose of 2.5 mg/kg AD is optimal but based on published data it is within a reasonable range for efficacy and tolerability.

Pivotal trial

The applicant has submitted a prospective multinational randomised clinical trial with 103 paediatric patients with frequently relapsing steroid sensitive nephrotic syndrome steroid dependent or independent.

According to the description 103 patients were treated with prednisone and 100 patients were actually treated with study medication (levamisole or placebo). Acceptable justification has been provided for the 3 patients not treated with study medication (1 relapse on prednisone, 2 late arrival of study medication). Only a small proportion (1 patient each group) was discontinued due to an adverse event. Five patients discontinued because of investigator's request. Two of those have not been reported appropriately as these were because of an adverse event (presence of ANCA, increased creatinine; see table 16.2.01.01 company dossier).

Randomisation can be considered successful with only slight differences between levamisole and placebo for age, steroid dependency, and the lowest dose of prednisone to prevent relapses. These minor differences are not expected to influence outcome data.

The included patients comprise a patient population which can be expected in daily practice. Most patients included were between 2-10 years of age, the majority was male, and a high proportion of the steroid dependency frequently relapsed. Any distinction between steroid dependent and non-steroid dependent patients in relation to mean steroid dose, steroid dose before relapse and frequency of relapses has not been made, while this is important to be able to interpret study results. The current proposed indication does not appropriately reflect the patient population included in the clinical trial. The study design does not reflect clinical practice for those patients who are steroid dependent in the steroid sensitive population because these patients are normally treated on a continuous low dose corticosteroid treatment for a longer period of time. Any distinction between steroid dependent and non-steroid dependent patients in relation to mean steroid dose, steroid dose before relapse and frequency of relapses has not been made. Further, although the study was designed to include patients with frequently relapsing SSNS, patients with and without frequently relapsing SSNS were included. There are several issues to be addressed concerning the number of patient with different grades of severity of diseases and number of relapses prior to inclusion.

A large proportion of patients was recruited in India (44%), which may be less relevant for the EU situation. In contrast with the European patient, the majority were non-Caucasians and steroid-independent. Steroid dependency may indicate a more severe disease state (97% in Caucasians). Only 6% used prior cyclophosphamide. It is not known why patients with prior cyclophosphamide were not excluded similar to patients unresponsive to cyclosporine or mycophenolate mofetil. However, prior use

of immunosuppressive drugs was low, consequently it is not known whether patients were indeed unresponsive to cyclosporine or mycophenolate mofetil. Remarkably, patients with 0 relapses in the past 6-12 months were included, which is questioned as this does not comply with the inclusion criteria. Further, the age group of 11-18 years was obviously underrepresented with only one patient above the age of 14.

The primary efficacy analysis of the ITT population indicated that one year of levamisole treatment resulted in a statistically significant longer time to relapse compared to placebo (34 patients in levamisole and 42 patients in the placebo group with a HR after 12 months of HR 0.564 (95% CI: 0.354-0.899)). However, as mentioned, there was an incomplete recording of corticosteroid intake which question the validity of the data, as any non-compliance to prednisone intake could initiate a relapse event and thus highly interfere with the primary analysis of the study. Further, the crossing of the KM curves is of interest, as for the first 50-100 days more patients relapsed on levamisole compared to placebo. It is agreed that there is an overall (statistically significant) benefit, and therefore it is agreed with the company that incorporating non-proportionality in the model is not necessary per se. However, it remains to be explained why there are more patients relapsing on levamisole compared to those on placebo during the first months of treatment. In this respect, several pre-planned subgroup analyses according to age, ethnicity, region, steroid dependency and prior cyclophosphamide use showed consistent results when compared with the overall primary analysis. Thus, results obtained from subgroup analyses could not clarify this observation. This raises the possibility that levamisole has no efficacy add-on corticosteroids. Possibly there may even be a negative interaction. Accordingly, treatment with levamisole may not be initiated at the beginning of corticosteroid therapy but rather at the end of the tapering phase but no data are available for a delayed treatment with levamisole. Also, the company has not identified the type of patients with an increased risk for early relapse, which might clarify the observed effect. Therefore, the patient characteristics of these early relapsing patients should be detailed and compared to the rest of the population. Also, data on duration of run-in has not been provided, while great variability could exist of time in run-in and randomisation.

Consistently, a lack of efficacy of levamisole during the steroid tapering phase was observed in the most important supportive study (BAPN 1991). In that study a treatment effect was visible not before prednisone was reduced to 0.25 mg/kg. Levamisole was initiated with two weeks delay in that study indicating that a delay may not be of concern when starting levamisole after a relapse.

At present the appropriate time of initation of levamisole in relation to steroid therapy is unknown and even a negative interaction between high dose corticosteroids and levamisole cannot be excluded.

Some issues pertaining to assessment of the primary endpoint are not entirely clear and should be further clarified as outlined in detail in the list of questions, section 6.

Further of notice with regard to the subgroup analyses is that almost all Caucasians were steroid dependent and recruited in European sites. It appears that the benefits are slightly less in these patients, as far as the observed HRs can be considered reliable, although significance levels for interactions have not been presented. Yet, the relevance of this difference is difficult to interpret. Since age group were redefined post hoc, an analysis for age groups as pre-defined should be provided. Efficacy in Caucasian patients (mainly from Europe) was numerically lower than in non-Caucasians (mainly India). In Europe without France efficacy of levamisole was not significantly better than placebo, whereas efficacy in France appeared to be better. There were some differences in France. E.g. only patients above 3 years were included that had passed a tablet swallow test. Patients in France were recruited later in the study than most of the other patients. Results of the pre-study swallow test should be provided by the applicant. It should further be discussed by the applicant, if children below 3

years of age had a different outcome (e.g. related to difficulties in swallowing) and if there was a correlation between time of randomisation and outcome in the study.

The time to relapse after prednisone cessation has been post hoc analysed as a secondary endpoint. This is a so called landmark analysis. A landmark analysis ”resets the clock” and starts survival analysis at a future time point chosen by the investigators, in this case when the prednisone dose scheme was ended for each patients at approximately 100-120 days after study randomisation. However, bias might be introduced as the number of patients at the time point the prednisone dose scheme was ended was different with only 32 patients still on treatment with levamisole and 29 patients on placebo. Approximately 40% of the patients were already out of the trial mainly because of reaching the primary endpoint. Therefore, although a beneficial effect could be shown (HR 0.373 (95% CI: 0.200-0.699 and p=0.0021)), the clinical relevance of this analysis and finding is highly questioned and should be explained further.

Relapse was identified by a dipstick method and confirmed with a laboratory urinary albumin assessment if last mentioned method was available. However, the proportion of relapses as confirmed by laboratory measurement has not been specified. Further it is described that an independent data monitoring committee was responsible for evaluating the clinical endpoints. Any data on investigator observed relapses in comparison to data from the independent monitoring committee have not been presented.

Several exploratory analyses have been performed on the prednisone intake during the study. The first analysis was to evaluate the prednisone dose at time of relapse between levamisole and placebo. The relevance of this analysis can be questioned as the prednisone regimen was fixed and ended at approximately 100-120 days after study entry, and thus the average prednisone dose at time of relapse appears a sort of alternative analysis of the primary analysis as it is dependent on the average time to relapse. For this analysis a higher mean dose of prednisone for levamisole treated subjects compared to placebo was found at time of relapse (not statistically tested), while strangely enough the median prednisone dose was 0 for both treatment groups. The clinical value of the expression in differences in mean and median dose levels is not understood. Further, as already mentioned, there was an incomplete recording of corticosteroid intake, further questioning the validity of this analysis. And in this respect, a better representation of this type of end point would be a comparison between the total cumulative use of corticosteroids during the fixed dose period between the different treatment groups. In a further analysis, no differences were found between the prednisone dose at time of relapse and the lowest prednisone dose to prevent relapse prior to the study. However, only p values were presented without any presentation of mean or median values of prednisone dose. Likewise, for the comparison between the prednisone dose administered prior to the dose at time of relapse and the lowest prednisone dose to prevent relapse prior to the study no mean or median prednisone values have been reported while a significant difference was found (significant p values). Although, the relevance of these analyses are questioned, these mean (or median) values should still be reported for completeness and understanding.

The 2012 KDIGO GN-guideline recommends to change the 3 months alternate day prednisone regimen to a QD administration during episodes of upper respiratory tract and other infections to reduce the risk for relapse. This recommendation is not included in the study protocol but it should be outlined how steroid administration during such episodes in the study was handled.

It appears that the company has tested the usability of the tablets also in the younger patients and that no patients experienced any problems. However, any details on whether, how and in how many patients specific testing has been performed has not been described. Further, any details on

medication errors have not been provided. This is considered important as the distinguishability has been questioned.

Only 5 patients have been included in the cohort extension phase. This is considered limited, though reasons not to include patients in the cohort extension phase are acceptable (distance to hospital and availability of commercial levamisole). Fourteen patients were not followed after study termination. The reasons for this have not been mentioned.

Supportive data

Several studies with levamisole, described in literature, have been submitted in the current dossier to support efficacy of levamisole in SNSS. Five studies enrolled children with frequently relapsing (Rashid, Weiss) and steroid-dependent SSNS (Abeyagunawardena; Al-Saran; BAPN; Rashid; Weiss) and compared treatment of levamisole to placebo (or background therapy). The study of Dayal included patients with SDNS in remission. Also these studies used a similar dose of levamisole as the pivotal study. Therefore, in terms of patients included and levamisole dose used they could partly support the pivotal study. The five studies reported a beneficial effect of treatment with levamisole. However, several concerns could be identified which question the relevance of these findings. First, all studies except for BAPN have been performed outside of Europa, in particular in Asia, which question the relevance to the EU setting and EU type of patients. Further, the study of BAPN showed a comparable K-M pattern as to the pivotal study. The most important study was the BAPN 1991 trial with 31 children with frequently relapsing corticosteroid sensitive and dependent nephrotic syndrome randomized to levamisole and 30 children to placebo for a 6 month treatment period. Any efficacy over time data for the other placebo controlled studies have not been presented and thus it is not known whether a similar time dependent effect would be present. Further, the study of BPAN, Donia and Rashid were 6 months or shorter, while the pivotal study evaluated levamisole treatment for a year. The study of Weiss and Dayal, apart from that they did not demonstrate a statistical beneficial effect, may not be relevant to support the current pivotal study as in these studies a different dosing of twice weekly was used, while in the other studies levamisole 2.5 mg/kg was administered on alternate days like in the pivotal study. Furthermore, the design of the studies may not be sufficient for current standards, with regard e.g. blinding, number of locations, and time of performance of the studies. For instance, the BAPN study was the only multicentre study and study of Weiss was a dual centre study. A discussion in a meta-analysis of Cochrane (2013) considered the quality of the evidence to be low, due to unclear allocation concealment and no blinding in most studies, and considered that significant heterogeneity between studies exists.

There are no conclusive data indicating that there is a persistent treatment effect of levamisole after discontinuation of therapy.

3.4.7. Conclusions on clinical efficacy

In summary, there may be a reasonable place for levamisole in the treatment of frequently relapsing steroid sensitive nephrotic syndrome in paediatric patients. There is a medical need for steroid sparing drugs although neither a steroid sparing effect nor a reduction of steroid associated toxicity has been demonstrated for levamisole in well conducted trials. Dose selection (2.5 mg/kg AD) can be accepted although it is not clear that in fact the optimal dose has been selected.

There are two studies indicating that paediatric patients with steroid sensitive nephrotic syndrome may have a lower relapse rate with levamisole compared to placebo, the pivotal 12 month trial and the supportive 6 month BAPN 1991 trial. The pivotal multi-centre, double-blind, placebo-controlled,

randomised trial demonstrated the efficacy and safety of levamisole with the objective to evaluate whether levamisole could prevent relapses in comparison to not treat with levamisole after one year of treatment for children between 2 and 18 with SSNS. Although the primary efficacy analysis of the ITT population indicated that one year of levamisole treatment resulted in a statistically significant longer time to relapse compared to placebo, incomplete background corticosteroid intake recordings question the validity of these findings.

Both trials consistently indicate that there is no treatment effect during the phase of steroid tapering after a relapse. This crossing of K-M curves are of interest. Any clarification for this has currently not been recognised, including subgroup analyses. Even a negative interaction between steroids and levamisole cannot be excluded. It may even be preferable to initiate levamisole therapy not before the end of tapering of prednisone (e.g. at a dose level of 0.25 mg/kg prednisone) but this was not investigated in the pivotal trial. Further, the clinical relevance of performed secondary and exploratory analyses is questioned.

In the pivotal trial, frequently relapsing disease was an inclusion criterion but the wording of the indication does not make this restriction. The wording of the indication should reflect the patients included in the pivotal trial.

Due to methodological drawbacks the other supportive studies cannot provide reliable evidence for efficacy of levamisole in the treatment of SSNS

Overall, the efficacy can currently not be well determined due to major issues in design, conduct, and interpretation of the study result.

3.4.8. Clinical safety

Patient exposure

The table below displays **overall exposure** of levamisole based on the submitted data.

Subjects receiving levamisole (n=50) were on average 194.2 ±135.9 (9-418) days on **trial medication**. The levamisole dose at the start of the trial had a mean of 2.427 ±0.2448 (1.92-2.78) mg/kg/AD. The placebo group (n=49) received trial medication for a mean of 149.8 ±94.94 (13-376) days.

Table S1: Clinical trial exposure in number of patients and patient-years

		Pivotal study		RCT from literature (n=7)		Other literature references (n=28)	
		patients	Patients-years	patients	Patients-years	patients	Patients years
Mean duration of exposure	<6 mnths	25	12.5	31	15.5	49	24.5
	6 - 12mnths	12	6	61	30.5	108	54
	12 mnths	6	6	107	107	133	133
	> 12 mnths	7	7			363	363
	Not specified					*413*	
Total (mean)		**50**	**31.5 (0.67)**	**199**	**153 (0.77)**	**621**	**542.5 (0.88)**
Dose (every other day)	2.5 mg/kg	50		145		654	
	< 2.5 mg/kg					26	
	>2.5 mg/kg					9	
	other			54		333	

Adverse events

In the pivotal RCT, more subjects in the levamisole group (n=29, 58.0%) experienced a **TEAE** than in the placebo group (n=19, 38.0%). Most frequently observed AEs reported at a higher frequency for levamisole were pyrexia (20% vs 12%), cough (14% vs 12%), increased aspartate aminotransferase (12% vs 0%), and neutropenia (8.0% vs 2.0%). Frequently observed AEs of nasopharyngitis was reported more in the placebo group than for levamisole (18% vs 20%). All except one subject out of 5 (80.0%) in the *cohort* experienced a TEAE. The most frequently reported TEAE in the cohort was headache (3 events).

The **most common drug-related TEAEs** were increased aspartate aminotransferase (AST) (12.0%), neutropenia (8.0%) and positive antineutrophil cytoplasmic antibody (ANCA) (4.0%). Other drug-related TEAEs were less common and occurred in not more than one subject. While infections and infestations had the highest incidence as TEAEs in the RCT (32.0% versus 4.0%), only some of these TEAEs were related to levamisole treatment.

In addition to safety data from the pivotal study, in the table below 44 literature studies or literature case reports ranging from 1980 to 2015 were evaluated. Also, in the named patient program of France 13 patients (out of 200) reported a related AE.

Table S2: Summary AEs from all sources

AE	SOC	Reported Frequency literature	Clinical trial	Named patient programs
Neutropenia (including agranulocytosis)	Blood and lymphatic system disorders	3%	8%	1%
pANCA positive (with or without vasculitis symptoms)	Investigations / Immune system disorder	1%	5%	1%
Mild increase in liver enzymes (AST/ALT)	Investigations / hepatobiliary disorder	0.3%	10%	0.5%
Significant increase in liver enzymes (AST/ALT)	Investigations / Hepatobiliary disorder	-	2%	
Cutaneous reactions (rash, pruritis and other skin reactions)	Skin and cutaneous tissue disorders	1%	6%	1%
Infections (mouth, respiratory, scalp)	Infections and infestations	1%	6%	
Gastrointestinal disturbances (nausea, vomiting, GI upset)	Gastrointestinal disorders	0.5%	1%	
Others (single reports) **- Myalgia** **- Increase blood creatinine** **- Pain in extremity** **- Vertigo** **- personality change** **- Acute Lymphatic Leukaemia** **- Arthralgia** **- Heel pain** **- erythema** **- fever**				

Serious adverse events and deaths

Serious adverse events are displayed in the table below.

Serious adverse events in the pivotal study

In the RCT, the occurrence of **hospitalisations** was 8.0% in subjects treated with levamisole versus 0% in subjects treated with placebo. Most hospitalisations (4 out of 5 in RCT) occurred within the first 3 months after start of levamisole (overall range 7-215 days). One subject in the cohort study was hospitalised (after 372 days of treatment).Hospitalisations in subjects who were being treated with levamisole were assessed as unrelated to study treatment.

Neutropenia (<1000 106/L) was reported as serious TEAE in 8.0% of subjects treated with levamisole (versus 0% in placebo group). In 4 out of 5 neutropenias, levamisole was discontinued. An additional neutropenia was reported in the follow-up phase, in which the dose was decreased. All

serious neutropenias were considered related to levamisole. Neutropenia occurred 90-426 days after start of levamisole.

Table S3: Serious adverse events in the pivotal study

Study phase	SAE (PT)	RCT treatment	Follow-up / Cohort treatment	Treatment duration (days)	Relationship	Stopped drug?	Outcome
Before start study medication	Neutropenia	NA	NA	0	Unrelated	NA	Resolved
In-trial	Neutropenia	Levamisole	NA	351	Possible	Yes, relapse	Resolved*
	Neutropenia	Levamisole	NA	131	Possible	Yes, relapse	Resolved
	Neutropenia	Levamisole	NA	90	Probable	Yes, relapse	Resolved
	Neutropenia	Levamisole	NA	225	Possible	No	Resolved
	Hospitalisation, hypovolemia / nephrotic syndrome	Levamisole	NA	70	Unlikely	Yes, relapse	Resolved
	Hospitalisation, pyrexia / headache / vomiting / rash	Levamisole	NA	13	Unrelated	No, but 2 weeks later for relapse	Resolved
	Hospitalisation, hypothermia / asthenia / infection	Levamisole	NA	7	Unknown	No	Resolved
	Hospitalisation, pneumonia	Levamisole	NA	215	Unknown	Yes, relapse	Resolved
	Hospitalisation, abdominal pain	Levamisole	NA	36	Unrelated	No	Resolved
Follow-up	Neutropenia	Levamisole	Levamisole	426	Probable	No, decreased dose	Resolved**
Cohort	Hospitalisation, acute pyelonephritis	Levamisole	Levamisole	372	Unrelated	No	Resolved**

* Neutropenia reported twice and then resolved
** Ongoing at time of reporting but resolved during follow-up
NA: not applicable

Significant AEs are defined according to the ICH E3 guideline (26) as marked haematological and other laboratory abnormalities other than those meeting the criteria for SAE as well as any events that led to an intervention, including discontinuation of study treatment, dose reduction, or significant additional treatment. Ten discontinuations and 3 marked laboratory abnormalities reported as TEAEs were considered significant TEAEs. These included 3 cases of significant neutropenia (1000-1500 106/L), 2 cases of positive ANCA, and 2 cases of increased AST. Single reported significant TEAEs included increased blood creatinine, positive hepatitis B antigen, gastroenteritis, helminthic infection, myalgia and acute renal failure.

All serious and significant TEAEs, except the positive hepatitis B antigen, resolved over time.

No **deaths** have been reported.

Table S4: Significant adverse events

Study phase	Significant TEAE (PT)	RCT treatment	Follow-up / Cohort treatment	Treatment duration (days)	Relationship	Stopped drug?	Outcome
In-trial	ANCA positive / pain in extremity	Levamisole	NA	168	Possible	Yes	Resolved
	Increased blood creatinine	Levamisole	NA	115	Probable	Yes	Resolved
	Hepatitis B antigen positive	Levamisole	NA	66	Unrelated	Yes	Ongoing
	Gastroenteritis	Levamisole	NA	30	Unrelated	Yes, relapse	Resolved
	ANCA positive	Levamisole	NA	272	Possible	Yes, relapse	Resolved
	Increased AST	Levamisole	NA	293	Possible	No	Resolved
	Anal pruritus / helminthic infection	Placebo	NA	13	Unrelated	Yes	Resolved
	Neutropenia	Placebo	NA	198	Unrelated	Yes	Resolved
Follow-up	Neutropenia	Levamisole	Levamisole	365	Definite	Yes	Resolved
	Increased AST / increased ALT	Placebo	Levamisole	597	Possible	Yes	Resolved
	Myalgia	Placebo	Levamisole	468	Probable	Yes	Resolved
Cohort	Acute renal failure	Levamisole	Levamisole	666	Unlikely	No	Resolved
	Neutropenia	Levamisole	Levamisole	729	Probable	No	Resolved

AEs with discontinued study medication due to relapse of SSNS were not included
NA: not applicable

Laboratory findings

Clinically significant drug-related **laboratory abnormalities** included increased AST, increased blood creatinine, and decreased neutrophil count.

The occurrence of **neutropenia** per treatment phase is shown in the table below. Serious neutropenia (<1000 10^6/l) occurred in 4 subjects (8.0%) treated with levamisole versus none in placebo, while significant neutropenia (1000-1500 10^6/l) was observed in 4.0% of subjects treated with placebo. Two cohort subjects had decreased neutrophil count, although neutropenia was reported only after repeated low neutrophil counts were observed while the subject received levamisole treatment.

Table S5: Classification neutropenia clinical study

Neutropenia	RCT		Follow-up		Cohort	
	Levamisole	Placebo	Levamisole	None	Levamisole	None
Serious (<1000 10^6/L)	8.0% (6/50)	0.0%	2.0% (2/100)	0.0%	20.0% (1/5)	0.0%
Significant (1000-1500 10^6/L)	0.0%	4.0% (2/50)	1.0% (1/100)	2.0% (2/100)	0.0%	20.0% (1/5)

Increased AST was reported as a drug-related TEAE in the RCT in 6 subjects treated with levamisole. Two subjects had exceptionally high levels of AST. One patient had a positive hepatitis B antigen test with AST levels of 83 and 112 U/l (2-3x ULN). The presence of hepatitis B was not considered related to levamisole treatment. Another patient had unexplained high levels of AST as high as 1036 and 1121 U/l (25-28x ULN). No positive hepatitis B antigen was found at baseline. The high levels disappeared without any action. For other subjects, increased AST ranged from 44 to 94 U/l (1-2x ULN). All abnormal values resolved spontaneously. During follow-up phase, levamisole was discontinued for increased **AST and ALT** in one subject. No laboratory values were reported on the date of discontinuation.

Levamisole was discontinued due to **increased blood creatinine** in one patient in the RCT. Blood creatinine was consistently increased (63-81 µmol/l) above baseline (43 µmol/l), although it was not above reference values. Other abnormalities or clinical signs were absent. Blood creatinine returned to baseline values after discontinuation of levamisole. One cohort subject had increased blood creatinine (150 µmol/l) reported as acute renal failure. This resolved without any further action. It was considered unlikely related to levamisole.

Safety in special populations

N/A

Immunological events

Haematological abnormalities (including neutropenia, leukopenia and agranulocytosis) have been described in the pivotal clinical trial, literature and named-patient programs to occur in 8, 3 and 1% of the patients, respectively, and most certainly have an immune-mediated cause. In most patients antibodies against leukocytes and/or granulocytes have been found. If undetected, abnormalities will worsen. Haematological abnormalities resolve without sequelae after levamisole treatment is reduced or withdrawn. Starting treatment again (at a lower dosage) may be considered.

Antineutrophil cytoplasmic antibodies (pANCA) positive vasculitis, sometimes resulting in dermal necrosis, or pANCA positive blood levels without symptoms have been reported in the clinical trial (4% vs 0%), literature (1%) and the named patient program (1%). The mechanism is most likely immune-mediated. Vasculitis can eventually lead to very severe skin reactions, which may not recover without sequela. If treatment is stopped immediately when a vasculitis is observed, recovery without sequela is common.

Safety related to drug-drug interactions and other interactions

Discontinuation due to AES

In a total of 13 subjects, levamisole treatment was **withdrawn** due to an AE versus 2 patients in the placebo group. Levamisole was discontinued for a TEAE concurrent with a relapse in RCT in 14.0%, for a TEAE alone in RCT in 6.0% or for a TEAE alone in follow-up in 3.0% of subjects.

Most drug-related TEAEs that led to withdrawal in the study were **known ADRs** concurrent with levamisole treatment (including positive ANCA, neutropenia, increased AST/ALT and myalgia) Increased blood creatinine was not a previously suspected ADR. For no obvious reason, above baseline levels of blood creatinine were sustained during levamisole treatment. After withdrawal, blood creatinine levels returned to baseline.

Neutropenia (<1000 106/L) was reported as serious TEAE in 8.0% of subjects treated with levamisole (versus 0% in placebo group). In 4 out of 5 neutropenias, levamisole was discontinued.

3.4.9. Discussion on clinical safety

The safety data obtained have been presented based on the safety data from the pivotal study, data from literature and serious adverse events reported in the named patient program in France. Any data on the named-patient program of the Netherlands have not been presented and the exact number in the France program is not specified. As levamisole has been used since approximately 1980 for the off

label use in children with nephrotic syndrome, the exposure as currently submitted may be underestimated. Further, additional experience has been obtained with other indications for levamisole, but these data have not been included in the current submission.

The total number of 890 patients exposed to levamisole can be considered reasonable, although these data have mainly been obtained from literature data for which it is uncertain to what extent they have been systematically followed and reported. The number of patients treated with levamisole in controlled studies appears somewhat limited with a total of 249 patients of which only 50 were from the pivotal study. Long term safety data for 12 months and beyond is mainly obtained from literature in controlled studies (n=107 for 12 months) and other literature sources (n=363). Systematically long term follow-up for adverse events is restricted to the pivotal study with a limited number of patients of only 12 patients been treated for 6-12 months and 6 patients for 12 months in the placebo controlled period. The applicant has not mentioned the number of patients and time of exposure in the named patient programs, for which it can be expected that these data are also systematically obtained.

In the pivotal study AEs were reported at a higher frequency for patients treated with levamisole (n=29, 58.0%) than placebo (n=19, 38.0%). Most frequently observed AEs reported at a higher frequency for levamisole were pyrexia (20% vs 12%), cough (14% vs 12%), increased aspartate aminotransferase (12% vs 0%), and neutropenia (8.0% vs 2.0%). Frequently observed AEs of nasopharyngitis was reported more in the placebo group than for levamisole (18% vs 20%). It should be indicated whether this would increase the risk of relapse as it is known that infections could increase this risk. All SAEs were reported in the levamisole group (12 SAEs in 10 subjects) and none in the placebo group. Remarkably, all hospitalisations occurred with levamisole treatment but were assessed as unrelated to study medication. In this respect, it is reassuring that all reasons for hospitalisation were different, so no clear pattern on this could be identified. Instead, the 5 events of neutropenia were considered to be related to study medication. Reassuring is that all except the positive hepatitis B antigen serious and significant adverse events resolved over time. Reassuring is that no deaths were reported.

The most common drug-related TEAEs were increased aspartate aminotransferase (AST) (7 out of 8 found in Indian patients, 12.0%, one severe), neutropenia (8.0%) and positive antineutrophil cytoplasmic antibody (ANCA) (4.0%). Liver abnormalities and blood cell abnormalities are known side effects of levamisole treatment as they were already reported during earlier studies and during named patient programs, although in a considerably lower frequency of 0.3 and 0.5% for liver abnormalities (AST/ALT) and 3% and 1% for blood cell abnormalities (neutropenia), respectively. Consistently, increased AST and decreased neutrophil count have also been identified as laboratory abnormalities in the current pivotal studies. Further, two cases of creatinine increase have been observed and were considered unrelated to study medication. However, this may be questioned as these abnormalities resolved after discontinuation of levamisole. Therefore, it is proposed to include this as an additional identified risk. Also ANCA is a known side effect and also reported at a lower frequency in literature (1%) and named patient program (1%). Further related AEs reported are cutaneous reactions, infections and gastro-intestinal symptoms. These have been obtained from 44 literature studies or literature case reports ranging from 1980 to 2015. In the named patient program of France 13 patients (out of probably 200 patients) reported a drug-related AE. Those were generally similar as the related AEs already identified.

The total number of discontinuations due to adverse events in the pivotal study appear acceptable, although it is not entirely clear how many patients discontinued due to AEs as this has not been systematically been described. The most prominent adverse event that has led to treatment discontinuation is neutropenia (4 out of 5 patients with neutropenia), and most likely have an

immunological cause since antibodies have been found in most cases (although not reported in detail). It is known that they may be severe as they may worsen if left untreated and thus need regular monitoring, though they resolve with discontinuation of treatment. Another 4 patients discontinued due to other AEs in the pivotal study, due to positive ANCA, neutropenia, increased AST/ALT and myalgia. However, as mentioned, overall discontinuation data have not exactly been presented. Of note, the data are also inconsistent with the general disposition table indicating that only 1 patient each in the levamisole arm and placebo arm discontinued study due to an AE. The applicant should clarify this inconsistency and better describe discontinuations due to AEs.

Data of treatment with levamisole longer than 12 months come from the cohort phase, although these data are not controlled and this only included 5 patients, which substantially limits any possible evaluation on this. The reported AEs were generally similar to those reported in the controlled phase, except for single reports of acute pyelonephritis, arthralgia, migraine, acute renal failure and epistaxis. However, these were all not considered related to study drug. Any data on reproductive and developmental toxicity have not been assessed as the long term data base is probably too limited for such an assessment.

The presentation of AEs during the follow-up period is considered also of less relevance as patients were also unblinded and uncontrolled. Apparently 48 patients were still treated with levamisole at follow-up of which twenty one subjects reported an AE. Apart from AEs already known, single reports of skin reactions, vomiting, herpes virus infection, increased ALT, and myalgia were described as drug-related during the follow-up period, while not being reported during the controlled period.

3.4.10. Conclusions on clinical safety

The safety profile has been investigated in the pivotal study, literature and named patient programs. In general, the systematically evaluated limited controlled data from the pivotal study demonstrate that the most prominent drug related adverse events are neutropenia, liver enzyme abnormalities and ANCA, which are known AEs from literature and named patients program although reported at a higher frequency in the pivotal study. In general levamisole can be considered well tolerated with an acceptable number of discontinuations (although this should be exactly reported) with neutropenia is the most prominent AE leading to discontinuation and requiring regular monitoring. Although a considerable number of patients have been treated for 12 months of longer, known systematically evaluated long term safety data (from the pivotal study) are limited.

3.5. Risk management plan

Summary of safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table R1: Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	Potential for medication errors Neutropenia, Leucopenia and agranulocytosis pANCA positive vasculitis Increase in aminotransferases
Important potential risks	Off-label use in adults Pregnancy, breastfeeding or planned pregnancy
Missing information	None

3.5.1. Discussion on safety specification

It is supported that there is a potential for medication errors as the current differences in the 4 strengths tablets are only size and inscription. Further, as discussed in the safety section, drug related adverse events of neutropenia, leucopenia and agranulocytosis, pANCA positive vasculitis, and increase in aminotransferases have been identified, although this is systematically evaluated in a limited number of placebo controlled data in the pivotal study.

Creatinine increase has been observed in 2 patients, and whether this is related to levamisole treatment remains unclear. Therefore, this should be included as a potential risk.

Systematically long term follow-up for adverse events is restricted to the pivotal study with a limited number of patients of only 12 patients been treated for 6-12 months and 6 patients for 12 months in the placebo controlled period. Therefore, long term safety is limited and should be included in the safety specifications.

The likelihood of misuse of illegal purposes is limited. Further, there is a potential risk for off label use in adults as, although even less frequent, SSNS also occurs in adults.

3.5.2. Conclusions on the safety specification

Having considered the data in the safety specification the CHMP considers that the following issues should be addressed:

- The CHMP considers that the possible risk on drop in eGFR and the uncertainty on long term safety should also be safety concern.

3.6. Pharmacovigilance system

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

4. Orphan medicinal products

Orphan designation

On 28 October 2005, orphan designation (EU/3/05/324) was granted by the European Commission to ACE Pharmaceuticals BV, The Netherlands, for levamisol hydrochloride for the treatment of nephrotic syndrome.

According to the conclusion of the COMP the prevalence of neprotic syndrome is 1 per 10000 individuals in the EU.

Similarity

The application did not contain a critical report pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, addressing the possible similarity with authorised orphan medicinal products. As there are currently no authorised products for this condition which have obtained maintenance of the Orphan Designation and therefore a 10yr Market Exclusivity, there is no need for a similarity report.

5. Benefit risk assessment

5.1. Therapeutic Context

5.1.1. Disease or condition

Nephrotic syndrome is characterised by glomerular leakage of proteins, which result in symptoms of hypoproteinaemia and generalised oedema. In children, the incidence of nephrotic syndrome in Europe, North America and New Zealand is about 2/100,000 children younger than 18 years, and a prevalence of 16 per 100 000 children. There is clear geographical variation, with a higher incidence reported in South Asian populations (3–9 per 100 000 children).

Levamisole is indicated for the treatment of Steroid Sensitive Nephrotic syndrome (SSNS) in children and adolescents from 2 years up to 18 years.

The aim of the treatment of SSNS in children is to induce and maintain complete remission with resolution of proteinuria and edema, maintenance of kidney function without encountering serious adverse effects of therapy. However, after a first episode of relapse, mostly starting in children between 2-5 years, a large proportion experience a relapsing course with recurrent episodes of oedema and proteinuria, which needs treatment to achieve remission, either periodically or continuously depending on severity of disease with associated risk for future relapses.

5.1.2. Available therapies and unmet medical need

The first-line treatment for children presenting with idiopathic nephrotic syndrome is oral corticosteroids. Of children who present with their first episode of nephrotic syndrome, 80% to 90% will achieve remission with corticosteroid therapy, and have steroid-sensitive nephrotic syndrome (SSNS). By treating relapses with periodically regimens of corticosteroids or prevent further relapses with continuously low dose corticosteroid (steroid dependent) many patients experience significant adverse effects, including impaired linear growth, behavioural changes, obesity, Cushing's syndrome, hypertension, ophthalmological disorders, impaired glucose tolerance, and reduced bone mineral density. Therefore, limiting the long-term adverse effects of corticosteroid treatment by secondary corticosteroid sparing agents is an important objective.

Possible off label second line therapies to prevent the risk of relapses include alkylating agents (cyclophosphamide and chlorambucil), levamisole, calcineurin inhibitors (cyclosporine, tacrolimus), MMF and rituximab. Clinical guidelines do not recommend the one agent over another, in particular due to omission of robust clinical comparison data and due to differences in their safety profile. In the Netherlands cyclosporine is the only registered drug for steroid resistant (SRNS) or steroid dependent (SDNS) nephrotic syndrome. According to the applicant, an advantage of levamisole over other treatments would be that it has less severe side effects.

5.1.3. Main clinical study

Demonstrating reductions in (corticosteroid free) relapses could be considered of clinical relevance, particularly considering that commonly a relapse needs further treatment with corticosteroids which have been associated with considerable side effects. Limiting the long-term adverse effects of corticosteroid treatment is an important objective.

The applicant has submitted one multi-centre, double-blind, placebo-controlled, randomised trial in which a number of 100 children from 2 to 18 years of age with steroid sensitive nephrotic syndrome (75% steroid dependent) were treated for 12 months with levamisole or placebo to evaluate whether levamisole could reduce the time to relapse. The commonly used off-label dose of 2.5 mg/kg of levamisole has been used in this study. The specific tablets for children were coated to mask the bitter taste of levamisole, which should last for at least 30 seconds to secure blinding.

Patients were randomized between levamisole and placebo and stratified by region, steroid dependency and prior cyclofosfamide use; Caucasians are found to be more steroid dependent than patients from India.

At inclusion, patients were directly treated with a down titration scheme of prednisone according to the protocol of the French Society of Nephrology Corticosteroids. Various corticosteroid down-titration schemes exist and the French variant is one commonly used in clinical practice. The study does not directly address the possibility of a steroid-sparing effect, in particular for those dependent on corticosteroid treatment due to the fixed down titration scheme of prednisone. Those patients who are steroid dependent were off corticosteroid treatment after approximately 100-120 days without continuation of low dose corticosteroid treatment.

Further, the study does not address whether levamisole would be more appropriate to use than other secondary treatment option such as cyclosporine, cyclophosphamide, and mycophenolate mofetil.

5.2. Favourable effects

The pivotal study demonstrated that one year of levamisole treatment resulted in a lower number of relapses in the levamisole group (n=34) when compared with the placebo group (n=42), with a statistically significant longer time to relapse (HR 0.564; 95% confidence interval (CI): 0.354-0.899 and p=0.0160) compared to placebo. This was demonstrated in a population of children between 2 to 16 years with a mean age of 6 years, in the majority male, with a large proportion of steroid dependency (75%), especially amongst Caucasians who were all recruited in Europe, while half of the population was recruited in India, and almost none priorly treated with second line drugs.

Subgroup analyses according to age, ethnicity, region, steroid dependency and prior cyclophosphamide use demonstrated comparable results as the main analysis. Some less effect could be observed in Caucasians, steroid dependent patients and Europe. No statistical assessment for interactions has been mentioned. Of note: all patients in Europe were Caucasian, and were mostly steroid dependent.

5.3. Uncertainties and limitations about favourable effects

The applicant mentions that the average quantity of steroids administered during the study was not evaluated due to incomplete recording of steroid intake. This questions the validity of the primary analysis, in particular during the period when patients were still down titrated with prednisone. Non-compliance to prednisone intake could initiate a relapse event and thus highly interfere with the primary analysis of the study. Also, this questions the value of exploratory analyses performed on comparison of prednisone intake between treatment arms and compared to intake before study entry (see also further below).

A large proportion of the included steroid sensitive patients were classified as steroid dependent at baseline and were not treated with (low dose) steroids during the study after the corticosteroid down titration scheme had ended. Therefore, the recurrence rate in the placebo might not be necessary comparable with the rate to be expected in optimally treated patients in clinical practice.

For the primary analysis, the Kaplan Meier curves show that the hazards of the occurrence of relapse changed over time with a higher hazard rate of relapse with levamisole in the beginning of treatment. A crossing of lines is observed after about 100-120 days of treatment and the effect of levamisole improves compared to placebo towards the end of one year of treatment. Subgroup analyses provided similar K-M curves. The initial period in which levamisole appears to be less effective coincide with the remaining prednisone treatment. A smaller (n=70) multicentre study in the UK, with a shorter follow-up (112 days) also showed and effect in the K-M lines after discontinuation of steroid treatment (BAPN, Lancet, 1991). Further analyses which could clarify the observed initial effect have not been provided.

A secondary analysis demonstrated a significant longer time to relapse after prednisone cessation (starting 100-120 days after run-in) for levamisole compared to placebo (HR 0.373 (95% CI: 0.200-0.699 and p=0.0021)). However, this landmark analysis, started when the prednisone dose scheme was ended for each patients at approximately 100-120 days after study randomisation. This might potentially introduce serious flaws like “survivor treatment bias” which make this result difficult to interpret, e.g. approximately 40% of patients were already out of trial mainly because of reaching the primary endpoint (32 patients still on treatment with levamisole and 29 patients on placebo).

Four small literature studies including 40 to 70 patients reported a beneficial effect of treatment with levamisole. Of those, four of those were performed outside Europe, four were single centre, three were 6 months or less, and publication ranged from 1991 to 2006. In two additional studies where

levamisole was dosed two times a week no significant effect was detected. One other study found no difference between cyclophosphamide and levamisole.

There are discrepancies in objectives and outcomes as described in the study protocols (SAP, study protocol version 6.0) and clinical study report which needs to be justified further.

The exact mechanism of action of levamisole is unknown according to the company. Two mechanisms are expected to contribute to the effect of levamisole in steroid sensitive nephrotic syndrome, immunomodulation and the direct action on kidney podocytes mediated by the glucocorticoid signalling pathway. Further, large proportions on the information on pharmacokinetic properties have not been described and no reliable pharmacokinetic data are available in the intended patient group, and as such reliable safety exposure data is lacking.

The prednisone dose at time of relapse had a mean of 28.2 ±37.4 (0-120) mg/m^2/AD in the levamisole group (n=34) and a mean of 16.1 ±25.53 (0-120) mg/m^2/AD in the placebo group (n=42), and strangely enough a median dose of 0 in both treatment groups. This is not understood and needs further justification.

Any data on the use of the tablets with regard to testing of usability and medication errors have not been provided. The company states that no patients experienced any problems with regard to usability.

5.4. Unfavourable effects

In the pivotal RCT, more subjects in the levamisole group (n=29, 58.0%) experienced **adverse events** than in the placebo group (n=19, 38.0%). Most frequently observed AEs reported at a higher frequency for levamisole were pyrexia (20% vs 12%), cough (14% vs 12%), increased aspartate aminotransferase (12% vs 0%), and neutropenia (8.0% vs 2.0%). Frequently observed AEs of nasopharyngitis were reported more in the placebo group than for levamisole (18% vs 20%).

All **serious AEs** were reported in the levamisole group (12 SAEs in 10 subjects) and none in the placebo group.

The most common **drug-related AE** was **increased aspartate aminotransferase (AST)** (12.0%). Two subjects had exceptionally high levels of AST (25-28x ULN). All abnormal values resolved spontaneously except for 1 case in the follow-up phase. **Neutropenia** was reported in 8.0% in levamisole treated patients. These most likely have an immunological cause since antibodies have been found in most cases (although not reported in detail). Increased AST and neutropenia are generally serious adverse events and may worsen if left untreated. Therefore, these led to treatment discontinuation in 4 out of 5 cases (1 placebo) in the RCT. **Positive antineutrophil cytoplasmic antibody (pANCA)** was reported in 4.0% in levamisole treated patients. The mechanism is most likely immune-mediated. PANCA mediated vasculitis can eventually lead to very severe skin reactions, which may not recover without sequela. Both patients discontinued (1 had relapse) and the AE resolved.

No **deaths** have been reported.

5.5. Uncertainties and limitations about unfavourable effects

Long term safety data for 12 months and beyond is mainly obtained from literature in controlled studies (n=107 for 12 months) and other literature sources (n=363). Systematically long term follow-

up for adverse events is restricted to the pivotal study with a limited number of patients of only 12 patients been treated for 6-12 months and 6 patients for 12 months in the placebo controlled period. A limited number of five patients were on 12 to 18 months open label long term treatment with levamisole during the cohort phase of the pivotal study. In relation to this, data on non-clinical safety aspects have currently only limitedly described.

The most common drug-related AEs of increased aspartate aminotransferase (AST), neutropenia and positive antineutrophil cytoplasmic antibody (pANCA) were reported at a lower frequency in literature (0.3%, 3%, 1%) and named patient program (0.5%, 1%, 1%) than in the pivotal study (12%, 8%, 4%). One patient in the RCT had a very high AST increase at two occasions in the RCT for unknown reasons. Hence, further clarification is requested.

Two cases of creatinine increase have been observed and were considered unrelated to study medication. However, these abnormalities resolved after discontinuation of levamisole. It is proposed to include this as potential risk, see RMP. Further, the mechanism and the relevance are unclear and need further clarification.

Serious adverse events of hospitalisations occurred with levamisole treatment but were assessed as unrelated to study medication. In this respect, it is reassuring that all reasons for hospitalisation were different, so no clear pattern on this could be identified.

No data is presented confirming the metabolic pathways for elimination of levamisole and its metabolites and no data is presented whether levamisole and its metabolites may interfere with other metabolic pathways (inhibition and/or induction) at CYP enzyme level and transporter level. Considering that the patient group will likely receive concomitant treatment and medications, the lack of these data is of concern as the interaction potential is unclear.

Frequently observed AEs of nasopharyngitis was reported more in the placebo group than for levamisole (18% vs 20%). It is uncertain whether this could increase the risk of relapse as it is known that infections could increase this risk.

The histological finding of perivascular cellular infiltration indicates an immunological origin of the neurological adverse events. Neurologic symptoms in humans are rare, and most often were observed when levamisole was administered together with 5-FU. In most cases foci resembling demyelination were visible in brain imaging. These probably correspond to cellular infiltrates seen histologically. It is not known whether neurologic events are dose-dependent or whether they will also develop over time with normal therapeutic doses. It should be noted that levamisole is usually administered for one or a few days only in the standard anti-helminthic indication so that experience with longer-term use is limited. However, one case in a child with a single but inadvertently too high dose of levamisole was reported. The Applicant should discuss whether certain clinical signs could be indicative for (beginning) neurological impairment and could be monitored.

Regarding cardiac effects of levamisole, the mechanism is unclear. In animals, the effects can be observed early after initiation of treatment and are most pronounced after IV injection. This argues for an acute effect which is independent from the immune system and which may depend on high plasma concentrations. The nature of the effects (extrasystoles, prolongation of AV conduction) argues for an effect on certain ion channels, e.g. calcium channels. In part, in particular in respect to AV conduction, a cholinergic effect of levamisole could also play a role (it should be noted that levamisole's anthelminthic action depends on activation of cholinergic receptors in the worms). For further clarification, the applicant should analyse the ECG recordings of the study patients in more detail and report also minor findings which could give a hint to the underlying mechanism and to the clinical

relevance. Furthermore, the Applicant should provide information whether there were observations in the trial which could be indicative for cholinergic effects in children.

5.6. Effects Table

Table BR1. Effects Table for levamisole for the treatment of Steroid Sensitive Nephrotic syndrome (SSNS) in children and adolescents from 2 years up to 18 years (data cut-off: December 2012 (RCT) – October 2013 (cohort).

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
Primary endpoint	Time to relapse	n	Levamisole 34 out of N=50	Placebo 42 out of N=50	HR 0.546 (95% CI: 0.354-0.899) at 12 months K-M curves crossed at 100-120 days	
Secondary endpoint	Time to relapse after prednisone cessation	N	Levamisole N=32	Placebo N=29	HR 0.373 (95% CI: 0.200-0.699) Landmark analysis; differences in baseline and number of patients as analysis started at 100-120 days	
Unfavourable Effects						
Immunological effects (likely)	Neutropenia	%	8%	1%	Serious events, 4 out of 5 treatment discontinuations	
	pANCA	%	4%	0%	Both discontinued treatment (1 during relapse)	
Liver effects	Increase in AST	%	12%	0%	2 serious increases, no treatment discontinuations during RCT	

Abbreviations: AST= aspartate aminotransferase**,** pANCA= positive antineutrophil cytoplasmic antibody
Notes:

5.7. Benefit-risk assessment and discussion

5.7.1. Importance of favourable and unfavourable effects

Patients with steroid sensitive nephrotic syndrome (SSNS) are treated with corticosteroids at time of relapse. In steroid dependent patients prophylaxis with corticosteroid is necessary to reduce the number of relapses. This prevents associated symptoms and indirectly long term renal and mortality outcome. However, continued long term corticosteroid treatment comes with several (long term) serious side effects. Several second line preventive treatment options, to reduce the steroid burden, have been recommended in clinical guidelines for off label use (although cyclosporine has an indication in the Netherlands) in these children without any clear preferences due to differences in the safety

profile, also including serious side effects, and limited comparative data. Levamisole has been recognized as one of these treatment options in international and national guidelines. Due to the low incidence of the disease an orphan designation for levamisole has been assigned already in 2005 (Orphan Designation EU/3/05/324), however, this will be reassessed by COMP in case of a positive outcome at CHMP.

Levamisole has been used for some decades for SSNS. The few small studies found in literature, currently submitted by the applicant, show some efficacy when levamisole is treated on alternate days. However, the studies have relevant limitations in design, number of patients included, reporting of data and relevance to the European setting. A recent Cochrane systemic meta-analysis (2013) also concluded that the quality of the evidence was low, due to unclear allocation concealment, blinding problems, and significant heterogeneity between studies. Despite these limited data, levamisole has been used in clinical practice as a secondary treatment option.

The current submitted pivotal study to support the indication of SNSS in children from 2 to 18 years and including the largest number of patients until now (n=100) has demonstrated that number of relapses could be reduced after 12 months in patients dominantly with a steroid dependent nephrotic syndrome. Submission of one confirmatory study (with additional supportive literature data) could be acceptable considering the orphan status of the disease. However, a serious uncertainty is that corticosteroid intake in the down-titration scheme has not been appropriately followed. This limits a clear assessment and interpretation of the primary study results, in particular for the period that patients were on a down-titration scheme of prednisone. While this also seriously questions whether the study has been conducted according to GCP recommendations, a GCP inspection has currently been requested by EMA and will start early 2017. An inspection conclusion would probably be available at the earliest when the Central Procedure has been steadily progressed. Furthermore, the relevance of the submitted study for clinical practice has limitations for steroid dependent patients in the study. In daily clinical practice these patients would likely to be treated with a continuous low dose of corticosteroid for a longer period of time to prevent relapse. Therefore, this does not directly answer the question whether levamisole could provide an alternative to reduce time to relapse and consequently the prednisone burden in those patients. Such as study would need comparison between treatment arms on accumulative prednisone dose and relapse rates during a certain amount of time to answer such a hypothesis, although feasibility of such a study is subject to discussion. The applicant should discuss the implications of this for the proposed labelling. Of further interest is that the primary analysis data over time (K-M curve) demonstrates a crossing of lines with an initial worse effect of levamisole compared to placebo, mainly during the prednisone down-titration background therapy. This effect could currently not be clarified by any subgroup analyses or any other way.

Short-term safety data have been partly obtained from literature data of prospective studies, case reports and retrospective reviews, due to off label experience with levamisole in treating nephrotic syndrome. However, whether these adverse events have been systematically monitored and reported remains questionable, in particular taking the notion that common drug-related events were reported at a lower frequency than in the pivotal study. The current pivotal study was placebo controlled and therefore provided systematic reporting of adverse events. In general, these safety data are limited due to the limited number of patients included, in particular for long term use, which is common when it concerns paediatric data, but also because of the low incidence of the disease. Despite this limitation, some confirmation has been obtained on known adverse events of which neutropenia, increased AST, and pANCA are most frequently drug associated reported adverse events, but are also considered serious with neutropenia often leading to treatment discontinuation. Guidelines already recommend regular patient monitoring and SmPC recommendations could similarly request for regular

monitoring, therefore, these adverse events could be timely identified and appropriate measures could be taken, which is often interruption or discontinuation of therapy.

Systematically obtained long term safety data is limited and is therefore uncertain, while long term use of levamisole is likely indicated when a positive benefit risk could be identified. Also, uncertainty exists on observed large reduction in GFR upon levamisole treatment, for which any association with the underlying disease cannot be excluded. These safety limitations have currently not been included in the in the RMP, but are requested to be addressed. This is also important considering that limitations and uncertainties exist on the pre-clinical, pharmacokinetic and pharmacodynamic data submitted.

5.7.2. Balance of benefits and risks

In conclusion, the conduct and the obtained efficacy results of the pivotal study have currently some uncertainties to determine whether levamisole could be beneficial as a treatment option in children with SNSS, dominantly SDNS. Levamisole has some serious side effects which appear to be manageable, although long term safety remains uncertain, while levamisole is likely to be indicated for long term use when the benefit risk is positive. Levamisole is currently used as secondary treatment option in clinical practice.

Overall, the benefit/risk is currently undetermined.

5.7.3. Additional considerations on the benefit-risk balance

A study in France as registered on clinicaltrials.gov is currently conducted named: *"A Multicenter, Randomised, Double-blind Placebo-controlled Trial Assessing the Efficiency of Levamisole for Maintaining Remission After the First Flare of Steroid Sensitive Nephrotic Syndrome in Children."* This concerns a less severe patient population than for the current submitted pivotal study.

Also, levamisole is currently investigated for warm antibody autoimmune haemolytic anaemia as well.

5.8. Conclusions

Currently the overall B/R of Elmisol (levamisole) is negative.